Treatment and Prevention of Cancer Using HER3 Antigen-Binding Molecules

This application claims priority from GB 1913079.8 filed 11 September 2019, the contents and elements of which are herein incorporated by reference for all purposes.

Field of the Invention

The present invention relates to the fields of molecular biology, more specifically antibody technology and methods of medical treatment and prophylaxis.

Background to the Invention

Increased HER3 expression is linked to poor prognosis in multiple solid tumors, including breast, gastric, head & neck, pancreatic, ovarian, and lung cancers. HER3-mediated signalling has adverse consequences for tumour progression; HER3 upregulation is associated with resistance to anti-HER2 and anti-EGFR therapy, and solid tumors refractory to anti-PD-1 therapy have been shown to have higher HER3 expression compared to responders to anti-PD-1 therapy.

HER3-binding antibodies are described e.g. in Zhang et al., Acta Biochimica et Biophysica Sinica (2016) 48(1): 39–48. The anti-HER3 antibody LJM-716 binds to an epitope on subdomains II and IV of the HER3 extracellular domain, locking HER3 in the inactive conformation (Garner et al., Cancer Res (2013) 73: 6024–6035). MM-121 (also known as seribantumab) has been shown to inhibit HER3-mediated signalling by blocking binding of heregulin (HRG) to HER3 (Schoeberl et al., Sci. Signal. (2009) 2(77): ra31). Patritumab (also known as U-1287 and AMG-888) also blocks binding of heregulins to HER3 (see e.g. Shimizu et al. Cancer Chemother Pharmacol. (2017) 79(3):489–495. RG7116 (also known as lumretuzumab and RO-5479599) recognises an epitope in subdomain I of the HER3 extracellular domain (see e.g. Mirschberger et al. Cancer Research (2013) 73(16) 5183-5194). KTN3379 binds to HER3 through interaction with amino acid residues in subdomain III (corresponding to the following positions of SEQ ID NO:1: Gly476, Pro477, Arg481, Gly452, Arg475, Ser450, Gly420, Ala451, Gly419, Arg421, Thr394, Leu423, Arg426, Gly427, Lys356, Leu358, Leu358, Lys356, Ala330, Lys329 and Gly337), and Met310, Glu311 and Pro328 of subdomain II (see Lee et al., Proc Natl Acad Sci U S A. 2015 Oct 27; 112(43):13225). AV-203 (also known as CAN-017) has been shown to block binding of NRG1 to HER3 and to promote HER3 degradation (see Meetze et al., Eur J Cancer 2012; 48:126). REGN1400 also inhibits binding of ligand to HER3 (see Zhang et al., Mol Cancer Ther (2014) 13:1345–1355). RG7597 (duligotuzumab) is a dual action Fab (DAF) capable of binding to both HER3 and EGFR, and binds to subdomain III of HER3 (see Schaefer et al., Cancer Cell (2011) 20(4):472-486). MM-111 and MM-141 are bispecific antibodies having HER3-binding arms which inhibit HRG ligand binding to HER3 (see McDonagh et al. Mol Cancer Ther (2012) 11:582–593 and Fitzgerald et al., Mol Cancer Ther (2014) 13:410-425).

Summary of the Invention

The present invention provides an antigen-binding molecule which is capable of binding to HER3 according to any embodiment described herein, for use in a method of treating or preventing a cancer in a subject, wherein the cancer comprises cells characterised by HER3 ligand expression/overexpression.

Also provided is the use of an antigen-binding molecule which is capable of binding to HER3 according to any embodiment described herein, in the manufacture of a medicament for use in a method of treating or preventing a cancer in a subject, wherein the cancer comprises cells characterised by HER3 ligand expression/overexpression.

Also provided is a method of treating or preventing a cancer in a subject, wherein the cancer comprises cells characterised by HER3 ligand expression/overexpression, wherein the method comprises administering a therapeutically or prophylactically effective amount of an antigen-binding molecule which is capable of binding to HER3 according to any embodiment described herein to the subject.

In some embodiments in accordance with the various aspects of the invention, the cancer comprises cells having a mutation resulting in increased expression of a ligand for HER3.

More particularly, the present invention provides an antigen-binding molecule which is capable of binding to HER3 for use in a method of treating or preventing a cancer in a subject, wherein the cancer comprises cells having a mutation resulting in increased expression of a ligand for HER3, and wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:43

HC-CDR2 having the amino acid sequence of SEQ ID NO:46

HC-CDR3 having the amino acid sequence of SEQ ID NO:51; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:91

LC-CDR2 having the amino acid sequence of SEQ ID NO:94

LC-CDR3 having the amino acid sequence of SEQ ID NO:99.

Also provided is the use of an antigen-binding molecule which is capable of binding to HER3 in the manufacture of a medicament for use in a method of treating or preventing a cancer in a subject, wherein the cancer comprises cells having a mutation resulting in increased expression of a ligand for HER3, and wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:43

HC-CDR2 having the amino acid sequence of SEQ ID NO:46

HC-CDR3 having the amino acid sequence of SEQ ID NO:51; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:91

LC-CDR2 having the amino acid sequence of SEQ ID NO:94

LC-CDR3 having the amino acid sequence of SEQ ID NO:99.

Also provided is a method of treating or preventing a cancer in a subject, wherein the cancer comprises cells having a mutation resulting in increased expression of a ligand for HER3, wherein the method comprises administering a therapeutically or prophylactically effective amount of an antigen-binding

molecule which is capable of binding to HER3 to the subject, and wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:43

HC-CDR2 having the amino acid sequence of SEQ ID NO:46

HC-CDR3 having the amino acid sequence of SEQ ID NO:51; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:91

LC-CDR2 having the amino acid sequence of SEQ ID NO:94

LC-CDR3 having the amino acid sequence of SEQ ID NO:99.

In some embodiments in accordance with the various aspects of the invention, the ligand for HER3 comprises an amino acid sequence having at least 60% sequence identity to the EGF-like domain of an NRG.

In some embodiments the cancer comprising cells having an NRG gene fusion. In some embodiments the NRG gene fusion is selected from *CLU-NRG1, CD74-NRG1, DOC4-NRG1, SLC3A2-NRG1, RBPMS-NRG1, WRN-NRG1, SDC4-NRG1, RAB2IL1-NRG1, VAMP2-NRG1, KIF13B-NRG1, THAP7-NRG1, SMAD4-NRG1, MDK-NRG1, TNC-NRG1, DIP2B-NRG1, MRPL13-NRG1, PARP8-NRG1, ROCK1-NRG1, DPYSL2-NRG1, ATP1B1-NRG1, CDH6-NRG1, APP-NRG1, AKAP13-NRG1, THBS1-NRG1, FOXA1-NRG1, PDE7A- NRG1, RAB3IL1-NRG1, CDK1-NRG1, BMPRIB-NRG1, TNFRSF10B-NRG1, MCPH1-NRG1 and SLC12A2-NRG2.* In some embodiments the NRG gene fusion is selected from *CLU-NRG1, CD74-NRG1, SLC3A2-NRG1 or VAMP2-NRG1.*

In some embodiments the cancer derives from the lung, breast, head, neck, kidney, ovary, pancreas, prostate, uterus, gallbladder, colon, rectum, bladder, soft tissue or nasopharynx.

In some embodiments the cancer is selected from lung cancer, non-small cell lung cancer, lung adenocarcinoma, invasive mucinous lung adenocarcinoma, lung squamous cell carcinoma, breast cancer, breast carcinoma, breast invasive carcinoma, head and neck cancer, head and neck squamous cell carcinoma, renal cancer, renal clear cell carcinoma, ovarian cancer, ovarian serous cystadenocarcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, prostate cancer, prostate adenocarcinoma, endometrial cancer, uterine carcinosarcoma, gallbladder cancer, cholangiocarcinoma, colorectal cancer, bladder cancer, urothelial bladder cancer, sarcoma, soft tissue sarcoma, neuroendocrine tumor and neuroendocrine tumor of the nasopharynx. In some embodiments the cancer is selected from lung cancer, non-small cell lung cancer, lung adenocarcinoma, invasive mucinous lung adenocarcinoma and lung squamous cell carcinoma.

In some embodiments the antigen-binding molecule comprises:

(i) a VH region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:48; and

(ii) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

In some embodiments the antigen-binding molecule comprises:

a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:36; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:83.

In some embodiments the antigen-binding molecule comprises:

a VH region incorporating the following framework regions (FRs):

HC-FR1 having the amino acid sequence of SEQ ID NO:53

HC-FR2 having the amino acid sequence of SEQ ID NO:59

HC-FR3 having the amino acid sequence of SEQ ID NO:66

HC-FR4 having the amino acid sequence of SEQ ID NO:71.

In some embodiments the antigen-binding molecule comprises:

a VL region incorporating the following framework regions (FRs):

LC-FR1 having the amino acid sequence of SEQ ID NO:104

LC-FR2 having the amino acid sequence of SEQ ID NO:110

LC-FR3 having the amino acid sequence of SEQ ID NO:120

LC-FR4 having the amino acid sequence of SEQ ID NO:125.

In some embodiments the antigen-binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171.

In some embodiments the antigen-binding molecule comprises a light chain comprising the amino acid sequence of SEQ ID NO:177.

Description

Known anti-HER3 antibodies fall broadly into two classes. Antibodies of the first class bind to domains I and/or III of HER3, and thereby competitively inhibit ligand binding to HER3. Seribantumab (MM-121) is a representative member of this class, and other members include patritumab (U3–1287 or AMG-888), lumretuzumab (RG-7116), AV-203, GSK2849330 and REGN1400. Antibodies of the second class lock HER3 in an inactive conformation, through binding to the interface between domains II and IV, or between domains II and III. LJM-716 is a representative example of this class, as is KTN3379.

The present invention relates to novel HER3-binding molecules having improved properties as compared to known anti-HER3 antibodies.

The inventors undertook the targeted generation of antigen-binding molecules which bind to particular regions of interest in the extracellular region of HER3. The HER3-binding molecules of the present invention are provided with combinations of desirable biophysical and/or functional properties as compared to antigen-binding molecules disclosed in the prior art.

In embodiments of the present invention the antigen binding molecules are capable of binding to the subdomain II of the extracellular region of HER3 (SEQ ID NO:16), and inhibit association of the bound HER3 molecule with interaction partners.

In particular, HER3-binding antigen-binding molecules described herein are demonstrated to bind to an epitope of HER3 providing for (i) potent inhibition of association of HER3 with interaction partners (e.g. EGFR, HER2) and (ii) high-affinity binding to HER3 both in the presence and absence of NRG ligand. This unique combination of properties provides for strong inhibition of downstream signalling and exceptional anti-cancer activity against a wide range of cancers.

HER3

HER3 (also known e.g. as ERBB3 LCCS2, MDA-BF-1) is the protein identified by UniProt P21860. Alternative splicing of mRNA encoded by the human *ERBB3* gene yields five different isoforms: isoform 1 (UniProt: P21860-1, v1; SEQ ID NO:1); isoform 2 (UniProt: P21860-2; SEQ ID NO:2), which comprises a different sequence to SEQ ID NO:1 from position 141, and which lacks amino acid sequence corresponding to positions 183 to 1342 of SEQ ID NO:1; isoform 3 (UniProt: P21860-3; SEQ ID NO:3), which comprises the substitution C331F relative to SEQ ID NO:1, and which lacks the amino acid sequence corresponding to positions 332 to 1342 of SEQ ID NO:1; isoform 4 (UniProt: P21860-4; SEQ ID NO:4), which lacks the amino acid sequence corresponding to positions 1 to 59 of SEQ ID NO:1; and isoform 5 (UniProt: P21860-5; SEQ ID NO:5), which lacks the amino acid sequence corresponding to positions 1 to 643 of SEQ ID NO:1.

The N-terminal 19 amino acids of SEQ ID NOs:1 to 3 constitute a signal peptide, and so the mature form of HER3 isoforms 1, 2 and 3 (i.e. after processing to remove the signal peptide) have the amino acid sequences shown in SEQ ID NOs:6, 7 and 8, respectively.

The structure and function of HER3 is described e.g. in Cho and Leahy Science (2002) 297 (5585):1330-1333, Singer et al., Journal of Biological Chemistry (2001) 276, 44266-44274, Roskoski et al., Pharmacol. Res. (2014) 79: 34–74, Bazley and Gullick Endocrine-Related Cancer (2005) S17-S27 and Mujoo et al., Oncotarget (2014) 5(21):10222-10236, each of which are hereby incorporated by reference in their entirety. HER3 is a single-pass transmembrane ErbB receptor tyrosine kinase having an N-terminal extracellular region (SEQ ID NO:9) comprising two leucine-rich subdomains (domains I and III, shown in SEQ ID NOs:15 and 17, respectively) and two cysteine-rich subdomains (domains II and IV, shown in SEQ ID NOs:16 and 18, respectively). Domain II comprises a β hairpin dimerisation loop (SEQ ID NO:19) which is involved in intermolecular interaction with other HER receptor molecules. The extracellular region is linked via a transmembrane region (SEQ ID NO:10) to a cytoplasmic region (SEQ ID NO:11). The

cytoplasmic region comprises a juxtamembrane segment (SEQ ID NO:12), a protein kinase domain (SEQ ID NO:13), and a C-terminal segment (SEQ ID NO:14).

Signalling through HER3 involves receptor homodimerisation (i.e. with other HER3 receptors) or heterodimerisation (with other HER receptors, e.g. HER2) and consequent autophosphorylation by the protein kinase domain of tyrosines of the cytoplasmic region. The phosphorylated tyrosine residues recruit adaptor/effector proteins (e.g. Grb2 and phospholipase Cγ (PLCγ), containing src homology domain 2 (SH2) or phosphotyrosine binding (PTB) domains.

Signalling through HER3 can be activated in a ligand-dependent or ligand-independent manner. In the absence of ligand, HER3 receptor molecules are normally expressed at the cell surface as monomers with a conformation which prevents receptor dimerisation in which the dimerisation loop of subdomain II makes intramolecular contact with a pocket on subdomain IV. Binding of a HER3 ligand such as a neuregulin (NRG), e.g. NRG1 (also known as heregulin, HRG) or NRG2 to subdomains I and III of the extracellular region causes a conformational change which results in the exposure of the dimerisation loop of subdomain II, facilitating receptor dimerisation and signalling. Some cancer-associated mutations in HER3 may disrupt interaction of subdomains II and IV required for the formation of the inactive 'closed' conformation and thereby cause constitutive presentation of the dimerisation loop and activation of HER3-mediated signalling in the absence of ligand binding (see e.g. in Jaiswal et al., Cancer Cell (2013) 23(5): 603-617).

In this specification "HER3" refers to HER3 from any species and includes HER3 isoforms, fragments, variants (including mutants) or homologues from any species.

As used herein, a "fragment", "variant" or "homologue" of a protein may optionally be characterised as having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of the reference protein (e.g. a reference isoform). In some embodiments fragments, variants, isoforms and homologues of a reference protein may be characterised by ability to perform a function performed by the reference protein.

A "fragment" generally refers to a fraction of the reference protein. A "variant" generally refers to a protein having an amino acid sequence comprising one or more amino acid substitutions, insertions, deletions or other modifications relative to the amino acid sequence of the reference protein, but retaining a considerable degree of sequence identity (e.g. at least 60%) to the amino acid sequence of the reference protein. An "isoform" generally refers to a variant of the reference protein expressed by the same species as the species of the reference protein (e.g. HER3 isoforms 1 to 5 are all isoforms of one another). A "homologue" generally refers to a variant of the reference protein produced by a different species as compared to the species of the reference protein. For example, human HER3 isoform 1 (P21860-1, v1; SEQ ID NO:1) and Rhesus macaque HER3 (UniProt: F7HEH3-1, v2; SEQ ID NO:20) are homologues of one another. Homologues include orthologues.

A “fragment” of a reference protein may be of any length (by number of amino acids), although may optionally be at least 20% of the length of the reference protein (that is, the protein from which the fragment is derived) and may have a maximum length of one of 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the length of the reference protein.

A fragment of HER3 may have a minimum length of one of 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1100, 1200 amino acids, and may have a maximum length of one of 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 amino acids.

In some embodiments, the HER3 is HER3 from a mammal (e.g. a primate (rhesus, cynomolgous, non-human primate or human) and/or a rodent (e.g. rat or murine) HER3). Isoforms, fragments, variants or homologues of HER3 may optionally be characterised as having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of an immature or mature HER3 isoform from a given species, e.g. human.

Isoforms, fragments, variants or homologues may optionally be functional isoforms, fragments, variants or homologues, e.g. having a functional property/activity of the reference HER3 (e.g. human HER3 isoform 1), as determined by analysis by a suitable assay for the functional property/activity. For example, an isoform, fragment, variant or homologue of HER3 may display association with one or more of: HER2, NRG1 (type I, II, III, IV, V or VI) or NRG2 (α or β).

In some embodiments, the HER3 comprises, or consists of, an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to one of SEQ ID NOs:1 to 8.

In some embodiments, a fragment of HER3 comprises, or consists of, an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to one of SEQ ID NOs:9 to 19, e.g. one of 9, 16 or 19.

Regions of particular interest on the target molecule

The antigen-binding molecules of the present invention were specifically designed to target regions of HER3 of particular interest. In a two-step approach, HER3 regions to be targeted were selected following analysis for predicted antigenicity, function and safety. Antibodies specific for the target regions of HER3 were then prepared using peptides corresponding to the target regions as immunogens to raise specific monoclonal antibodies, and subsequent screening identified antibodies capable of binding to HER3 in the native state. This approach provides exquisite control over the antibody epitope.

The antigen-binding molecules of the present invention may be defined by reference to the region of HER3 to which they bind. The antigen-binding molecules of the present invention may bind to a particular region of interest of HER3. In some embodiments the antigen-binding molecule may bind to a linear epitope of HER3, consisting of a contiguous sequence of amino acids (i.e. an amino acid primary

sequence). In some embodiments, the antigen-binding molecule may bind to a conformational epitope of HER3, consisting of a discontinuous sequence of amino acids of the amino acid sequence.

In some embodiments, the antigen-binding molecule of the present invention binds to HER3. In some embodiments, the antigen-binding molecule binds to the extracellular region of HER3 (e.g. the region shown in SEQ ID NO:9). In some embodiments, the antigen-binding molecule binds to subdomain II of the extracellular region of HER3 (e.g. the region shown in SEQ ID NO:16).

In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:229. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:229. In some embodiments, the antigen-binding molecule binds to the regions of HER3 shown in SEQ ID NOs:230 and 231. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the regions of HER3 shown in SEQ ID NOs:230 and 231. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:230. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:230. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:231. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:231.

In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:23. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:23. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:21. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:21. In some embodiments the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:19. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:19. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:22. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:22.

In some embodiments, the antigen-binding molecule does not bind to the region of HER3 corresponding to positions 260 to 279 of SEQ ID NO:1. In some embodiments the antigen-binding molecule does not contact an amino acid residue of the region of HER3 corresponding to positions 260 to 279 of SEQ ID NO:1. In some embodiments, the antigen-binding molecule does not bind to the region of HER3 shown in SEQ ID NO:23. In some embodiments the antigen-binding molecule does not contact an amino acid residue of the region of HER3 shown in SEQ ID NO:23.

The region of a peptide/polypeptide to which an antibody binds can be determined by the skilled person using various methods well known in the art, including X-ray co-crystallography analysis of antibody-antigen complexes, peptide scanning, mutagenesis mapping, hydrogen-deuterium exchange analysis by mass spectrometry, phage display, competition ELISA and proteolysis-based ‘protection’ methods. Such

methods are described, for example, in Gershoni et al., BioDrugs, 2007, 21(3):145-156, which is hereby incorporated by reference in its entirety.

In some embodiments the antigen-binding molecule is capable of binding the same region of HER3, or an overlapping region of HER3, to the region of HER3 which is bound by an antibody comprising the VH and VL sequences of one of antibody clones 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93, 10A6, 4-35-B2 or 4-35-B4 described herein. In some embodiments the antigen-binding molecule is capable of binding the same region of HER3, or an overlapping region of HER3, to the region of HER3 which is bound by an antibody comprising the VH and VL sequences of one of antibody clones 10D1_c89, 10D1_c90 or 10D1_c91. In some embodiments the antigen-binding molecule is capable of binding the same region of HER3, or an overlapping region of HER3, to the region of HER3 which is bound by an antibody comprising the VH and VL sequences of antibody clone 10D1_c89.

As used herein, a “peptide” refers to a chain of two or more amino acid monomers linked by peptide bonds. A peptide typically has a length in the region of about 2 to 50 amino acids. A “polypeptide” is a polymer chain of two or more peptides. Polypeptides typically have a length greater than about 50 amino acids.

In some embodiments, the antigen-binding molecule of the present invention is capable of binding to a polypeptide comprising, or consisting of, the amino acid sequence of one of SEQ ID NOs:1, 3, 4, 6 or 8.

In some embodiments, the antigen-binding molecule is capable of binding to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:9. In some embodiments, the antigen-binding molecule is capable of binding to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:16.

In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:229. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequences of SEQ ID NOs:230 and 231. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:230. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:231. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:23. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:21. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:19. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:22.

In some embodiments, the antigen-binding molecule is not capable of binding to a peptide consisting of the amino acid sequence corresponding to positions 260 to 279 of SEQ ID NO:1. In some embodiments, the antigen-binding molecule is not capable of binding to a peptide consisting of the amino acid sequence of SEQ ID NO:23.

The ability of an antigen-binding molecule to bind to a given peptide/polypeptide can be analysed by methods well known to the skilled person, including analysis by ELISA, immunoblot (e.g. western blot), immunoprecipitation, Surface Plasmon Resonance (SPR; see e.g. Hearty et al., Methods Mol Biol (2012) 907:411-442) or Bio-Layer Interferometry (see e.g. Lad et al., (2015) J Biomol Screen 20(4): 498-507).

In embodiments where the antigen binding molecule is capable of binding to a peptide/polypeptide comprising a reference amino acid sequence, the peptide/polypeptide may comprise one or more additional amino acids at one or both ends of the reference amino acid sequence. In some embodiments the peptide/polypeptide comprises e.g. 1-5, 1-10, 1-20, 1-30, 1-40, 1-50, 5-10, 5-20, 5-30, 5-40, 5-50, 10-20, 10-30, 10-40, 10-50, 20-30, 20-40 or 20-50 additional amino acids at one or both ends of the reference amino acid sequence.

In some embodiments the additional amino acid(s) provided at one or both ends (i.e. the N-terminal and C-terminal ends) of the reference sequence correspond to the positions at the ends of the reference sequence in the context of the amino acid sequence of HER3. By way of example, where the antigen-binding molecule is capable of binding to a peptide comprising the sequence of SEQ ID NO:23 and an additional two amino acids at the C-terminal end of SEQ ID NO:23, the additional two amino acids may be threonine and lysine, corresponding to positions 278 and 279 of SEQ ID NO:1.

In some embodiments the antigen-binding molecule is capable of binding to a peptide/polypeptide which is bound by an antibody comprising the VH and VL sequences of one of antibody clones 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93, 10A6, 4-35-B2 or 4-35-B4 described herein. In some embodiments the antigen-binding molecule is capable of binding to a peptide/polypeptide which is bound by an antibody comprising the VH and VL sequences of one of antibody clones 10D1_c89, 10D1_c90 or 10D1_c91. In some embodiments the antigen-binding molecule is capable of binding to a peptide/polypeptide which is bound by an antibody comprising the VH and VL sequences of antibody clone 10D1_c89.

Antigen-binding molecules

The present invention provides antigen-binding molecules capable of binding to HER3.

An “antigen-binding molecule” refers to a molecule which is capable of binding to a target antigen, and encompasses monoclonal antibodies, polyclonal antibodies, monospecific and multispecific antibodies (e.g., bispecific antibodies), and antibody fragments (e.g. Fv, scFv, Fab, scFab, $F(ab')_2$, Fab_2, diabodies,

triabodies, scFv-Fc, minibodies, single domain antibodies (e.g. VhH), etc.), as long as they display binding to the relevant target molecule(s).

The antigen-binding molecule of the present invention comprises a moiety capable of binding to a target antigen(s). In some embodiments, the moiety capable of binding to a target antigen comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL) of an antibody capable of specific binding to the target antigen. In some embodiments, the moiety capable of binding to a target antigen comprises or consists of an aptamer capable of binding to the target antigen, e.g. a nucleic acid aptamer (reviewed, for example, in Zhou and Rossi Nat Rev Drug Discov. 2017 16(3):181-202). In some embodiments, the moiety capable of binding to a target antigen comprises or consists of a antigen-binding peptide/polypeptide, e.g. a peptide aptamer, thioredoxin, monobody, anticalin, Kunitz domain, avimer, knottin, fynomer, atrimer, DARPin, affibody, nanobody (i.e. a single-domain antibody (sdAb)) affilin, armadillo repeat protein (ArmRP), OBody or fibronectin – reviewed e.g. in Reverdatto et al., Curr Top Med Chem. 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also e.g. Boersma et al., J Biol Chem (2011) 286:41273-85 and Emanuel et al., Mabs (2011) 3:38-48).

The antigen-binding molecules of the present invention generally comprise an antigen-binding domain comprising a VH and a VL of an antibody capable of specific binding to the target antigen. The antigen-binding domain formed by a VH and a VL may also be referred to herein as an Fv region.

An antigen-binding molecule may be, or may comprise, an antigen-binding polypeptide, or an antigen-binding polypeptide complex. An antigen-binding molecule may comprise more than one polypeptide which together form an antigen-binding domain. The polypeptides may associate covalently or non-covalently. In some embodiments the polypeptides form part of a larger polypeptide comprising the polypeptides (e.g. in the case of scFv comprising VH and VL, or in the case of scFab comprising VH-CH1 and VL-CL).

An antigen-binding molecule may refer to a non-covalent or covalent complex of more than one polypeptide (e.g. 2, 3, 4, 6, or 8 polypeptides), e.g. an IgG-like antigen-binding molecule comprising two heavy chain polypeptides and two light chain polypeptides.

The antigen-binding molecules of the present invention may be designed and prepared using the sequences of monoclonal antibodies (mAbs) capable of binding to HER3. Antigen-binding regions of antibodies, such as single chain variable fragment (scFv), Fab and $F(ab')_2$ fragments may also be used/provided. An “antigen-binding region” is any fragment of an antibody which is capable of binding to the target for which the given antibody is specific.

Antibodies generally comprise six complementarity-determining regions CDRs; three in the heavy chain variable (VH) region: HC-CDR1, HC-CDR2 and HC-CDR3, and three in the light chain variable (VL) region: LC-CDR1, LC-CDR2, and LC-CDR3. The six CDRs together define the paratope of the antibody, which is the part of the antibody which binds to the target antigen.

The VH region and VL region comprise framework regions (FRs) either side of each CDR, which provide a scaffold for the CDRs. From N-terminus to C-terminus, VH regions comprise the following structure: N term-[HC-FR1]-[HC-CDR1]-[HC-FR2]-[HC-CDR2]-[HC-FR3]-[HC-CDR3]-[HC-FR4]-C term; and VL regions comprise the following structure: N term-[LC-FR1]-[LC-CDR1]-[LC-FR2]-[LC-CDR2]-[LC-FR3]-[LC-CDR3]-[LC-FR4]-C term.

There are several different conventions for defining antibody CDRs and FRs, such as those described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991), Chothia et al., J. Mol. Biol. 196:901-917 (1987), and VBASE2, as described in Retter et al., Nucl. Acids Res. (2005) 33 (suppl 1): D671-D674. The CDRs and FRs of the VH regions and VL regions of the antibody clones described herein were defined according to the international IMGT (ImMunoGeneTics) information system (LeFranc et al., Nucleic Acids Res. (2015) 43 (Database issue):D413-22), which uses the IMGT V-DOMAIN numbering rules as described in Lefranc et al., Dev. Comp. Immunol. (2003) 27:55-77.

In some embodiments, the antigen-binding molecule comprises the CDRs of an antigen-binding molecule which is capable of binding to HER3. In some embodiments, the antigen-binding molecule comprises the FRs of an antigen-binding molecule which is capable of binding to HER3. In some embodiments, the antigen-binding molecule comprises the CDRs and the FRs of an antigen-binding molecule which is capable of binding to HER3. That is, in some embodiments the antigen-binding molecule comprises the VH region and the VL region of an antigen-binding molecule which is capable of binding to HER3.

In some embodiments the antigen-binding molecule comprises a VH region and a VL region which is, or which is derived from, the VH/VL region of a HER3-binding antibody clone described herein (i.e. anti-HER3 antibody clones 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93, 10D1, 10A6, 4-35-B2 or 4-35-B4; e.g. 10D1_c89, 10D1_c90 or 10D1_c91; e.g. 10D1_c89).

In some embodiments the antigen-binding molecule comprises a VH region according to one of (1) to (10) below:

(1) (10D1 derived) a VH region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:43

HC-CDR2 having the amino acid sequence of SEQ ID NO:46

HC-CDR3 having the amino acid sequence of SEQ ID NO:51,

or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(2) (10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c87, 10D1_c92, 10D1_c93) a VH region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:44
HC-CDR3 having the amino acid sequence of SEQ ID NO:47,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(3) (10D1_c85v1, 10D1_c85v2) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:45
HC-CDR3 having the amino acid sequence of SEQ ID NO:47,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(4) (10D1_c85o1) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:45
HC-CDR3 having the amino acid sequence of SEQ ID NO:49,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(5) (10D1_c85o2) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:45
HC-CDR3 having the amino acid sequence of SEQ ID NO:50,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(6) (10D1_c89, 10D1_c90) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:45
HC-CDR3 having the amino acid sequence of SEQ ID NO:48,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(7) (10D1_c91) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:42
HC-CDR2 having the amino acid sequence of SEQ ID NO:45
HC-CDR3 having the amino acid sequence of SEQ ID NO:48,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(8) (10A6) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:158

HC-CDR2 having the amino acid sequence of SEQ ID NO:159
HC-CDR3 having the amino acid sequence of SEQ ID NO:160,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(9) (4-35-B2) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:128
HC-CDR2 having the amino acid sequence of SEQ ID NO:129
HC-CDR3 having the amino acid sequence of SEQ ID NO:130,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

(10) (4-35-B4) a VH region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:144
HC-CDR2 having the amino acid sequence of SEQ ID NO:145
HC-CDR3 having the amino acid sequence of SEQ ID NO:146,
or a variant thereof in which one or two or three amino acids in one or more of HC-CDR1, HC-CDR2, or HC-CDR3 are substituted with another amino acid.

In some embodiments the antigen-binding molecule comprises a VH region according to one of (11) to (24) below:

(11) (10D1) a VH region incorporating the following FRs:
HC-FR1 having the amino acid sequence of SEQ ID NO:55
HC-FR2 having the amino acid sequence of SEQ ID NO:58
HC-FR3 having the amino acid sequence of SEQ ID NO:69
HC-FR4 having the amino acid sequence of SEQ ID NO:73,
or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(12) (10D1_c75, 10D1_c92) a VH region incorporating the following FRs:
HC-FR1 having the amino acid sequence of SEQ ID NO:52
HC-FR2 having the amino acid sequence of SEQ ID NO:56
HC-FR3 having the amino acid sequence of SEQ ID NO:61
HC-FR4 having the amino acid sequence of SEQ ID NO:70,
or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(13) (10D1_c76, 10D1_c77, 10D1_c78v1) a VH region incorporating the following FRs:
HC-FR1 having the amino acid sequence of SEQ ID NO:52
HC-FR2 having the amino acid sequence of SEQ ID NO:56
HC-FR3 having the amino acid sequence of SEQ ID NO:62
HC-FR4 having the amino acid sequence of SEQ ID NO:70,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(14) (10D1_c78v2) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:52

HC-FR2 having the amino acid sequence of SEQ ID NO:57

HC-FR3 having the amino acid sequence of SEQ ID NO:62

HC-FR4 having the amino acid sequence of SEQ ID NO:70,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(15) (10D1_11B) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:224

HC-FR2 having the amino acid sequence of SEQ ID NO:60

HC-FR3 having the amino acid sequence of SEQ ID NO:63

HC-FR4 having the amino acid sequence of SEQ ID NO:70,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(16) (10D1_c85v1) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:52

HC-FR2 having the amino acid sequence of SEQ ID NO:56

HC-FR3 having the amino acid sequence of SEQ ID NO:64

HC-FR4 having the amino acid sequence of SEQ ID NO:70,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(17) (10D1_c85v2, 10D1_c85o1, 10D1_c85o2) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:52

HC-FR2 having the amino acid sequence of SEQ ID NO:57

HC-FR3 having the amino acid sequence of SEQ ID NO:64

HC-FR4 having the amino acid sequence of SEQ ID NO:70,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(18) (10D1_c87, 10D1_c93) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:52

HC-FR2 having the amino acid sequence of SEQ ID NO:56

HC-FR3 having the amino acid sequence of SEQ ID NO:65

HC-FR4 having the amino acid sequence of SEQ ID NO:70,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(19) (10D1_c89) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:53

HC-FR2 having the amino acid sequence of SEQ ID NO:59

HC-FR3 having the amino acid sequence of SEQ ID NO:66

HC-FR4 having the amino acid sequence of SEQ ID NO:71,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(20) (10D1_c90) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:54

HC-FR2 having the amino acid sequence of SEQ ID NO:59

HC-FR3 having the amino acid sequence of SEQ ID NO:67

HC-FR4 having the amino acid sequence of SEQ ID NO:71,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(21) (10D1_c91) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:53

HC-FR2 having the amino acid sequence of SEQ ID NO:59

HC-FR3 having the amino acid sequence of SEQ ID NO:68

HC-FR4 having the amino acid sequence of SEQ ID NO:72,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(22) (10A6) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:161

HC-FR2 having the amino acid sequence of SEQ ID NO:162

HC-FR3 having the amino acid sequence of SEQ ID NO:163

HC-FR4 having the amino acid sequence of SEQ ID NO:73,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(23) (4-35-B2) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:131

HC-FR2 having the amino acid sequence of SEQ ID NO:132

HC-FR3 having the amino acid sequence of SEQ ID NO:133

HC-FR4 having the amino acid sequence of SEQ ID NO:134,

or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

(24) (4-35-B4) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:147
HC-FR2 having the amino acid sequence of SEQ ID NO:148
HC-FR3 having the amino acid sequence of SEQ ID NO:149
HC-FR4 having the amino acid sequence of SEQ ID NO:73,
or a variant thereof in which one or two or three amino acids in one or more of HC-FR1, HC-FR2, HC-FR3, or HC-FR4 are substituted with another amino acid.

In some embodiments the antigen-binding molecule comprises a VH region comprising the CDRs according to one of (1) to (10) above, and the FRs according to one of (11) to (24) above.

In some embodiments the antigen-binding molecule comprises a VH region according to one of (25) to (41) below:

(25) a VH region comprising the CDRs according to (1) and the FRs according to (11), (12), (13), (14), (15), (16), (17), (18), (19), (20) or (21).

(26) a VH region comprising the CDRs according to (2) and the FRs according to (11).

(27) a VH region comprising the CDRs according to (2) and the FRs according to (12).

(28) a VH region comprising the CDRs according to (2) and the FRs according to (13).

(29) a VH region comprising the CDRs according to (2) and the FRs according to (14).

(30) a VH region comprising the CDRs according to (2) and the FRs according to (15).

(31) a VH region comprising the CDRs according to (2) and the FRs according to (18).

(32) a VH region comprising the CDRs according to (3) and the FRs according to (16).

(33) a VH region comprising the CDRs according to (3) and the FRs according to (17).

(34) a VH region comprising the CDRs according to (4) and the FRs according to (17).

(35) a VH region comprising the CDRs according to (5) and the FRs according to (17).

(36) a VH region comprising the CDRs according to (6) and the FRs according to (19).

(37) a VH region comprising the CDRs according to (6) and the FRs according to (20).

(38) a VH region comprising the CDRs according to (7) and the FRs according to (21).

(39) a VH region comprising the CDRs according to (8) and the FRs according to (22).

(40) a VH region comprising the CDRs according to (9) and the FRs according to (23).

(41) a VH region comprising the CDRs according to (10) and the FRs according to (24).

In some embodiments the antigen-binding molecule comprises a VH region according to one of (42) to (61) below:

(42) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:24.

(43) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:25.

(44) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:26.

(45) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:27.

(46) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:28.

(47) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:29.

(48) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:30.

(49) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:31.

(50) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:32.

(51) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:33.

(52) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:34.

(53) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:35.

(54) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:36.

(55) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:37.

(56) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:38.

(57) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:39.

(58) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:40.

(59) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:127.

(60) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:143.

(61) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:157.

In some embodiments the antigen-binding molecule comprises a VL region according to one of (62) to (71) below:

(62) (10D1 derived) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:91

LC-CDR2 having the amino acid sequence of SEQ ID NO:94

LC-CDR3 having the amino acid sequence of SEQ ID NO:99;

or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(63) (10D1, 10D1_c75, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c87, 10D1_c89, 10D1_c91, 10D1_c93) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95;

or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(64) (10D1_c76) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:89

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95;

or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(65) (10D1_c77) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:90

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:96;

or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(66) (10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:93

LC-CDR3 having the amino acid sequence of SEQ ID NO:95;

or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(67) (10D1_c90) a VL region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:88
LC-CDR2 having the amino acid sequence of SEQ ID NO:92
LC-CDR3 having the amino acid sequence of SEQ ID NO:97;
or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(68) (10D1_c92) a VL region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:88
LC-CDR2 having the amino acid sequence of SEQ ID NO:92
LC-CDR3 having the amino acid sequence of SEQ ID NO:98;
or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(69) (10A6) a VL region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:165
LC-CDR2 having the amino acid sequence of SEQ ID NO:166
LC-CDR3 having the amino acid sequence of SEQ ID NO:167;
or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(70) (4-35-B2) a VL region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:136
LC-CDR2 having the amino acid sequence of SEQ ID NO:137
LC-CDR3 having the amino acid sequence of SEQ ID NO:138;
or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

(71) (4-35-B4) a VL region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:151
LC-CDR2 having the amino acid sequence of SEQ ID NO:152
LC-CDR3 having the amino acid sequence of SEQ ID NO:153;
or a variant thereof in which one or two or three amino acids in one or more of LC-CDR1, LC-CDR2 or LC-CDR3 are substituted with another amino acid.

In some embodiments the antigen-binding molecule comprises a VL region according to one of (72) to (86) below:

(72) (10D1) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:106
LC-FR2 having the amino acid sequence of SEQ ID NO:113
LC-FR3 having the amino acid sequence of SEQ ID NO:123

LC-FR4 having the amino acid sequence of SEQ ID NO:126,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(73) (10D1_c75) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:100

LC-FR2 having the amino acid sequence of SEQ ID NO:107

LC-FR3 having the amino acid sequence of SEQ ID NO:114

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(74) (10D1_c76) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:101

LC-FR2 having the amino acid sequence of SEQ ID NO:108

LC-FR3 having the amino acid sequence of SEQ ID NO:115

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(75) (10D1_c77) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:102

LC-FR2 having the amino acid sequence of SEQ ID NO:108

LC-FR3 having the amino acid sequence of SEQ ID NO:116

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(76) (10D1_c78v1, 10D1_c78v2, 10D1_11B) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:103

LC-FR2 having the amino acid sequence of SEQ ID NO:108

LC-FR3 having the amino acid sequence of SEQ ID NO:117

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(77) (10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:103

LC-FR2 having the amino acid sequence of SEQ ID NO:108

LC-FR3 having the amino acid sequence of SEQ ID NO:118

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(78) (10D1_c87) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:103

LC-FR2 having the amino acid sequence of SEQ ID NO:109

LC-FR3 having the amino acid sequence of SEQ ID NO:119

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(79) (10D1_c89) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:104

LC-FR2 having the amino acid sequence of SEQ ID NO:110

LC-FR3 having the amino acid sequence of SEQ ID NO:120

LC-FR4 having the amino acid sequence of SEQ ID NO:125,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(80) (10D1_c90) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:105

LC-FR2 having the amino acid sequence of SEQ ID NO:110

LC-FR3 having the amino acid sequence of SEQ ID NO:121

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(81) (10D1_c91) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:104

LC-FR2 having the amino acid sequence of SEQ ID NO:111

LC-FR3 having the amino acid sequence of SEQ ID NO:122

LC-FR4 having the amino acid sequence of SEQ ID NO:125,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(82) (10D1_c92) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:100

LC-FR2 having the amino acid sequence of SEQ ID NO:112

LC-FR3 having the amino acid sequence of SEQ ID NO:114

LC-FR4 having the amino acid sequence of SEQ ID NO:124,

or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(83) (10D1_c93) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:103
LC-FR2 having the amino acid sequence of SEQ ID NO:108
LC-FR3 having the amino acid sequence of SEQ ID NO:119
LC-FR4 having the amino acid sequence of SEQ ID NO:124,
or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(84) (10A6) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:168
LC-FR2 having the amino acid sequence of SEQ ID NO:169
LC-FR3 having the amino acid sequence of SEQ ID NO:170
LC-FR4 having the amino acid sequence of SEQ ID NO:142,
or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(85) (4-35-B2) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:139
LC-FR2 having the amino acid sequence of SEQ ID NO:140
LC-FR3 having the amino acid sequence of SEQ ID NO:141
LC-FR4 having the amino acid sequence of SEQ ID NO:142,
or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

(86) (4-35-B4) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:154
LC-FR2 having the amino acid sequence of SEQ ID NO:155
LC-FR3 having the amino acid sequence of SEQ ID NO:156
LC-FR4 having the amino acid sequence of SEQ ID NO:142,
or a variant thereof in which one or two or three amino acids in one or more of LC-FR1, LC-FR2, LC-FR3, or LC-FR4 are substituted with another amino acid.

In some embodiments the antigen-binding molecule comprises a VL region comprising the CDRs according to one of (62) to (71) above, and the FRs according to one of (72) to (86) above.

In some embodiments the antigen-binding molecule comprises a VL region according to one of (87) to (102) below:

(87) a VL region comprising the CDRs according to (62) and the FRs according to (72), (73), (74), (75), (76), (77), (78), (79), (80), (81), (82), or (83).

(88) a VL region comprising the CDRs according to (63) and the FRs according to (72).

(89) a VL region comprising the CDRs according to (63) and the FRs according to (73).

(90) a VL region comprising the CDRs according to (63) and the FRs according to (76).

(91) a VL region comprising the CDRs according to (63) and the FRs according to (78).

(92) a VL region comprising the CDRs according to (63) and the FRs according to (79).

(93) a VL region comprising the CDRs according to (63) and the FRs according to (81).

(94) a VL region comprising the CDRs according to (63) and the FRs according to (83).

(95) a VL region comprising the CDRs according to (64) and the FRs according to (74).

(96) a VL region comprising the CDRs according to (65) and the FRs according to (75).

(97) a VL region comprising the CDRs according to (66) and the FRs according to (77).

(98) a VL region comprising the CDRs according to (67) and the FRs according to (80).

(99) a VL region comprising the CDRs according to (68) and the FRs according to (82).

(100) a VL region comprising the CDRs according to (69) and the FRs according to (84).

(101) a VL region comprising the CDRs according to (70) and the FRs according to (85).

(102) a VL region comprising the CDRs according to (71) and the FRs according to (86).

In some embodiments the antigen-binding molecule comprises a VL region according to one of (103) to (119) below:

(103) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:74.

(104) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:75.

(105) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:76.

(106) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:77.

(107) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:78.

(108) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:79.

(109) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:80.

(110) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:81.

(111) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:82.

(112) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:83.

(113) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:84.

(114) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:85.

(115) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:86.

(116) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:87.

(117) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:135.

(118) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:150.

(119) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:164.

In some embodiments the antigen-binding molecule comprises a VH region according to any one of (1) to (61) above, and a VL region according to any one of (62) to (119) above.

In embodiments in accordance with the present invention in which one or more amino acids are substituted with another amino acid, the substitutions may be conservative substitutions, for example according to the following Table. In some embodiments, amino acids in the same block in the middle column are substituted. In some embodiments, amino acids in the same line in the rightmost column are substituted:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

In some embodiments, substitution(s) may be functionally conservative. That is, in some embodiments the substitution may not affect (or may not substantially affect) one or more functional properties (e.g. target binding) of the antigen-binding molecule comprising the substitution as compared to the equivalent unsubstituted molecule.

The VH and VL region of an antigen-binding region of an antibody together constitute the Fv region. In some embodiments, the antigen-binding molecule according to the present invention comprises, or consists of, an Fv region which binds to HER3. In some embodiments the VH and VL regions of the Fv are provided as single polypeptide joined by a linker region, i.e. a single chain Fv (scFv).

In some embodiments the antigen-binding molecule of the present invention comprises one or more regions of an immunoglobulin heavy chain constant sequence. In some embodiments the immunoglobulin

heavy chain constant sequence is, or is derived from, the heavy chain constant sequence of an IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM.

In some embodiments the immunoglobulin heavy chain constant sequence is human immunoglobulin G 1 constant (IGHG1; UniProt: P01857-1, v1; SEQ ID NO:171). Positions 1 to 98 of SEQ ID NO:171 form the CH1 region (SEQ ID NO:172). Positions 99 to 110 of SEQ ID NO:171 form a hinge region between CH1 and CH2 regions (SEQ ID NO:173). Positions 111 to 223 of SEQ ID NO:171 form the CH2 region (SEQ ID NO:174). Positions 224 to 330 of SEQ ID NO:171 form the CH3 region (SEQ ID NO:175).

The exemplified antigen-binding molecules may be prepared using pFUSE-CHIg-hG1, which comprises the substitutions D356E, L358M (positions numbered according to EU numbering) in the CH3 region. The amino acid sequence of the CH3 region encoded by pFUSE-CHIg-hG1 is shown in SEQ ID NO:176. It will be appreciated that CH3 regions may be provided with further substitutions in accordance with modification to an Fc region of the antigen-binding molecule as described herein.

In some embodiments a CH1 region comprises or consists of the sequence of SEQ ID NO:172, or a sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:172. In some embodiments a CH1-CH2 hinge region comprises or consists of the sequence of SEQ ID NO:173, or a sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:173. In some embodiments a CH2 region comprises or consists of the sequence of SEQ ID NO:174, or a sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:174. In some embodiments a CH3 region comprises or consists of the sequence of SEQ ID NO:175 or 176, or a sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:175 or 176.

In some embodiments the antigen-binding molecule of the present invention comprises one or more regions of an immunoglobulin light chain constant sequence. In some embodiments the immunoglobulin light chain constant sequence is human immunoglobulin kappa constant (IGKC; Cκ; UniProt: P01834-1, v2; SEQ ID NO:177). In some embodiments the immunoglobulin light chain constant sequence is a human immunoglobulin lambda constant (IGLC; Cλ), e.g. IGLC1, IGLC2, IGLC3, IGLC6 or IGLC7. In some embodiments a CL region comprises or consists of the sequence of SEQ ID NO:177, or a sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:177.

The VL and light chain constant (CL) region, and the VH region and heavy chain constant 1 (CH1) region of an antigen-binding region of an antibody together constitute the Fab region. In some embodiments the antigen-binding molecule comprises a Fab region comprising a VH, a CH1, a VL and a CL (e.g. Cκ or

Cλ). In some embodiments the Fab region comprises a polypeptide comprising a VH and a CH1 (e.g. a VH-CH1 fusion polypeptide), and a polypeptide comprising a VL and a CL (e.g. a VL-CL fusion polypeptide). In some embodiments the Fab region comprises a polypeptide comprising a VH and a CL (e.g. a VH-CL fusion polypeptide) and a polypeptide comprising a VL and a CH (e.g. a VL-CH1 fusion polypeptide); that is, in some embodiments the Fab region is a CrossFab region. In some embodiments the VH, CH1, VL and CL regions of the Fab or CrossFab are provided as single polypeptide joined by linker regions, i.e. as a single chain Fab (scFab) or a single chain CrossFab (scCrossFab).

In some embodiments, the antigen-binding molecule of the present invention comprises, or consists of, a Fab region which binds to HER3.

In some embodiments, the antigen-binding molecule described herein comprises, or consists of, a whole antibody which binds to HER3. As used herein, "whole antibody" refers to an antibody having a structure which is substantially similar to the structure of an immunoglobulin (Ig). Different kinds of immunoglobulins and their structures are described e.g. in Schroeder and Cavacini J Allergy Clin Immunol. (2010) 125(202): S41-S52, which is hereby incorporated by reference in its entirety.

Immunoglobulins of type G (i.e. IgG) are ~150 kDa glycoproteins comprising two heavy chains and two light chains. From N- to C-terminus, the heavy chains comprise a VH followed by a heavy chain constant region comprising three constant domains (CH1, CH2, and CH3), and similarly the light chain comprise a VL followed by a CL. Depending on the heavy chain, immunoglobulins may be classed as IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE, or IgM. The light chain may be kappa (κ) or lambda (λ).

In some embodiments, the antigen-binding molecule described herein comprises, or consists of, an IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE, or IgM which binds to HER3.

In some embodiments, the antigen-binding molecule of the present invention is at least monovalent binding for HER3. Binding valency refers to the number of binding sites in an antigen-binding molecule for a given antigenic determinant. Accordingly, in some embodiments the antigen-binding molecule comprises at least one binding site for HER3.

In some embodiments the antigen-binding molecule comprises more than one binding site for HER3, e.g. 2, 3 or 4 binding sites. The binding sites may be the same or different. In some embodiments the antigen-binding molecule is e.g. bivalent, trivalent or tetravalent for HER3.

Aspects of the present invention relate to multispecific antigen-binding molecules. By "multispecific" it is meant that the antigen-binding molecule displays specific binding to more than one target. In some embodiments the antigen-binding molecule is a bispecific antigen-binding molecule. In some embodiments the antigen-binding molecule comprises at least two different antigen-binding domains (i.e. at least two antigen-binding domains, e.g. comprising non-identical VHs and VLs).

In some embodiments the antigen-binding molecule binds to HER3 and another target (e.g. an antigen other than HER3), and so is at least bispecific. The term “bispecific” means that the antigen-binding molecule is able to bind specifically to at least two distinct antigenic determinants.

It will be appreciated that an antigen-binding molecule according to the present invention (e.g. a multispecific antigen-binding molecule) may comprise antigen-binding molecules capable of binding to the targets for which the antigen-binding molecule is specific. For example, an antigen-binding molecule which is capable of binding to HER3 and an antigen other than HER3 may comprise: (i) an antigen-binding molecule which is capable of binding to HER3, and (ii) an antigen-binding molecule which is capable of binding to an antigen other than HER3.

It will also be appreciated that an antigen-binding molecule according to the present invention (e.g. a multispecific antigen-binding molecule) may comprise antigen-binding polypeptides or antigen-binding polypeptide complexes capable of binding to the targets for which the antigen-binding molecule is specific. For example, an antigen-binding molecule according to the invention may comprise e.g. (i) an antigen-binding polypeptide complex capable of binding to HER3, comprising a light chain polypeptide (comprising the structure VL-CL) and a heavy chain polypeptide (comprising the structure VH-CH1-CH2-CH3), and (ii) an antigen-binding polypeptide complex capable of binding to an antigen other than HER3, comprising a light chain polypeptide (comprising the structure VL-CL) and a heavy chain polypeptide (comprising the structure VH-CH1-CH2-CH3).

In some embodiments, a component antigen-binding molecule of a larger antigen-binding molecule (e.g. a multispecific antigen-biding molecule) may be referred to e.g. as an “antigen-binding domain” or “antigen-binding region” of the larger antigen-binding molecule.

In some embodiments the antigen-binding molecule comprises an antigen-binding molecule capable of binding to HER3, and an antigen-binding molecule capable of binding to an antigen other than HER3. In some embodiments, the antigen other than HER3 is an immune cell surface molecule. In some embodiments, the antigen other than HER3 is a cancer cell antigen. In some embodiments the antigen other than HER3 is a receptor molecule, e.g. a cell surface receptor. In some embodiments the antigen other than HER3 is a cell signalling molecule, e.g. a cytokine, chemokine, interferon, interleukin or lymphokine. In some embodiments the antigen other than HER3 is a growth factor or a hormone.

A cancer cell antigen is an antigen which is expressed or over-expressed by a cancer cell. A cancer cell antigen may be any peptide/polypeptide, glycoprotein, lipoprotein, glycan, glycolipid, lipid, or fragment thereof. A cancer cell antigen’s expression may be associated with a cancer. A cancer cell antigen may be abnormally expressed by a cancer cell (e.g. the cancer cell antigen may be expressed with abnormal localisation), or may be expressed with an abnormal structure by a cancer cell. A cancer cell antigen may be capable of eliciting an immune response. In some embodiments, the antigen is expressed at the cell surface of the cancer cell (i.e. the cancer cell antigen is a cancer cell surface antigen). In some embodiments, the part of the antigen which is bound by the antigen-binding molecule described herein is displayed on the external surface of the cancer cell (i.e. is extracellular). The cancer cell antigen may be a

cancer-associated antigen. In some embodiments the cancer cell antigen is an antigen whose expression is associated with the development, progression or severity of symptoms of a cancer. The cancer-associated antigen may be associated with the cause or pathology of the cancer, or may be expressed abnormally as a consequence of the cancer. In some embodiments, the cancer cell antigen is an antigen whose expression is upregulated (e.g. at the RNA and/or protein level) by cells of a cancer, e.g. as compared to the level of expression by comparable non-cancerous cells (e.g. non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be preferentially expressed by cancerous cells, and not expressed by comparable non-cancerous cells (e.g. non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be the product of a mutated oncogene or mutated tumor suppressor gene. In some embodiments, the cancer-associated antigen may be the product of an overexpressed cellular protein, a cancer antigen produced by an oncogenic virus, an oncofetal antigen, or a cell surface glycolipid or glycoprotein.

In some embodiments the antigen other than HER3 is an antigen expressed by cells of a HER3-associated cancer. A HER3-associated cancer may be a cancer expressing HER3 (e.g. expressing HER3 protein at the cell surface); such cancers may be referred to as “HER3-positive” cancers. HER3-associated cancers include cancers for which HER3 gene/protein expression is a risk factor for, and/or is positively associated with, the onset, development, progression or severity of symptoms of the cancer, and/or metastasis. HER3-associated cancers include those described in Zhang et al., Acta Biochimica et Biophysica Sinica (2016) 48(1):39–48 and Sithanandam and Anderson Cancer Gene Ther (2008) 15(7):413-448, both of which are hereby incorporated by reference in their entirety. In some embodiments a HER3-associated cancer may be a lung cancer (e.g. NSCLC), melanoma, breast cancer, pancreatic cancer, prostate cancer, ovarian cancer, gastric cancer, colon cancer or oral cavity cancer.

An immune cell surface molecule may be any peptide/polypeptide, glycoprotein, lipoprotein, glycan, glycolipid, lipid, or fragment thereof expressed at or on the cell surface of an immune cell. In some embodiments, the part of the immune cell surface molecule which is bound by the antigen-binding molecule of the present invention is on the external surface of the immune cell (i.e. is extracellular). The immune cell surface molecule may be expressed at the cell surface of any immune cell. In some embodiments, the immune cell may be a cell of hematopoietic origin, e.g. a neutrophil, eosinophil, basophil, dendritic cell, lymphocyte, or monocyte. The lymphocyte may be e.g. a T cell, B cell, natural killer (NK) cell, NKT cell or innate lymphoid cell (ILC), or a precursor thereof (e.g. a thymocyte or pre-B cell). In some embodiments the immune cell surface molecule may be a costimulatory molecule (e.g. CD28, OX40, 4-1BB, ICOS or CD27) or a ligand thereof. In some embodiments the immune cell surface molecule may be a checkpoint molecule (e.g. PD-1, CTLA-4, LAG-3, TIM-3, VISTA, TIGIT or BTLA) or a ligand thereof.

Multispecific antigen-binding molecules according to the invention may be provided in any suitable format, such as those formats described in described in Brinkmann and Kontermann MAbs (2017) 9(2): 182-212, which is hereby incorporated by reference in its entirety. Suitable formats include those shown in Figure 2 of Brinkmann and Kontermann MAbs (2017) 9(2): 182-212: antibody conjugates, e.g. IgG_2, $F(ab')_2$ or

CovX-Body; IgG or IgG-like molecules, e.g. IgG, chimeric IgG, κλ-body common HC; CH1/CL fusion proteins, e.g. scFv2-CH1/CL, VHH2-CH1/CL; 'variable domain only' bispecific antigen-binding molecules, e.g. tandem scFv (taFV), triplebodies, diabodies (Db), dsDb, Db(kih), DART, scDB, dsFv-dsFv, tandAbs, triple heads, tandem dAb/VHH, tertravalent dAb.VHH; Non-Ig fusion proteins, e.g. $scFv_2$-albumin, scDb-albumin, taFv-albumin, taFv-toxin, miniantibody, DNL-Fab_2, DNL-Fab_2-scFv, DNL-Fab_2-IgG-$cytokine_2$, ImmTAC (TCR-scFv); modified Fc and CH3 fusion proteins, e.g. scFv-Fc(kih), scFv-Fc(CH3 charge pairs), scFv-Fc (EW-RVT), scFv-fc (HA-TF), scFv-Fc (SEEDbody), taFv-Fc(kih), scFv-Fc(kih)-Fv, Fab-Fc(kih)-scFv, Fab-scFv-Fc(kih), Fab-scFv-Fc(BEAT), Fab-scFv-Fc (SEEDbody), DART-Fc, scFv-CH3(kih), TriFabs; Fc fusions, e.g. Di-diabody, scDb-Fc, taFv-Fc, scFv-Fc-scFv, HCAb-VHH, Fab-scFv-Fc, $scFv_4$-Ig, $scFv_2$-Fcab; CH3 fusions, e.g. Dia-diabody, scDb-CH3; IgE/IgM CH2 fusions, e.g. scFv-EHD2-scFv, scFvMHD2-scFv; Fab fusion proteins, e.g. Fab-scFv (bibody), Fab-$scFv_2$ (tribody), Fab-Fv, Fab-dsFv, Fab-VHH, orthogonal Fab-Fab; non-Ig fusion proteins, e.g. DNL-Fab_3, DNL-Fab_2-scFv, DNL-Fab_2-IgG-$cytokine_2$; asymmetric IgG or IgG-like molecules, e.g. IgG(kih), IgG(kih) common LC, ZW1 IgG common LC, Biclonics common LC, CrossMab, CrossMab(kih), scFab-IgG(kih), Fab-scFab-IgG(kih), orthogonal Fab IgG(kih), DuetMab, CH3 charge pairs + CH1/CL charge pairs, hinge/CH3 charge pairs, SEED-body, Duobody, four-in-one-CrossMab(kih), LUZ-Y common LC; LUZ-Y scFab-IgG, FcFc*; appended and Fc-modified IgGs, e.g. IgG(kih)-Fv, IgG HA-TF-Fv, IgG(kih)scFab, scFab-Fc(kih)-scFv2, scFab-Fc(kih)-scFv, half DVD-Ig, DVI-Ig (four-in-one), CrossMab-Fab; modified Fc and CH3 fusion proteins, e.g. Fab-Fc(kih)-scFv, Fab-scFv-Fc(kih), Fab-scFv-Fc(BEAT), Fab-scFv-Fc-SEEDbody, TriFab; appended IgGs - HC fusions, e.g. IgG-HC, scFv, IgG-dAb, IgG-taFV, IgG-CrossFab, IgG-orthogonal Fab, IgG-(CαCβ) Fab, scFv-HC-IgG, tandem Fab-IgG (orthogonal Fab) Fab-IgG(CαCβ Fab), Fab-IgG(CR3), Fab-hinge-IgG(CR3); appended IgGs - LC fusions, e.g. IgG-scFv(LC), scFv(LC)-IgG, dAb-IgG; appended IgGs - HC and LC fusions, e.g. DVD-Ig, TVD-Ig, CODV-Ig, $scFv_4$-IgG, Zybody; Fc fusions, e.g. Fab-scFv-Fc, $scFv_4$-Ig; F(ab')2 fusions, e.g. $F(ab')_2$-$scFv_2$; CH1/CL fusion proteins e.g. $scFv_2$-CH1-hinge/CL; modified IgGs, e.g. DAF (two-in one-IgG), DutaMab, Mab^2; and non-Ig fusions, e.g. DNL-Fab_4-IgG.

The skilled person is able to design and prepare bispecific antigen-binding molecules. Methods for producing bispecific antigen-binding molecules include chemically crosslinking of antigen-binding molecules or antibody fragments, e.g. with reducible disulphide or non-reducible thioether bonds, for example as described in Segal and Bast, 2001. Production of Bispecific Antigen-binding molecules. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16, which is hereby incorporated by reference in its entirety. For example, *N*-succinimidyl-3-(-2-pyridyldithio)-propionate (SPDP) can be used to chemically crosslink e.g. Fab fragments via hinge region SH- groups, to create disulfide-linked bispecific $F(ab)_2$ heterodimers.

Other methods for producing bispecific antigen-binding molecules include fusing antibody-producing hybridomas e.g. with polyethylene glycol, to produce a quadroma cell capable of secreting bispecific antibody, for example as described in D. M. and Bast, B. J. 2001. Production of Bispecific Antigen-binding molecules. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16.

Bispecific antigen-binding molecules according to the present invention can also be produced recombinantly, by expression from e.g. a nucleic acid construct encoding polypeptides for the antigen-

binding molecules, for example as described in Antibody Engineering: Methods and Protocols, Second Edition (Humana Press, 2012), at Chapter 40: Production of Bispecific Antigen-binding molecules: Diabodies and Tandem scFv (Hornig and Färber-Schwarz), or French, How to make bispecific antigen-binding molecules, Methods Mol. Med. 2000; 40:333-339, the entire contents of both of which are hereby incorporated by reference. For example, a DNA construct encoding the light and heavy chain variable domains for the two antigen-binding fragments (i.e. the light and heavy chain variable domains for the antigen-binding fragment capable of binding HER3, and the light and heavy chain variable domains for the antigen-binding fragment capable of binding to another target protein), and including sequences encoding a suitable linker or dimerization domain between the antigen-binding fragments can be prepared by molecular cloning techniques. Recombinant bispecific antibody can thereafter be produced by expression (e.g. *in vitro*) of the construct in a suitable host cell (e.g. a mammalian host cell), and expressed recombinant bispecific antibody can then optionally be purified.

Fc regions

In some embodiments the antigen-binding molecules of the present invention comprise an Fc region.

In IgG, IgA and IgD isotypes an Fc region is composed of CH2 and CH3 regions from one polypeptide, and CH2 and CH3 regions from another polypeptide. The CH2 and CH3 regions from the two polypeptides together form the Fc region. In IgM and IgE isotypes the Fc regions contain three constant domains (CH2, CH3 and CH4), and CH2 to CH4 from the two polypeptides together form the Fc region.

In preferred embodiments in accordance with the various aspects of the present disclosure an Fc region comprises two polypeptides, each polypeptide comprising a CH2 region and a CH3 region.

In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising modification in one or more of the CH2 and CH3 regions promoting association of the Fc region. Recombinant co-expression of constituent polypeptides of an antigen-binding molecule and subsequent association leads to several possible combinations. To improve the yield of the desired combinations of polypeptides in antigen-binding molecules in recombinant production, it is advantageous to introduce in the Fc regions modification(s) promoting association of the desired combination of heavy chain polypeptides. Modifications may promote e.g. hydrophobic and/or electrostatic interaction between CH2 and/or CH3 regions of different polypeptide chains. Suitable modifications are described e.g. in Ha et al., Front. Immnol (2016) 7:394, which is hereby incorporated by reference in its entirety.

In some embodiments the antigen-binding molecule of the present invention comprises an Fc region comprising paired substitutions in the CH3 regions of the Fc region according to one of the following formats, as shown in Table 1 of Ha et al., Front. Immnol (2016) 7:394: KiH, KiH_{s-s}, HA-TF, ZW1, 7.8.60, DD-KK, EW-RVT, $EW\text{-}RVT_{s-s}$, SEED or A107.

In some embodiments, the Fc region comprises the “knob-into-hole” or “KiH” modification, e.g. as described e.g. in US 7,695,936 and Carter, J Immunol Meth 248, 7-15 (2001). In such embodiments, one of the CH3 regions of the Fc region comprises a “knob” modification, and the other CH3 region comprises

a “hole” modification. The “knob” and “hole” modifications are positioned within the respective CH3 regions so that the “knob” can be positioned in the “hole” in order to promote heterodimerisation (and inhibit homodimerisation) of the polypeptides and/or stabilise heterodimers. Knobs are constructed by substituting amino acids having small chains with those having larger side chains (e.g. tyrosine or tryptophan). Holes are created by substituting amino acids having large side chains with those having smaller side chains (e.g. alanine or threonine).

In some embodiments, one of the CH3 regions of the Fc region of the antigen-binding molecule of the present invention comprises the substitution (numbering of positions/substitutions in the Fc, CH2 and CH3 regions herein is according to the EU numbering system as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991) T366W, and the other CH3 region of the Fc region comprises the substitution Y407V. In some embodiments, one of the CH3 regions of the Fc region of the antigen-binding molecule comprises the substitution T366W, and the other CH3 region of the Fc region comprises the substitutions T366S and L368A. In some embodiments, one of the CH3 regions of the Fc region of the antigen-binding molecule comprises the substitution T366W, and the other CH3 region of the Fc region comprises the substitutions Y407V, T366S and L368A.

In some embodiments, the Fc region comprises the “DD-KK” modification as described e.g. in WO 2014/131694 A1. In some embodiments, one of the CH3 regions comprises the substitutions K392D and K409D, and the other CH3 region of the Fc region comprises the substitutions E356K and D399K. The modifications promote electrostatic interaction between the CH3 regions.

In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region modified as described in Labrijn et al., Proc Natl Acad Sci U S A. (2013) 110(13):5145-50, referred to as ‘Duobody’ format. In some embodiments one of the CH3 regions comprises the substitution K409R, and the other CH3 region of the Fc region comprises the substitution K405L.

In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising the “EEE-RRR” modification as described in Strop et al., J Mol Biol. (2012) 420(3):204-19. In some embodiments one of the CH3 regions comprises the substitutions D221E, P228E and L368E, and the other CH3 region of the Fc region comprises the substitutions D221R, P228R and K409R.

In some embodiments, the antigen-binding molecule comprises an Fc region comprising the “EW-RVT” modification described in Choi et al., Mol Cancer Ther (2013) 12(12):2748–59. In some embodiments one of the CH3 regions comprises the substitutions K360E and K409W, and the other CH3 region of the Fc region comprises the substitutions Q347R, D399V and F405T.

In some embodiments, one of the CH3 regions comprises the substitution S354C, and the other CH3 region of the Fc region comprises the substitution Y349C. Introduction of these cysteine residues results in formation of a disulphide bridge between the two CH3 regions of the Fc region, further stabilizing the heterodimer (Carter (2001), J Immunol Methods 248, 7-15).

In some embodiments, the Fc region comprises the "KiH$_{S-S}$" modification. In some embodiments one of the CH3 regions comprises the substitutions T366W and S354C, and the other CH3 region of the Fc region comprises the substitutions T366S, L368A, Y407V and Y349C.

In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising the "SEED" modification as described in Davis et al., Protein Eng Des Sel (2010) 23(4):195–202, in which β-strand segments of human IgG1 CH3 and IgA CH3 are exchanged.

In some embodiments, one of the CH3 regions comprises the substitutions S364H and F405A, and the other CH3 region of the Fc region comprises the substitutions Y349T and T394F (see e.g. Moore et al., MAbs (2011) 3(6):546–57).

In some embodiments, one of the CH3 regions comprises the substitutions T350V, L351Y, F405A and Y407V, and the other CH3 region of the Fc region comprises the substitutions T350V, T366L, K392L and T394W (see e.g. Von Kreudenstein et al., MAbs (2013) 5(5):646–54).

In some embodiments, one of the CH3 regions comprises the substitutions K360D, D399M and Y407A, and the other CH3 region of the Fc region comprises the substitutions E345R, Q347R, T366V and K409V (see e.g. Leaver-Fay et al., Structure (2016) 24(4):641–51).

In some embodiments, one of the CH3 regions comprises the substitutions K370E and K409W, and the other CH3 region of the Fc region comprises the substitutions E357N, D399V and F405T (see e.g. Choi et al., PLoS One (2015) 10(12):e0145349).

Fc-mediated functions include Fc receptor binding, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), formation of the membrane attack complex (MAC), cell degranulation, cytokine and/or chemokine production, and antigen processing and presentation.

Modifications to antibody Fc regions that influence Fc-mediated functions are known in the art, such as those described e.g. in Wang et al., Protein Cell (2018) 9(1):63-73, which is hereby incorporated by reference in its entirety. Exemplary Fc region modifications known to influence antibody effector function are summarised in Table 1 of Wang et al., Protein Cell (2018) 9(1):63-73.

The combination of substitutions F243L/R292P/Y300L/V305I/P396L is described in Stavenhagen et al. Cancer Res. (2007) to increase binding to FcγRIIIa, and thereby enhance ADCC. The combination of substitutions S239D/I332E or S239D/I332E/A330L is described in Lazar et al., Proc Natl Acad Sci USA. (2006)103:4005–4010 to increase binding to FcγRIIIa, and thereby increase ADCC. The combination of substitutions S239D/I332E/A330L is also described to decrease binding to FcγRIIb, and thereby increase ADCC. The combination of substitutions S298A/E333A/K334A is described in Shields et al., J Biol Chem. (2001) 276:6591–6604 to increase binding to FcγRIIIa, and thereby increase ADCC. The combination of

substitutions L234Y/L235Q/G236W/S239M/H268D/D270E/S298A in one heavy chain, and the combination of substitutions D270E/K326D/A330M/K334E in the other heavy chain, is described in Mimoto et al., MAbs. (2013): 5:229–236 to increase binding to FcγRIIIa, and thereby increase ADCC. The combination of substitutions G236A/S239D/I332E is described in Richards et al., Mol Cancer Ther. (2008) 7:2517–2527 to increase binding to FcγRIIa and to increase binding to FcγRIIIa, and thereby increase ADCP.

The combination of substitutions K326W/E333S is described in Idusogie et al. J Immunol. (2001) 166(4):2571-5 to increase binding to C1q, and thereby increase CDC. The combination of substitutions S267E/H268F/S324T is described in Moore et al. MAbs. (2010) 2(2):181-9 to increase binding to C1q, and thereby increase CDC. The combination of substitutions described in Natsume et al., Cancer Res. (2008) 68(10):3863-72 is reported to increase binding to C1q, and thereby increase CDC. The combination of substitutions E345R/E430G/S440Y is described in Diebolder et al. Science (2014) 343(6176):1260-3 to increase hexamerisation, and thereby increase CDC.

The combination of substitutions M252Y/S254T/T256E is described in Dall'Acqua et al. J Immunol. (2002) 169:5171–5180 to increase binding to FcRn at pH 6.0, and thereby increase antigen-binding molecule half-life. The combination of substitutions M428L/N434S is described in Zalevsky et al. Nat Biotechnol. (2010) 28:157–159 to increase binding to FcRn at pH 6.0, and thereby increase antigen-binding molecule half-life.

Where a heavy chain constant region/Fc region/CH2-CH3 region/CH2 region/CH3 region is described herein as comprising position(s)/substitution(s) "corresponding to" reference position(s)/substitution(s), equivalent position(s)/substitution(s) in homologous heavy chain constant regions/Fc regions/CH2-CH3 regions/CH2 regions/CH3 regions are contemplated.

Where an Fc region is described as comprising specific position(s)/substitution(s), the position(s)/substitution(s) may be present in one or both of the polypeptide chains which together form the Fc region.

Unless otherwise specified, positions herein refer to positions of human immunoglobulin constant region amino acid sequences numbered according to the EU numbering system as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991. By way of illustration, the substitutions L242C and K334C in human IgG1 correspond to L>C substitution at position 125, and K>C substitution at position 217 of the human IgG1 constant region numbered according to SEQ ID NO:171.

Homologous heavy chain constant regions are heavy chain constant regions comprising an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the heavy chain constant region of Human IgG1 (i.e. the amino acid sequence shown in SEQ ID NO:171). Homologous Fc regions are Fc regions comprised of polypeptides comprising an amino acid sequence having at least 60%, preferably

one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to CH2-CH3 region of Human IgG1 (i.e. the amino acid sequences shown in SEQ ID NO:174 and 175). Homologous CH2 regions are CH2 regions comprising an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to CH2 region of Human IgG1 (i.e. the amino acid sequence shown in SEQ ID NO:174). Homologous CH3 regions are CH3 regions comprising an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to CH3 region of Human IgG1 (i.e. the amino acid sequence shown in SEQ ID NO:175).

Corresponding positions to those identified in human IgG1 can be identified by sequence alignment which can be performed e.g. using sequence alignment software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960).

In some embodiments the antigen-binding molecule of the present invention comprises an Fc region comprising modification to increase an Fc-mediated function. In some embodiments the Fc region comprises modification to increase ADCC. In some embodiments the Fc region comprises modification to increase ADCP. In some embodiments the Fc region comprises modification to increase CDC. An antigen-binding molecule comprising an Fc region comprising modification to increase an Fc-mediated function (e.g. ADCC, ADCP, CDC) induces an increased level of the relevant effector function as compared to an antigen-binding molecule comprising the corresponding unmodified Fc region.

In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising modification to increase affinity for one or more Fc receptors (e.g. FcγRIIa, FcγRIIIa). Modifications increasing affinity for Fc receptors can increase Fc-mediated effector function such as antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular phagocytosis (ADCP). In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising modification to reduce affinity for C1q; such modification reducing complement-dependent cytotoxicity (CDC), which can be desirable. In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising modification to increase hexamer formation. Modifications to the Fc region capable of increasing affinity for one or more Fc receptors, reducing affinity for C1q and/or increasing hexamer formation are described e.g. in Saxena and Wu Front Immunol. (2016) 7:580, which is hereby incorporated by reference in its entirety. In some embodiments the antigen-binding molecule of the present invention comprises an Fc region comprising CH2/CH3 comprising one or more of the substitutions shown in Table 1 of Saxena and Wu Front Immunol. (2016) 7:580.

In some embodiments the antigen-binding molecule of the present invention comprises an Fc comprising modification to increase binding to an Fc receptor. In some embodiments the Fc region comprises modification to increase binding to an Fcγ receptor. In some embodiments the Fc region comprises modification to increase binding to one or more of FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa and FcγRIIIb. In some embodiments the Fc region comprises modification to increase binding to FcγRIIIa. In

some embodiments the Fc region comprises modification to increase binding to FcγRIIa. In some embodiments the Fc region comprises modification to increase binding to FcγRIIb. In some embodiments the Fc region comprises modification to increase binding to FcRn. In some embodiments the Fc region comprises modification to increase binding to a complement protein. In some embodiments the Fc region comprises modification to increase or reduce binding to C1q. In some embodiments the Fc region comprises modification to promote hexamerisation of the antigen-binding molecule. In some embodiments the Fc region comprises modification to increase antigen-binding molecule half-life. In some embodiments the Fc region comprises modification to increase co-engagement.

In this specification an “Fcγ receptor” may be from any species, and includes isoforms, fragments, variants (including mutants) or homologues from any species. Similarly, “FcγRI”, “FcγRIIa”, “FcγRIIb”, “FcγRIIc", “FcγRIIIa” and “FcγRIIIb” refer respectively to FcγRI/FcγRIIa/FcγRIIb/FcγRIIc/FcγRIIIa/FcγRIIIb from any species, and include isoforms, fragments, variants (including mutants) or homologues from any species. Humans have six different classes of Fc γ receptor (mouse orthologues are shown in brackets): FcγRI (mFcγRI), FcγRIIa (mFcγRIII), FcγRIIb (mFcγRIIb), FcγRIIc, FcγRIIIa (mFcγRIV) and FcγRIIIb. Variant Fc γ receptors include e.g. the 158V and 158F polymorphs of human FcγRIIIa, and the 167H and 167R polymorphs of human FcγRIIa.

In some embodiments the antigen-binding molecule of the present invention comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, or a CH2-CH3 region, comprising) one or more (e.g. 1, 2, 3, 4, 5, 6, 7 or 8) of the following: C at the position corresponding to position 242; C at the position corresponding to position 334; A at the position corresponding to position 236; D at the position corresponding to position 239; E at the position corresponding to position 332; L at the position corresponding to position 330; K at the position corresponding to position 345; and G at the position corresponding to position 430.

In some embodiments the antigen-binding molecule of the present invention comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, or a CH2-CH3 region, comprising) one or more (e.g. 1, 2, 3, 4, 5, 6, 7 or 8) of the following substitutions (or corresponding substitutions): L242C, K334C, G236A, S239D, I332E, A330L, E345K, and E430G.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a C at the position corresponding to position 242. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a C at the position corresponding to position 334. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a C at the position corresponding to position 242 and a C at the position corresponding to position 334.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region,

comprising) an A at the position corresponding to position 236. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a D at the position corresponding to position 239. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) an A at the position corresponding to position 236, and a D at the position corresponding to position 239.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) an E at the position corresponding to position 332. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) an A at the position corresponding to position 236, a D at the position corresponding to position 239, and an E at the position corresponding to position 332.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) an L at the position corresponding to position 330. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) an A at the position corresponding to position 236, a D at the position corresponding to position 239, an E at the position corresponding to position 332, and an L at the position corresponding to position 330.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH3 region, comprising) a K at the position corresponding to position 345. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH3 region, comprising) a G at the position corresponding to position 430. In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a K at the position corresponding to position 345, and a G at the position corresponding to position 430.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a C at the position corresponding to position 242, a C at the position corresponding to position 334, an A at the position corresponding to position 236, and a D at the position corresponding to position 239.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a C at the position corresponding to position 242, a C at the position corresponding to position 334, an A at the position corresponding to position 236, a D at the position corresponding to position 239, and an E at the position corresponding to position 332.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) a C at the position corresponding to position 242, a C at the position corresponding to position 334, an A at the position corresponding to position 236, a D at the position corresponding to position 239, an E at the position corresponding to position 332, and an L at the position corresponding to position 330.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, or a CH2-CH3 region, comprising) a C at the position corresponding to position 242, a C at the position corresponding to position 334, a K at the position corresponding to position 345, and a G at the position corresponding to position 430.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution L242C (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution K334C (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution L242C (or an equivalent substitution) and the substitution K334C (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution G236A (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution S239D (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution G236A (or an equivalent substitution), and the substitution S239D (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution I332E (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution G236A (or an equivalent substitution), the substitution S239D (or an equivalent substitution), and the substitution I332E (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution A330L (or an equivalent substitution). In some embodiments the Fc region

comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution G236A (or an equivalent substitution), the substitution S239D (or an equivalent substitution), the substitution I332E (or an equivalent substitution), and the substitution A330L (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH3 region, comprising) the substitution E345K (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH3 region, comprising) the substitution E430G (or an equivalent substitution). In some embodiments the Fc region comprises (e.g. comprises one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution E345K (or an equivalent substitution), and the substitution E430G (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution L242C (or an equivalent substitution), the substitution K334C (or an equivalent substitution), the substitution G236A (or an equivalent substitution), and the substitution S239D (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution L242C (or an equivalent substitution), the substitution K334C (or an equivalent substitution), the substitution G236A (or an equivalent substitution), the substitution S239D (or an equivalent substitution), and the substitution I332E (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, a CH2-CH3 region, or a CH2 region, comprising) the substitution L242C (or an equivalent substitution), the substitution K334C (or an equivalent substitution), the substitution G236A (or an equivalent substitution), the substitution S239D (or an equivalent substitution), the substitution I332E (or an equivalent substitution), and the substitution A330L (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, or a CH2-CH3 region, comprising) the substitution L242C (or an equivalent substitution), the substitution K334C (or an equivalent substitution), the substitution E345K (or an equivalent substitution), and the substitution E430G (or an equivalent substitution).

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, or a CH2-CH3 region, comprising) one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12) of the following: L at the position corresponding to

position 243, P at the position corresponding to position 292, L at the position corresponding to position 300, I at the position corresponding to position 305 and L at the position corresponding to position 396; D at the position corresponding to position 239 and E at the position corresponding to position 332; D at the position corresponding to position 239, E at the position corresponding to position 332 and L at the position corresponding to position 330; A at the position corresponding to position 298, A at the position corresponding to position 333 and A at the position corresponding to position 334; Y at the position corresponding to position 234, Q at the position corresponding to position 235, W at the position corresponding to position 236, M at the position corresponding to position 239, D at the position corresponding to position 268, E at the position corresponding to position 270 and A at the position corresponding to position 298; E at the position corresponding to position 270, D at the position corresponding to position 326, M at the position corresponding to position 330 and E at the position corresponding to position 334; A at the position corresponding to position 236, D at the position corresponding to position 239 and E at the position corresponding to position 332; W at the position corresponding to position 326 and S at the position corresponding to position 333; E at the position corresponding to position 267, F at the position corresponding to position 268 and T at the position corresponding to position 324; R at the position corresponding to position 345, G at the position corresponding to position 430 and Y at the position corresponding to position 440; Y at the position corresponding to position 252, T at the position corresponding to position 254 and E at the position corresponding to position 256; and L at the position corresponding to position 428 and S at the position corresponding to position 434.

In some embodiments the antigen-binding molecule comprises an Fc region comprising (e.g. comprising one more polypeptides comprising a heavy chain constant region, or a CH2-CH3 region, comprising) one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12) of the following combinations of substitutions (or corresponding substitutions): F243L/R292P/Y300L/V305I/P396L; S239D/I332E; S239D/I332E/A330L; S298A/E333A/K334A; L234Y/L235Q/G236W/S239M/H268D/D270E/S298A; D270E/K326D/A330M/K334E; G236A/S239D/I332E; K326W/E333S; S267E/H268F/S324T; E345R/E430G/S440Y; M252Y/S254T/T256E; and M428L/N434S.

Polypeptides

The present invention also provides polypeptide constituents of antigen-binding molecules. The polypeptides may be provided in isolated or substantially purified form.

The antigen-binding molecule of the present invention may be, or may comprise, a complex of polypeptides.

In the present specification where a polypeptide comprises more than one domain or region, it will be appreciated that the plural domains/regions are preferably present in the same polypeptide chain. That is, the polypeptide comprises more than one domain or region is a fusion polypeptide comprising the domains/regions.

In some embodiments a polypeptide according to the present invention comprises, or consists of, a VH as described herein. In some embodiments a polypeptide according to the present invention comprises, or consists of, a VL as described herein.

In some embodiments, the polypeptide additionally comprises one or more antibody heavy chain constant regions (CH). In some embodiments, the polypeptide additionally comprises one or more antibody light chain constant regions (CL).In some embodiments, the polypeptide comprises a CH1, CH2 region and/or a CH3 region of an immunoglobulin (Ig).

In some embodiments the polypeptide comprises one or more regions of an immunoglobulin heavy chain constant sequence. In some embodiments the polypeptide comprises a CH1 region as described herein. In some embodiments the polypeptide comprises a CH1-CH2 hinge region as described herein. In some embodiments the polypeptide comprises a CH2 region as described herein. In some embodiments the polypeptide comprises a CH3 region as described herein. In some embodiments the polypeptide comprises a CH2-CH3 region as described herein.

In some embodiments the polypeptide comprises a CH3 region comprising any one of the following amino acid substitutions/combinations of amino acid substitutions (shown e.g. in Table 1 of Ha et al., Front. Immnol (2016) 7:394, incorporated by reference hereinabove): T366W; T366S, L368A and Y407V; T366W and S354C; T366S, L368A, Y407V and Y349C; S364H and F405A; Y349T and T394F; T350V, L351Y, F405A and Y407V; T350V, T366L, K392L and T394W; K360D, D399M and Y407A; E345R, Q347R, T366V and K409V; K409D and K392D; D399K and E356K; K360E and K409W; Q347R, D399V and F405T; K360E, K409W and Y349C; Q347R, D399V, F405T and S354C; K370E and K409W; and E357N, D399V and F405T.

In some embodiments the CH2 and/or CH3 regions of the polypeptide comprise one or more amino acid substitutions for promoting association of the polypeptide with another polypeptide comprising a CH2 and/or CH3 region.

In some embodiments the polypeptide comprises one or more regions of an immunoglobulin light chain constant sequence. In some embodiments the polypeptide comprises a CL region as described herein.

In some embodiments, the polypeptide according to the present invention comprises a structure from N- to C-terminus according to one of the following:

(i) VH
(ii) VL
(iii) VH-CH1
(iv) VL-CL
(v) VL-CH1
(vi) VH-CL
(vii) VH-CH1-CH2-CH3

(viii) VL-CL-CH2-CH3

(ix) VL-CH1-CH2-CH3

(x) VH-CL-CH2-CH3

Also provided by the present invention are antigen-binding molecules composed of the polypeptides of the present invention. In some embodiments, the antigen-binding molecule of the present invention comprises one of the following combinations of polypeptides:

(A) VH + VL

(B) VH-CH1 + VL-CL

(C) VL-CH1 + VH-CL

(D) VH-CH1-CH2-CH3 + VL-CL

(E) VH-CL-CH2-CH3 + VL-CH1

(F) VL-CH1-CH2-CH3 + VH-CL

(G) VL-CL-CH2-CH3 + VH-CH1

(H) VH-CH1-CH2-CH3 + VL-CL-CH2-CH3

(I) VH-CL-CH2-CH3 + VL-CH1-CH2-CH3

In some embodiments the antigen-binding molecule comprises more than one of a polypeptide of the combinations shown in (A) to (I) above. By way of example, with reference to (D) above, in some embodiments the antigen-binding molecule comprises two polypeptides comprising the structure VH-CH1-CH2-CH3, and two polypeptides comprising the structure VL-CL.

In some embodiments, the antigen-binding molecule of the present invention comprises one of the following combinations of polypeptides:

(J) VH (anti-HER3) + VL (anti-HER3)

(K) VH (anti-HER3)-CH1 + VL (anti-HER3)-CL

(L) VL (anti-HER3)-CH1 + VH (anti-HER3)-CL

(M) VH (anti-HER3)-CH1-CH2-CH3 + VL (anti-HER3)-CL

(N) VH (anti-HER3)-CL-CH2-CH3 + VL (anti-HER3)-CH1

(O) VL (anti-HER3)-CH1-CH2-CH3 + VH (anti-HER3)-CL

(P) VL (anti-HER3)-CL-CH2-CH3 + VH (anti-HER3)-CH1

(Q) VH (anti-HER3)-CH1-CH2-CH3 + VL (anti-HER3)-CL-CH2-CH3

(R) VH (anti-HER3)-CL-CH2-CH3 + VL (anti-HER3)-CH1-CH2-CH3

Wherein: “VH(anti-HER3)” refers to the VH of an antigen-binding molecule capable of binding to HER3 as described herein, e.g. as defined in one of (1) to (61) above; “VL(anti-HER3)” refers to the VL of an antigen-binding molecule capable of binding to HER3 as described herein, e.g. as defined in one of (62) to (119) above.

In some embodiments the polypeptide comprises or consists of an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of one of SEQ ID NOs:187 to 223.

Linkers and additional sequences

In some embodiments the antigen-binding molecules and polypeptides of the present invention comprise a hinge region. In some embodiments a hinge region is provided between a CH1 region and a CH2 region. In some embodiments a hinge region is provided between a CL region and a CH2 region. In some embodiments the hinge region comprises, or consists of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:173.

In some embodiments the antigen-binding molecules and polypeptides of the present invention comprise one or more linker sequences between amino acid sequences. A linker sequence may be provided at one or both ends of one or more of a VH, VL, CH1-CH2 hinge region, CH2 region and a CH3 region of the antigen-binding molecule/polypeptide.

Linker sequences are known to the skilled person, and are described, for example in Chen et al., Adv Drug Deliv Rev (2013) 65(10): 1357-1369, which is hereby incorporated by reference in its entirety. In some embodiments, a linker sequence may be a flexible linker sequence. Flexible linker sequences allow for relative movement of the amino acid sequences which are linked by the linker sequence. Flexible linkers are known to the skilled person, and several are identified in Chen et al., Adv Drug Deliv Rev (2013) 65(10): 1357-1369. Flexible linker sequences often comprise high proportions of glycine and/or serine residues.

In some embodiments, the linker sequence comprises at least one glycine residue and/or at least one serine residue. In some embodiments the linker sequence consists of glycine and serine residues. In some embodiments, the linker sequence has a length of 1-2, 1-3, 1-4, 1-5 or 1-10 amino acids.

The antigen-binding molecules and polypeptides of the present invention may additionally comprise further amino acids or sequences of amino acids. For example, the antigen-binding molecules and polypeptides may comprise amino acid sequence(s) to facilitate expression, folding, trafficking, processing, purification or detection of the antigen-binding molecule/polypeptide. For example, the antigen-binding molecule/polypeptide may comprise a sequence encoding a His, (e.g. 6XHis), Myc, GST, MBP, FLAG, HA, E, or Biotin tag, optionally at the N- or C- terminus of the antigen-binding molecule/polypeptide. In some embodiments the antigen-binding molecule/polypeptide comprises a detectable moiety, e.g. a fluorescent, lunminescent, immuno-detectable, radio, chemical, nucleic acid or enzymatic label.

The antigen-binding molecules and polypeptides of the present invention may additionally comprise a signal peptide (also known as a leader sequence or signal sequence). Signal peptides normally consist of

a sequence of 5-30 hydrophobic amino acids, which form a single alpha helix. Secreted proteins and proteins expressed at the cell surface often comprise signal peptides.

The signal peptide may be present at the N-terminus of the antigen-binding molecule/polypeptide, and may be present in the newly synthesised antigen-binding molecule/polypeptide. The signal peptide provides for efficient trafficking and secretion of the antigen-binding molecule/polypeptide. Signal peptides are often removed by cleavage, and thus are not comprised in the mature antigen-binding molecule/polypeptide secreted from the cell expressing the antigen-binding molecule/polypeptide.

Signal peptides are known for many proteins, and are recorded in databases such as GenBank, UniProt, Swiss-Prot, TrEMBL, Protein Information Resource, Protein Data Bank, Ensembl, and InterPro, and/or can be identified/predicted e.g. using amino acid sequence analysis tools such as SignalP (Petersen et al., 2011 Nature Methods 8: 785-786) or Signal-BLAST (Frank and Sippl, 2008 Bioinformatics 24: 2172-2176).

In some embodiments, the signal peptide of the antigen-binding molecule/polypeptide of the present invention comprises, or consists of, an amino acid sequence having at least 80%, 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of one of SEQ ID NOs:178 to 186.

Labels and conjugates

In some embodiments the antigen-binding molecules of the present invention additionally comprise a detectable moiety.

In some embodiments the antigen-binding molecule comprises a detectable moiety, e.g. a fluorescent label, phosphorescent label, luminescent label, immuno-detectable label (e.g. an epitope tag), radiolabel, chemical, nucleic acid or enzymatic label. The antigen-binding molecule may be covalently or non-covalently labelled with the detectable moiety.

Fluorescent labels include e.g. fluorescein, rhodamine, allophycocyanin, eosine and NDB, green fluorescent protein (GFP) chelates of rare earths such as europium (Eu), terbium (Tb) and samarium (Sm), tetramethyl rhodamine, Texas Red, 4-methyl umbelliferone, 7-amino-4-methyl coumarin, Cy3, and Cy5. Radiolabels include radioisotopes such as Iodine^{123}, Iodine^{125}, Iodine^{126}, Iodine^{131}, Iodine^{133}, Bromine^{77}, Technetium^{99m}, Indium^{111}, Indium^{113m}, Gallium^{67}, Gallium^{68}, Ruthenium^{95}, Ruthenium^{97}, Ruthenium^{103}, Ruthenium^{105}, Mercury^{207}, Mercury^{203}, Rhenium^{99m}, Rhenium^{101}, Rhenium^{105}, Scandium^{47}, Tellurium^{121m}, Tellurium^{122m}, Tellurium^{125m}, Thulium^{165}, Thuliuml^{167}, Thulium^{168}, Copper^{67}, Fluorine^{18}, Yttrium^{90}, Palladium^{100}, Bismuth^{217} and Antimony^{211}. Luminescent labels include as radioluminescent, chemiluminescent (e.g. acridinium ester, luminol, isoluminol) and bioluminescent labels. Immuno-detectable labels include haptens, peptides/polypeptides, antibodies, receptors and ligands such as biotin, avidin, streptavidin or digoxigenin. Nucleic acid labels include aptamers. Enzymatic labels include e.g. peroxidase, alkaline phosphatase, glucose oxidase, beta-galactosidase and luciferase.

In some embodiments the antigen-binding molecules of the present invention are conjugated to a chemical moiety. The chemical moiety may be a moiety for providing a therapeutic effect. Antibody-drug conjugates are reviewed e.g. in Parslow et al., Biomedicines. 2016 Sep; 4(3):14. In some embodiments, the chemical moiety may be a drug moiety (e.g. a cytotoxic agent). In some embodiments, the drug moiety may be a chemotherapeutic agent. In some embodiments, the drug moiety is selected from calicheamicin, DM1, DM4, monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), SN-38, doxorubicin, duocarmycin, D6.5 and PBD.

Particular exemplary embodiments of the antigen-binding molecules

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:187; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:188.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:189; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:190.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:191; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:192.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:193; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:195.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:194; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:195.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:196; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:195.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:197; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:199.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:198; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:199.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:200; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:201.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:202; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:203.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:204; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:205.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:206; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:207.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:208; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:209.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:210; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:211.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:212; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:213.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:214; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:215.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:216; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:217.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:218; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:219.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:220; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:221.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:222; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:223.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:225; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:207.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:226; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:207.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:227; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:217.

In some embodiments the antigen-binding molecule comprises, or consists of:

(i) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:228; and

(ii) two polypeptides comprising, or consisting of, an amino acid sequence having at least 70%, preferably one of 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of NO:217.

Functional properties of the antigen-binding molecules

The antigen-binding molecules described herein may be characterised by reference to certain functional properties. In some embodiments, the antigen-binding molecule described herein may possess one or more of the following properties:

binds to HER3 (e.g. human, mouse, rat or cynomolgus macaque HER3);
does not bind to EGFR and/or HER2;
binds to HER3-expressing cells;
binds to subdomain II of the extracellular region of HER3;
binds to HER3 when HER3 is in open and closed conformations;
binds to HER3 independently of NRG;
does not compete with MM-121 and/or LJM-716 for binding to HER3;
does not compete with M-05-74 and/or M-08-11 for binding to HER3;
inhibits interaction between HER3 and an interaction partner for HER3 (e.g. HER3, HER2, EGFR, HER4, HGFR, IGF1R and/or cMet);
inhibits HER3-mediated signalling;
inhibits proliferation of HER3-expressing cells (e.g. in response to stimulation with NRG);
inhibits PI3K/AKT/mTOR and/or MAPK signalling by HER3-expressing cells (e.g. in response to stimulation with NRG);
binds to an activatory Fcγ receptor (e.g. FcγRIIIa);
increased binding to an activatory Fcγ receptor;
increased binding to an activatory Fcγ receptor as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
decreased binding to an inhibitory Fcγ receptor as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
increased binding to an activatory Fcγ receptor over an inhibitory Fcγ receptor as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
increased or decreased binding to a complement protein (e.g. C1q) as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
increased hexamerisation as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
increased ADCC activity as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
increased ADCP activity as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;
increased or decreased CDC activity as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;

similar or increased thermostability as compared to an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175;

increases killing of HER3-expressing cells;

reduces the number/proportion of HER3-expressing cells;

and

inhibits the development and/or progression of cancer *in vivo*.

The antigen-binding molecules described herein preferably display specific binding to HER3. As used herein, "specific binding" refers to binding which is selective for the antigen, and which can be discriminated from non-specific binding to non-target antigen. An antigen-binding molecule that specifically binds to a target molecule preferably binds the target with greater affinity, and/or with greater duration than it binds to other, non-target molecules.

The ability of a given polypeptide to bind specifically to a given molecule can be determined by analysis according to methods known in the art, such as by ELISA, Surface Plasmon Resonance (SPR; see e.g. Hearty et al., Methods Mol Biol (2012) 907:411-442), Bio-Layer Interferometry (see e.g. Lad et al., (2015) J Biomol Screen 20(4): 498-507), flow cytometry, or by a radiolabeled antigen-binding assay (RIA) enzyme-linked immunosorbent assay. Through such analysis binding to a given molecule can be measured and quantified. In some embodiments, the binding may be the response detected in a given assay.

In some embodiments, the extent of binding of the antigen-binding molecule to an non-target molecule is less than about 10% of the binding of the antibody to the target molecule as measured, e.g. by ELISA, SPR, Bio-Layer Interferometry or by RIA. Alternatively, binding specificity may be reflected in terms of binding affinity where the antigen-binding molecule binds with a dissociation constant (K_D) that is at least 0.1 order of magnitude (i.e. 0.1×10^n, where n is an integer representing the order of magnitude) greater than the K_D of the antigen-binding molecule towards a non-target molecule. This may optionally be one of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, or 2.0.

In some embodiments, the antigen-binding molecule displays binding to human HER3, mouse HER3, rat HER3 and/or cynomolgus macaque (*Macaca fascicularis*) HER3. That is, in some embodiments the antigen-binding molecule is cross-reactive for human HER3, mouse HER3, rat HER3 and/or cynomolgus macaque HER3. In some embodiments the antigen-binding molecule of the present invention displays cross-reactivity with HER3 of a non-human primate. Cross-reactivity to HER3 in model species allows *in vivo* exploration of efficacy in syngeneic models without relying on surrogate molecules.

In some embodiments the antigen-binding molecule binds to human HER3, mouse HER3, rat HER3 and/or cynomolgus macaque HER3; and does not bind to HER2 and/or EGFR (e.g. human HER2 and/or human EGFR).

In some embodiments, the antigen-binding molecule does not display specific binding to EGFR (e.g. human EGFR). In some embodiments, the antigen-binding molecule does not display specific binding to HER2 (e.g. human HER2). In some embodiments, the antigen-binding molecule does not display specific binding to (i.e. does not cross-react with) a member of the EGFR family of proteins other than HER3. In some embodiments, the antigen-binding molecule does not display specific binding to EGFR, HER2 and/or HER4.

In some embodiments, the antigen-binding molecule of the invention binds to HER3 (e.g. human HER3) with a K_D of 10 μM or less, preferably one of ≤5 μM, ≤2 μM, ≤1 μM, ≤500 nM, ≤400 nM, ≤300 nM, ≤200 nM, ≤100 nM, ≤95 nM, ≤90 nM, ≤85 nM, ≤80 nM, ≤75 nM, ≤70 nM, ≤65 nM, ≤60 nM, ≤55 nM, ≤50 nM, ≤45 nM, ≤40 nM, ≤35 nM, ≤30 nM, ≤25 nM, ≤20 nM, ≤15 nM, ≤12.5 nM, ≤10 nM, ≤9 nM, ≤8 nM, ≤7 nM, ≤6 nM, ≤5 nM, ≤4 nM ≤3 nM, ≤2 nM, ≤1 nM, ≤900 pM, ≤800 pM, ≤700 pM, ≤600 pM, ≤500 pM, ≤400 pM, ≤300 pM, ≤200 pM, ≤100 pM, ≤90 pM, ≤80 pM, ≤70 pM, ≤60 pM, ≤50 pM, ≤40 pM, ≤30 pM, ≤20 pM, ≤10 pM, ≤9 pM, ≤8 pM, ≤7 pM, ≤6 pM, ≤5 pM, ≤4 pM, ≤3 pM, ≤2 pM, ≤1 pM.

The antigen-binding molecules of the present invention may bind to a particular region of interest of HER3. The antigen-binding region of an antigen-binding molecule according to the present invention may bind to a linear epitope of HER3, consisting of a contiguous sequence of amino acids (i.e. an amino acid primary sequence). In some embodiments, the antigen-binding molecule may bind to a conformational epitope of HER3, consisting of a discontinuous sequence of amino acids of the amino acid sequence.

In some embodiments, the antigen-binding molecule of the present invention binds to HER3. In some embodiments, the antigen-binding molecule binds to the extracellular region of HER3 (e.g. the region shown in SEQ ID NO:9). In some embodiments, the antigen-binding molecule binds to subdomain II of the extracellular region of HER3 (e.g. the region shown in SEQ ID NO:16).

In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:229. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:229. In some embodiments, the antigen-binding molecule binds to the regions of HER3 shown in SEQ ID NOs:230 and 231. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the regions of HER3 shown in SEQ ID NOs:230 and 231. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:230. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:230. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:231. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:231. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:23. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:23. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:21. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:21. In some embodiments the antigen-binding molecule binds to the region of HER3 shown in SEQ

ID NO:19. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:19. In some embodiments, the antigen-binding molecule binds to the region of HER3 shown in SEQ ID NO:22. In some embodiments the antigen-binding molecule contacts one or more amino acid residues of the region of HER3 shown in SEQ ID NO:22.

In some embodiments, the antigen-binding molecule of the present invention is capable of binding to a polypeptide comprising, or consisting of, the amino acid sequence of one of SEQ ID NOs:1, 3, 4, 6 or 8. In some embodiments, the antigen-binding molecule is capable of binding to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:9. In some embodiments, the antigen-binding molecule is capable of binding to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:16. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:229. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequences of SEQ ID NO:230 and 231. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:230. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:231. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:23. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:21. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:19. In some embodiments, the antigen-binding molecule is capable of binding to a peptide/polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:22.

In some embodiments, the antigen-binding molecule does not bind to the region of HER3 corresponding to positions 260 to 279 of SEQ ID NO:1. In some embodiments the antigen-binding molecule does not contact an amino acid residue of the region of HER3 corresponding to positions 260 to 279 of SEQ ID NO:1. In some embodiments, the antigen-binding molecule does not bind to the region of HER3 shown in SEQ ID NO:23. In some embodiments the antigen-binding molecule does not contact an amino acid residue of the region of HER3 shown in SEQ ID NO:23. In some embodiments, the antigen-binding molecule is not capable of binding to a peptide consisting of the amino acid sequence corresponding to positions 260 to 279 of SEQ ID NO:1. In some embodiments, the antigen-binding molecule is not capable of binding to a peptide consisting of the amino acid sequence of SEQ ID NO:23.

As used herein, a “peptide” refers to a chain of two or more amino acid monomers linked by peptide bonds. A peptide typically has a length in the region of about 2 to 50 amino acids. A “polypeptide” is a polymer chain of two or more peptides. Polypeptides typically have a length greater than about 50 amino acids.

The ability of an antigen-binding molecule to bind to a given peptide/polypeptide can be analysed by methods well known to the skilled person, including analysis by ELISA, immunoblot (e.g. western blot), immunoprecipitation, surface plasmon resonance and biolayer interferometry.

Ligand binding to HER3 promotes conformational changes that enables HER3 to homo- or heterodimerise, resulting in activation of downstream pathways. HER3 demonstrates 'closed' and 'open' conformations. By closed conformation it is meant that HER3 is in a tethered conformation and is unavailable for receptor homo- or heterodimerisation. By open conformation it is meant that HER3 is in an extended conformation and is available for receptor homo- or heterodimerisation.

In some embodiments the antigen-binding molecule is capable of binding to HER3 when HER3 is in the open conformation. In some embodiments the antigen-binding molecule is capable of binding to HER3 when HER3 is in the closed conformation. In some embodiments the antigen-binding molecule is capable of binding to HER3 when HER3 is in the open and/or closed conformation. In some embodiments the antigen-binding molecule is capable of binding to the HER3 ectodomain when HER3 is in the open and/or closed conformation. In some embodiments the antigen-binding molecule is capable of binding to the HER3 dimerisation arm when HER3 is in the open and/or closed conformation. Binding to the dimerisation arm enables an antigen-binding molecule to prevent interaction between HER3 and an interaction partner for HER3, e.g. as described herein.

In some embodiments the antigen-binding molecule is capable of binding to HER3 in the presence and/or absence of a ligand for HER3. In some embodiments the antigen-binding molecule is capable of binding to HER3 independently of a ligand for HER3. In some embodiments the ligand is NRG, NRG-1 and/or NRG-2. HER3 is activated by ligand binding to its extracellular domain which promotes conformational changes that enables HER3 to homo- or heterodimerise. Binding of an antigen-binding molecule to HER3 independently of ligand binding allows the antigen-binding molecule to inhibit the action of HER3 in both ligand-absent and ligand-present conformational states. In some embodiments the antigen-binding molecule does not compete with ligand binding to HER3. In some embodiments the antigen-binding molecule does not bind to HER3 at the ligand binding site.

In some embodiments, the antigen-binding molecule binds to HER3 similarly well in the presence or absence of ligand for HER3 (i.e. irrespective of whether HER3 is provided in the ligand-bound or unbound form).

In some embodiments, the antigen-binding molecule binds to HER3 in the presence of a ligand for HER3 with an affinity which is similar to the affinity of binding of the antigen-binding molecule to HER3 in the absence of ligand for HER3. Example 8.10 and Figures 78A and 78B of the present disclosure demonstrate that 10D1F binds to human HER3 with sub-picomolar affinity both when HER3 is provided in the NRG1-bound form, and in the absence of NRG1.

Herein, a binding affinity which is 'similar' to a reference binding affinity means a binding affinity which is within 50%, e.g. within one of 40%, 45%, 30%, 25%, 20% 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%,

11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the reference binding affinity, as determined under comparable conditions.

In some embodiments, the antigen-binding molecule binds to HER3 in the presence of a ligand for HER3 (e.g. NRG1 or NRG2) with a K_D which is within 50%, e.g. within one of 40%, 45%, 30%, 25%, 20% 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the K_D of the antigen-binding molecule for binding to HER3 in the absence of the ligand (as determined under comparable conditions).

In some embodiments, the antigen-binding molecule binds to HER3 in the presence of a ligand for HER3 (e.g. NRG1 or NRG2) with a K_{on} which is within 50%, e.g. within one of 40%, 45%, 30%, 25%, 20% 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the K_{on} of the antigen-binding molecule for binding to HER3 in the absence of the ligand (as determined under comparable conditions).

In some embodiments, the antigen-binding molecule binds to HER3 in the presence of a ligand for HER3 (e.g. NRG1 or NRG2) with a K_{off} which is within 50%, e.g. within one of 40%, 45%, 30%, 25%, 20% 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the K_{off} of the antigen-binding molecule for binding to HER3 in the absence of the ligand (as determined under comparable conditions).

In some embodiments the antigen-binding molecule is capable of binding the same region of HER3, or an overlapping region of HER3, to the region of HER3 which is bound by an antibody comprising the VH and VL sequences of one of clones 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93, 10A6, 4-35-B2 or 4-35-B4. In some embodiments the antigen-binding molecule is capable of binding the same region of HER3, or an overlapping region of HER3, to the region of HER3 which is bound by an antibody comprising the VH and VL sequences of one of clones 10D1_c89, 10D1_c90 or 10D1_c91. In some embodiments the antigen-binding molecule is capable of binding the same region of HER3, or an overlapping region of HER3, to the region of HER3 which is bound by an antibody comprising the VH and VL sequences of clone 10D1_c89.

The region of a peptide/polypeptide to which an antibody binds can be determined by the skilled person using various methods well known in the art, including X-ray co-crystallography analysis of antibody-antigen complexes, peptide scanning, mutagenesis mapping, hydrogen-deuterium exchange analysis by mass spectrometry, phage display, competition ELISA and proteolysis-based 'protection' methods. Such methods are described, for example, in Gershoni et al., BioDrugs, 2007, 21(3):145-156, which is hereby incorporated by reference in its entirety. Such methods can also be used to determine whether an antigen-binding molecule is capable of binding to proteins in different conformations.

In some embodiments the antigen-binding molecule of the present invention does not bind to HER3 in the same region of HER3, or an overlapping region of HER3, as an antibody comprising the VH and VL sequences of anti-HER3 antibody clone MM-121 (described e.g. in Schoeberl et al., Sci. Signal. (2009)

2(77): ra31) and/or LJM-716 (described e.g. Garner et al., Cancer Res (2013) 73: 6024–6035). In some embodiments the antigen-binding molecule of the present invention does not display competition with an antibody comprising the VH and VL sequences of anti-HER3 antibody clone MM-121 and/or LJM-716 for binding to HER3, e.g. as determined by SPR analysis.

In some embodiments the antigen-binding molecule of the present invention binds to HER3 in a region which is accessible to an antigen-binding molecule (i.e., an extracellular antigen-binding molecule) when HER3 is expressed at the cell surface (i.e. in or at the cell membrane). In some embodiments the antigen-binding molecule is capable of binding to HER3 expressed at the cell surface of a cell expressing HER3. In some embodiments the antigen-binding molecule is capable of binding to HER3-expressing cells (e.g. HER3+ cells, e.g. HER3+ cancer cells).

The ability of an antigen-binding molecule to bind to a given cell type can be analysed by contacting cells with the antigen-binding molecule, and detecting antigen-binding molecule bound to the cells, e.g. after a washing step to remove unbound antigen-binding molecule. The ability of an antigen-binding molecule to bind to immune cell surface molecule-expressing cells and/or cancer cell antigen-expressing cells can be analysed by methods such as flow cytometry and immunofluorescence microscopy.

The antigen-binding molecule of the present invention may be an antagonist of HER3. In some embodiments, the antigen-binding molecule is capable of inhibiting a function or process (e.g. interaction, signalling or other activity) mediated by HER3 and/or a binding partner for HER3 (e.g. HER3 (i.e. in the case of homodimerisation), HER2, EGFR, HER4, HGFR, IGF1R and/or cMet). Herein, 'inhibition' refers to a reduction, decrease or lessening relative to a control condition.

In some embodiments the antigen-binding molecule of the present invention is capable of inhibiting interaction between HER3 and an interaction partner for HER3. An interaction partner for HER3 may be expressed by the same cell as the HER3. An interaction partner or HER3 may be expressed at the cell surface (i.e. in or at the cell membrane). In some embodiments an interaction partner for HER3 may be a member of the EGFR family of proteins, e.g. HER3, HER2, EGFR, HER4, HGFR, IGF1R and/or cMet. In some embodiments an interaction partner for HER3 may be IGF1R and/or cMet. Interaction between HER3 and an interaction partner for HER3 may result in the formation of a polypeptide complex. Interaction between HER3 and an interaction partner for HER3 to form a polypeptide complex may be referred to as multimerisation. Where multimerisation is between polypeptide monomers multimerisation may be referred to as dimerisation.

In some embodiments the antigen-binding molecule is capable of inhibiting interaction between HER3 monomers. In some embodiments the antigen-binding molecule is capable of inhibiting interaction between HER3 and HER2. In some embodiments the antigen-binding molecule is capable of inhibiting interaction between HER3 and EGFR. In some embodiments the antigen-binding molecule is capable of inhibiting interaction between HER3 and HER4. In some embodiments the antigen-binding molecule is capable of inhibiting interaction between HER3 and HGFR. In some embodiments the antigen-binding

molecule is capable of inhibiting interaction between HER3 and IGF1R. In some embodiments the antigen-binding molecule is capable of inhibiting interaction between HER3 and cMet.

Inhibition of interaction may be achieved by binding of the antigen-binding molecule to a region of HER3 required for interaction between HER3 and an interaction partner for HER3 (e.g. the dimerisation loop of HER3 shown in SEQ ID NO:19). In some embodiments the antigen-binding molecule contacts one or more residues of HER3 necessary for interaction between HER3 and an interaction partner for HER3; in this way the antigen-binding molecule makes the region unavailable, thereby inhibiting interaction. In some embodiments the antigen-binding molecule binds to HER3 in a manner which inhibits/prevents interaction between HER3 and an interaction partner for HER3. In some embodiments the antigen-binding molecule inhibits/prevents access of the interaction partner for HER3 to the region of HER3 required for interaction between HER3 and the interaction partner for HER3; this may be achieved in cases even where the antigen-binding molecule does not contact the region of HER3 required for interaction between HER3 and the interaction partner for HER3, e.g. through steric inhibition of access of the interaction partner for HER3 to the region of HER3 required for interaction between HER3 and the interaction partner.

In some embodiments the antigen-binding molecule is capable of inhibiting homodimerisation of HER3 monomers. In some embodiments the antigen-binding molecule is capable of inhibiting dimerisation between HER3 and HER2. In some embodiments the antigen-binding molecule is capable of inhibiting dimerisation between HER3 and EGFR. In some embodiments the antigen-binding molecule is capable of inhibiting dimerisation between HER3 and HER4. In some embodiments the antigen-binding molecule is capable of inhibiting dimerisation between HER3 and HGFR. In some embodiments the antigen-binding molecule is capable of inhibiting dimerisation between HER3 and IGF1R. In some embodiments the antigen-binding molecule is capable of inhibiting dimerisation between HER3 and cMet.

The ability of an antigen-binding molecule to inhibit interaction between two factors can be determined for example by analysis of interaction in the presence of, or following incubation of one or both of the interaction partners with, the antibody/fragment. Assays for determining whether a given antigen-binding molecule is capable of inhibiting interaction between two interaction partners include competition ELISA assays and analysis by SPR. In some embodiments the antigen-binding molecule is a competitive inhibitor of interaction between HER3 and an interaction partner for HER3.

In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting interaction between HER3 and an interaction partner for HER3 (e.g. HER3, HER2, EGFR, HER4, HGFR, IGF1R and/or cMet) to less than less than 1 times, e.g. ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the level of interaction between HER3 and the interaction partner for HER3 in the absence of the antigen-binding molecule (or in the presence of an appropriate control antigen-binding molecule), in a suitable assay.

The ability of an antigen-binding molecule to inhibit interaction between interaction partners can also be determined by analysis of the downstream functional consequences of such interaction. For example, downstream functional consequences of interaction between HER3 and interaction partners for HER3 include PI3K/AKT/mTOR and/or MAPK signalling. For example, the ability of an antigen-binding molecule to inhibit interaction of HER3 and an interaction partner for HER3 may be determined by analysis of PI3K/AKT/mTOR and/or MAPK signalling following treatment with NRG in the presence of the antigen-binding molecule. PI3K/AKT/mTOR and/or MAPK signalling can be detected and quantified e.g. using antibodies capable of detecting phosphorylated members of the signal transduction pathways.

The ability of an antigen-binding molecule to inhibit interaction of HER3 and an interaction partner for HER3 can also be determined by analysing proliferation of cells expressing HER3 following treatment with NRG in the presence of the antigen-binding molecule. Cell proliferation can be determined e.g. by detecting changes in number of cells over time, or by *in vitro* analysis of incorporation of ^{3}H-thymidine or by CFSE dilution assay, e.g. as described in Fulcher and Wong, Immunol Cell Biol (1999) 77(6): 559-564, hereby incorporated by reference in entirety.

In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting proliferation of cells harbouring mutation to BRAF V600, e.g. cells comprising the BRAF V600E or V600K mutation (see Example 10).

In some embodiments the antigen-binding molecule inhibits HER3-mediated signalling. HER3-mediated signalling can be analysed e.g. using an assay of a correlate of HER3-mediated signalling, e.g. cell proliferation, and/or phosphorylation of one or more signal transduction molecules of the PI3K/AKT/mTOR and/or MAPK signal transduction pathways.

In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting PI3K/AKT/mTOR and/or MAPK signalling by HER3-expressing cells. The level of PI3K/AKT/mTOR and/or MAPK signalling may be analysed by detection and quantification of the level of phosphorylation of one or more of the components of the PI3K/AKT/mTOR and/or MAPK pathways, e.g. following stimulation with NRG (see Example 4.3).

In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting proliferation of HER3-expressing cells, e.g. in response to stimulation with NRG. In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting proliferation of HER3-expressing cells to less than less than 1 times, e.g. ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the level of proliferation of HER3-expressing cells in the absence of the antigen-binding molecule (or in the presence of an appropriate control antigen-binding molecule), in a suitable assay.

In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting PI3K/AKT/mTOR and/or MAPK signalling by HER3-expressing cells to less than less than 1 times, e.g.

≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the level of signalling by HER3-expressing cells in the absence of the antigen-binding molecule (or in the presence of an appropriate control antigen-binding molecule), in a suitable assay.

HER3-mediated signalling can be investigated *in vitro*, e.g. as described in Example 8.9, or *in vivo*, e.g. as described in Example 11.

ADCC activity can be analysed e.g. according to the methods described in Yamashita et al., Scientific Reports (2016) 6:19772 (hereby incorporated by reference in its entirety), or by ^{51}Cr release assay as described e.g. in Jedema et al., Blood (2004) 103: 2677–82 (hereby incorporated by reference in its entirety). ADCC activity can also be analysed using the Pierce LDH Cytotoxicity Assay Kit, in accordance with the manufacturer's instructions (as described in Example 5 herein).

ADCP can be analysed e.g. according to the method described in Kamen et al., J Immunol (2017) 198 (1 Supplement) 157.17 (hereby incorporated by reference in its entirety).

The ability to induce CDC can be analysed e.g. using a C1q binding assay, e.g. as described in Schlothauer et al., Protein Engineering, Design and Selection (2016), 29(10):457–466 (hereby incorporated by reference in its entirety).

Thermostability of antigen-binding molecules can be analysed by methods well known to the skilled person, including Differential Scanning Fuorimetry and Differential Scanning Calorimetry (DSC), which are described e.g. in He et al., J Pharm Sci. (2010) which is hereby incorporated by reference in its entirety. Thermostability may be reflected in terms of a melting temperature (T_m), unfolding temperature or disassembly temperature (expressed e.g. in °C or F°).

In some embodiments, an antigen-binding molecule comprising an Fc region as described herein binds to an activatory Fcγ receptor (e.g. hFcγRIIa (e.g. hFcγRIIa167H, hFcγRIIa167R), hFcγRIIIa (e.g. hFcγRIIIa158V, hFcγRIIIa158F), mFcγRIV, mFcγRIII) with an affinity of binding which is greater than 1 times, e.g. greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or greater than 20 times the affinity of binding to the activatory Fcγ receptor by an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175. In some embodiments the K_D of the antigen-binding molecule comprising an Fc region described herein for binding to the activatory Fcγ receptor is less than 1 times, e.g. less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.07, 0.06 or less than 0.05 times the K_D of an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175 for the activatory Fcγ receptor.

In some embodiments, the antigen-binding molecule comprising an Fc region as described herein binds to an activatory Fcγ receptor (e.g. hFcγRIIa (e.g. hFcγRIIa167H, hFcγRIIa167R), hFcγRIIIa (e.g. hFcγRIIIa158V, hFcγRIIIa158F), mFcγRIV, mFcγRIII) with a K_D of 1000 nM or less, preferably one of

≤500 nM, ≤100 nM, ≤75 nM, ≤50 nM, ≤40 nM, ≤30 nM, ≤20 nM, ≤15 nM, ≤12.5 nM, ≤10 nM, ≤9 nM, ≤8 nM, ≤7 nM, ≤6 nM, ≤5 nM, ≤4 nM ≤3 nM, ≤2 nM or ≤1 nM.

In some embodiments, an antigen-binding molecule comprising an Fc region as described herein binds to an FcRn (e.g. hFcRn, mFcRn) with an affinity of binding which is greater than 1 times, e.g. greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or greater than 20 times the affinity of binding to the FcRn by an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175. In some embodiments the K_D of the antigen-binding molecule comprising an Fc region described herein for binding to the FcRn is less than 1 times, e.g. less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.07, 0.06 or less than 0.05 times the K_D of an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175 for the FcRn.

In some embodiments, the antigen-binding molecule comprising an Fc region as described herein binds to an FcRn (e.g. hFcRn, mFcRn) with a K_D of 1000 nM or less, preferably one of ≤500 nM, ≤100 nM, ≤75 nM, ≤50 nM, ≤40 nM, ≤30 nM, ≤20 nM, ≤15 nM, ≤12.5 nM, ≤10 nM, ≤9 nM, ≤8 nM, ≤7 nM, ≤6 nM, ≤5 nM, ≤4 nM ≤3 nM, ≤2 nM or ≤1 nM.

In some embodiments, an antigen-binding molecule comprising an Fc region as described herein binds to an inhibitory Fcγ receptor (e.g. hFcγRIIb mFcγRIIb) with an affinity of binding which is less than 1 times, e.g. less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or less than 0.1 times the affinity of binding to the inhibitory Fcγ receptor by an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175. In some embodiments the K_D of the antigen-binding molecule comprising an Fc region described herein for binding to the inhibitory Fcγ receptor is greater than 1 times, e.g. greater than 2, 3, 4, 5, 6, 7, 8, 9 or greater than 10 times the K_D of an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175 for the inhibitory Fcγ receptor.

In some embodiments, the antigen-binding molecule comprising an Fc region as described herein binds to an inhibitory Fcγ receptor (e.g. hFcγRIIb mFcγRIIb) with a K_D 1 nM or greater, preferably one of ≥ 5 nM, ≥ 10 nM, ≥ 50 nM, ≥ 100 nM, ≥ 500 nM, ≥ 1000 nM, ≥ 2000 nM, ≥ 3000 nM, ≥ 4000 nM or ≥ 5000 nM.

In some embodiments the selectivity of binding for an activatory Fcγ receptor (e.g. hFcγRIIa) relative to an inhibitory Fcγ receptor (e.g. hFcγRIIb) for an antigen-binding molecule comprising an Fc region as described herein is greater than 1 times, e.g. greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or greater than 20 times selectivity of binding displayed by an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175.

In some embodiments, an antigen-binding molecule comprising an Fc region as described herein displays ADCC which is greater than 1 times, e.g. greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or greater than 20 times the ADCC displayed by an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175.

In some embodiments, the EC50 (ng/ml) determined for an antigen-binding molecule comprising an Fc region as described herein in an assay of ADCC activity less than 1 times, e.g. less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or less than 0.1 times the EC50 (ng/ml) determined for an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175.

In some embodiments, the EC50 (ng/ml) for an antigen-binding molecule comprising an Fc region as described herein in an assay of ADCC activity is 500 ng/ml or less, preferably one of ≤400 ng/ml, ≤300 ng/ml, ≤200 ng/ml, ≤100 ng/ml, ≤90 ng/ml, ≤80 ng/ml, ≤70 ng/ml, ≤60 ng/ml, ≤50 ng/ml, ≤40 ng/ml, ≤30 ng/ml, ≤20 ng/ml, or ≤10 ng/ml.

In some embodiments, an antigen-binding molecule comprising an Fc region as described herein may have a melting temperature, unfolding temperature or disassembly temperature which is which is ≥ 0.75 times and ≤ 1.25 times, e.g. ≥ 0.8 times and ≤ 1.2 times, ≥ 0.85 times and ≤ 1.15 times, ≥ 0.9 times and ≤ 1.1 times, ≥ 0.91 times and ≤ 1.09 times, ≥ 0.92 times and ≤ 1.08 times, ≥ 0.93 times and ≤ 1.07 times, ≥ 0.94 times and ≤ 1.06 times, ≥ 0.95 times and ≤ 1.05 times, ≥ 0.96 times and ≤ 1.04 times, ≥ 0.97 times and ≤ 1.03 times, ≥ 0.98 times and ≤ 1.02 times, or ≥ 0.99 times and ≤ 1.01 times the melting temperature, unfolding temperature or disassembly temperature of an equivalent antigen-binding molecule having an Fc region comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:174-175.

In some embodiments, the antigen-binding molecule of the present invention is capable of increasing killing of HER3-expressing cells. Killing of HER3-expressing cells may be increased through an effector function of the antigen-binding molecule. In embodiments wherein antigen-binding molecule comprises an Fc region the antigen-binding molecule may increasing killing of HER3-expressing cells through one or more of complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).

An antigen-binding molecule which is capable of increasing killing of HER3-expressing cells can be identified by observation of an increased level of killing of HER3-expressing cells in the presence of – or following incubation of the HER3-expressing cells with – the antigen-binding molecule, as compared to the level of cell killing detected in the absence of the antigen-binding molecule (or in the presence of an appropriate control antigen-binding molecule), in an appropriate assay. Assays of CDC, ADCC and ADCP are well known the skilled person. The level of killing of HER3-expressing cells can also be determined by measuring the number/proportion of viable and/or non-viable HER3-expressing cells following exposure to different treatment conditions.

In some embodiments, the antigen-binding molecule of the present invention is capable of increasing killing of HER3-expressing cells (e.g. HER3-expressing cancer cells) to more than 1 times, e.g. ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6

times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of killing observed in the absence of the antigen-binding molecule (or in the presence of an appropriate control antigen-binding molecule).

In some embodiments, the antigen-binding molecule of the present invention is capable of reducing the number of HER3-expressing cells (e.g. HER3-expressing cancer cells) to less than less than 1 times, e.g. ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the number of HER3-expressing cells (e.g. HER3-expressing cancer cells) detected following incubation in the absence of the antigen-binding molecule (or following incubation in the presence of an appropriate control antigen-binding molecule), in a comparable assay.

In some embodiments, the antigen-binding molecule of the present invention inhibits the development and/or progression of cancer *in vivo*.

In some embodiments the antigen-binding molecule causes an increase in the killing of cancer cells, e.g. by effector immune cells. In some embodiments the antigen-binding molecule causes a reduction in the number of cancer cells *in vivo*, e.g. as compared to an appropriate control condition. In some embodiments the antigen-binding molecule inhibits tumor growth, e.g. as determined by measuring tumor size/volume over time.

The antigen-binding molecule of the present invention may be analysed for the ability to inhibit development and/or progression of cancer in an appropriate *in vivo* model, e.g. cell line-derived xenograft model. The cell line-derived xenograft model may be derived from HER3-expressing cancer cells. In some embodiments the model is an N87 cell-derived model, a SNU16 cell-derived model, a FaDu cell-derived model, an OvCAR8 cell-derived model, a HCC95 cell-derived model, an A549 cell-derived model, an ACHN cell-derived model or a HT29 cell-derived model.

The cancer may be a HER3-associated cancer as described herein (i.e. cancers for which HER3 gene/protein expression is a risk factor for, and/or is positively associated with, the onset, development, progression or severity of symptoms of the cancer, and/or metastasis). The cancer may comprise HER3-expressing cells. In some embodiments the cancer comprises a HER3+ tumor.

In some embodiments, administration of an antigen-binding molecule according to the present invention may cause one or more of: inhibition of the development/progression of the cancer, a delay to/prevention of onset of the cancer, a reduction in/delay to/prevention of tumor growth, a reduction in/delay to/prevention of metastasis, a reduction in the severity of the symptoms of the cancer, a reduction in the number of cancer cells, a reduction in tumour size/volume, and/or an increase in survival (e.g. progression free survival), e.g. as determined in an appropriate HER3-expressing cancer cell line-derived xenograft model.

In some embodiments, the antigen-binding molecule of the present invention is capable of inhibiting tumor growth in a HER3-expressing cancer cell line-derived xenograft model to less than less than 1 times, e.g. ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the tumor growth observed in the absence of treatment with the antigen-binding molecule (or following treatment with an appropriate negative control antigen-binding molecule).

Chimeric antigen receptors (CARs)

The present invention also provides Chimeric Antigen Receptors (CARs) comprising the antigen-binding molecules or polypeptides of the present invention.

CARs are recombinant receptors that provide both antigen-binding and T cell activating functions. CAR structure and engineering is reviewed, for example, in Dotti et al., Immunol Rev (2014) 257(1), hereby incorporated by reference in its entirety. CARs comprise an antigen-binding region linked to a cell membrane anchor region and a signalling region. An optional hinge region may provide separation between the antigen-binding region and cell membrane anchor region, and may act as a flexible linker.

The CAR of the present invention comprises an antigen-binding region which comprises or consists of the antigen-binding molecule of the present invention, or which comprises or consists of a polypeptide according to the invention.

The cell membrane anchor region is provided between the antigen-binding region and the signalling region of the CAR and provides for anchoring the CAR to the cell membrane of a cell expressing a CAR, with the antigen-binding region in the extracellular space, and signalling region inside the cell. In some embodiments, the CAR comprises a cell membrane anchor region comprising or consisting of an amino acid sequence which comprises, consists of, or is derived from, the transmembrane region amino acid sequence for one of CD3-ζ, CD4, CD8 or CD28. As used herein, a region which is 'derived from' a reference amino acid sequence comprises an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the reference sequence.

The signalling region of a CAR allows for activation of the T cell. The CAR signalling regions may comprise the amino acid sequence of the intracellular domain of CD3-ζ, which provides immunoreceptor tyrosine-based activation motifs (ITAMs) for phosphorylation and activation of the CAR-expressing T cell. Signalling regions comprising sequences of other ITAM-containing proteins such as FcγRI have also been employed in CARs (Haynes et al., 2001 J Immunol 166(1):182-187). Signalling regions of CARs may also comprise co-stimulatory sequences derived from the signalling region of co-stimulatory molecules, to facilitate activation of CAR-expressing T cells upon binding to the target protein. Suitable co-stimulatory molecules include CD28, OX40, 4-1BB, ICOS and CD27. In some cases CARs are engineered to provide for co-stimulation of different intracellular signalling pathways. For example, signalling associated with CD28 costimulation preferentially activates the phosphatidylinositol 3-kinase

(P13K) pathway, whereas the 4-1BB-mediated signalling is through TNF receptor associated factor (TRAF) adaptor proteins. Signalling regions of CARs therefore sometimes contain co-stimulatory sequences derived from signalling regions of more than one co-stimulatory molecule. In some embodiments, the CAR of the present invention comprises one or more co-stimulatory sequences comprising or consisting of an amino acid sequence which comprises, consists of, or is derived from, the amino acid sequence of the intracellular domain of one or more of CD28, OX40, 4-1BB, ICOS and CD27.

An optional hinge region may provide separation between the antigen-binding domain and the transmembrane domain, and may act as a flexible linker. Hinge regions may be derived from IgG1. In some embodiments, the CAR of the present invention comprises a hinge region comprising or consisting of an amino acid sequence which comprises, consists of, or is derived from, the amino acid sequence of the hinge region of IgG1.

Also provided is a cell comprising a CAR according to the invention. The CAR according to the present invention may be used to generate CAR-expressing immune cells, e.g. CAR-T or CAR-NK cells. Engineering of CARs into immune cells may be performed during culture, *in vitro*.

The antigen-binding region of the CAR of the present invention may be provided with any suitable format, e.g. scFv, scFab, etc.

Nucleic acids and vectors

The present invention provides a nucleic acid, or a plurality of nucleic acids, encoding an antigen-binding molecule, polypeptide or CAR according to the present invention.

In some embodiments, the nucleic acid is purified or isolated, e.g. from other nucleic acid, or naturally-occurring biological material. In some embodiments the nucleic acid(s) comprise or consist of DNA and/or RNA.

The present invention also provides a vector, or plurality of vectors, comprising the nucleic acid or plurality of nucleic acids according to the present invention.

The nucleotide sequence may be contained in a vector, e.g. an expression vector. A “vector” as used herein is a nucleic acid molecule used as a vehicle to transfer exogenous nucleic acid into a cell. The vector may be a vector for expression of the nucleic acid in the cell. Such vectors may include a promoter sequence operably linked to the nucleotide sequence encoding the sequence to be expressed. A vector may also include a termination codon and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express a peptide or polypeptide from a vector according to the invention.

The term “operably linked” may include the situation where a selected nucleic acid sequence and regulatory nucleic acid sequence (e.g. promoter and/or enhancer) are covalently linked in such a way as to place the expression of nucleic acid sequence under the influence or control of the regulatory

sequence (thereby forming an expression cassette). Thus a regulatory sequence is operably linked to the selected nucleic acid sequence if the regulatory sequence is capable of effecting transcription of the nucleic acid sequence. The resulting transcript(s) may then be translated into a desired peptide(s)/polypeptide(s).

Suitable vectors include plasmids, binary vectors, DNA vectors, mRNA vectors, viral vectors (e.g. gammaretroviral vectors (e.g. murine Leukemia virus (MLV)-derived vectors), lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, vaccinia virus vectors and herpesvirus vectors), transposon-based vectors, and artificial chromosomes (e.g. yeast artificial chromosomes).

In some embodiments, the vector may be a eukaryotic vector, e.g. a vector comprising the elements necessary for expression of protein from the vector in a eukaryotic cell. In some embodiments, the vector may be a mammalian vector, e.g. comprising a cytomegalovirus (CMV) or SV40 promoter to drive protein expression.

Constituent polypeptides of an antigen-binding molecule according to the present invention may be encoded by different nucleic acids of the plurality of nucleic acids, or by different vectors of the plurality of vectors.

Cells comprising/expressing the antigen-binding molecules and polypeptides

The present invention also provides a cell comprising or expressing an antigen-binding molecule, polypeptide or CAR according to the present invention. Also provided is a cell comprising or expressing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the invention.

The cell may be a eukaryotic cell, e.g. a mammalian cell. The mammal may be a primate (rhesus, cynomolgous, non-human primate or human) or a non-human mammal (e.g. rabbit, guinea pig, rat, mouse or other rodent (including any animal in the order Rodentia), cat, dog, pig, sheep, goat, cattle (including cows, e.g. dairy cows, or any animal in the order Bos), horse (including any animal in the order Equidae), donkey, and non-human primate).

The present invention also provides a method for producing a cell comprising a nucleic acid(s) or vector(s) according to the present invention, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present invention into a cell. In some embodiments, introducing an isolated nucleic acid(s) or vector(s) according to the invention into a cell comprises transformation, transfection, electroporation or transduction (e.g. retroviral transduction).

The present invention also provides a method for producing a cell expressing/comprising an antigen-binding molecule, polypeptide or CAR according to the present invention, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present invention in a cell. In some embodiments, the methods additionally comprise culturing the cell under conditions suitable for expression of the nucleic acid(s) or vector(s) by the cell. In some embodiments, the methods are performed *in vitro*.

The present invention also provides cells obtained or obtainable by the methods according to the present invention.

Producing the antigen-binding molecules and polypeptides

Antigen-binding molecules and polypeptides according to the invention may be prepared according to methods for the production of polypeptides known to the skilled person.

Polypeptides may be prepared by chemical synthesis, e.g. liquid or solid phase synthesis. For example, peptides/polypeptides can by synthesised using the methods described in, for example, Chandrudu et al., Molecules (2013), 18: 4373-4388, which is hereby incorporated by reference in its entirety.

Alternatively, antigen-binding molecules and polypeptides may be produced by recombinant expression. Molecular biology techniques suitable for recombinant production of polypeptides are well known in the art, such as those set out in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition), Cold Spring Harbor Press, 2012, and in Nat Methods. (2008); 5(2): 135-146 both of which are hereby incorporated by reference in their entirety. Methods for the recombinant production of antigen-binding molecules are also described in Frenzel et al., Front Immunol. (2013); 4: 217 and Kunert and Reinhart, Appl Microbiol Biotechnol. (2016) 100: 3451–3461, both of which are hereby incorporated by reference in their entirety.

In some cases the antigen-binding molecule of the present invention are comprised of more than one polypeptide chain. In such cases, production of the antigen-binding molecules may comprise transcription and translation of more than one polypeptide, and subsequent association of the polypeptide chains to form the antigen-binding molecule.

For recombinant production according to the invention, any cell suitable for the expression of polypeptides may be used. The cell may be a prokaryote or eukaryote. In some embodiments the cell is a prokaryotic cell, such as a cell of archaea or bacteria. In some embodiments the bacteria may be Gram-negative bacteria such as bacteria of the family Enterobacteriaceae, for example Escherichia coli. In some embodiments, the cell is a eukaryotic cell such as a yeast cell, a plant cell, insect cell or a mammalian cell, e.g. CHO, HEK (e.g. HEK293), HeLa or COS cells. In some embodiments, the cell is a CHO cell that transiently or stably expresses the polypeptides.

In some cases the cell is not a prokaryotic cell because some prokaryotic cells do not allow for the same folding or post-translational modifications as eukaryotic cells. In addition, very high expression levels are possible in eukaryotes and proteins can be easier to purify from eukaryotes using appropriate tags. Specific plasmids may also be utilised which enhance secretion of the protein into the media.

In some embodiments polypeptides may be prepared by cell-free-protein synthesis (CFPS), e.g. according using a system described in Zemella et al. Chembiochem (2015) 16(17): 2420-2431, which is hereby incorporated by reference in its entirety.

Production may involve culture or fermentation of a eukaryotic cell modified to express the polypeptide(s) of interest. The culture or fermentation may be performed in a bioreactor provided with an appropriate supply of nutrients, air/oxygen and/or growth factors. Secreted proteins can be collected by partitioning culture media/fermentation broth from the cells, extracting the protein content, and separating individual proteins to isolate secreted polypeptide(s). Culture, fermentation and separation techniques are well known to those of skill in the art, and are described, for example, in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition; incorporated by reference herein above).

Bioreactors include one or more vessels in which cells may be cultured. Culture in the bioreactor may occur continuously, with a continuous flow of reactants into, and a continuous flow of cultured cells from, the reactor. Alternatively, the culture may occur in batches. The bioreactor monitors and controls environmental conditions such as pH, oxygen, flow rates into and out of, and agitation within the vessel such that optimum conditions are provided for the cells being cultured.

Following culturing the cells that express the antigen-binding molecule/polypeptide(s), the polypeptide(s) of interest may be isolated. Any suitable method for separating proteins from cells known in the art may be used. In order to isolate the polypeptide it may be necessary to separate the cells from nutrient medium. If the polypeptide(s) are secreted from the cells, the cells may be separated by centrifugation from the culture media that contains the secreted polypeptide(s) of interest. If the polypeptide(s) of interest collect within the cell, protein isolation may comprise centrifugation to separate cells from cell culture medium, treatment of the cell pellet with a lysis buffer, and cell disruption e.g. by sonification, rapid freeze-thaw or osmotic lysis.

It may then be desirable to isolate the polypeptide(s) of interest from the supernatant or culture medium, which may contain other protein and non-protein components. A common approach to separating protein components from a supernatant or culture medium is by precipitation. Proteins of different solubilities are precipitated at different concentrations of precipitating agent such as ammonium sulfate. For example, at low concentrations of precipitating agent, water soluble proteins are extracted. Thus, by adding different increasing concentrations of precipitating agent, proteins of different solubilities may be distinguished. Dialysis may be subsequently used to remove ammonium sulfate from the separated proteins.

Other methods for distinguishing different proteins are known in the art, for example ion exchange chromatography and size chromatography. These may be used as an alternative to precipitation, or may be performed subsequently to precipitation.

Once the polypeptide(s) of interest have been isolated from culture it may be desired or necessary to concentrate the polypeptide(s). A number of methods for concentrating proteins are known in the art, such as ultrafiltration or lyophilisation.

Compositions

The present invention also provides compositions comprising the antigen-binding molecules, polypeptides, CARs, nucleic acids, expression vectors and cells described herein.

The antigen-binding molecules, polypeptides, CARs, nucleic acids, expression vectors and cells described herein may be formulated as pharmaceutical compositions or medicaments for clinical use and may comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The composition may be formulated for topical, parenteral, systemic, intracavitary, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, intratumoral, subcutaneous, intradermal, intrathecal, oral or transdermal routes of administration which may include injection or infusion.

Suitable formulations may comprise the antigen-binding molecule in a sterile or isotonic medium. Medicaments and pharmaceutical compositions may be formulated in fluid, including gel, form. Fluid formulations may be formulated for administration by injection or infusion (e.g. via catheter) to a selected region of the human or animal body.

In some embodiments the composition is formulated for injection or infusion, e.g. into a blood vessel or tumor.

In accordance with the invention described herein methods are also provided for the production of pharmaceutically useful compositions, such methods of production may comprise one or more steps selected from: producing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein; isolating an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein; and/or mixing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

For example, a further aspect the invention described herein relates to a method of formulating or producing a medicament or pharmaceutical composition for use in the treatment of a disease/condition (e.g. a cancer), the method comprising formulating a pharmaceutical composition or medicament by mixing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

Therapeutic and prophylactic applications

The antigen-binding molecules, polypeptides, CARs, nucleic acids, expression vectors, cells and compositions described herein find use in therapeutic and prophylactic methods.

The present invention provides an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein for use in a method of medical treatment or prophylaxis. Also provided is the use of an antigen-binding molecule, polypeptide,

CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein in the manufacture of a medicament for treating or preventing a disease or condition. Also provided is a method of treating or preventing a disease or condition, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

The methods may be effective to reduce the development or progression of a disease/condition, alleviation of the symptoms of a disease/condition or reduction in the pathology of a disease/condition. The methods may be effective to prevent progression of the disease/condition, e.g. to prevent worsening of, or to slow the rate of development of, the disease/condition. In some embodiments the methods may lead to an improvement in the disease/condition, e.g. a reduction in the symptoms of the disease/condition or reduction in some other correlate of the severity/activity of the disease/condition. In some embodiments the methods may prevent development of the disease/condition a later stage (e.g. a chronic stage or metastasis).

It will be appreciated that the articles of the present invention may be used for the treatment/prevention of any disease/condition that would derive therapeutic or prophylactic benefit from a reduction in the number and/or activity of cells expressing HER3. For example, the disease/condition may be a disease/condition in which cells expressing HER3 are pathologically implicated, e.g. a disease/condition in which an increased number/proportion of cells expressing HER3 is positively associated with the onset, development or progression of the disease/condition, and/or severity of one or more symptoms of the disease/condition, or for which an increased number/proportion of cells expressing HER3, is a risk factor for the onset, development or progression of the disease/condition.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present invention is a disease/condition characterised by an increase in the number/proportion/activity of cells expressing HER3, e.g. as compared to the number/proportion/activity of cells expressing HER3 in the absence of the disease/condition.

In some embodiments the disease/condition to be treated/prevented is a cancer.

The cancer may be any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor. The cancer may be benign or malignant and may be primary or secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells and may be located in any tissue. The cancer may be of tissues/cells derived from e.g. the adrenal gland, adrenal medulla, anus, appendix, bladder, blood, bone, bone marrow, brain, breast, cecum, central nervous system (including or excluding the brain) cerebellum, cervix, colon, duodenum, endometrium, epithelial cells (e.g. renal epithelia), gallbladder, oesophagus, glial cells, heart, ileum, jejunum, kidney, lacrimal glad, larynx, liver, lung, lymph, lymph node, lymphoblast, maxilla, mediastinum, mesentery, myometrium, nasopharynx, omentum, oral cavity, ovary, pancreas, parotid gland, peripheral nervous system, peritoneum, pleura, prostate, salivary gland, sigmoid colon, skin, small intestine, soft tissues,

spleen, stomach, testis, thymus, thyroid gland, tongue, tonsil, trachea, uterus, vulva, and/or white blood cells.

Tumors to be treated may be nervous or non-nervous system tumors. Nervous system tumors may originate either in the central or peripheral nervous system, e.g. glioma, medulloblastoma, meningioma, neurofibroma, ependymoma, Schwannoma, neurofibrosarcoma, astrocytoma and oligodendroglioma. Non-nervous system cancers/tumors may originate in any other non-nervous tissue, examples include melanoma, mesothelioma, lymphoma, myeloma, leukemia, Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), cutaneous T-cell lymphoma (CTCL), chronic lymphocytic leukemia (CLL), hepatoma, epidermoid carcinoma, prostate carcinoma, breast cancer, lung cancer , colon cancer, ovarian cancer, pancreatic cancer, thymic carcinoma, NSCLC, hematologic cancer and sarcoma.

HER3 and its association with and role in cancer is reviewed e.g. in Karachaliou et al., BioDrugs. (2017) 31(1):63-73 and Zhang et al., Acta Biochimica et Biophysica Sinica (2016) 48(1): 39–48, both of which are hereby incorporated by reference in their entirety.

In some embodiments, a cancer is selected from: a cancer comprising cells expressing HER3, a solid tumor, breast cancer, breast carcinoma, ductal carcinoma, gastric cancer, gastric carcinoma, gastric adenocarcinoma, colorectal cancer, colorectal carcinoma, colorectal adenocarcinoma, head and neck cancer, squamous cell carcinoma of the head and neck (SCCHN), lung cancer, lung adenocarcinoma, squamous cell lung carcinoma, ovarian cancer, ovarian carcinoma, ovarian serous adenocarcinoma, kidney cancer, renal cell carcinoma, renal clear cell carcinoma, renal cell adenocarcinoma, renal papillary cell carcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, cervical cancer, cervical squamous cell carcinoma, skin cancer, melanoma, esophageal cancer, esophageal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, uterine cancer, uterine corpus endometrial carcinoma, thyroid cancer, thyroid carcinoma, pheochromocytoma, paraganglioma, bladder cancer, bladder urothelial carcinoma, prostate cancer, prostate adenocarcinoma, sarcoma and thymoma.

In some embodiments the cancer to be treated in accordance with the present invention is selected from: a HER3-expressing cancer, gastric cancer (e.g. gastric carcinoma, gastric adenocarcinoma, gastrointestinal adenocarcinoma), head and neck cancer (e.g. head and neck squamous cell carcinoma), breast cancer, ovarian cancer (e.g. ovarian carcinoma), lung cancer (e.g. NSCLC, lung adenocarcinoma, squamous lung cell carcinoma), melanoma, prostate cancer, oral cavity cancer (e.g. oropharyngeal cancer), renal cancer (e.g. renal cell carcinoma) or colorectal cancer (e.g. colorectal carcinoma), oesophageal cancer, pancreatic cancer, a solid cancer and/or a liquid cancer.

The treatment/prevention may be aimed at one or more of: delaying/preventing the onset/progression of symptoms of the cancer, reducing the severity of symptoms of the cancer, reducing the survival/growth/invasion/metastasis of cells of the cancer, reducing the number of cells of the cancer and/or increasing survival of the subject.

In some embodiments, the cancer to be treated/prevented comprises cells expressing an EGFR family member (e.g. HER3, EGFR, HER2 or HER4), and/or cells expressing a ligand for an EGFR family member. In some embodiments, the cancer to be treated/prevented is a cancer which is positive for an EGFR family member. In some embodiments, the cancer over-expresses an EGFR family member and/or a ligand for an EGFR family member. Overexpression of can be determined by detection of a level of expression which is greater than the level of expression by equivalent non-cancerous cells/non-tumor tissue.

Expression may be determined by any suitable means. Expression may be gene expression or protein expression. Gene expression can be determined e.g. by detection of mRNA encoding HER3, for example by quantitative real-time PCR (qRT-PCR). Protein expression can be determined e.g. by for example by antibody-based methods, for example by western blot, immunohistochemistry, immunocytochemistry, flow cytometry, or ELISA.

In some embodiments, the cancer to be treated/prevented comprises cells expressing HER3. In some embodiments, the cancer to be treated/prevented is a cancer which is positive for HER3. In some embodiments, the cancer over-expresses HER3. Overexpression of HER3 can be determined by detection of a level of expression of HER3 which is greater than the level of expression by equivalent non-cancerous cells/non-tumor tissue.

In some embodiments, a patient may be selected for treatment described herein based on the detection of a cancer expressing HER3, or overexpressing HER3, e.g. in a sample obtained from the subject.

In some embodiments, the cancer to be treated/prevented comprises cells expressing a ligand for HER3 (e.g. NRG1 and/or NRG2). In some embodiments, the cancer to be treated/prevented comprises cells expressing a level of expression of NRG1 and/or NRG2 which is greater than the level of expression by equivalent non-cancerous cells/non-tumor tissue.

HER3-binding antigen-binding molecules described herein are demonstrated to bind to HER3 with extremely high affinity when HER3 is bound by NRG (i.e. when HER3 is provided in the ‘open’ conformation), and also when HER3 is not bound by NRG (i.e. when HER3 is provided in the ‘closed’ conformation).

Thus the antigen-binding molecules of the present invention are particularly useful for the treatment/prevention of cancers characterised by HER3 ligand expression/overexpression, for example cancers/tumors comprising cells expressing/overexpressing a ligand for HER3.

In some embodiments, the cancer to be treated in accordance with the present invention comprises cells harbouring a genetic variant (e.g. a mutation) which causes increased (gene and/or protein) expression of a ligand for HER3, relative to comparable cells harbouring a reference allele not comprising the genetic variant (e.g. a non-mutated, or ‘wildtype’ allele). The genetic variant may be or comprise insertion,

deletion, substitution to, or larger-scale translocation/rearrangement of, the nucleotide sequence relative to the reference allele.

A mutation 'resulting in' increased expression of a ligand for HER3 may be known or predicted to cause, or may be associated with, increased gene/protein expression of a ligand for HER3. Mutations resulting in increased expression of a ligand for HER3 may be referred to as 'activating' mutations.

A mutation which causes increased expression of a ligand for HER3 may result in gene or protein expression of a ligand for HER3 which is not expressed by, and/or not encoded by genomic nucleic acid of, an equivalent cell not harbouring the mutation. That is, the ligand for HER3 may be a neoantigen arising as a result of the mutation, and thus 'increased expression' may be from no expression. By way of illustration, a cell comprising *CD47-NRG1* gene fusion displays increased expression of the CD47-NRG1 fusion polypeptide encoded by the gene fusion relative to cells lacking the *CD47-NRG1* gene fusion.

A mutation which causes increased expression of a ligand for HER3 may result in increased gene or protein expression of a ligand for HER3 which is expressed by, and/or which is encoded by genomic nucleic acid of, an equivalent cell not comprising the mutation. By way of illustration, a cell may comprise a mutation resulting in an increase in the level of transcription of nucleic acid encoding NRG1 relative to level of transcription of nucleic acid encoding NRG1 by an equivalent cell not comprising the mutation.

In some embodiments, a mutation which causes increased expression of a ligand for HER3 may cause an increase in gene expression of a ligand for HER3 relative to an equivalent cell not comprising the mutation. In some embodiments, a mutation which causes increased expression of a ligand for HER3 may cause an increase in protein expression of a ligand for HER3 relative to an equivalent cell not comprising the mutation.

In some embodiments, a mutation which causes increased expression of a ligand for HER3 may cause an increase in the level of a ligand for HER3 on or at the cell surface of a cell comprising the mutation, relative to an equivalent cell not comprising the mutation. In some embodiments, a mutation which causes increased expression of a ligand for HER3 may cause an increase in the level of a secretion of a ligand for HER3 from a cell comprising the mutation, relative to an equivalent cell not comprising the mutation.

Cells having increased expression of a ligand for HER3 relative to the level of expression of the ligand for HER3 by a reference cell (e.g. as a result of mutation) may be described as 'overexpressing' the ligand for HER3, or having 'upregulated expression' of the ligand for HER3. For example, a cancer comprising cells harbouring a mutation resulting in increased expression of a ligand for HER3 relative to equivalent cells lacking the mutation may be described as a cancer comprising cells displaying overexpression/upregulated expression of the ligand for HER3. In some embodiments, the reference cell lacking the mutation may be a non-cancerous cell (e.g. of equivalent cell type) or a cancerous cell (e.g. of equivalent cancer type).

Herein, a 'ligand for HER3' is generally intended to refer to molecule capable of binding to HER3 through the ligand binding region of HER3 formed by domains I and III of HER3. In some embodiments, a ligand for HER3 binds to HER3 via interaction with domains I and/or III of HER3. Exemplary ligands for HER3 include Neuregulins such as NRG1 and NRG2, which bind to HER3 via interaction between their EGF-like domains and the ligand binding region of HER3.

The HER3 ligand is preferably able to bind and trigger signalling through the HER3 receptor and/or receptor complexes comprising HER3. As will be clear from the present disclosure, receptor complexes comprising HER3 may further comprise an interaction partner for HER3 as described herein, e.g. HER3, HER2, EGFR, HER4, HGFR, IGF1R and/or cMet).

In some embodiments the ligand for HER3 is able to bind to HER3 receptor/receptor complex expressed by a cell other than the cell having increased expression of the HER3 ligand. For example, in some embodiments the ligand for HER3 is able to bind to a HER3-expressing cancer cell.

In some embodiments the ligand for HER3 is able to bind to HER3 receptor/receptor complex expressed by the cell having increased expression of the HER3 ligand.

In some embodiments the cancer to be treated comprises (i) cells expressing HER3, and (ii) cells expressing a ligand for HER3 (e.g. having increased expression of a ligand for HER3, e.g. as a consequence of mutation resulting in increased expression of a ligand for HER3).

In some embodiments the cancer to be treated comprises cells which (i) express HER3 and (ii) which also express a ligand for HER3 (e.g. which have increased expression of a ligand for HER3, e.g. as a consequence of mutation resulting in increased expression of a ligand for HER3).

In some embodiments, the ligand for HER3 comprises, or consists of, the amino acid sequence a HER3-binding region of a ligand for HER3, or an amino acid sequence derived from a HER3-binding region of a ligand for HER3. An amino acid sequence which is derived from a HER3-binding region of a ligand for HER3 may comprise at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the amino acid sequence from which it is derived.

In some embodiments, the ligand for HER3 comprises an EGF-like domain capable of binding to HER3, or a HER3-binding fragment thereof. In some embodiments, a HER3-binding EGF-like domain/fragment is, or is derived from, an EGF family member (e.g. heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor-α (TGF-α), amphiregulin (AR), epiregulin (EPR), epigen, betacellulin (BTC), NRG1, NRG2, NRG3 or NRG4).

EGF family members contain one or more repeats of the conserved amino acid sequence shown in SEQ ID NO:240, which contains six cysteine residues that form three intramolecular disulfide bonds, providing three structural loops required for high-affinity binding to their cognate receptors (see Harris et al.

Experimental Cell Research (2003) 284(1): 2-13). In some embodiments, a ligand for HER3 comprises one or more copies of an amino acid sequence conforming to the consensus sequence shown in SEQ ID NO:240.

Exemplary ligands for HER3 include Neuregulins (NRGs). Neuregulins include NRG1, NRG2, NRG3 and NRG4. The amino acid sequence of human NRG1 (alpha isoform) is shown in SEQ ID NO:232. The alpha isoform and several other isoforms of human NRG1 (including alpha1a isoform (see UnitProt: Q02297-2), alpha2b isoform (see UnitProt: Q02297-3) and alpha3 isoform (see UnitProt: Q02297-4)) comprise the EGF-like domain shown in SEQ ID NO:233, through which they bind to HER3. The amino acid sequence of human NRG2 (isoform 1) is shown in SEQ ID NO:234. Isoform 1 and several other isoforms of human NRG2 (including isoform 3 (see UniProt:O14511-3), isoform 5 (see UniProt:O14511-5), isoform 6 (see UniProt:O14511-6), isoform DON-1B (see UniProt:O14511-7) and isoform DON-1R (see UniProt:O14511-8)) comprise the EGF-like domain shown in SEQ ID NO:235, through which they bind to HER3. The amino acid sequence of human NRG3 is shown in SEQ ID NO:236, and the EGF-like domain of human NRG3 shown in SEQ ID NO:237. The amino acid sequence of human NRG4 is shown in SEQ ID NO:238, and the EGF-like domain of human NRG3 shown in SEQ ID NO:239. In some embodiments, an NRG is selected from NRG1, NRG2, NRG3 and NRG4. In some embodiments, an NRG is selected from NRG1 and NRG2.

In some embodiments an EGF-like domain/fragment comprises, or consists of, an amino acid sequence having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the EGF-like domain of an NRG (NRG1, NRG2, NRG3 or NRG4). In some embodiments an EGF-like domain/fragment comprises, or consists of, an amino acid sequence having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to one of SEQ ID NOs:233, 235, 237 or 239.

In some embodiments, the ligand for HER3 is an NRG (e.g. NRG1, NRG2, NRG3 or NRG4; e.g. NRG1 or NRG2), or comprises an amino acid sequence derived from an amino acid sequence of an NRG (i.e. comprises an amino acid sequence having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to an amino acid sequence of an NRG.

In some embodiments, the ligand for HER3 comprises, or consists of, an amino acid sequence having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the HER3-binding region of a ligand for HER3 (e.g. an NRG, e.g. NRG1, NRG2, NRG3 or NRG4; e.g. NRG1 or NRG2). In some embodiments, a ligand for HER3 comprises, or consists of, an amino acid sequence having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the EGF-like domain of an NRG (e.g. NRG1, NRG2, NRG3 or NRG4; e.g. NRG1 or NRG2).

In some embodiments a ligand for HER3 is not an EGFR family protein (e.g. HER3, HER2, EGFR, HER4, HGFR, IGF1R, cMet).

In some embodiments, the mutation resulting in increased expression of a ligand for HER3 is an NRG gene fusion. In some embodiments, the ligand for HER3 is the product of (i.e. a polypeptide encoded by) an NRG gene fusion. In some embodiments the cancer comprises cells having an NRG gene fusion.

As used herein, an "NRG gene fusion" refers to a genetic variant encoding a polypeptide comprising (i) an amino acid sequence of an NRG protein (e.g. NRG1, NRG2, NRG3 or NRG4; e.g. NRG1 or NRG2), and (ii) an amino acid sequence of a protein other than the NRG protein.

It will be appreciated that an NRG gene fusion preferably encodes a HER3 ligand as described herein. In some embodiments, an NRG gene fusion encodes a polypeptide comprising a HER3-binding region of an NRG protein. In some embodiments, an NRG gene fusion encodes a polypeptide comprising the EGF-like domain of an NRG protein, or an amino acid sequence which is capable of binding to HER3 and having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the EGF-like domain of an NRG protein.

In some embodiments, an NRG gene fusion encodes a fusion polypeptide comprising a transmembrane domain. In some embodiments, an NRG gene fusion encodes a fusion polypeptide comprising the transmembrane domain of a protein other than the NRG protein.

In some embodiments, an NRG gene fusion is an *NRG1* gene fusion. In some embodiments, the *NRG1* gene fusion encodes a polypeptide comprising the EGF-like domain of NRG1, or an amino acid sequence which is capable of binding to HER3 and having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the EGF-like domain of NRG1.

NRG1 gene fusions are described e.g. in WO 2018/182422 A1, WO 2019/051155 A1, Dhanasekaran et al., Nat Commun. (2014) 5: 5893, Drilon et al., Cancer Discov. (2018) 8(6):686-695, Nagasaka et al., Journal of Thoracic Oncology (2019) 14(8):1354-1359 and Jonna et al., Clin Cancer Res. (2019) 25(16):4966-4972, all of which are hereby incorporated by reference in their entirety. The diversity of *NRG1* gene fusions may result from *NRG1* being located on chromosome 8, which is particularly susceptible to genomic translocation events (Adélaïde et al., Genes Chromosomes Cancer. (2003) 37(4):333-45).

In some embodiments, an *NRG1* gene fusion is selected from *CLU-NRG1, CD74-NRG1, DOC4-NRG1, SLC3A2-NRG1, RBPMS-NRG1, WRN-NRG1, SDC4-NRG1, RAB2IL1-NRG1, VAMP2-NRG1, KIF13B-NRG1, THAP7-NRG1, SMAD4-NRG1, MDK-NRG1, TNC-NRG1, DIP2B-NRG1, MRPL13-NRG1, PARP8-NRG1, ROCK1-NRG1, DPYSL2-NRG1, ATP1B1-NRG1, CDH6-NRG1, APP-NRG1, AKAP13-NRG1, THBS1-NRG1, FOXA1-NRG1, PDE7A- NRG1, RAB3IL1-NRG1, CDK1-NRG1, BMPRIB-NRG1, TNFRSF10B-NRG1, and MCPH1-NRG1*. In some embodiments, an *NRG1* gene fusion is *CLU-NRG1.*

CD74-NRG1 gene fusion is described e.g. in Fernandez-Cuesta et al. Cancer Discov. (2014) 4:415–22 and Nakaoku et al., Clin Cancer Res (2014) 20:3087-93. *DOC4-NRG1* gene fusion is described e.g. in Liu et al., Oncogene. (1999) 18(50):7110-4 and Wang et al., Oncogene. (1999) 18(41):5718-21. *SLC3A2-NRG1* gene fusion is described e.g. in Nakaoku et al., Clin Cancer Res (2014) 20:3087-93, Shin et al., Oncotarget (2016) 7:69450–65 and Shin et al., Mol Cancer Ther. (2018) 17(9):2024-2033. *RBPMS-NRG1*, *WRN-NRG1*, *RAB2IL1-NRG1* and *SDC4-NRG1* gene fusions are described e.g. in Dhanasekaran et al., Nat Commun. (2014) 5: 5893. *VAMP2-NRG1* gene fusion is described e.g. in Jung et al., J Thorac Oncol. (2015) 10(7):1107-11 and Shim et al., J Thorac Oncol. (2015) 10(8):1156-62. *KIF13B-NRG1* gene fusion is described e.g. in Xia et al., Int J Surg Pathol. (2017) 25(3):238-240. *SMAD4-NRG1, AKAP13-NRG1, THBS1-NRG1, FOXA1-NRG1, PDE7A- NRG1, RAB3IL1-NRG1 and THAP7-NRG1* gene fusions are described e.g. in Drilon et al., Cancer Discov. (2018) 8(6):686-695. *MDK-NRG1, TNC-NRG1, DIP2B-NRG1, MRPL13-NRG1, PARP8-NRG1, ROCK1-NRG1 and DPYSL2-NRG1* gene fusions are described e.g. in Jonna et al., Clin Cancer Res. (2019) 25(16):4966-4972. *ATP1B1-NRG1* gene fusion is described e.g. in Drilon et al., Cancer Discov. (2018) 8(6):686-695 and Jones et al., Annals of Oncology (2017) 28:3092–3097. *CLU-NRG1* gene fusion is described e.g. in Drilon et al., Cancer Discov. (2018) 8(6):686-695 and Nagasaka et al., Journal of Thoracic Oncology (2019) 14(8):1354-1359.

In some embodiments, an NRG gene fusion is an *NRG2* gene fusion. In some embodiments, the *NRG2* gene fusion encodes a polypeptide comprising the EGF-like domain of NRG2, or an amino acid sequence which is capable of binding to HER3 and having at least 60% (e.g. 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) amino acid sequence identity to the EGF-like domain of NRG2.

NRG2 gene fusions include *SLC12A2-NRG2* described e.g. in WO 2015/093557 A1, and *ZNF208-NRG2* described in Dupain et al., Mol Ther. (2019) 27(1):200-218.

A cancer comprising cells having a mutation which results in increased expression of a ligand for HER3 (e.g. comprising cells having an NRG gene fusion, e.g. an *NRG1* gene fusion or an *NRG2* gene fusion) can be any cancer described herein. In some embodiments, such cancer may be of tissues/cells derived from the lung, breast, head, neck, kidney, ovary, pancreas, prostate, uterus, gallbladder, colon, rectum, bladder, soft tissue or nasopharynx.

In some embodiments, a cancer comprising cells having a mutation which results in increased expression of a ligand for HER3 (e.g. comprising cells having an NRG gene fusion, e.g. an *NRG1* gene fusion or an *NRG2* gene fusion) is selected from: lung cancer, non-small cell lung cancer, lung adenocarcinoma, invasive mucinous lung adenocarcinoma, lung squamous cell carcinoma, breast cancer, breast carcinoma, breast invasive carcinoma, head and neck cancer, head and neck squamous cell carcinoma, renal cancer, renal clear cell carcinoma, ovarian cancer, ovarian serous cystadenocarcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, prostate cancer, prostate adenocarcinoma, endometrial cancer, uterine carcinosarcoma, gallbladder cancer, cholangiocarcinoma, colorectal cancer, bladder cancer, urothelial bladder cancer, sarcoma, soft tissue sarcoma, neuroendocrine tumor and neuroendocrine tumor of the nasopharynx.

In particular embodiments, the cancer to be treated in accordance with the present invention is lung cancer (e.g. non-small cell lung cancer, lung adenocarcinoma, invasive mucinous lung adenocarcinoma or lung squamous cell carcinoma) comprising cells having an *NRG1* gene fusion.

It will be appreciated that in embodiments herein, cancers comprising cells having specified characteristics may be or comprise tumors comprising cells having those characteristics.

As is common in the art, a cancer/tumor comprising cells having specified characteristics may be referred to herein simply as a cancer/tumor having those characteristics. By way of illustration, a cancer/tumor comprising cells having an NRG1 gene fusion may be referred to simply as “a cancer/tumor comprising NRG1 gene fusion”, or “an NRG1 gene fusion cancer/tumor”.

Administration of the articles of the present invention is preferably in a "therapeutically effective” or “prophylactically effective” amount, this being sufficient to show therapeutic or prophylactic benefit to the subject. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease/condition and the particular article administered. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disease/disorder to be treated, the condition of the individual subject, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington’s Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Administration may be alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. The antigen-binding molecule or composition described herein and a therapeutic agent may be administered simultaneously or sequentially.

In some embodiments, the methods comprise additional therapeutic or prophylactic intervention, e.g. for the treatment/prevention of a cancer. In some embodiments, the therapeutic or prophylactic intervention is selected from chemotherapy, immunotherapy, radiotherapy, surgery, vaccination and/or hormone therapy. In some embodiments, the therapeutic or prophylactic intervention comprises leukapheresis. In some embodiments the therapeutic or prophylactic intervention comprises a stem cell transplant.

In some embodiments the antigen-binding molecule is administered in combination with an agent capable of inhibiting signalling mediated by an EGFR family member.

Accordingly, the invention provides compositions comprising an article according to the present invention (e.g. an antigen-binding molecule according to the invention) and another agent capable of inhibiting signalling mediated by an EGFR family member (e.g. EGFR, HER2, HER3 or HER4). Also provided is the use of such compositions in methods of medical treatment and prophylaxis of diseases/conditions described herein.

Also provided are methods for treating/preventing diseases/conditions described herein comprising administering articles of the present invention an article according to the present invention (e.g. an antigen-binding molecule according to the invention) and another agent capable of inhibiting signalling mediated by an EGFR family member.

Agents capable of inhibiting signalling mediated by EGFR family members are known in the art, and include e.g. small molecule inhibitors (e.g. tyrosine kinase inhibitors), monoclonal antibodies (and antigen-binding fragments thereof), peptide/polypeptide inhibitors (e.g. decoy ligands/receptors or peptide aptamers) and nucleic acids (e.g. antisense nucleic acid, splice-switching nucleic acids or nucleic acid aptamers). Inhibitors of signalling mediated by EGFR family members include agents that inhibit signalling through a direct effect on an EGFR family member, an interaction partner therefore, and/or a downstream factor involved in signalling mediated by the EGFR family member.

In some embodiments the antagonist of signalling mediated by an EGFR family member inhibits signalling mediated by one or more of EGFR, HER2, HER4 and HER3. Inhibitors of signalling mediated by EGFR family members are described e.g. in Yamaoka et al., Int. J. Mol. Sci. (2018), 19, 3491, which is hereby incorporated by reference in its entirety. In some embodiments the antagonist is a pan-ErbB inhibitor. In some embodiments the antagonist is an inhibitor of signalling mediated by EGFR (e.g. cetuximab, panitumumab, gefitinib, erlotinib, lapatinib, afatinib, brigatinib, icotinib, osimertinib, zalutumumab, vandetanib, necitumumab, nimotuzumab, dacomitinib, duligotuzumab or matuzumab). In some embodiments the antagonist is an inhibitor of signalling mediated by HER2 (e.g. trastuzumab, pertuzumab, lapatinib, neratinib, afatinib, dacomitinib, MM-111, MCLA-128 or margetuximab). In some embodiments the antagonist is an inhibitor of signalling mediated by HER3 (e.g. seribantumab, lumretuzumab, elgemtumab, KTN3379, AV-203, GSK2849330, REGN1400, MP-RM-1, EV20, duligotuzumab, MM-111, istiratumab, MCLA-128, patritumab, EZN-3920, RB200 or U3-1402). In some embodiments the antagonist is an inhibitor of signalling mediated by HER4 (e.g. lapatinib, ibrutinib, afatinib, dacomitinib or neratinib).

In some embodiments the antagonist of signalling mediated by an EGFR family member inhibits a downstream effector of signalling by an EGFR family member. Downstream effectors of signalling by an EGFR family members include e.g. PI3K, AKT, KRAS, BRAF, MEK/ERK and mTOR. In some embodiments, the antagonist of signalling mediated by an EGFR family member is an inhibitor of the MAPK/ERK pathway. In some embodiments, the antagonist of signalling mediated by an EGFR family member is an inhibitor of the PI3K/ATK/mTOR pathway. In some embodiments the antagonist is a PI3K inhibitor (e.g. pictilisib, buparlisib, idelalisib, copanlisib or duvelisib). In some embodiments the antagonist is an AKT inhibitor (e.g. MK-2206, AZD5363, ipatasertib, VQD-002, perifosine or miltefosine). In some embodiments the antagonist is a BRAF inhibitor (e.g. vemurafenib, dabrafenib, SB590885, XL281, RAF265, encorafenib, GDC-0879, PLX-4720, sorafenib, or LGX818). In some embodiments the antagonist is a MEK/ERK inhibitor (e.g. trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, PD035901, or TAK-733). In some embodiments the antagonist is a mTOR inhibitor (e.g. rapamycin, deforolimus, temsirolimus, everolimus, ridaforolimus or sapanisertib).

In some embodiments, the cancer to be treated in accordance with an aspect of the present invention (including monotherapy or combination therapy) is a cancer which is resistant to treatment with an antagonist of signalling mediated by an EGFR family member (e.g. EGFR, HER2, HER4 and/or HER3), e.g. an antagonist as described in the preceding three paragraphs. In some embodiments the subject to be treated has a cancer which is resistant to treatment with an antagonist of signalling mediated by an EGFR family member. In some embodiments the subject to be treated has a cancer which has developed resistance to treatment with an antagonist of signalling mediated by an EGFR family member. In some embodiments the subject to be treated has a cancer which previously responded to treatment with an antagonist of signalling mediated by an EGFR family member, and which is now resistant to treatment with the antagonist. In some embodiments the subject to be treated has a cancer which has relapsed and/or progressed following treatment with an antagonist of signalling mediated by an EGFR family member. In some embodiments the subject to be treated has a cancer which initially responded to treatment with an antagonist of signalling mediated by an EGFR family member, but later progressed on said treatment.

In some embodiments a subject to be treated in accordance with the present invention may have been determined to have (i.e. may have been diagnosed as having) a cancer comprising cells having a mutation which causes increased expression of a ligand for HER3 (e.g. as described herein). In some embodiments the methods of the invention may comprise determining whether a subject has a cancer comprising cells having a mutation which causes increased expression of a ligand for HER3. In some embodiments, the methods comprise analysing nucleic acid from cells of a cancer. In some embodiments the methods comprise detecting a mutation which causes increased expression of a ligand for HER3.

The skilled person is readily able to identify cancers and subjects described herein. Such cancers and subjects may be identified e.g. through monitoring of the development/progression of the cancer (and/or correlates thereof) over time e.g. during the course of treatment with an antagonist of signalling mediated by an EGFR family member. In some embodiments, identification of such subjects/cancers may comprise analysis of a sample (e.g. a biopsy), e.g. *in vitro*. In some embodiments the cancer may be determined to comprise cells having a mutation which is associated with reduced susceptibility and/or resistance to treatment with the antagonist. In some embodiments the cancer may be determined to comprise cells having upregulated expression of an EGFR family member.

In particular embodiments, the cancer to be treated is a cancer which is resistant to treatment with an antagonist of signalling mediated by EGFR and/or HER2. In some embodiments the subject to be treated has a cancer which is resistant to treatment with an antagonist of signalling mediated by EGFR and/or HER2. In some embodiments the subject to be treated has a cancer which has developed resistance to treatment with an antagonist of signalling mediated by EGFR and/or HER2. In some embodiments the subject to be treated has a cancer which previously responded to treatment with an antagonist of signalling mediated by EGFR and/or HER2, and which is now resistant to treatment with the antagonist. In some embodiments the subject to be treated has a cancer which has relapsed and/or progressed following treatment with an antagonist of signalling mediated by EGFR and/or HER2. In some

embodiments the subject to be treated has a cancer which initially responded to treatment with an antagonist of signalling mediated by EGFR and/or HER2, but later progressed on said treatment.

In particular embodiments, the cancer to be treated comprises mutation conferring resistance to treatment with an inhibitor of BRAF. In some embodiments, the mutation is mutation at BRAF V600. In some embodiments, the mutation is BRAF V600E or V600K.

In particular embodiments, the cancer to be treated comprises mutation conferring resistance to treatment with an inhibitor of BRAF (e.g. mutation at BRAF V600), and the treatment comprises administration of vemurafenib or darafenib.

In some embodiments the antigen-binding molecule is administered in combination with an agent capable of inhibiting signalling mediated by an immune checkpoint molecule. In some embodiments the immune checkpoint molecule is e.g. PD-1, CTLA-4, LAG-3, VISTA, TIM-3, TIGIT or BTLA. In some embodiments the antigen-binding molecule is administered in combination with an agent capable of promoting signalling mediated by a costimulatory receptor. In some embodiments the costimulatory receptor is e.g. CD28, CD80, CD40L, CD86, OX40, 4-1BB, CD27 or ICOS.

Accordingly, the invention provides compositions comprising an article according to the present invention (e.g. an antigen-binding molecule according to the invention) and an agent capable of inhibiting signalling mediated by an immune checkpoint molecule. Also provided are compositions comprising the articles of the present invention and an agent capable of promoting signalling mediated by a costimulatory receptor. Also provided is the use of such compositions in methods of medical treatment and prophylaxis of diseases/conditions described herein.

Also provided are methods for treating/preventing diseases/conditions described herein comprising administering articles of the present invention an article according to the present invention (e.g. an antigen-binding molecule according to the invention) and an agent capable of inhibiting signalling mediated by an immune checkpoint molecule. Also provided are methods for treating/preventing diseases/conditions described herein comprising administering articles of the present invention an article according to the present invention (e.g. an antigen-binding molecule according to the invention) and an agent capable of promoting signalling mediated by a costimulatory receptor.

Agents capable of inhibiting signalling mediated by immune checkpoint molecules are known in the art, and include e.g. antibodies capable of binding to immune checkpoint molecules or their ligands, and inhibiting signalling mediated by the immune checkpoint molecule. Other agents capable of inhibiting signalling mediated by an immune checkpoint molecule include agents capable of reducing gene/protein expression of the immune checkpoint molecule or a ligand for the immune checkpoint molecule (e.g. through inhibiting transcription of the gene(s) encoding the immune checkpoint molecule/ligand, inhibiting post-transcriptional processing of RNA encoding the immune checkpoint molecule/ligand, reducing stability of RNA encoding the immune checkpoint molecule/ligand, promoting degradation of RNA encoding the immune checkpoint molecule/ligand, inhibiting post-translational processing of the immune

checkpoint molecule/ligand, reducing stability the immune checkpoint molecule/ligand, or promoting degradation of the immune checkpoint molecule/ligand), and small molecule inhibitors.

Agents capable of promoting signalling mediated by costimulatory receptors are known in the art, and include e.g. agonist antibodies capable of binding to costimulatory receptors and triggering or increasing signalling mediated by the costimulatory receptor. Other agents capable of promoting signalling mediated by costimulatory receptors include agents capable of increasing gene/protein expression of the costimulatory receptor or a ligand for the costimulatory receptor (e.g. through promoting transcription of the gene(s) encoding the costimulatory receptor/ligand, promoting post-transcriptional processing of RNA encoding the costimulatory receptor/ligand, increasing stability of RNA encoding the costimulatory receptor/ligand, inhibiting degradation of RNA encoding the costimulatory receptor/ligand, promoting post-translational processing of the costimulatory receptor/ligand, increasing stability the costimulatory receptor/ligand, or inhibiting degradation of the costimulatory receptor/ligand), and small molecule agonists.

In particular embodiments the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by PD-1. The agent capable of inhibiting signalling mediated by PD-1 may be a PD-1- or PD-L1-targeted agent. The agent capable of inhibiting signalling mediated by PD-1 may e.g. be an antibody capable of binding to PD-1 or PD-L1 and inhibiting PD-1-mediated signalling.

In some embodiments, the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by CTLA-4. The agent capable of inhibiting signalling mediated by CTLA-4 may be a CTLA-4-targeted agent, or an agent targeted against a ligand for CTLA-4 such as CD80 or CD86. In some embodiments, the agent capable of inhibiting signalling mediated by CTLA-4 may e.g. be an antibody capable of binding to CTLA-4, CD80 or CD86 and inhibiting CTLA-4-mediated signalling.

In some embodiments, the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by LAG-3. The agent capable of inhibiting signalling mediated by LAG-3 may be a LAG-3-targeted agent, or an agent targeted against a ligand for LAG-3 such as MHC class II. In some embodiments, the agent capable of inhibiting signalling mediated by LAG-3 may e.g. be an antibody capable of binding to LAG-3 or MHC class II and inhibiting LAG-3-mediated signalling.

In some embodiments, the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by VISTA. The agent capable of inhibiting signalling mediated by VISTA may be a VISTA-targeted agent, or an agent targeted against a ligand for VISTA such as VSIG-3 or VSIG-8. In some embodiments, the agent capable of inhibiting signalling mediated by VISTA may e.g. be an antibody capable of binding to VISTA, VSIG-3 or VSIG-8 and inhibiting VISTA-mediated signalling.

In some embodiments, the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by TIM-3. The agent capable of inhibiting signalling mediated by TIM-3 may be a TIM-3-targeted agent, or an agent targeted against a ligand for TIM-3 such as Galectin 9. In some embodiments, the agent capable of inhibiting signalling mediated by TIM-3 may e.g. be an antibody capable of binding to TIM-3 or Galectin 9 and inhibiting TIM-3-mediated signalling.

In some embodiments, the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by TIGIT. The agent capable of inhibiting signalling mediated by TIGIT may be a TIGIT-targeted agent, or an agent targeted against a ligand for TIGIT such as CD113, CD112 or CD155. In some embodiments, the agent capable of inhibiting signalling mediated by TIGIT may e.g. be an antibody capable of binding to TIGIT, CD113, CD112 or CD155 and inhibiting TIGIT-mediated signalling.

In some embodiments, the antigen-binding molecule of the present invention is administered in combination with an agent capable of inhibiting signalling mediated by BTLA. The agent capable of inhibiting signalling mediated by BTLA may be a BTLA-targeted agent, or an agent targeted against a ligand for BTLA such as HVEM. In some embodiments, the agent capable of inhibiting signalling mediated by BTLA may e.g. be an antibody capable of binding to BTLA or HVEM and inhibiting BTLA -mediated signalling.

In some embodiments methods employing a combination of an antigen-binding molecule of the present invention and an agent capable of inhibiting signalling mediated by an immune checkpoint molecule (e.g. PD-1) provide an improved treatment effect as compared to the effect observed when either agent is used as a monotherapy. In some embodiments the combination of an antigen-binding molecule of the present invention and an agent capable of inhibiting signalling mediated by an immune checkpoint molecule (e.g. PD-1) provide a synergistic (i.e. super-additive) treatment effect.

Simultaneous administration refers to administration of the antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition and therapeutic agent together, for example as a pharmaceutical composition containing both agents (combined preparation), or immediately after each other and optionally via the same route of administration, e.g. to the same artery, vein or other blood vessel. Sequential administration refers to administration of one of the antigen-binding molecule/composition or therapeutic agent followed after a given time interval by separate administration of the other agent. It is not required that the two agents are administered by the same route, although this is the case in some embodiments. The time interval may be any time interval.

Chemotherapy and radiotherapy respectively refer to treatment of a cancer with a drug or with ionising radiation (e.g. radiotherapy using X-rays or γ-rays). The drug may be a chemical entity, e.g. small molecule pharmaceutical, antibiotic, DNA intercalator, protein inhibitor (e.g. kinase inhibitor), or a biological agent, e.g. antibody, antibody fragment, aptamer, nucleic acid (e.g. DNA, RNA), peptide,

polypeptide, or protein. The drug may be formulated as a pharmaceutical composition or medicament. The formulation may comprise one or more drugs (e.g. one or more active agents) together with one or more pharmaceutically acceptable diluents, excipients or carriers.

A treatment may involve administration of more than one drug. A drug may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. For example, the chemotherapy may be a co-therapy involving administration of two drugs, one or more of which may be intended to treat the cancer.

The chemotherapy may be administered by one or more routes of administration, e.g. parenteral, intravenous injection, oral, subcutaneous, intradermal or intratumoral.

The chemotherapy may be administered according to a treatment regime. The treatment regime may be a pre-determined timetable, plan, scheme or schedule of chemotherapy administration which may be prepared by a physician or medical practitioner and may be tailored to suit the patient requiring treatment. The treatment regime may indicate one or more of: the type of chemotherapy to administer to the patient; the dose of each drug or radiation; the time interval between administrations; the length of each treatment; the number and nature of any treatment holidays, if any etc. For a co-therapy a single treatment regime may be provided which indicates how each drug is to be administered.

Chemotherapeutic drugs may be selected from: Abemaciclib, Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, Acalabrutinib, AC-T, Adcetris (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine, Adriamycin (Doxorubicin Hydrochloride), Afatinib Dimaleate, Afinitor (Everolimus), Akynzeo (Netupitant and Palonosetron Hydrochloride), Aldara (Imiquimod), Aldesleukin, Alecensa (Alectinib), Alectinib, Alemtuzumab, Alimta (Pemetrexed Disodium), Aliqopa (Copanlisib Hydrochloride), Alkeran for Injection (Melphalan Hydrochloride), Alkeran Tablets (Melphalan), Aloxi (Palonosetron Hydrochloride), Alunbrig (Brigatinib), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Amifostine, Aminolevulinic Acid, Anastrozole, Aprepitant, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysanthemi, Atezolizumab, Avastin (Bevacizumab), Avelumab, Axicabtagene Ciloleucel, Axitinib, Azacitidine, Bavencio (Avelumab), BEACOPP, Becenum (Carmustine), Beleodaq (Belinostat), Belinostat, Bendamustine Hydrochloride, BEP, Besponsa (Inotuzumab Ozogamicin) , Bevacizumab, Bexarotene, Bexxar (Tositumomab and Iodine I 131 Tositumomab), Bicalutamide, BiCNU (Carmustine), Bleomycin, Blinatumomab, Blincyto (Blinatumomab), Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab Vedotin, Brigatinib, BuMel, Busulfan, Busulfex (Busulfan), Cabazitaxel, Cabometyx (Cabozantinib-S-Malate), Cabozantinib-S-Malate, CAF, Calquence (Acalabrutinib), Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carac (Fluorouracil--Topical), Carboplatin, CARBOPLATIN-TAXOL, Carfilzomib, Carmubris (Carmustine), Carmustine, Carmustine Implant, Casodex (Bicalutamide), CEM, Ceritinib, Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, CEV, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Cladribine, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar

(Clofarabine), CMF, Cobimetinib, Cometriq (Cabozantinib-S-Malate), Copanlisib Hydrochloride, COPDAC, COPP, COPP-ABV, Cosmegen (Dactinomycin), Cotellic (Cobimetinib), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cyramza (Ramucirumab), Cytarabine, Cytarabine Liposome, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Daratumumab, Darzalex (Daratumumab), Dasatinib, Daunorubicin Hydrochloride, Daunorubicin Hydrochloride and Cytarabine Liposome, Decitabine, Defibrotide Sodium, Defitelio (Defibrotide Sodium), Degarelix, Denileukin Diftitox, Denosumab, DepoCyt (Cytarabine Liposome), Dexamethasone, Dexrazoxane Hydrochloride, Dinutuximab, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine), Durvalumab, Efudex (Fluorouracil--Topical), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Elotuzumab, Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Empliciti (Elotuzumab), Enasidenib Mesylate, Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysanthemi), Ethyol (Amifostine), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Evomela (Melphalan Hydrochloride), Exemestane, 5-FU (Fluorouracil Injection), 5-FU (Fluorouracil--Topical), Fareston (Toremifene), Farydak (Panobinostat), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Fluoroplex (Fluorouracil--Topical), Fluorouracil Injection, Fluorouracil--Topical, Flutamide, Folex (Methotrexate), Folex PFS (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRI-CETUXIMAB, FOLFIRINOX, FOLFOX, Folotyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gardasil 9 (Recombinant HPV Nonavalent Vaccine), Gazyva (Obinutuzumab), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, GEMCITABINE-OXALIPLATIN, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gilotrif (Afatinib Dimaleate), Gleevec (Imatinib Mesylate), Gliadel (Carmustine Implant), Gliadel wafer (Carmustine Implant), Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate), Hemangeol (Propranolol Hydrochloride), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Nonavalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hydrea (Hydroxyurea), Hydroxyurea, Hyper-CVAD, Ibrance (Palbociclib), Ibritumomab Tiuxetan, Ibrutinib, ICE, Iclusig (Ponatinib Hydrochloride), Idamycin (Idarubicin Hydrochloride), Idarubicin Hydrochloride, Idelalisib, Idhifa (Enasidenib Mesylate), Ifex (Ifosfamide), Ifosfamide, Ifosfamidum (Ifosfamide), IL-2 (Aldesleukin), Imatinib Mesylate, Imbruvica (Ibrutinib), Imfinzi (Durvalumab), Imiquimod, Imlygic (Talimogene Laherparepvec), Inlyta (Axitinib), Inotuzumab Ozogamicin, Interferon Alfa-2b, Recombinant, Interleukin-2 (Aldesleukin), Intron A (Recombinant Interferon Alfa-2b), Iodine I 131 Tositumomab and Tositumomab, Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Irinotecan Hydrochloride Liposome, Istodax (Romidepsin), Ixabepilone, Ixazomib Citrate, Ixempra (Ixabepilone), Jakafi (Ruxolitinib Phosphate), JEB, Jevtana (Cabazitaxel), Kadcyla (Ado-Trastuzumab Emtansine), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Keytruda (Pembrolizumab), Kisqali (Ribociclib), Kymriah (Tisagenlecleucel), Kyprolis (Carfilzomib), Lanreotide Acetate, Lapatinib Ditosylate, Lartruvo (Olaratumab), Lenalidomide, Lenvatinib Mesylate, Lenvima (Lenvatinib Mesylate), Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Leustatin (Cladribine), Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Lomustine, Lonsurf

(Trifluridine and Tipiracil Hydrochloride), Lupron (Leuprolide Acetate), Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lynparza (Olaparib), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Megestrol Acetate, Mekinist (Trametinib), Melphalan, Melphalan Hydrochloride, Mercaptopurine, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Methylnaltrexone Bromide, Mexate (Methotrexate), Mexate-AQ (Methotrexate), Midostaurin, Mitomycin C, Mitoxantrone Hydrochloride, Mitozytrex (Mitomycin C), MOPP, Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C), Myleran (Busulfan), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Necitumumab, Nelarabine, Neosar (Cyclophosphamide), Neratinib Maleate, Nerlynx (Neratinib Maleate), Netupitant and Palonosetron Hydrochloride, Neulasta (Pegfilgrastim), Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilandron (Nilutamide), Nilotinib, Nilutamide, Ninlaro (Ixazomib Citrate), Niraparib Tosylate Monohydrate, Nivolumab, Nolvadex (Tamoxifen Citrate), Nplate (Romiplostim), Obinutuzumab, Odomzo (Sonidegib), OEPA, Ofatumumab, OFF, Olaparib, Olaratumab, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase), Ondansetron Hydrochloride, Onivyde (Irinotecan Hydrochloride Liposome), Ontak (Denileukin Diftitox), Opdivo (Nivolumab), OPPA, Osimertinib, Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, PAD, Palbociclib, Palifermin, Palonosetron Hydrochloride, Palonosetron Hydrochloride and Netupitant, Pamidronate Disodium, Panitumumab, Panobinostat, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, PCV, PEB, Pegaspargase, Pegfilgrastim, Peginterferon Alfa-2b, PEG-Intron (Peginterferon Alfa-2b), Pembrolizumab, Pemetrexed Disodium, Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Portrazza (Necitumumab), Pralatrexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Propranolol Hydrochloride, Provenge (Sipuleucel-T), Purinethol (Mercaptopurine), Purixan (Mercaptopurine), [No Entries], Radium 223 Dichloride, Raloxifene Hydrochloride, Ramucirumab, Rasburicase, R-CHOP, R-CVP, Recombinant Human Papillomavirus (HPV) Bivalent Vaccine, Recombinant Human Papillomavirus (HPV) Nonavalent Vaccine, Recombinant Human Papillomavirus (HPV) Quadrivalent Vaccine, Recombinant Interferon Alfa-2b, Regorafenib, Relistor (Methylnaltrexone Bromide), R-EPOCH, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Ribociclib, R-ICE, Rituxan (Rituximab), Rituxan Hycela (Rituximab and Hyaluronidase Human), Rituximab, Rituximab and Hyaluronidase Human, Rolapitant Hydrochloride, Romidepsin, Romiplostim, Rubidomycin (Daunorubicin Hydrochloride), Rubraca (Rucaparib Camsylate), Rucaparib Camsylate, Ruxolitinib Phosphate, Rydapt (Midostaurin), Sclerosol Intrapleural Aerosol (Talc), Siltuximab, Sipuleucel-T, Somatuline Depot (Lanreotide Acetate), Sonidegib, Sorafenib Tosylate, Sprycel (Dasatinib), STANFORD V, Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Sylatron (Peginterferon Alfa-2b), Sylvant (Siltuximab), Synribo (Omacetaxine Mepesuccinate), Tabloid (Thioguanine), TAC, Tafinlar (Dabrafenib), Tagrisso (Osimertinib), Talc, Talimogene Laherparepvec, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targretin (Bexarotene), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Tecentriq (Atezolizumab), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid (Thalidomide), Thioguanine, Thiotepa, Tisagenlecleucel, Tolak (Fluorouracil--Topical), Topotecan Hydrochloride, Toremifene, Torisel

(Temsirolimus), Tositumomab and Iodine I 131 Tositumomab, Totect (Dexrazoxane Hydrochloride), TPF, Trabectedin, Trametinib, Trastuzumab, Treanda (Bendamustine Hydrochloride), Trifluridine and Tipiracil Hydrochloride, Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Unituxin (Dinutuximab), Uridine Triacetate, VAC, Valrubicin, Valstar (Valrubicin), Vandetanib, VAMP, Varubi (Rolapitant Hydrochloride), Vectibix (Panitumumab), VeIP, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, Venclexta (Venetoclax), Venetoclax, Verzenio (Abemaciclib), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, VIP, Vismodegib, Vistogard (Uridine Triacetate), Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Vyxeos (Daunorubicin Hydrochloride and Cytarabine Liposome), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), XELIRI, XELOX, Xgeva (Denosumab), Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Yescarta (Axicabtagene Ciloleucel), Yondelis (Trabectedin), Zaltrap (Ziv-Aflibercept), Zarxio (Filgrastim), Zejula (Niraparib Tosylate Monohydrate), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zofran (Ondansetron Hydrochloride), Zoladex (Goserelin Acetate), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), Zydelig (Idelalisib), Zykadia (Ceritinib) and Zytiga (Abiraterone Acetate).

In some embodiments the antigen-binding molecule of the invention is administered in combination with one or more of: trastuzumab, cetuximab, cisplatin, 5-FU or capecitabine. In some embodiments the antigen-binding molecule of the invention is administered in combination with trastuzumab and cisplatin, and 5-FU or capecitabine.

In some embodiments the antigen-binding molecule of the invention is administered in combination with cetuximab. Administration in combination with cetuximab is contemplated in particular for the treatment of head and neck cancer (e.g. head and neck squamous cell carcinoma).

Multiple doses of the antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition may be provided. One or more, or each, of the doses may be accompanied by simultaneous or sequential administration of another therapeutic agent.

Multiple doses may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days).

Methods of detection

The invention also provides the articles of the present invention for use in methods for detecting, localizing or imaging HER3, or cells expressing HER3.

The antigen-binding molecules described herein may be used in methods that involve the antigen-binding molecule to HER3. Such methods may involve detection of the bound complex of the antigen-binding molecule and HER3.

As such, a method is provided, comprising contacting a sample containing, or suspected to contain, HER3, and detecting the formation of a complex of the antigen-binding molecule and HER3. Also provided is a method comprising contacting a sample containing, or suspected to contain, a cell expressing HER3, and detecting the formation of a complex of the antigen-binding molecule and a cell expressing HER3.

Suitable method formats are well known in the art, including immunoassays such as sandwich assays, e.g. ELISA. The methods may involve labelling the antigen-binding molecule, or target(s), or both, with a detectable moiety, e.g. a fluorescent label, phosphorescent label, luminescent label, immuno-detectable label, radiolabel, chemical, nucleic acid or enzymatic label as described herein. Detection techniques are well known to those of skill in the art and can be selected to correspond with the labelling agent.

Methods of this kind may provide the basis of methods for the diagnostic and/or prognostic evaluation of a disease or condition, e.g. a cancer. Such methods may be performed *in vitro* on a patient sample, or following processing of a patient sample. Once the sample is collected, the patient is not required to be present for the *in vitro* method to be performed, and therefore the method may be one which is not practised on the human or animal body. In some embodiments the method is performed *in vivo*.

Detection in a sample may be used for the purpose of diagnosis of a disease/condition (e.g. a cancer), predisposition to a disease/condition, or for providing a prognosis (prognosticating) for a disease/condition, e.g. a disease/condition described herein. The diagnosis or prognosis may relate to an existing (previously diagnosed) disease/ condition.

Such methods may involve detecting or quantifying HER3 or cells expressing HER3, e.g. in a patient sample. Where the method comprises quantifying the relevant factor, the method may further comprise comparing the determined amount against a standard or reference value as part of the diagnostic or prognostic evaluation. Other diagnostic/prognostic tests may be used in conjunction with those described herein to enhance the accuracy of the diagnosis or prognosis or to confirm a result obtained by using the tests described herein.

A sample may be taken from any tissue or bodily fluid. The sample may comprise or may be derived from: a quantity of blood; a quantity of serum derived from the individual's blood which may comprise the fluid portion of the blood obtained after removal of the fibrin clot and blood cells; a tissue sample or biopsy; pleural fluid; cerebrospinal fluid (CSF); or cells isolated from said individual. In some embodiments, the sample may be obtained or derived from a tissue or tissues which are affected by the disease/condition (e.g. tissue or tissues in which symptoms of the disease manifest, or which are involved in the pathogenesis of the disease/condition).

The present invention also provides methods for selecting/stratifying a subject for treatment with a HER3-targeted agent. In some embodiments a subject is selected for treatment/prevention in accordance with

the invention, or is identified as a subject which would benefit from such treatment/prevention, based on detection/quantification of HER3, or cells expressing HER3, e.g. in a sample obtained from the individual.

Subjects

The subject in accordance with aspects the invention described herein may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient. A subject may have been diagnosed with a disease or condition requiring treatment (e.g. a cancer), may be suspected of having such a disease/condition, or may be at risk of developing/contracting such a disease/condition.

In embodiments according to the present invention the subject is preferably a human subject. In some embodiments, the subject to be treated according to a therapeutic or prophylactic method of the invention herein is a subject having, or at risk of developing, a cancer. In embodiments according to the present invention, a subject may be selected for treatment according to the methods based on characterisation for certain markers of such disease/condition.

Kits

In some aspects of the invention described herein a kit of parts is provided. In some embodiments the kit may have at least one container having a predetermined quantity of an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

In some embodiments, the kit may comprise materials for producing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

The kit may provide the antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition together with instructions for administration to a patient in order to treat a specified disease/condition.

In some embodiments the kit may further comprise at least one container having a predetermined quantity of another therapeutic agent (e.g. anti-infective agent or chemotherapy agent). In such embodiments, the kit may also comprise a second medicament or pharmaceutical composition such that the two medicaments or pharmaceutical compositions may be administered simultaneously or separately such that they provide a combined treatment for the specific disease or condition. The therapeutic agent may also be formulated so as to be suitable for injection or infusion to a tumor or to the blood.

Sequence identity

As used herein, “sequence identity” refers to the percent of nucleotides/amino acid residues in a subject sequence that are identical to nucleotides/amino acid residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum percent sequence identity

between the sequences. Pairwise and multiple sequence alignment for the purposes of determining percent sequence identity between two or more amino acid or nucleic acid sequences can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960), T-coffee (Notredame et al. 2000, J. Mol. Biol. (2000) 302, 205-217), Kalign (Lassmann and Sonnhammer 2005, BMC Bioinformatics, 6(298)) and MAFFT (Katoh and Standley 2013, Molecular Biology and Evolution, 30(4) 772–780 software. When using such software, the default parameters, e.g. for gap penalty and extension penalty, are preferably used.

Sequences

SEQ ID NO:	DESCRIPTION	SEQUENCE
1	Human HER3 isoform 1 (UniProt: P21860-1, v1)	MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLE IVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHA LRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCW GPGSEDCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVP RCPQPLVYNKLTFQLEPNPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMC EPCGGLCPKACEGTGSGSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNV FRTVREITGYLNIQSWPPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGR IYISANRQLCYHHSLNWTKVLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQC LSCRNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDTCAQCAHFR DGPHCVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKTHLTM ALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETELRK LKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLC PGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLL KSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWELMT FGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDP PRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLN RPRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASESSEGHVT GSEAELQEKVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPDTHL KGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSD LSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASEQGYEEMR AFQGPGHQAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT
2	Human HER3 isoform 2 (UniProt: P21860-2)	MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLE IVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHA LRQLRLTQLTGQFPMVPSGLTPQPAQDWYLLDDDPRLLTLSASSKVPVTLAAV
3	Human HER3 isoform 3 (UniProt: P21860-3)	MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLE IVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHA LRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCW GPGSEDCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVP RCPQPLVYNKLTFQLEPNPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMC EPCGGLCPKAF
4	Human HER3 isoform 4 (UniProt: P21860-4)	MGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNT NSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCK GRCWGPGSEDCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSG ACVPRCPQPLVYNKLTFQLEPNPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNG LKMCEPCGGLCPKACEGTGSGSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDP EKLNVFRTVREITGYLNIQSWPPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKE ISAGRIYISANRQLCYHHSLNWTKVLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGP GPGQCLSCRNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDTCA QCAHFRDGPHCVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIG KTHLTMALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIF KETELRKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHI VRLLGLCPGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNL AARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVT VWELMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFT RMARDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALS LPVGTLNRPRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASE SSEGHVTGSEAELQEKVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGY VMPDTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYE YMDVGSDLSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASE QGYEEMRAFQGPGHQAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT

5	Human HER3 isoform 5 (UniProt: P21860-5)	MALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETEL RKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLG LCPGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNV LLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWEL MTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMA RDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVG TLNRPRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASESSEG HVTGSEAELQEKVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMP DTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMD VGSDLSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASEQGY EEMRAFQGPGHQAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT
6	Mature human HER3 isoform 1 (UniProt: P21860-1, v1 positions 20 to 1342)	SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIREVT GYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIE KNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKTICAPQC NGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEPNP HTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMCEPCGGLCPKACEGTGSGS RFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNVFRTVREITGYLNIQSWPPH MHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGRIYISANRQLCYHHSLNWTK VLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSCRNYSRGGVCVTHCNF LNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDTCAQCAHFRDGPHCVSSCPHGVLGAK GPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKTHLTMALTVIAGLVVIFMMLGGTF LYWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVHKGVWI PEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTQYLPLGSLLD HVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVADFGVADLLPP DDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEK GERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDPPRYLVIKRESGPGIAPGPEP HGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSSGYMPM NQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQEKVSMCRSRSR SRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPDTHLKGTPSSREGTLSSVGLSS VLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQSCPLHPVPI MPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASEQGYEEMRAFQGPGHQAPHVHYAR LKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT
7	Mature human HER3 isoform 2 (UniProt: P21860-2 positions 20 to 183)	SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIREVT GYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTGQFPMVPSG LTPQPAQDWYLLDDDPRLLTLSASSKVPVTLAAV
8	Mature human HER3 isoform 3 (UniProt: P21860-3 positions 20 to 331)	SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIREVT GYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIE KNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKTICAPQC NGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEPNP HTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMCEPCGGLCPKAF
9	Human HER3 isoform 1 extracellular region (UniProt: P21860-1, v1 positions 20 to 643)	SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIREVT GYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIE KNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKTICAPQC NGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEPNP HTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMCEPCGGLCPKACEGTGSGS RFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNVFRTVREITGYLNIQSWPPH MHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGRIYISANRQLCYHHSLNWTK VLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSCRNYSRGGVCVTHCNF LNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDTCAQCAHFRDGPHCVSSCPHGVLGAK GPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKTHLT
10	Human HER3 isoform 1 transmembrane domain (UniProt: P21860-1, v1 positions 644 to 664)	MALTVIAGLVVIFMMLGGTFL
11	Human HER3 isoform 1 cytoplasmic domain (UniProt: P21860-1, v1 positions 665 to 1342)	YWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVHKGVWI PEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTQYLPLGSLLD HVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVADFGVADLLPP DDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEK GERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDPPRYLVIKRESGPGIAPGPEP HGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSSGYMPM NQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQEKVSMCRSRSR SRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPDTHLKGTPSSREGTLSSVGLSS VLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQSCPLHPVPI MPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASEQGYEEMRAFQGPGHQAPHVHYAR LKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT

12	Human HER3 isoform 1 juxtamembrane segment (UniProt: P21860-1, v1 positions 665 to 708)	YWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETE
13	Human HER3 isoform 1 protein kinase domain (UniProt: P21860-1, v1 positions 709 to 966)	LRKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDH AHIVRLLGLCPGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEH GMVHRNLAARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKY THQSDVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCW MIDENIRPTFKELANEFT
14	Human HER3 isoform 1 C terminal segment (UniProt: P21860-1, v1 positions 967 to 1342)	RMARDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALS LPVGTLNRPRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASE SSEGHVTGSEAELQEKVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGY VMPDTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYE YMDVGSDLSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASE QGYEEMRAFQGPGHQAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT
15	Human HER3 extracellular region subdomain I (UniProt: P21860-1, v1 positions 20 to 183)	SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIREVT GYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIE KNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSC
16	Human HER3 extracellular region subdomain II (UniProt: P21860-1, v1 positions 184 to 329)	PPCHEVCKGRCWGPGSEDCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFA CRHFNDSGACVPRCPQPLVYNKLTFQLEPNPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPD KMEVDKNGLKMCEPCGGLCPK
17	Human HER3 extracellular region subdomain III (UniProt: P21860-1, v1 positions 330 to 495)	ACEGTGSGSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNVFRTVREITGY LNIQSWPPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGRIYISANRQLC YHHSLNWTKVLRGPTEERLDIKHNRPRRDCVA
18	Human HER3 extracellular region subdomain IV (UniProt: P21860-1, v1 positions 496 to 643)	EGKVCDPLCSSGGCWGPGPGQCLSCRNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQ PMEGTATCNGSGSDTCAQCAHFRDGPHCVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQ GCKGPELQDCLGQTLVLIGKTHLT
19	Human HER3 extracellular region subdomain II dimerisation loop (UniProt: P21860-1, v1 positions 261 to 278)	QPLVYNKLTFQLEPNPH
20	Rhesus macaque HER3 (UniProt: F7HEH3-1, v2)	MGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNT NSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWKDIVRDQDAEIVVKDNGRSCPLCHEVCK GRCWGPGPEDCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSG ACVPRCPQPLVYNKLTFQLEPNPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNG LKMCEPCGGLCPKACEGTGSGSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDP EKLNVFRTVREITGYLNIQSWPPHMYNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKE ISAGRIYISANRQLCYHHSLNWTKVLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGP GPGQCLSCRNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDTCA QCAHFRDGPHCVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIG KTHLTMALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIF KETELRKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKIIEDKSGRQSFQAVTDHMLAIGSLDHAHIV RLLGLCPGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLA ARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTV WELMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTR MARDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLP VGTLNRPRGSQSLLSPSSGYMPMNQGNLGEAFQESAVSGSSEWCPRPVSLHPMPRGCLASESS EGHVTGSEAELQEKVSTCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVM PDTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPRPPRPSSLEELGYEYM DVGSDLSASLGSTQSCPLHPVPVMPTAGTTPDEDYEYMNRQRGGSGPGGDYAAMGACPASEQ

		GYEEMRAFQGPGHQAPHVHYAHLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT
21	Epitope recognised by anti-HER3 antibody clone 10A6	YNKLTFQLEPNPH
22	Epitope recognised by anti-HER3 antibody clone 4-35-B2 and 4-35-B4	PRCPQPLVYNKLTF
23	Composite sequence of epitopes recognised by anti-HER3 antibody clones 4-35-B2, 4-35-B4 and 10A6	PRCPQPLVYNKLTFQLEPNPH
24	10D1 heavy chain variable region	DVQLQESGPDLVKPSQSLSLTCTVTGYSITSGYSWHWIRQFPGNKLEWMGSIHYSGGTNYNPSL KSRISITRDTSKNQFFLQLNSVTTEDTATYFCARMTTAPRYPFDYWGQGTTLTVSS
25	10D1_c75 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPTLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
26	10D1_c76 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
27	10D1_c77 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
28	10D1_c78v1 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
29	10D1_c78v2 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGkGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
30	10D1_11B heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVYGYSITSGYSWHWIRQPPGKGLEWIGSIHYSGGTNYNPSLK SRVTISRDTSKNQFSLKLSSVTAADTAVYYCARMTTAPRYPFDYWGQGTLVTVSS
31	10D1_c85v1 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
32	10D1_c85v2 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGKGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
33	10D1_c85o1 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGKGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARETTAPRYPFDYWGQGTLVTVSS
34	10D1_c85o2 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGKGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARGTTAPRYPFDYWGQGTLVTVSS
35	10D1_c87 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLRLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
36	10D1_c89 heavy chain variable region	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLVTISADTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTTVTVSS
37	10D1_c90 heavy chain variable region	QVQLQESGPGLVKPSQTLFLTCTVSGYSITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLVTISVDTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTTVTVSS
38	10D1_c91 heavy chain variable region	QVQLQESGPGLVKPSQTLSLTCTVSGYYITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLATISADTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTAVTVSS
39	10D1_c92 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPTLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
40	10D1_c93 heavy chain variable region	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLRLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSS
41	10D1, 10D1_c75, 10D1_c76,	GYSITSGYS

	10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c92, 10D1_c93 heavy chain CDR1	
42	10D1_c91 heavy chain CDR1	GYYITSGYS
43	10D1 derived consensus heavy chain CDR1	GYX$_1$ITSGYS wherein X_1 = S or Y
44	10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c87, 10D1_c92, 10D1_c93 heavy chain CDR2	IHYSGGT
45	10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c89, 10D1_c90, 10D1_c91 heavy chain CDR2	IRYSGGT
46	10D1 derived consensus heavy chain CDR2	IX$_2$YSGGT wherein X_2 = H or R
47	10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c87, 10D1_c92, 10D1_c93 heavy chain CDR3	ARMTTAPRYPFDY
48	10D1_c89, 10D1_c90, 10D1_c91 heavy chain CDR3	ARMTTAPWYPFDY
49	10D1_c85o1 heavy chain CDR3	ARETTAPRYPFDY
50	10D1_c85o2 heavy chain CDR3	ARGTTAPRYPFDY
51	10D1 derived consensus heavy chain CDR3	ARX$_3$TTAPX$_4$YPFDY wherein X_3 = M, E or G; X_4 = R or W
52	10D1_c75 10D1_c76, 10D1_c77, 10D1_c78v1,	DVQLQEWGAGLLKPSETLSLTCAVT

	10D1_c78v2, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c92, 10D1_c93 heavy chain FR1	
53	110D1_c89, 10D1_c91 heavy chain FR1	QVQLQESGPGLVKPSQTLSLTCTVS
54	10D1_c90 heavy chain FR1	QVQLQESGPGLVKPSQTLFLTCTVS
55	10D1 heavy chain FR1	DVQLQESGPDLVKPSQSLSLTCTVT
56	10D1_c75 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c85v1, 10D1_c87, 10D1_c92, 10D1_c93 heavy chain FR2	WHWIRQFPGNGLEWIGS
57	10D1_c78v2, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2 heavy chain FR2	WHWIRQFPGKGLEWIGS
58	10D1 heavy chain FR2	WHWIRQFPGNKLEWMGS
59	10D1_c89, 10D1_c90, 10D1_c91 heavy chain FR2	WHWIRQHPGKGLEWIGS
60	10D1_11B heavy chain FR2	WHWIRQPPGKGLEWIGS
61	10D1_c75, 10D1_c92 heavy chain FR3	NYNPTLKSRITISRDTSKNQFSLKLSSVTAADTAVYFC
62	10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2 heavy chain FR3	NYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYFC
63	10D1_11B heavy chain FR3	NYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYC
64	10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2 heavy chain FR3	NYNPSLKSRITISRDTSKNQFSLKLGSVTAADTAVYFC
65	10D1_c87, 10D1_c93 heavy chain FR3	NYNPSLKSRITISRDTSKNQFSLRLSSVTAADTAVYFC
66	10D1_c89 heavy chain FR3	DYNPSLKSLVTISADTSKNQFSLKLSSVTAADTAVYYC
67	10D1_c90 heavy chain FR3	DYNPSLKSLVTISVDTSKNQFSLKLSSVTAADTAVYYC
68	10D1_c91 heavy chain FR3	DYNPSLKSLATISADTSKNQFSLKLSSVTAADTAVYYC
69	10D1 heavy chain FR3	NYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYFC
70	10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2,	WGQGTLVTVSS

	10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c92, 10D1_c93 heavy chain FR4	
71	10D1_c89, 10D1_c90 heavy chain FR4	WGQGTTVTVSS
72	10D1_c91 heavy chain FR4	WGQGTAVTVSS
73	10D1, 4-35-B4, 10A6 heavy chain FR4	WGQGTTLTVSS
74	10D1 light chain variable region	DIVMTQSQKFMSTSVGDRVSVTCKASQIVGSNVAWYQQKPGQSPKPLIYSASYRYSGVPDRFTA SGSGTDFTLTITNVQSEDLAEYFCQQYSSHPLTFGAGTKLELK
75	10D1_c75 light chain variable region	DIVMTQSPSSLSASVGDLVTITCKASQIVGSNVAWYQMKPGKSPKPLIYSASYLYFGVPSRFSGSG SGTDFTLTISSLQPEDVAEYFCQQYSSHPLTFGPGTKVEIK
76	10D1_c76 light chain variable region	DIVMTQSPSSLSASGGDRVTITCKASQIVGYNVAWYQQKPGKSPKPLIYSASYLYSDVPSRFSAS GSGTDFTLTISSLQPEDVAEYFCQQYSSHPLTFGPGTKVEIK
77	10D1_c77 light chain variable region	VIVMTQSPSSLSASVGDRVTITCKASQIVGPNVAWYQQKPGKSPKPLIYSASYGYSDVPSRFSGS GSGTDFTLTISSLQPEDVAEYFCQQYSTHPLTFGPGTKVEIK
78	10D1_c78v1, 10D1_c78v2, 10D1_11B light chain variable region	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSASYGYSDVPSRFSGS GSGTDFTLTISSLRPEDVATYYCQQYSSHPLTFGPGTKVEIK
79	10D1_c85v1, 10D1_c85v2 light chain variable region	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSARYQYSGVPFRFSGS GSGTDFTLTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIK
80	10D1_c85o1 light chain variable region	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSARYQYSGVPFRFSGS GSGTDFTLTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIK
81	10D1_c85o2 light chain variable region	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSARYQYSGVPFRFSGS GSGTDFTLTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIK
82	10D1_c87 light chain variable region	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQMPGKSPEPLIYSASYLYSDVPSRFSGS GSGTDFTMTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIK
83	10D1_c89 light chain variable region	DIQMTQSPSSVSASVGDRVTITCKASQIVGSNVAWYQQKPGKAPEPLIYSASYLYSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQQYSSHPLTFGQGTKLEIK
84	10D1_c90 light chain variable region	DIQMTQSPSSVSASVGDRVTFTCKASQIVGSNVAWYQQKPGKAPEPLIYSASYLYSSVPSRFSGS GSGTEFTMTISSLEPEDFATYYCQQYTTHPLTFGPGTKVEIK
85	10D1_c91 light chain variable region	DIQMTQSPSSVSASVGDRVTITCKASQIVGSNVAWYQQKPGKAPMPLIYSASYGYSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQQYSSHPLTFGQGTKLEIK
86	10D1_c92 light chain variable region	DIVMTQSPSSLSASVGDLVTITCKASQIVGSNVAWYQMKLGKSPKPLIYSASYLYFGVPSRFSGSG SGTDFTLTISSLQPEDVAEYFCQQYFSHPLTFGPGTKVEIK
87	10D1_c93 light chain variable region	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSASYLYSDVPSRFSGS GSGTDFTMTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIK
88	10D1, 10D1_c75, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1,	QIVGSN

	10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93 light chain CDR1	
89	10D1_c76 light chain CDR1	QIVGYN
90	10D1_c77 light chain CDR1	QIVGPN
91	10D1 derived consensus light chain CDR1	QIVGX_5N wherein X_5 = S, Y or P
92	10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93 light chain CDR2	SAS
93	10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2 light chain CDR2	SAR
94	10D1 derived consensus light chain CDR2	SAX_6 wherein X_6 = S or R
95	10D1, 10D1_c75, 10D1_c76, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c91, 10D1_c93 light chain CDR3	QQYSSHPLT
96	10D1_c77 light chain CDR3	QQYSTHPLT
97	10D1_c90 light chain CDR3	QQYTTHPLT
98	10D1_c92 light chain CDR3	QQYFSHPLT
99	10D1 derived consensus light chain CDR3	QQYX_7X_8HPLT wherein X_7 = S, T or F; X_8 = S or T
100	10D1_c75, 10D1_c92 light chain FR1	DIVMTQSPSSLSASVGDLVTITCKAS
101	10D1_c76 light chain FR1	DIVMTQSPSSLSASGGDRVTITCKAS
102	10D1_c77 light chain FR1	VIVMTQSPSSLSASVGDRVTITCKAS
103	10D1_c78v1,	DIVMTQSPSSLSASVGDRVTITCKAS

	10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c93 light chain FR1	
104	10D1_c89, 10D1_c91 light chain FR1	DIQMTQSPSSVSASVGDRVTITCKAS
105	10D1_c90 light chain FR1	DIQMTQSPSSVSASVGDRVTFTCKAS
106	10D1 light chain FR1	DIVMTQSQKFMSTSVGDRVSVTCKAS
107	10D1_c75 light chain FR2	VAWYQMKPGKSPKPLIY
108	10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c93 light chain FR2	VAWYQQKPGKSPKPLIY
109	10D1_c87 light chain FR2	VAWYQQMPGKSPEPLIY
110	10D1_c89, 10D1_c90 light chain FR2	VAWYQQKPGKAPEPLIY
111	10D1_c91 light chain FR2	VAWYQQKPGKAPMPLIY
112	10D1_c92 light chain FR2	VAWYQMKLGKSPKPLIY
113	10D1 light chain FR2	VAWYQQKPGQSPKPLIY
114	10D1_c75, 10D1_c92 light chain FR3	YLYFGVPSRFSGSGSGTDFTLTISSLQPEDVAEYFC
115	10D1_c76 light chain FR3	YLYSDVPSRFSASGSGTDFTLTISSLQPEDVAEYFC
116	10D1_c77 light chain FR3	YGYSDVPSRFSGSGSGTDFTLTISSLQPEDVAEYFC
117	10D1_c78v1, 10D1_c78v2, 10D1_11B light chain FR3	YGYSDVPSRFSGSGSGTDFTLTISSLRPEDVATYYC
118	10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2 light chain FR3	YQYSGVPFRFSGSGSGTDFTLTISSLQPEDVATYYC
119	10D1_c87, 10D1_c93 light chain FR3	YLYSDVPSRFSGSGSGTDFTMTISSLQPEDVATYYC
120	10D1_c89 light chain FR3	YLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
121	10D1_c90 light chain FR3	YLYSSVPSRFSGSGSGTEFTMTISSLEPEDFATYYC
122	10D1_c91 light chain FR3	YGYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
123	10D1 light chain FR3	YRYSGVPDRFTASGSGTDFTLTITNVQSEDLAEYFC
124	10D1_c75, 10D1_c76,	FGPGTKVEIK

	10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c90, 10D1_c92, 10D1_c93 light chain FR4	
125	10D1_c89, 10D1_c91 light chain FR4	FGQGTKLEIK
126	10D1 light chain FR4	FGAGTKLELK
127	4-35-B2 heavy chain variable region	EIQLQQSGPELVKPGASVKVSCKASGYSFTDYNMYWVKQSHGKSLEWIGHINPYNGGTTYNQKF KGRATLTVDKSSSTAFMHLNSLTSEDSAVYFCVSLRWGAMDYWGQGTSVTVSS
128	4-35-B2 heavy chain CDR1	GYSFTDYN
129	4-35-B2 heavy chain CDR2	INPYNGGT
130	4-35-B2 heavy chain CDR3	VSLRWGAMDY
131	4-35-B2 heavy chain FR1	EIQLQQSGPELVKPGASVKVSCKAS
132	4-35-B2 heavy chain FR2	MYWVKQSHGKSLEWIGH
133	4-35-B2 heavy chain FR3	TYNQKFKGRATLTVDKSSSTAFMHLNSLTSEDSAVYFC
134	4-35-B2 heavy chain FR4	WGQGTSVTVSS
135	4-35-B2 light chain variable region	QIVLTQSPALMSASPGEKVTMTCSASSSVSYMYWYQQKPRSSPKPWIYLTSNLASGVPARFSGS GSGTSYSLTISSMEAEDAATYYCQQWNSNPYTFGGGTKLEIK
136	4-35-B2 light chain CDR1	SSVSY
137	4-35-B2 light chain CDR2	LTS
138	4-35-B2 light chain CDR3	QQWNSNPYT
139	4-35-B2 light chain FR1	QIVLTQSPALMSASPGEKVTMTCSAS
140	4-35-B2 light chain FR2	MYWYQQKPRSSPKPWIY
141	4-35-B2 light chain FR3	NLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYC
142	4-35-B2, 4-35-B4, 10A6 light chain FR4	FGGGTKLEIK
143	4-35-B4 heavy chain variable region	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHWVKQRPDQGLEWIGKIIDPANGNTNYDPKF QGKATITADTSSNTAYLQLSSLSSEDTAVYFCARGLHWGQGTTLTVSS
144	4-35-B4 heavy chain CDR1	GFNIKDTY
145	4-35-B4 heavy chain CDR2	IDPANGNT
146	4-35-B4 heavy chain CDR3	ARGLH
147	4-35-B4 heavy chain FR1	EVQLQQSGAELVKPGASVKLSCTAS
148	4-35-B4 heavy chain FR2	IHWVKQRPDQGLEWIGK
149	4-35-B4 heavy chain FR3	NYDPKFQGKATITADTSSNTAYLQLSSLSSEDTAVYFC
150	4-35-B4 light chain variable region	DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGVPARF SGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPYTFGGGTKLEIK

151	4-35-B4 light chain CDR1	KSVSTSGYSY
152	4-35-B4 light chain CDR2	LAS
153	4-35-B4 light chain CDR3	QHSRELPYT
154	4-35-B4 light chain FR1	DIVLTQSPASLAVSLGQRATISCRAS
155	4-35-B4 light chain FR2	MHWYQQKPGQPPKLLIY
156	4-35-B4 light chain FR3	NLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYC
157	10A6 heavy chain variable region	DVQLQESGPGLVKPSQSLSLTCSVTGNFITSGYFWNWIRQFPGNKLEWMGFISYDGSNNYKPSL KNRISITRDTSKNQFFLKLNSVTTEDTATYYCARENYGFGFDYWGQGTTLTVSS
158	10A6 heavy chain CDR1	GNFITSGYF
159	10A6 heavy chain CDR2	ISYDGSN
160	10A6 heavy chain CDR3	ARENYGFGFDY
161	10A6 heavy chain FR1	DVQLQESGPGLVKPSQSLSLTCSVT
162	10A6 heavy chain FR2	WNWIRQFPGNKLEWMGF
163	10A6 heavy chain FR3	NYKPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYC
164	10A6 light chain variable region	DIVLTQSPSSLPVSIGEKVTMSCKSSQSLLYSDNQKNYLAWYQQKPGQSPKLLIYWASTWKSGVP DRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYFTFPWTFGGGTKLEIK
165	10A6 light chain CDR1	QSLLYSDNQKNY
166	10A6 light chain CDR2	WAS
167	10A6 light chain CDR3	QQYFTFPWT
168	10A6 light chain FR1	DIVLTQSPSSLPVSIGEKVTMSCKSS
169	10A6 light chain FR2	LAWYQQKPGQSPKLLIY
170	10A6 light chain FR3	TWKSGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYC
171	Human IgG1 constant region (IGHG1; UniProt:P01857-1, v1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
172	CH1 IgG1 (positions 1-98 of P01857-1, v1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
173	Hinge IgG1 (positions 99-110 of P01857-1, v1)	EPKSCDKTHTCP
174	CH2 IgG1 (positions 111-223 of P01857-1, v1)	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
175	CH3 IgG1 (positions 224-330 of P01857-1, v1)	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
176	CH3 (D356E, L358M; positions numbered according to EU numbering)	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
177	Cκ CL (IGCK; UniProt: P01834-1, v2)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
178	10A6 heavy chain SignalP	MKVLSLLYLLTAIPGILS

179	10D1 heavy chain SignalP	MRVLILLCLFTAFPGILS
180	10D1 light chain SignalP	MESQTQVFVYMLLWLSGVDG
181	4-35-B2 heavy chain SignalP	MEWSWIFLFLLSGTTGVHS
182	4-35-B2 light chain SignalP	MDFQVQIFSFLLMSASVMMSRG
183	4-35-B4 heavy chain SignalP	MKCSWVIFFLMAVVTGVNS
184	4-35-B4 light chain SignalP	METDTLLLWVLLLWVPGSTG
185	10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93 heavy chain SignalP	MELGLRWVFLIATLAGARC
186	10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93 light chain SignalP	MDMRVPAQLLGLLLLWLRGARC
187	10D1_c75 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPTLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
188	10D1_c75 VL-Cκ	DIVMTQSPSSLSASVGDLVTITCKASQIVGSNVAWYQMKPGKSPKPLIYSASYLYFGVPSRFSGSG SGTDFTLTISSLQPEDVAEYFCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
189	10D1_c76 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
190	10D1_c76 VL-Cκ	DIVMTQSPSSLSASGGDRVTITCKASQIVGYNVAWYQQKPGKSPKPLIYSASYLYSDVPSRFSAS GSGTDFTLTISSLQPEDVAEYFCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC

191	10D1_c77 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
192	10D1_c77 VL-Cκ	VIVMTQSPSSLSASVGDRVTITCKASQIVGPNVAWYQQKPGKSPKPLIYSASYGYSDVPSRFSGS GSGTDFTLTISSLQPEDVAEYFCQQYSTHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
193	10D1_c78v1 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
194	10D1_c78v2 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGkGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
195	10D1_c78v1, 10D1_c78v2, 10D1_11B VL-Cκ	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSASYGYSDVPSRFSGS GSGTDFTLTISSLRPEDVATYYCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
196	10D1_11B VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVYGYSITSGYSWHWIRQPPGKGLEWIGSIHYSGGTNYNPSLK SRVTISRDTSKNQFSLKLSSVTAADTAVYYCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
197	10D1_c85v1 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
198	10D1_c85v2 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGKGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
199	10D1_c85v1, 10D1_c85v2 VL-Cκ	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSARYQYSGVPFRFSGS GSGTDFTLTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
200	10D1_c85o1 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGKGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARETTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
201	10D1_c85o1 VL-Cκ	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSARYQYSGVPFRFSGS GSGTDFTLTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
202	10D1_c85o2 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGKGLEWIGSIRYSGGTNYNPSLK SRITISRDTSKNQFSLKLGSVTAADTAVYFCARGTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV

		TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
203	10D1_c85o2 VL-Cκ	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSARYQYSGVPFRFSGS GSGTDFTLTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
204	10D1_c87 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLRLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
205	10D1_c87 VL-Cκ	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQMPGKSPEPLIYSASYLYSDVPSRFSGS GSGTDFTMTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
206	10D1_c89 VH-CH1-CH2-CH3	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLVTISADTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
207	10D1_c89 VL-Cκ	DIQMTQSPSSVSASVGDRVTITCKASQIVGSNVAWYQQKPGKAPEPLIYSASYLYSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQQYSSHPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
208	10D1_c90 VH-CH1-CH2-CH3	QVQLQESGPGLVKPSQTLFLTCTVSGYSITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLVTISVDTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
209	10D1_c90 VL-Cκ	DIQMTQSPSSVSASVGDRVTFTCKASQIVGSNVAWYQQKPGKAPEPLIYSASYLYSSVPSRFSGS GSGTEFTMTISSLEPEDFATYYCQQYTTHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
210	10D1_c91 VH-CH1-CH2-CH3	QVQLQESGPGLVKPSQTLSLTCTVSGYYITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLATISADTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTAVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
211	10D1_c91 VL-Cκ	DIQMTQSPSSVSASVGDRVTITCKASQIVGSNVAWYQQKPGKAPMPLIYSASYGYSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQQYSSHPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
212	10D1_c92 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPTLK SRITISRDTSKNQFSLKLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
213	10D1_c92 VL-Cκ	DIVMTQSPSSLSASVGDLVTITCKASQIVGSNVAWYQMKLGKSPKPLIYSASYLYFGVPSRFSGSG SGTDFTLTISSLQPEDVAEYFCQQYFSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
214	10D1_c93 VH-CH1-CH2-CH3	DVQLQEWGAGLLKPSETLSLTCAVTGYSITSGYSWHWIRQFPGNGLEWIGSIHYSGGTNYNPSLK SRITISRDTSKNQFSLRLSSVTAADTAVYFCARMTTAPRYPFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

215	10D1_c93 VL-Cκ	DIVMTQSPSSLSASVGDRVTITCKASQIVGSNVAWYQQKPGKSPKPLIYSASYLYSDVPSRFSGS GSGTDFTMTISSLQPEDVATYYCQQYSSHPLTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
216	10D1 VH-CH1-CH2-CH3	DVQLQESGPDLVKPSQSLSLTCTVTGYSITSGYSWHWIRQFPGNKLEWMGSIHYSGGTNYNPSL KSRISITRDTSKNQFFLQLNSVTTEDTATYFCARMTTAPRYPFDYWGQGTTLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
217	10D1 VL-Cκ	DIVMTQSQKFMSTSVGDRVSVTCKASQIVGSNVAWYQQKPGQSPKPLIYSASYRYSGVPDRFTA SGSGTDFTLTITNVQSEDLAEYFCQQYSSHPLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC
218	4-35-B2 VH-CH1-CH2-CH3	EIQLQQSGPELVKPGASVKVSCKASGYSFTDYNMYWVKQSHGKSLEWIGHINPYNGGTTYNQKF KGRATLTVDKSSSTAFMHLNSLTSEDSAVYFCVSLRWGAMDYWGQGTSVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
219	4-35-B2 VL-Cκ	QIVLTQSPALMSASPGEKVTMTCSASSSVSYMYWYQQKPRSSPKPWIYLTSNLASGVPARFSGS GSGTSYSLTISSMEAEDAATYYCQQWNSNPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC
220	4-35-B4 VH-CH1-CH2-CH3	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHWVKQRPDQGLEWIGKIIDPANGNTNYDPKF QGKATITADTSSNTAYLQLSSLSSEDTAVYFCARGLHWGQGTTLTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
221	4-35-B4 VL-Cκ	DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGVPARF SGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
222	10A6 VH-CH1-CH2-CH3	DVQLQESGPGLVKPSQSLSLTCSVTGNFITSGYFWNWIRQFPGNKLEWMGFISYDGSNNYKPSL KNRISITRDTSKNQFFLKLNSVTTEDTATYYCARENYGFGFDYWGQGTTLTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
223	10A6 VL-Cκ	DIVLTQSPSSLPVSIGEKVTMSCKSSQSLLYSDNQKNYLAWYQQKPGQSPKLLIYWASTWKSGVP DRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYFTFPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
224	10D1_11B heavy chain FR1	DVQLQEWGAGLLKPSETLSLTCAVY
225	10D1F VH-CH1-CH2-CH3 (GASDALIE; LCKC) (10D1F.FcB)	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLVTISADTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLAGPDVFCFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPLPEECTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
226	10D1F VH-CH1-CH2-CH3 (N297Q)	QVQLQESGPGLVKPSQTLSLTCTVSGYSITSGYSWHWIRQHPGKGLEWIGSIRYSGGTDYNPSLK SLVTISADTSKNQFSLKLSSVTAADTAVYYCARMTTAPWYPFDYWGQGTTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
227	10D1 VH-CH1-CH2-CH3 (GASDALIE; LCKC)	DVQLQESGPDLVKPSQSLSLTCTVTGYSITSGYSWHWIRQFPGNKLEWMGSIHYSGGTNYNPSL KSRISITRDTSKNQFFLQLNSVTTEDTATYFCARMTTAPRYPFDYWGQGTTLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLAGPDVFCFPPKPKDTLMISRTPE

		VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPLPEECTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
228	10D1 VH-CH1-CH2-CH3 (GASD)	DVQLQESGPDLVKPSQSLSLTCTVTGYSITSGYSWHWIRQFPGNKLEWMGSIHYSGGTNYNPSL KSRISITRDTSKNQFFLQLNSVTTEDTATYFCARMTTAPRYPFDYWGQGTTLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLAGPDVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
229	HER3 binding site for 10D1-derived clones	CFGPNPNQCCHDECAGGC
230	Binding site motif 1	PNPNQ
231	Binding site motif 2	DECAG
232	Human NRG1 (UniProt:Q02297-1, v3)	MSERKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPRLKEMKSQESAAGSKLVLRCETSSEY SSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRINKASLADSGEYMCKVISKLGNDSASANITIV ESNEIITGMPASTEGAYVSSESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECF MVKDLSNPSRYLCKCQPGFTGARCTENVPMKVQNQEKAEELYQKRVLTITGICIALLVVGIMCVVA YCKTKKQRKKLHDRLRQSLRSERNNMMNIANGPHHPNPPPENVQLVNQYVSKNVISSEHIVEREA ETSFSTSHYTSTAHHSTTVTQTPSHSWSNGHTESILSESHSVIVMSSVENSRHSSPTGGPRGRLN GTGGPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSPKSPPSEMSPPV SSMTVSMPSMAVSPFMEEERPLLLVTPPRLREKKFDHHPQQFSSFHHNPAHDSNSLPASPLRIVE DEEYETTQEYEPAQEPVKKLANSRRAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGED TPFLGIQNPLAASLEATPAFRLADSRTNPAGRFSTQEEIQARLSSVIANQDPIAV
233	Human NRG1 EGF-like domain (positions 178-222 of UniProt:Q02297-1)	HLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCQPGFTGARCT
234	Human NRG2 (UniProt:O14511-1, v1)	MRQVCCSALPPPPLEKGRCSSYSDSSSSSSERSSSSSSSSSESGSSSRSSSNNSSISRPAAPPE PRPQQQPQPRSPAARRAAARSRAAAAGGMRRDPAPGFSMLLFGVSLACYSPSLKSVQDQAYKA PVVVEGKVQGLVPAGGSSSNSTREPPASGRVALVKVLDKWPLRSGGLQREQVISVGSCVPLERN QRYIFFLEPTEQPLVFKTAFAPLDTNGKNLKKEVGKILCTDCATRPKLKKMKSQTGQVGEKQSLKC EAAAGNPQPSYRWFKDGKELNRSRDIRIKYGNGRKNSRLQFNKVKVEDAGEYVCEAENILGKDT VRGRLYVNSVSTTLSSWSGHARKCNETAKSYCVNGGVCYYIEGINQLSCKCPNGFFGQRCLEKL PLRLYMPDPKQKAEELYQKRVLTITGICVALLVVGIVCVVAYCKTKKQRKQMHNHLRQNMCPAHQ NRSLANGPSHPRLDPEEIQMADYISKNVPATDHVIRRETETTFSGSHSCSPSHHCSTATPTSSHR HESHTWSLERSESLTSDSQSGIMLSSVGTSKCNSPACVEARARRAAAYNLEERRRATAPPYHDS VDSLRDSPHSERYVSALTTPARLSPVDFHYSLATQVPTFEITSPNSAHAVSLPPAAPISYRLAEQQ PLLRHPAPPGPGPGPGPGPGPGADMQRSYDSYYYPAAGPGPRRGTCALGGSLGSLPASPFRIP EDDEYETTQECAPPPPPRPRARGASRRTSAGPRRWRRSRLNGLAAQRARAARDSLSLSSGSGG GSASASDDDADDADGALAAESTPFLGLRGAHDALRSDSPPLCPAADSRTYYSLDSHSTRASSRH SRGPPPRAKQDSAPL
235	Human NRG2 EGF-like domain (positions 341-382 of UniProt:O14511-1)	HARKCNETAKSYCVNGGVCYYIEGINQLSCKCPNGFFGQRCL
236	Human NRG3 (UniProt:B9EGV5-1, v1)	MSEGAAAASPPGAASAAAASAEEGTAAAAAAAAAGGGPDGGGEGAAEPPRELRCSDCIVWNRQ QTWLCVVPLFIGFIGLGLSLMLLKWIVVGSVKEYVPTDLVDSKGMGQDPFFLSKPSSFPKAMETTT TTTSTTSPATPSAGGAASSRTPNRISTRLTTITRAPTRFPGHRVPIRASPRSTTARNTAAPATVPST TAPFFSSSTLGSRPPVPGTPSTQAMPSWPTAAYATSSYLHDSTPSWTLSPFQDAASSSSSSSSS ATTTTPETSTSPKFHTTTYSTERSEHFKPCRDKDLAYCLNDGECFVIETLTGSHKHCRCKEGYQG VRCDQFLPKTDSILSDPNHLGIEFMESEEVYQRQVLSISCIIFGIVIVGMFCAAFYFKSKKQAKQIQE QLKVPQNGKSYSLKASSTMAKSENLVKSHVQLQNYSKVERHPVTALEKMMESSFVGPQSFPEVP SPDRGSQSVKHHRSLSSCCSPGQRSGMLHRNAFRRTPPSPRSRLGGIVGPAYQQLEESRIPDQ DTIPCQGYSSSGLKTQRNTSINMQLPSRETNPYFNSLEQKDLVGYSSTRASSVPIIPSVGLEETCL QMPGISEVKSIKWCKNSYSADVVNVSIPVSDCLIAEQQEVKILLETVQEQIRILTDARRSEDYELASV ETEDSASENTAFLPLSPTAKSEREAQFVLRNEIQRDSALTK
237	Human NRG3 EGF-like domain (positions 286-329 of UniProt:B9EGV5-1)	HFKPCRDKDLAYCLNDGECFVIETLTGSHKHCRCKEGYQGVRCD
238	Human NRG4 (UniProt:Q8WWG1-1, v1)	MPTDHEEPCGPSHKSFCLNGGLCYVIPTIPSPFCRCVENYTGARCEEVFLPGSSIQTKSNLFEAFV ALAVLVTLIIGAFYFLCRKGHFQRASSVQYDINLVETSSTSAHHSHEQH

239	Human NRG4 EGF-like domain (positions 5-46 of UniProt:Q8WWG1-1)	HEEPCGPSHKSFCLNGGLCYVIPTIPSPFCRCVENYTGARCE
240	EGF family consensus motif	$C(X)_7C(X)_{4-5}C(X)_{10-13}CXC(X)_8GXRC$ wherein 'X' is any amino acid, and wherein the number indicates the number of residues of the preceding bracket (i.e. '$(X)_7$' = 'XXXXXXX')

Numbered paragraphs

The following numbered paragraphs (paras) provide further statements of features and combinations of features which are contemplated in connection with the present invention:

1. An antigen-binding molecule, optionally isolated, which is capable of binding to HER3 in extracellular region subdomain II.

2. The antigen-binding molecule according to para 1, wherein the antigen-binding molecule inhibits interaction between HER3 and an interaction partner for HER3.

3. The antigen-binding molecule according to para 1 or para 2, wherein the antigen-binding molecule is capable of binding to a polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO:16.

4. The antigen-binding molecule according to any one of paras 1 to 3, wherein the antigen-binding molecule is capable of binding to a polypeptide comprising the amino acid sequence of SEQ ID NO:23 or SEQ ID NO:229.

5. The antigen-binding molecule according to any one of paras 1 to 4, wherein the antigen-binding molecule is capable of binding to a polypeptide comprising the amino acid sequence of SEQ ID NO:21 or SEQ ID NO:229.

6. The antigen-binding molecule according to any one of paras 1 to 5, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:43

HC-CDR2 having the amino acid sequence of SEQ ID NO:46

HC-CDR3 having the amino acid sequence of SEQ ID NO:51; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:91

LC-CDR2 having the amino acid sequence of SEQ ID NO:94

LC-CDR3 having the amino acid sequence of SEQ ID NO:99.

7. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:44
HC-CDR3 having the amino acid sequence of SEQ ID NO:47; and
(ii) a light chain variable (VL) region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:88
LC-CDR2 having the amino acid sequence of SEQ ID NO:92
LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

8. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:
(i) a heavy chain variable (VH) region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:44
HC-CDR3 having the amino acid sequence of SEQ ID NO:47; and
(ii) a light chain variable (VL) region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:89
LC-CDR2 having the amino acid sequence of SEQ ID NO:92
LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

9. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:
(i) a heavy chain variable (VH) region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:44
HC-CDR3 having the amino acid sequence of SEQ ID NO:47; and
(ii) a light chain variable (VL) region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:90
LC-CDR2 having the amino acid sequence of SEQ ID NO:92
LC-CDR3 having the amino acid sequence of SEQ ID NO:96.

10. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:
(i) a heavy chain variable (VH) region incorporating the following CDRs:
HC-CDR1 having the amino acid sequence of SEQ ID NO:41
HC-CDR2 having the amino acid sequence of SEQ ID NO:44
HC-CDR3 having the amino acid sequence of SEQ ID NO:47; and
(ii) a light chain variable (VL) region incorporating the following CDRs:
LC-CDR1 having the amino acid sequence of SEQ ID NO:88
LC-CDR2 having the amino acid sequence of SEQ ID NO:92
LC-CDR3 having the amino acid sequence of SEQ ID NO:98.

11. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:47; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:93

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

12. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:49; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:93

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

13. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:50; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:93

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

14. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:48; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

15. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:48; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:97.

16. The antigen-binding molecule according to any one of paras 1 to 6, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:42

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:48; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

17. The antigen-binding molecule according to any one of paras 1 to 5, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:158

HC-CDR2 having the amino acid sequence of SEQ ID NO:159

HC-CDR3 having the amino acid sequence of SEQ ID NO:160; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:165

LC-CDR2 having the amino acid sequence of SEQ ID NO:166

LC-CDR3 having the amino acid sequence of SEQ ID NO:167.

18. The antigen-binding molecule according to any one of paras 1 to 4, wherein the antigen-binding molecule is capable of binding to a polypeptide comprising the amino acid sequence of SEQ ID NO:22.

19. The antigen-binding molecule according to any one of paras 1 to 4 or para 18, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:128

HC-CDR2 having the amino acid sequence of SEQ ID NO:129

HC-CDR3 having the amino acid sequence of SEQ ID NO:130; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:136

LC-CDR2 having the amino acid sequence of SEQ ID NO:137

LC-CDR3 having the amino acid sequence of SEQ ID NO:138.

20. The antigen-binding molecule according to any one of paras 1 to 4 or para 18, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:144

HC-CDR2 having the amino acid sequence of SEQ ID NO:145

HC-CDR3 having the amino acid sequence of SEQ ID NO:146; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:151

LC-CDR2 having the amino acid sequence of SEQ ID NO:152

LC-CDR3 having the amino acid sequence of SEQ ID NO:153.

21. The antigen-binding molecule according to any one of paras 1 to 4, wherein the antigen-binding molecule comprises:

(i) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:24; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:74;

or

(ii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:25; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:75;

or

(iii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:26; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:76;

or

(iv) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:27; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:77;

or

(v) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:28; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:78;

or

(vi) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:29; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:78;

or

(vii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:30; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:78;

or

(viii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:31; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:79;

or

(ix) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:32; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:79;

or

(x) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:33; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:80;

or

(xi) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:34; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:81;

or

(xii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:35; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:82;

or

(xiii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:36; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:83;

or

(xiv) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:37; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:84;

or

(xv) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:38; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:85;

or

(xvi) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:39; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:86;

or

(xvii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:40; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:87;

or

(xviii) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:127; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:135;

or

(xix) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:143; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:150;

or

(xx) a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:157; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:164.

22. The antigen-binding molecule according to any one of paras 1 to 21, wherein the antigen-binding molecule is capable of binding to human HER3 and one or more of mouse HER3, rat HER3 and cynomolgous macaque HER3.

23. An antigen-binding molecule, optionally isolated, comprising (i) an antigen-binding molecule according to any one of paras 1 to 22, and (ii) an antigen-binding molecule capable of binding to an antigen other than HER3.

24. The antigen-binding molecule according to any one of paras 1 to 23, wherein the antigen-binding molecule is capable of binding to cells expressing HER3 at the cell surface.

25. The antigen-binding molecule according to any one of paras 1 to 24, wherein the antigen-binding molecule is capable of inhibiting HER3-mediated signalling.

26. The antigen-binding molecule according to any one of paras 1 to 25, wherein the antigen-binding molecule comprises an Fc region, the Fc region comprising a polypeptide having: (i) C at the position corresponding to position 242, and C at the position corresponding to position 334, and (ii) one or more of: A at the position corresponding to position 236, D at the position corresponding to position 239, E at the position corresponding to position 332, L at the position corresponding to position 330, K at the position corresponding to position 345, and G at the position corresponding to position 430.

27. The antigen-binding molecule according to para 26 wherein the Fc region comprises a polypeptide having C at the position corresponding to position 242, C at the position corresponding to position 334, A at the position corresponding to position 236, D at the position corresponding to position 239, E at the position corresponding to position 332, and L at the position corresponding to position 330.

28. A chimeric antigen receptor (CAR) comprising an antigen-binding molecule according to any one of paras 1 to 27.

29. A nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an antigen-binding molecule according to any one of paras 1 to 27 or a CAR according to para 28.

30. An expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to para 29.

31. A cell comprising an antigen-binding molecule according to any one of paras 1 to 27, a CAR according to para 28, a nucleic acid or a plurality of nucleic acids according to para 29, or an expression vector or a plurality of expression vectors according to para 30.

32. A method comprising culturing a cell comprising a nucleic acid or a plurality of nucleic acids according to para 29, or an expression vector or a plurality of expression vectors according to para 30, under conditions suitable for expression of the antigen-binding molecule or CAR from the nucleic acid(s) or expression vector(s).

33. A composition comprising an antigen-binding molecule according to any one of paras 1 to 27, a CAR according to para 28, a nucleic acid or a plurality of nucleic acids according to para 29, an expression vector or a plurality of expression vectors according to para 30, or a cell according to para 31.

34. An antigen-binding molecule according to any one of paras 1 to 27, a CAR according to para 28, a nucleic acid or a plurality of nucleic acids according to para 29, an expression vector or a plurality of expression vectors according to para 30, a cell according to para 31, or a composition according to para 33 for use in a method of medical treatment or prophylaxis.

35. An antigen-binding molecule according to any one of paras 1 to 27, a CAR according to para 28, a nucleic acid or a plurality of nucleic acids according to para 29, an expression vector or a plurality of expression vectors according to para 30, a cell according to para 31, or a composition according to para 33, for use in a method of treatment or prevention of a cancer.

36. Use of an antigen-binding molecule according to any one of paras 1 to 27, a CAR according to para 28, a nucleic acid or a plurality of nucleic acids according to para 29, an expression vector or a plurality of expression vectors according to para 30, a cell according to para 31, or a composition according to para 33, in the manufacture of a medicament for use in a method of treatment or prevention of a cancer.

37. A method of treating or preventing a cancer, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule according to any one of paras 1 to 27, a CAR according to para 28, a nucleic acid or a plurality of nucleic acids according to para 29, an expression vector or a plurality of expression vectors according to para 30, a cell according to para 31, or a composition according to para 33.

38. The antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition for use according to para 34 or para 35, the use according to para 36 or the method according to para 37, wherein the method additionally comprises administration of an inhibitor of signalling mediated by an EGFR family member, optionally wherein the inhibitor of signalling mediated by an EGFR family member is an inhibitor of signalling mediated by HER2 and/or EGFR.

39. The antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition for use, the use or the method according to any one of paras 34 to para 38, wherein the cancer is selected from: a cancer comprising cells expressing an EGFR family member, a cancer comprising cells expressing HER3, a solid tumor, breast cancer, breast carcinoma, ductal carcinoma, gastric cancer, gastric carcinoma, gastric adenocarcinoma, colorectal cancer, colorectal carcinoma, colorectal adenocarcinoma, head and neck cancer, squamous cell carcinoma of the head and neck (SCCHN), lung cancer, lung adenocarcinoma, squamous cell lung carcinoma, ovarian cancer, ovarian carcinoma, ovarian serous adenocarcinoma, kidney cancer, renal cell carcinoma, renal clear cell carcinoma, renal cell adenocarcinoma, renal papillary cell carcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, cervical cancer,

cervical squamous cell carcinoma, skin cancer, melanoma, esophageal cancer, esophageal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, uterine cancer, uterine corpus endometrial carcinoma, thyroid cancer, thyroid carcinoma, pheochromocytoma, paraganglioma, bladder cancer, bladder urothelial carcinoma, prostate cancer, prostate adenocarcinoma, sarcoma and thymoma.

40. A method of inhibiting HER3-mediated signalling, comprising contacting HER3-expressing cells with an antigen-binding molecule according to any one of paras 1 to 27.

41. A method of reducing the number or activity of HER3-expressing cells, the method comprising contacting HER3-expressing cells with an antigen-binding molecule according to any one of paras 1 to 27.

42. An *in vitro* complex, optionally isolated, comprising an antigen-binding molecule according to any one of paras 1 to 27 bound to HER3.

43. A method comprising contacting a sample containing, or suspected to contain, HER3 with an antigen-binding molecule according to any one of paras 1 to 27, and detecting the formation of a complex of the antigen-binding molecule with HER3.

44. A method of selecting or stratifying a subject for treatment with a HER3-targeted agent, the method comprising contacting, *in vitro*, a sample from the subject with an antigen-binding molecule according to any one of paras 1 to 27 and detecting the formation of a complex of the antigen-binding molecule with HER3.

45. Use of an antigen-binding molecule according to any one of paras 1 to 27 as an *in vitro* or *in vivo* diagnostic or prognostic agent.

46. Use of an antigen-binding molecule according to any one of paras 1 to 27 in a method for detecting, localizing or imaging a cancer, optionally wherein the cancer is selected from: a cancer comprising cells expressing an EGFR family member, a cancer comprising cells expressing HER3, a solid tumor, breast cancer, breast carcinoma, ductal carcinoma, gastric cancer, gastric carcinoma, gastric adenocarcinoma, colorectal cancer, colorectal carcinoma, colorectal adenocarcinoma, head and neck cancer, squamous cell carcinoma of the head and neck (SCCHN), lung cancer, lung adenocarcinoma, squamous cell lung carcinoma, ovarian cancer, ovarian carcinoma, ovarian serous adenocarcinoma, kidney cancer, renal cell carcinoma, renal clear cell carcinoma, renal cell adenocarcinoma, renal papillary cell carcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, cervical cancer, cervical squamous cell carcinoma, skin cancer, melanoma, esophageal cancer, esophageal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, uterine cancer, uterine corpus endometrial carcinoma, thyroid cancer, thyroid carcinoma, pheochromocytoma, paraganglioma, bladder cancer, bladder urothelial carcinoma, prostate cancer, prostate adenocarcinoma, sarcoma and thymoma.

47. An antigen-binding molecule, optionally isolated, which is capable of binding to HER3, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:43

HC-CDR2 having the amino acid sequence of SEQ ID NO:46

HC-CDR3 having the amino acid sequence of SEQ ID NO:51; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:91

LC-CDR2 having the amino acid sequence of SEQ ID NO:94

LC-CDR3 having the amino acid sequence of SEQ ID NO:99.

48. The antigen-binding molecule according to para 47, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:48; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

49. The antigen-binding molecule according to para 47 or para 48, wherein the antigen-binding molecule comprises:

a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:36; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:83.

50. An antigen-binding molecule, optionally isolated, comprising (i) an antigen-binding molecule according to any one of paras 47 to 49, and (ii) an antigen-binding molecule capable of binding to an antigen other than HER3.

51. A chimeric antigen receptor (CAR) comprising an antigen-binding molecule according to any one of paras 47 to 50.

52. A nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an antigen-binding molecule according to any one of paras 47 to 50 or a CAR according to para 51.

53. An expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to para 52.

54. A cell comprising an antigen-binding molecule according to any one of paras 47 to 50, a CAR according to para 51, a nucleic acid or a plurality of nucleic acids according to para 52, or an expression vector or a plurality of expression vectors according to para 53.

55. A method comprising culturing a cell comprising a nucleic acid or a plurality of nucleic acids according to para 52, or an expression vector or a plurality of expression vectors according to para 53, under conditions suitable for expression of the antigen-binding molecule or CAR from the nucleic acid(s) or expression vector(s).

56. A composition comprising an antigen-binding molecule according to any one of paras 47 to 50, a CAR according to para 51, a nucleic acid or a plurality of nucleic acids according to para 52, an expression vector or a plurality of expression vectors according to para 53, or a cell according to para 54.

57. An antigen-binding molecule according to any one of paras 47 to 50, a CAR according to para 51, a nucleic acid or a plurality of nucleic acids according to para 52, an expression vector or a plurality of expression vectors according to para 53, a cell according to para 54, or a composition according to para 56 for use in a method of medical treatment or prophylaxis.

58. An antigen-binding molecule according to any one of paras 47 to 50, a CAR according to para 51, a nucleic acid or a plurality of nucleic acids according to para 52, an expression vector or a plurality of expression vectors according to para 53, a cell according to para 54, or a composition according to para 56, for use in a method of treatment or prevention of a cancer.

59. Use of an antigen-binding molecule according to any one of paras 47 to 50, a CAR according to para 51, a nucleic acid or a plurality of nucleic acids according to para 52, an expression vector or a plurality of expression vectors according to para 53, a cell according to para 54, or a composition according to para 56, in the manufacture of a medicament for use in a method of treatment or prevention of a cancer.

60. A method of treating or preventing a cancer, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule according to any one of paras 47 to 50, a CAR according to para 51, a nucleic acid or a plurality of nucleic acids according to para 52, an expression vector or a plurality of expression vectors according to para 53, a cell according to para 54, or a composition according to para 56.

61. The antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition for use according to para 57 or para 58, the use according to para 59 or the method according to para 60, wherein the method additionally comprises administration of an inhibitor of signalling mediated by an EGFR family member, optionally wherein the inhibitor of signalling mediated by an EGFR family member is an inhibitor of signalling mediated by HER2 and/or EGFR.

62. The antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition for use, the use or the method according to any one of paras 57 to para 61, wherein the cancer is selected from: a cancer comprising cells expressing an EGFR family member, a cancer comprising cells expressing HER3, a solid tumor, breast cancer, breast carcinoma, ductal carcinoma, gastric cancer, gastric carcinoma, gastric adenocarcinoma, colorectal cancer, colorectal carcinoma, colorectal adenocarcinoma, head and neck cancer, squamous cell carcinoma of the head and neck (SCCHN), lung cancer, lung adenocarcinoma, squamous cell lung carcinoma, ovarian cancer, ovarian carcinoma, ovarian serous adenocarcinoma, kidney cancer, renal cell carcinoma, renal clear cell carcinoma, renal cell adenocarcinoma, renal papillary cell carcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, cervical cancer, cervical squamous cell carcinoma, skin cancer, melanoma, esophageal cancer, esophageal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, uterine cancer, uterine corpus endometrial carcinoma, thyroid cancer, thyroid carcinoma, pheochromocytoma, paraganglioma, bladder cancer, bladder urothelial carcinoma, prostate cancer, prostate adenocarcinoma, sarcoma and thymoma.

63. A method of inhibiting HER3-mediated signalling, comprising contacting HER3-expressing cells with an antigen-binding molecule according to any one of paras 47 to 50.

64. A method of reducing the number or activity of HER3-expressing cells, the method comprising contacting HER3-expressing cells with an antigen-binding molecule according to any one of paras 47 to 50.

65. An *in vitro* complex, optionally isolated, comprising an antigen-binding molecule according to any one of paras 47 to 50 bound to HER3.

66. A method comprising contacting a sample containing, or suspected to contain, HER3 with an antigen-binding molecule according to any one of paras 47 to 50, and detecting the formation of a complex of the antigen-binding molecule with HER3.

67. A method of selecting or stratifying a subject for treatment with a HER3-targeted agent, the method comprising contacting, *in vitro*, a sample from the subject with an antigen-binding molecule according to any one of paras 47 to 50 and detecting the formation of a complex of the antigen-binding molecule with HER3.

68. Use of an antigen-binding molecule according to any one of paras 47 to 50 as an *in vitro* or *in vivo* diagnostic or prognostic agent.

69. Use of an antigen-binding molecule according to any one of paras 47 to 50 in a method for detecting, localizing or imaging a cancer, optionally wherein the cancer is selected from: a cancer comprising cells expressing an EGFR family member, a cancer comprising cells expressing HER3, a solid tumor, breast cancer, breast carcinoma, ductal carcinoma, gastric cancer, gastric carcinoma, gastric adenocarcinoma,

colorectal cancer, colorectal carcinoma, colorectal adenocarcinoma, head and neck cancer, squamous cell carcinoma of the head and neck (SCCHN), lung cancer, lung adenocarcinoma, squamous cell lung carcinoma, ovarian cancer, ovarian carcinoma, ovarian serous adenocarcinoma, kidney cancer, renal cell carcinoma, renal clear cell carcinoma, renal cell adenocarcinoma, renal papillary cell carcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, cervical cancer, cervical squamous cell carcinoma, skin cancer, melanoma, esophageal cancer, esophageal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, uterine cancer, uterine corpus endometrial carcinoma, thyroid cancer, thyroid carcinoma, pheochromocytoma, paraganglioma, bladder cancer, bladder urothelial carcinoma, prostate cancer, prostate adenocarcinoma, sarcoma and thymoma.

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The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise,” and variations such as “comprises” and “comprising,” will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent “about,” it will be understood that the particular value forms another embodiment.

Where a nucleic acid sequence is disclosed herein, the reverse complement thereof is also expressly contemplated.

Methods described herein may preferably performed *in vitro*. The term “*in vitro*” is intended to encompass procedures performed with cells in culture whereas the term “*in vivo*” is intended to encompass procedures with/on intact multi-cellular organisms.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures.

Figures 1A and 1B. Histograms showing staining of cells by anti-HER3 antibodies as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E) by (**1A, 1B**) anti-HER3 antibody clone 10D1 and (**1B**) anti-HER3 antibody clone LJM716.

Figures 2A and 2B. Histograms showing staining of cells by anti-HER3 antibodies as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E) by (**2A, 2B**) anti-HER3 antibody clone 4-35-B2 and (**2B**) anti-HER3 antibody clone LJM716.

Figures 3A and 3B. Histograms showing staining of cells by anti-HER3 antibodies as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E) by (**3A, 3B**) anti-HER3 antibody clone 4-35-B4 and (**3B**) anti-HER3 antibody clone LJM716.

Figure 4. Histograms showing staining of cells by anti-HER3 antibodies as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E) by anti-HER3 antibody clone 10A6.

Figures 5A and 5B. Graphs showing the results of ELISA analysis of binding of anti-HER3 antibody clone 10D1 to (**5A**) human, mouse, rat and cynomolgus macaque HER3, and (**5B**) human EGFR and human HER2. EC_{50} values are shown.

Figures 6A and 6B. Graphs showing the results of ELISA analysis of binding of anti-HER3 antibody clone 4-35-B2 to (**6A**) human, mouse, rat and cynomolgus macaque HER3, and (**6B**) human EGFR and human HER2.

Figures 7A and 7B. Graphs showing the results of ELISA analysis of binding of anti-HER3 antibody clone 4-35-B4 to (**7A**) human HER3, human EGFR and human HER2, and (**7B**) human, mouse, rat and cynomolgus macaque HER3.

Figure 8. Representative sensorgram showing the results of analysis of affinity of binding of anti-HER3 antibody clone 10D1 to human HER3. Kon, Koff and K_D are shown.

Figure 9. Representative sensorgram showing the results of analysis of affinity of binding of anti-HER3 antibody clone 4-35-B2 to human HER3.

Figure 10. Representative sensorgram showing the results of analysis of affinity of binding of anti-HER3 antibody clone 4-35-B4 to human HER3.

Figure 11. Graph showing the results of analysis of stability of anti-HER3 antibody clone 10D1 by Differential Scanning Fluorimetry analysis.

Figure 12. Graph showing the results of the analysis of recombinantly-expressed anti-HER3 antibody clone 10D1 by size exclusion chromatography.

Figure 13. Images showing the results of the analysis of anti-HER3 antibody clone 10D1 expression by SDS-PAGE and western blot. Lanes: M1 = TaKaRa protein marker Cat. No. 3452; M2 = GenScript protein marker Cat. No. M00521; 1 = reducing conditions; 2 = non-reducing conditions; P = positive control: human IgG1, Kappa (Sigma Cat. No. I5154). For western blot, the primary antibodies used were goat anti-human IgG-HRP (GenScript Cat No. A00166) and goat anti-human kappa-HRP (SouterhnBiotech Cat No. 2060-05).

Figure 14A and 14B. Representative sensorgram and table showing the results of analysis of competition between different anti-HER3 antibody clones for binding to HER3.

Figure 15. Graph showing the results of analysis of the inhibition of interaction between HER3 and HER2 by anti-HER3 antibody clone 10D1 as determined by ELISA.

Figures 16A and 16B. Table and histograms showing gene and protein expression of EGFR protein family members and their ligands by different cancer cell lines.

Figure 17. Images showing the results of analysis of the effect of anti-HER3 antibody clone 10D1 treatment on the HER3-medated signalling in N87 and FaDu cells by phospho-western blot. UN = untreated; T = treated with anti-HER3 antibody clone 10D1.

Figure 18. Images and graph showing the results of analysis of the effect of anti-HER3 antibody clone 10D1 treatment on the HER3-medated signalling in FaDu cells using the Phosphoprotein Antibody Array assay kit. Untreated = untreated FaDu cells; Treated = FaDu cells treated with anti-HER3 antibody clone 10D1.

Figures 19A and 19B. Graphs showing the percent confluence of cells relative to an untreated control condition (100%), for the indicated cells lines as determined by CCK8 assay, following incubation in the presence of anti-HER3 antibody clone 10D1. (**19A**) Shows the results obtained for N87 cells, and (**19B**) shows the results obtained for FaDu cells.

Figure 20. Graph showing the results of analysis of tumour volume over time in a N87 cell-line derived mouse gastric carcinoma model. Anti-HER3 antibody clone 10D1 was administered IP, biweekly

at 500 µg per dose for a total of 10 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 21. Graph showing the results of analysis of tumour volume over time in a N87 cell-line derived mouse gastric carcinoma model. Anti-HER3 antibody clone 4-35-B2 was administered IP, weekly at 11 mg/kg per dose for a total of 4 doses. A control treatment group received an equal amount of isotype control antibody (isotype).

Figure 22. Graph showing the results of analysis of tumour volume over time in a SNU16 cell-line derived mouse gastric carcinoma model. Anti-HER3 antibody clone 10D1 was administered IP, biweekly at 500 µg per dose for a total of 9 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 23. Graph showing the results of analysis of tumour volume over time in a FaDu cell-line derived mouse model of head and neck squamous cell carcinoma. Anti-HER3 antibody clone 10D1 was administered IP, weekly at 500 µg per dose for a total of 4 doses. Control treatment groups received an equal volume of PBS (vehicle), or the same dose of an isotype control antibody (isotype).

Figure 24. Graph showing the results of analysis of tumour volume over time in a FaDu cell-line derived mouse model of head and neck squamous cell carcinoma. Anti-HER3 antibody clone 10D1 was administered IP, biweekly at 500 µg per dose for a total of 8 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 25. Graph showing the results of analysis of tumour volume over time in an OvCAR8 cell-line derived mouse model of ovarian carcinoma. Anti-HER3 antibody clone 10D1 was administered IP, biweekly at 500 µg per dose for a total of 9 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 26. Graph showing the results of analysis of tumour volume over time in a HCC-95 cell-line derived mouse model of squamous lung cell carcinoma. Anti-HER3 antibody clone 10D1 was administered IP, biweekly at 500 µg per dose for a total of 4 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 27. Graph showing the results of analysis of tumour volume over time in an A549 cell-line derived mouse model of lung adenocarcinoma. Anti-HER3 antibody clone 10D1 was administered IP, biweekly at 500 µg per dose for a total of 10 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 28. Graph showing the results of analysis of tumour volume over time in an A549 cell-line derived mouse model of lung adenocarcinoma. Anti-HER3 antibody clone 4-35-B2 was administered IP, biweekly at 500 µg per dose for a total of 4 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 29. Graph showing the results of analysis of tumour volume over time in an ACHN cell-line derived mouse model of renal cell carcinoma. Anti-HER3 antibody clone 10D1 was administered IP, biweekly at 500 µg per dose for a total of 7 doses. A control treatment group received an equal volume of PBS (vehicle).

Figure 30. Histogram showing staining of cells by anti-HER3 antibody clone 10D1_c89 as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E).

Figure 31. Histogram showing staining of cells by anti-HER3 antibody clone 10D1_c90 as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E).

Figure 32. Histogram showing staining of cells by anti-HER3 antibody clone 10D1_c91 as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E).

Figures 33A and 33B. Graphs showing the results of ELISA analysis of binding of anti-HER3 antibody 10D1 variant clones to human HER3. (**33A**) shows binding of anti-HER3 antibody clones 10D1 (referred to as 10D1P), 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78, 10D1_11B (referred to as v11b78L), 10D1_c85, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c93, LJM716 and hIgG (negative control). (**33B**) shows the same data as 33A, but for clones 10D1_c89, 10D1_c90, 10D1_c91 and LJM716 only.

Figures 34A and 34B. Graphs showing the results of the analysis of recombinantly-expressed anti-HER3 antibody 10D1 variant clones by size exclusion chromatography. (**34A**) shows results for anti-HER3 antibody clones 10D1_c93, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78, 10D1_11B (referred to as C78 v11b), 10D1_c85, 10D1_c85o1, 10D1_c85o2, 10D1_c89, 10D1_c90, 10D1_c91 and 10D1_c93. (**34B**) shows the same data as 33A, but for clones 10D1_c89, 10D1_c90, 10D1_c91 and only.

Figures 35A to 35C. Graphs showing the results analysis of stability of anti-HER3 antibody 10D1 variant clones by Differential Scanning Fluorimetry analysis. (**35A**) shows results for anti-HER3 antibody clones LJM716 (also referred to as Elgemtumab), 10D1 (referred to as 10D1 (parental)), 10D1_c75, 10D1_c76, 10D1_c77 and 10D1_c78. (**35B**) shows results for 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_11B (referred to as c78_V11B), 10D1_c85 and 10D1_c85o1. (**35C**) shows results for 10D1_c90, 10D1_c91 and 10D1_c93.

Figures 36A to 36M. Representative sensorgrams showing the results of analysis of affinity of anti-HER3 antibody 10D1 variant clones to human HER3. Kon, Koff and K_D are shown. (**36A**) shows results for clone 10D1_c89, (**36B**) shows results for clone 10D1_c90, (**36C**) shows results for clone 10D1_c91, (**36D**) shows results for clone 10D1_c11B, (**36E**) shows results for clone 10D1_c85o2, (**36F**) shows

results for clone 10D1_c87, (**36G**) shows results for clone 10D1_c93, (**36H**) shows results for clone 10D1_c76, (**36I**) shows results for clone 10D1_c77, (**36J**) shows results for clone 10D1_c78, (**36K**) shows results for clone 10D1_c75, (**36L**) shows results for clone 10D1_c85, and (**36M**) shows results for clone 10D1_c85o1.

Figure 37. Table summarising properties of anti-HER3 antibody 10D1 variant clones relevant to safety and developability.

Figures 38A and 38B. Bio-Layer Interferometry (**38A**) and thermostability (**38B**) analysis of Fc-modified anti-HER3 antibody clone 10D1 comprising GASDALIE and LCKC substitutions in CH2 region. (**38A**) BLI shows a representative sensorgram showing the results of analysis of affinity of binding to FcγRIIIa by Fc-modified anti-HER3 antibody clone 10D1. Kon, Koff and K_D are shown.

Figures 39A and 39B. Representative sensorgrams showing results of analysis of affinity of binding to FcγRIIIa by (**39A**) non-Fc-modified anti-HER3 antibody clone 10D1 and (**39B**) Fc-modified anti-HER3 antibody clone 10D1 comprising GASD substitutions in CH2 region. Kon, Koff and K_D are shown.

Figure 40. Graph showing the results of analysis of stability of anti-HER3 antibody clone 10D1 GASD variant by Differential Scanning Fluorimetry analysis.

Figures 41A and 41B. Tables showing the binding affinity for mouse and human Fc receptors of anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB (GASDALIE-LCKC variant) compared to silent variant N297Q, isoform variants, and commercially available antibodies. ND = K_D Not Determined due to low binding affinity.

Figures 42A and 42B. Histograms showing staining of cells by anti-HER3 antibodies as determined by flow cytometry. Histograms show staining of HEK293 cells (which do not express HER3), or HEK293 HER3 overexpressing cells (HEK293 HER3 O/E) by (**42A**) anti-HER3 antibody clone 10D1F.FcA and (**42B**) anti-HER3 antibody clones 10D1 and LJM-716.

Figure 43. Graph showing the results of ELISA analysis of binding of anti-HER3 antibody clone 10D1F.FcA to human EGFR (HER1) and human HER2. EC_{50} values are shown.

Figure 44. Histogram showing staining of cells by anti-HER3 and anti-HER4 antibodies as determined by flow cytometry. Histogram shows staining of HEK293 HER4 overexpressing cells by anti-HER3 antibody clone 10D1F.FcA, anti-HER3 antibodies LJM-716 and MM-121, and commercial anti-HER4 antibody.

Figure 45. Graph showing the results of ELISA analysis of binding of anti-HER3 antibody clone 10D1F.FcA to human, mouse, rat and cynomolgus macaque HER3. EC_{50} values are shown.

Figures 46A and 46B. Representative sensorgrams showing the results of analysis of affinity of binding of anti-HER3 antibody clones (**46A**) 10D1F.FcA and (**46B**) 10D1F.FcB to human HER3. Kon, Koff and K_D are shown.

Figures 47A and 47B. Graph showing the results of analysis of stability of anti-HER3 antibody clones (**47A**) 10D1F.FcA and (**47B**) 10D1F.FcB by Differential Scanning Fluorimetry analysis.

Figures 48A and 48B. Graph showing the results of purity analysis of anti-HER3 antibody clones (**48A**) 10D1F.FcA and (**48B**) 10D1F.FcB by size exclusion chromatography.

Figures 49A and 49B. Representative sensorgram and table showing the results of analysis of competition for binding to HER3 between anti-HER3 antibody clone 10D1F.FcA and anti-HER3 antibodies M-05-74 and M-08-11.

Figure 50. Graph and tables showing the results of pharmacokinetic analysis of anti-HER3 antibody clone 10D1 in mice.

Figures 51A to 51F. Graphs showing the effect of anti-HER3 antibody clone 10D1 treatment on blood cell counts (**51A**), electrolyte indices (**51B**) and indices of hepatoxicity, nephrotoxicity and pancreatic toxicity (**51C-51F**) in mice. Left bars represent vehicle control, right bars represent 10D1 treatment. Dotted lines indicate the end points of the Charles River reference range. Indices of hepatoxicity, nephrotoxicity and pancreatic toxicity include alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine (CREA), alkaline phosphatase (ALP), glucose (GLU), calcium (CAL), total bilirubin (BIL), total protein (TPR) and albumin (ALB).

Figure 52. Graph and table showing the results of analysis of the inhibition of interaction between HER2 and HER3 by anti-HER3 antibody clone 10D1F.FcA and antibodies MM-121, LJM-716 and Roche M05 as determined by ELISA.

Figure 53. Graph and table showing the results of analysis of the inhibition of interaction between EGFR and HER3 by anti-HER3 antibody clone 10D1F.FcA and antibodies MM-121 and LJM716, as determined by ELISA.

Figure 54. Graph and table showing the results of analysis of the ability of anti-HER3 antibody clones 10D1F.FcA (10D1F.A), 10D1F.FcB (10D1F.B), 10D1F-hIgG1(N297Q) and anti-HER3 antibodies LJM-716 and Seribantumab (MM-121), to induce antibody-dependent cell-mediated cytotoxicity (ADCC). EC_{50} values are shown.

Figures 55A to 55C. Images showing the results of analysis of the effect of anti-HER3 antibody treatment on HER3-medated signalling in (**55A**) N87, (**55B**) FaDu and (**55C**) OvCar8 cells by phospho-western blot.

Figures 56A and 56B. Graph and tables showing the results of pharmacokinetic analysis of anti-HER3 antibody clones (**56A**) 10D1F.FcA and (**56B**) 10D1F.FcB in mice. Parameters: maximum concentration (C_{max}), T_{max}, AUC (0-336hr), AUC (0-infinity), Half-life ($t_{½}$), Clearance (CL), Volume of distribution at steady state (V_{ss}).

Figures 57A to 57D. Graph and tables showing the results of pharmacokinetic analysis of anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB at (**57A**) 10 mg/kg, (**57B**) 25 mg/kg, (**57C**) 100 mg/kg and (**57D**) 250 mg/kg in rats. Parameters: maximum concentration (C_{max}), T_{max}, AUC (0-336hr), AUC (0-infinity), Half-life ($t_{½}$), Clearance (CL), Volume of distribution at steady state (V_{ss}).

Figures 58A to 58F. Graphs showing the effect of treatment of anti-HER3 antibody clone 10D1F.FcA or 10D1F.FcB at 200 ug (~10 mg/kg), 500 ug (~25 mg/kg), 2 mg (~100 mg/kg), or 5 mg (~250 mg/kg) on (**58A, 58B**) red blood cell indices, (**58C**) white blood cell indices, (**58D**) hepatotoxicity, (**58E**) kidney and pancreatic indices, and (**58F**) electrolyte indices.

Figures 59A to 59D. Graphs showing the effect of treatment anti-HER3 antibody clone 10D1F.FcA on percentage tumour inhibition in *in vitro* mouse cancer models using N87 cells (gastric cancer), HCC95 cells (lung cancer), FaDu cells (head and neck cancer), SNU-16 cells (gastric cancer), A549 cells (lung cancer), OvCar8 cells (ovarian cancer), ACHN cells (kidney cancer) and HT29 cells (colorectal cancer) in comparison to (**59A & 59B**) anti-HER3 antibodies seribantumab and LJM-716 and (**59C & 59D**) EGFR family therapies cetuximab, trastuzumab and pertuzumab.

Figure 60. Graph showing the results of analysis of tumour volume over time in an A549 cell-line derived mouse model of lung adenocarcinoma after biweekly treatment with the indicated concentrations of antibodies for six weeks (n=6, vehicle control n=8). Antibody administration is indicated by triangles along x-axis.

Figure 61. Graph showing the results of analysis of tumour volume over time in a FaDu cell-line derived mouse model of head and neck squamous cell carcinoma after weekly treatment with the indicated concentrations of antibodies for six weeks (n=6). Antibody administration is indicated by triangles along x-axis.

Figure 62. Graph showing the results of analysis of tumour volume over time in a OvCAR8 cell-line derived mouse model of ovarian carcinoma after weekly treatment with the indicated concentrations of antibodies for six weeks (n=6). Antibody administration is indicated by triangles along x-axis.

Figures 63A to 63D. Box plots showing the results of analysis of pathway activation by gene set enrichment analysis, for cancer cell lines treated with 10D1F.FcA, LJM-716 or seribantumab in *in vitro* phosphorylation assays. **63A** shows the results obtained from N87 cells, **63B** shows the results obtained from A549 cells, **63C** shows the results obtained from OvCar8 cells and **63D** shows the results obtained from FaDu cells.

Figure 64. Images showing the results of analysis of the effect of anti-HER3 antibody treatment on HER3-medated signalling in A549 cells by phospho-western blot, at the indicated time points.

Figure 65. Graph showing the results of analysis of inhibiting of HER2:HER3 interaction by 10D1F.FcA or pertuzumab, as determined by PathHunter Pertuzumab Bioassay. IC50 (M) values are shown.

Figures 66A to 66C. Histograms showing the results of analysis of expression of EGFR, HER2 and HER3 by (**66A**) BCPAP (**66B**) BHT101 and (**66C**) SW1736 cells. 1 = unstained cells, 2 = isotype control, 3 = cetuximab, 4 = trastuzumab, and 5 = 10D1F.FcA.

Figures 67A to 67C. Graphs showing the results of analysis of the ability of different anti-ErbB antibodies to inhibit proliferation of $BRAF^{V600E}$ mutant thyroid cancer cell lines *in vitro*. **67A** shows the results obtained for BHT101 cells, **687** shows the results obtained for BCPAP cells, and **67C** shows the results obtained for SW1736 cells.

Figures 68A to 68C. Graphs showing the results of analysis of the ability of 10D1F.FcA alone, or in combation with vemurafenib, to inhibit proliferation of $BRAF^{V600E}$ mutant thyroid cancer cell lines *in vitro*. **68A** shows the results obtained for BHT101 cells, **68B** shows the results obtained for SW1736 cells, and **68C** shows the results obtained for BCPAP cells.

Figures 69A to 69C. Tables showing representative hematological profiles of BALB/c mice following administration of 10 mg/kg, 25 mg/kg, 100 mg/kg or 250 mg/kg of 10D1F.FcA or an equal volume of PBS. **69A** shows results of analysis of the red blood cell compartment, **69B** shows results of analysis of the white blood cell compartment, and **69C** shows results of analysis of correlates of liver, kidney and pancreas function, and levels of electrolytes. RBC = red blood cell, MVC = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, WBC = white blood cell, ALB = albumin, ALT = alanine aminotransferase, ALP = alkaline phosphatase, CREA = creatinine, BUN = blood urea nitrogen, GLU = glucagon, AMY = amylase, NA = sodium, K = potassium, P = phosphorus and CA = calcium.

Figures 70A to 70C. Tables showing representative hematological profiles of SD rats at the indicated time points, following administration of 250 mg/kg 10D1F.FcA or an equal volume of PBS. **70A** shows results of analysis of the red blood cell compartment, **70B** shows results of analysis of the white blood cell compartment, and **70C** shows results of analysis of correlates of liver, kidney and pancreas function, and levels of electrolytes. RBC = red blood cell, MVC = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, WBC = white blood cell, ALB = albumin, ALP = alkaline phosphatase, CREA = creatinine, BUN = blood urea nitrogen, GLU = glucagon, AMY = amylase, NA = sodium, K = potassium, P = phosphorus and CA = calcium.

Figure 71. Images showing the results of analysis of the effect of 10D1F.FcA treatment on HER3-medated signalling *in vivo* in cells of FaDu or OvCar8 cell-derived tumors, as determined by phospho-western blot.

Figure 72. Box blot showing the results of analysis of internalisation of different anti-ErbB antibodies by the indicated cell lines.

Figures 73A and 73B. Histograms and tables showing the results of analysis of internalisation of 10D1F.FcA or trastuzumab by the indicated cell lines at different time points, as determined by flow cytometry. **73B** shows median fluorescence intensity and percentages of PE-positive cells determined from the histograms shown in **73A**.

Figure 74. Graph showing the results of analysis of tumour volume over time in a N87 cell-line derived mouse model of gastric cancer after biweekly treatment with the indicated concentrations of the indicated anti-ErbB antibodies, for six weeks (n=6). Antibody administration is indicated by triangles along x-axis.

Figures 75A and 75B. Images showing immunohistochemical staining of malignant and normal human tissues using 10D1F.FcA. **75A** and **75B** shows staining of different tissues.

Figure 76. Images showing immunohistochemical staining of A549 tumor xenograft cryosections by 10D1F or a rabbit polyclonal anti-HER3 antibody, at the indicated magnifications. Secondary-only control stainings are shown.

Figures 77A and 77B. Bar charts showing the results of analysis of the ability of the indicated anti-ErbB antibodies to inhibit *in vitro* proliferation of the indicated cancer cell lines at the serum concentrations the antibodies reach at C_{max} following IP administration to mice at 25 mg/kg. **77A** and **77B** show results obtained using different cell lines.

Figures 78A to 78F. Representative sensorgrams showing results of analysis of affinity of binding to human HER3 by anti-HER3 antibodies 10D1F.A (**78A**, **78B**), MM-121 (**78C**, **78D**) and LJM-716 (**78E**, **78F**), in the absence of human NRG1 (**78A**, **78C**, **78E**), and in the presence of human NRG1 (**78B**, **78D**, **78F**). Kon, Koff and K_D are shown.

Figure 79. Graph showing the results of analysis of tumour volume over time in a patient-derived xenograft model of ovarian cancer comprising *CLU-NRG1* fusion, after biweekly treatment with 25 mg/kg of 10D1F or isotype-matched control antibody (n=10 per treatment group). Antibody administration is indicated by triangles along x-axis.

Examples

In the following Examples, the inventors describe the generation of novel anti-HER3 antibody clones targeted to specific regions of interest in the HER3 molecule, and the biophysical and functional characterisation and therapeutic evaluation of these antigen-binding molecules.

Example 1: HER3 target design and anti-HER3 antibody hybridoma production

The inventors selected two regions in the extracellular region of human HER3 (SEQ ID NO:9) for raising HER3-binding monoclonal antibodies.

1.1 Hybridoma production

Approximately 6 week old female BALB/c mice were obtained from InVivos (Singapore). Animals were housed under specific pathogen-free conditions and were treated in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

For hybridoma production, mice were immunized with proprietary mixtures of antigenic peptide, recombinant target protein or cells expressing the target protein.

Prior to harvesting the spleen for fusion, mice were either boosted with antigen mixture for three consecutive days or only for a single day. 24 h after the final boost total splenocytes were isolated and fused with the myeloma cell line P3X63.Ag8.653 (ATCC, USA), with PEG using ClonaCell-HY Hybridoma Cloning Kit, in accordance with the manufacturer's instructions (Stemcell Technologies, Canada).

Fused cells were cultured in ClonaCell-HY Medium C (Stemcell Technologies, Canada) overnight at 37°C in a 5% CO_2 incubator. The next day, fused cells were centrifuged and resuspended in 10 ml of ClonaCell-HY Medium C and then gently mixed with 90 ml of semisolid methylcellulose-based ClonaCell-HY Medium D (StemCell Technologies, Canada) containing HAT components, which combines the hybridoma selection and cloning into one step.

The fused cells were then plated into 96 well plates and allowed to grow at 37 °C in a 5% CO_2 incubator. After 7–10 days, single hybridoma clones were isolated and antibody producing hybridomas were selected by screening the supernatants by Enzyme-linked immunosorbent assay (ELISA) and Fluorescence-activated cell sorting (FACs).

1.2 Antibody variable region amplification and sequencing

Total RNA was extracted from hybridoma cells using TRIzol reagent (Life Technologies, Inc., USA) using manufacturer's protocol. Double-stranded cDNA was synthesized using SMARTer RACE 5'/3' Kit (Clontech™, USA) in accordance with the manufacturer's instructions. Briefly, 1 µg total RNA was used to generate full-length cDNA using 5′-RACE CDS primer (provided in the kit), and the 5′ adaptor (SMARTer II A primer) was then incorporated into each cDNA according to manufacturer's instructions. cDNA synthesis reactions contained: 5X First-Strand Buffer, DTT (20 mM), dNTP Mix (10 mM), RNase Inhibitor (40 U/µl) and SMARTScribe Reverse Transcriptase (100 U/µl).

The race-ready cDNAs were amplified using SeqAmp DNA Polymerase (Clontech™, USA). Amplification reactions contained SeqAmp DNA Polymerase, 2X Seq AMP buffer, 5' universal primer provided in the 5' SMARTer Race kit, that is complement to the adaptor sequence, and 3' primers that anneal to respective heavy chain or light chain constant region primer. The 5' constant region were designed based on previously reported primer mix either by Krebber et al. J. Immunol. Methods 1997; 201: 35-55, Wang et al. Journal of Immunological Methods 2000, 233; 167–177 or Tiller et al. Journal of Immunological Methods 2009; 350:183–193. The following thermal protocol was used: pre-denature cycle at 94°C for 1 min; 35 cycles of 94°C, 30 s, 55°C, 30 s and 72°C, 45 s; final extension at 72°C for 3 min.

The resulting VH and VL PCR products, approximately 550 bp, were cloned into pJET1.2/blunt vector using CloneJET PCR Cloning Kit (Thermo Scientific, USA) and used to transform highly competent *E.coli* DH5α. From the resulting transformants, plasmid DNA was prepared using Miniprep Kit (Qiagene, Germany) and sequenced. DNA sequencing was carried out by AITbiotech. These sequencing data were analyzed using the international IMGT (ImMunoGeneTics) information system (LeFranc et al., Nucleic Acids Res. (2015) 43 (Database issue):D413-22) to characterize the individual CDRs and framework sequences. The signal peptide at 5' end of the VH and VL was identified by SignalP (v 4.1; Nielsen, in Kihara, D (ed): Protein Function Prediction (Methods in Molecular Biology vol. 1611) 59-73, Springer 2017).

Four monoclonal anti-HER3 antibody clones were selected for further development: 10D1, 10A6, 4-35-B2, and 4-35-B4.

Humanised versions of 10D1 were designed *in silico* by grafting of complementarity determining regions (CDRs) into VH and VL comprising human antibody framework regions, and were further optimized for antigen binding by yeast display method.

For yeast display, humanized sequences were converted into single-chain fragment variable (scFv) format by polymerase chain reaction (PCR) and used as templates to generate mutant libraries by random mutagenesis. Mutant PCR libraries were then electroporated into yeast together with linearized pCTcon2 vector to generate yeast libraries. The libraries were stained with human HER3 antigen and sorted for top binders. After 4-5 rounds of sorting, individual yeast clones were sequenced to identify unique antibody sequences.

Antibody clone	VH/VL sequence	
10A6	VH = SEQ ID NO:157	
	VL = SEQ ID NO:164	
10D1	VH = SEQ ID NO:24	
	VL = SEQ ID NO:74	
10D1_c75	VH = SEQ ID NO:25	
	VL = SEQ ID NO:75	
10D1_c76	VH = SEQ ID NO:26	
	VL = SEQ ID NO:76	
10D1_c77	VH = SEQ ID NO:27	
	VL = SEQ ID NO:77	
10D1_c78v1	VH = SEQ ID NO:28	
	VL = SEQ ID NO:78	

10D1_c78v2	VH = SEQ ID NO:29	
	VL = SEQ ID NO:78	
10D1_11B	VH = SEQ ID NO:30	
	VL = SEQ ID NO:78	
10D1_c85v1	VH = SEQ ID NO:31	
	VL = SEQ ID NO:79	
10D1_c85v2	VH = SEQ ID NO:32	
	VL = SEQ ID NO:79	
10D1_c85o1	VH = SEQ ID NO:33	
	VL = SEQ ID NO:80	
10D1_c85o2	VH = SEQ ID NO:34	
	VL = SEQ ID NO:81	
10D1_c87	VH = SEQ ID NO:35	
	VL = SEQ ID NO:82	
10D1_c89	VH = SEQ ID NO:36	
	VL = SEQ ID NO:83	
10D1_c90	VH = SEQ ID NO:37	
	VL = SEQ ID NO:84	
10D1_c91	VH = SEQ ID NO:38	
	VL = SEQ ID NO:85	
10D1_c92	VH = SEQ ID NO:39	
	VL = SEQ ID NO:86	
10D1_c93	VH = SEQ ID NO:40	
	VL = SEQ ID NO:87	
4-35-B2	VH = SEQ ID NO:127	
	VL = SEQ ID NO:135	
4-35-B4	VH = SEQ ID NO:143	
	VL = SEQ ID NO:150	

Example 2: Antibody production and purification

2.1 Cloning VH and VL into Expression Vectors:

DNA sequences encoding the heavy and light chain variable regions of the anti-HER3 antibody clones were subcloned into the pmAbDZ_IgG1_CH and pmAbDZ_IgG1_CL (InvivoGen, USA) eukaryotic expression vectors for construction of human-mouse chimeric antibodies.

Alternatively, DNA sequence encoding the heavy and light chain variable regions of the anti-HER3 antibody clones were subcloned into the pFUSE-CHIg-hG1 and pFUSE2ss-CLIg-hk (InvivoGen, USA) eukaryotic expression vectors for construction of human-mouse chimeric antibodies. Human IgG1 constant region encoded by pFUSE-CHIg-hG1 comprises the substitutions D356E, L358M (positions numbered according to EU numbering) in the CH3 region relative to Human IgG1 constant region (IGHG1; UniProt:P01857-1, v1; SEQ ID NO:176). pFUSE2ss-CLIg-hk encodes human IgG1 light chain kappa constant region (IGCK; UniProt: P01834-1, v2).

Variable regions along with the signal peptides were amplified from the cloning vector using SeqAmp enzyme (Clontech™, USA) following the manufacturer's protocol. Forward and reverse primers having 15-20bp overlap with the appropriate regions within VH or VL plus 6 bp at 5' end as restriction sites were used. The DNA insert and the vector were digested with restriction enzyme recommended by the manufacturer to ensure no frameshift was introduced and ligated into its respective plasmid using T4 ligase enzyme (Thermo Scientific, USA). The molar ratio of 3:1 of DNA insert to vector was used for ligation.

2.2 Expression of antibodies in mammalian cells

Antibodies were expressed using either 1) Expi293 Transient Expression System Kit (Life Technologies, USA), or 2) HEK293-6E Transient Expression System (CNRC-NRC, Canada) following the manufacturer's instructions.

1) Expi293 Transient Expression System:

Cell line maintenance:

HEK293F cells (Expi293F) were obtained from Life Technologies, Inc (USA). Cells were cultured in serum-free, protein-free, chemically defined medium (Expi293 Expression Medium, Thermo Fisher, USA), supplemented with 50 IU/ml penicillin and 50 µg/ml streptomycine (Gibco, USA) at 37°C, in 8% CO_2 and 80% humidified incubators with shaking platform.

Transfection:

Expi293F cells were transfected with expression plasmids using ExpiFectamine 293 Reagent kit (Gibco, USA) according to its manufacturer's protocol. Briefly, cells at maintenance were subjected to a media exchange to remove antibiotics by spinning down the culture, cell pellets were re-suspended in fresh media without antibiotics at 1 day before transfection. On the day of transfection, 2.5 x 10^6/ml of viable cells were seeded in shaker flasks for each transfection. DNA-ExpiFectamine complexes were formed in serum-reduced medium, Opti-MEM (Gibco, USA), for 25 min at room temperature before being added to the cells. Enhancers were added to the transfected cells at 16-18 h post transfection. An equal amount of media was topped up to the transfectants at day 4 post-transfection to prevent cell aggregation. Transfectants were harvested at day 7 by centrifugation at 4000 x *g* for 15 min, and filtered through 0.22 µm sterile filter units.

2) HEK293-6E Transient Expression System

Cell line maintenance:

HEK293-6E cells were obtained from National Research Council Canada. Cells were cultured in serum-free, protein-free, chemically defined Freestyle F17 Medium (Invitrogen, USA), supplemented with 0.1% Kolliphor-P188 and 4 mM L-Glutamine (Gibco, USA) and 25 µg/ml G-418 at 37°C, in 5% CO_2 and 80% humidified incubators with shaking platform.

Transfection:

HEK293-6E cells were transfected with expression plasmids using PEIproTM (Polyplus, USA) according to its manufacturer's protocol. Briefly, cells at maintenance were subjected to a media exchange to remove antibiotics by centrifugation, cell pellets were re-suspended with fresh media without antibiotics at 1 day before transfection. On the day of transfection, 1.5-2 x 10^6 cells/ml of viable cells were seeded in shaker flasks for each transfection. DNA and PEIproTM were mixed to a ratio of 1:1 and the complexes were allowed to form in F17 medium for 5 min at RT before adding to the cells. 0.5% (w/v) of Tryptone N1 was fed to transfectants at 24-48 h post transfection. Transfectants were harvested at day 6-7 by centrifugation at 4000 x *g* for 15 min and the supernatant was filtered through 0.22 µm sterile filter units.

Cells were transfected with vectors encoding the following combinations of polypeptides:

Antigen-biding molecule	Polypeptides	Antibody
[1]	10D1 VH-CH1-CH2-CH3 (SEQ ID NO:216) + 10D1 VL-Cκ (SEQ ID NO:217)	anti-HER3 clone 10D1 IgG1
[2]	10A6 VH-CH1-CH2-CH3 (SEQ ID NO:222) + 10A6 VL-Cκ (SEQ ID NO:223)	anti-HER3 clone 10A6 IgG1
[3]	4-35-B2 VH-CH1-CH2-CH3 (SEQ ID NO:218) + 4-35-B2 VL-Cκ (SEQ ID NO:219)	anti-HER3 clone 4-35-B2 IgG1
[4]	4-35-B4 VH-CH1-CH2-CH3 (SEQ ID NO:220) + 4-35-B4 VL-Cκ (SEQ ID NO:221)	anti-HER3 clone 4-35-B4 IgG1
[5]	10D1_c75 VH-CH1-CH2-CH3 (SEQ ID NO:187) + 10D1_c75 VL-Cκ (SEQ ID NO:188)	anti-HER3 clone 10D1_c75 IgG1
[6]	10D1_c76 VH-CH1-CH2-CH3 (SEQ ID NO:189) + 10D1_c76 VL-Cκ (SEQ ID NO:190)	anti-HER3 clone 10D1_c76 IgG1
[7]	10D1_c77 VH-CH1-CH2-CH3 (SEQ ID NO:191) + 10D1_c77 VL-Cκ (SEQ ID NO:192)	anti-HER3 clone 10D1_c77 IgG1
[8]	10D1_c78v1 VH-CH1-CH2-CH3 (SEQ ID NO:193) + 10D1_c78v1 VL-Cκ (SEQ ID NO:195)	anti-HER3 clone 10D1_c78v1 IgG1
[9]	10D1_c78v1 VH-CH1-CH2-CH3 (SEQ ID NO:194) + 10D1_c78v2 VL-Cκ (SEQ ID NO:195)	anti-HER3 clone 10D1_c78v2 IgG1
[10]	10D1_11B VH-CH1-CH2-CH3 (SEQ ID NO:196) + 10D1_11B VL-Cκ (SEQ ID NO:195)	anti-HER3 clone 10D1_11B IgG1
[11]	10D1_c85v1 VH-CH1-CH2-CH3 (SEQ ID	anti-HER3 clone 10D1_c85v1 IgG1

	NO:197) + 10D1_c85v1 VL-Cκ (SEQ ID NO:199)	
[12]	10D1_c85v2 VH-CH1-CH2-CH3 (SEQ ID NO:198) + 10D1_c85v2 VL-Cκ (SEQ ID NO:199)	anti-HER3 clone 10D1_c85v2 IgG1
[13]	10D1_c85o1 VH-CH1-CH2-CH3 (SEQ ID NO:200) + 10D1_c85o1 VL-Cκ (SEQ ID NO:201)	anti-HER3 clone 10D1_c85o1 IgG1
[14]	10D1_c85o2 VH-CH1-CH2-CH3 (SEQ ID NO:202) + 10D1_c85o2 VL-Cκ (SEQ ID NO:203)	anti-HER3 clone 10D1_c85o2 IgG1
[15]	10D1_c87 VH-CH1-CH2-CH3 (SEQ ID NO:204) + 10D1_c87 VL-Cκ (SEQ ID NO:205)	anti-HER3 clone 10D1_c87 IgG1
[16]	10D1_c89 VH-CH1-CH2-CH3 (SEQ ID NO:206) + 10D1_c89 VL-Cκ (SEQ ID NO:207)	anti-HER3 clone 10D1_c89 IgG1
[17]	10D1_c90 VH-CH1-CH2-CH3 (SEQ ID NO:208) + 10D1_c90 VL-Cκ (SEQ ID NO:209)	anti-HER3 clone 10D1_c90 IgG1
[18]	10D1_c91 VH-CH1-CH2-CH3 (SEQ ID NO:210) + 10D1_c91 VL-Cκ (SEQ ID NO:211)	anti-HER3 clone 10D1_c91 IgG1
[19]	10D1_c92 VH-CH1-CH2-CH3 (SEQ ID NO:212) + 10D1_c92 VL-Cκ (SEQ ID NO:213)	anti-HER3 clone 10D1_c92 IgG1
[20]	10D1_c93 VH-CH1-CH2-CH3 (SEQ ID NO:214) + 10D1_c93 VL-Cκ (SEQ ID NO:215)	anti-HER3 clone 10D1_c93 IgG1

2.3 Antibody Purification

Affinity purification, buffer exchange and storage:

Antibodies secreted by the transfected cells into the culture supernatant were purified using liquid chromatography system AKTA Start (GE Healthcare, UK). Specifically, supernatants were loaded onto HiTrap Protein G column (GE Healthcare, UK) at a binding rate of 5 ml/min, followed by washing the column with 10 column volumes of washing buffer (20 mM sodium phosphate, pH 7.0). Bound mAbs were eluted with elution buffer (0.1 M glycine, pH 2.7) and the eluents were fractionated to collection tubes which contain appropriate amount of neutralization buffer (1 M Tris, pH 9). Neutralised elution buffer containing purified mAb were exchanged into PBS using 30K MWCO protein concentrators (Thermo Fisher, USA) or 3.5K MWCO dialysis cassettes (Thermo Fisher, USA). Monoclonal antibodies were sterilized by passing through 0.22 µm filter, aliquoted and snap-frozen in -80°C for storage.

2.4 Antibody-purity analysis

Size exclusion chromatography (SEC):

Antibody purity was analysed by size exclusion chromatography (SEC) using Superdex 200 10/30 GL columns (GE Healthcare, UK) in PBS running buffer, on a AKTA Explorer liquid chromatography system (GE Healthcare, UK). 150 µg of antibody in 500 µl PBS pH 7.2 was injected to the column at a flow rate of 0.75 ml/min at room temperature. Proteins were eluted according to their molecular weights.

The result for anti-HER3 antibody clone 10D1 ([1] of Example 2.2) is shown in Figure 12.

The results obtained for the different 10D1 variant clones are shown in Figure 34.

Sodium-Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE):

Antibody purity was also analysed by SDS-PAGE under reducing and non-reducing conditions according to standard methods. Briefly, 4%-20% TGX protein gels (Bio-Rad, USA) were used to resolve proteins using a Mini-Protean Electrophoresis System (Bio-Rad, USA). For non-reducing condition, protein samples were denatured by mixing with 2x Laemmli sample buffer (Bio-Rad, USA) and boiled at 95°C for 5-10 min before loading to the gel. For reducing conditions, 2x sample buffer containing 5% of β-mercaptoethanol (βME), or 40 mM DTT (dithiothreitol) was used. Electrophoresis was carried out at a constant voltage of 150V for 1 h in SDS running buffer (25 mM Tris, 192 mM glycine, 1% SDS, pH 8.3).

Western Blot:

Protein samples (30 µg) were fractionated by SDS-PAGE as described above and transferred to nitrocellulose membranes. Membranes were then blocked and immunoblotted with antibodies overnight at 4°C. After washing three times in PBS-Tween the membranes were then incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies. The results were visualized via a chemiluminescent Pierce ECL Substrate Western blot detection system (Thermo Scientific, USA) and exposure to autoradiography film (Kodak XAR film).

The primary antibodies used for detection were goat anti-human IgG-HRP (GenScript Cat No. A00166) and goat anti-human kappa-HRP (SouterhnBiotech Cat No. 2060-05).

The result for anti-HER3 antibody clone 10D1 ([1] of Example 2.2) is shown in Figure 13. 10D1 was easily expressed, purified and processed at high concentrations.

Example 3: Biophysical characterisation

3.1 Analysis of cell surface antigen-binding by flow cytometry

Wildtype HEK293 cells (which do not express high levels of HER3) and HEK293 cells transfected with vector encoding human HER3 (i.e. HEK 293 HER O/E cells) were incubated with 20 µg/ml of anti-HER3 antibody or isotype control antibody at 4°C for 1.5 hr. The anti-HER3 antibody clone LJM716 (described e.g. in Garner et al., Cancer Res (2013) 73: 6024–6035) was included in the analysis as a positive control.

The cells were washed thrice with FACS buffer (PBS with 5mM EDTA and 0.5% BSA) and resuspended in FITC-conjugated anti-FC antibody (Invitrogen, USA) for 40 min at 2-8°C. Cells were washed again and resuspended in 200 µL of FACS flow buffer (PBS with 5mM EDTA) for flow cytometric analysis using MACSQuant 10 (Miltenyi Biotec, Germany). After acquisition, all raw data were analyzed using Flowlogic software. Cells were gated using forward and side scatter profile percentage of positive cells was determined for native and overexpressing cell populations.

The results are shown in Figures 1 to 4 and 30 to 32. The anti-HER3 antibodies were shown to bind to human HER3 with high specificity. 10D1 and LJM716 were shown to bind to human HER3-expressing cells to a similar extent.

3.2 ELISAs for determining antibody specificity and cross-reactivity

ELISAs were used to determine the binding specificity of the antibodies. The antibodies were analysed for binding to human HER3 polypeptide, as well as respective mouse, rat and monkey homologues of HER3 (Sino Biological Inc., China). The antibodies were also analysed for their ability to bind to human EGFR and human HER2 (Sino Biological Inc., China).

ELISAs were carried out according to standard protocols. Briefly, 96-well plates (Nunc, Denmark) were coated with 0.1 µg/ml of target polypeptide in phosphate-buffered saline (PBS) for 16 h at 4°C. After blocking for 1 h with 1 % BSA in Tris buffer saline (TBS) at room temperature, the anti-HER3 antibody was serially diluted with the highest concentration being 10 µg/ml, and added to the plate. Post 1 h incubation at room temperature, plates were washed three times with TBS containing 0.05% Tween 20 (TBS-T) and were then incubated with a HRP-conjugated anti-His antibody (Life Technologies, Inc., USA) for 1 h at room temperature. After washing, plates were developed with colorimetric detection substrate 3,3′,5,5′-tetramethylbenzidine (Turbo-TMB; Pierce, USA) for 10 min. The reaction was stopped with 2M H_2SO_4, and OD was measured at 450 nM.

The results of the ELISAs are shown in Figures 5 to 7 and Figure 33.

Anti-HER3 antibody clone 10D1 was found not to bind to human HER2 or human EGFR even at high concentrations of the antibody (Figure 5A). Anti-HER3 antibody clone 10D1 was also found to display substantial cross-reactivity with mouse HER3, rat HER3 and cynomolgus macaque HER3 (Figure 5B).

Anti-HER3 antibody clone 4-35-B2 was found to bind to human HER2 and human EGFR (Figure 6A). Anti-HER3 antibody clone 4-35-B2 also displayed substantial cross-reactivity with mouse HER3, rat HER3 and cynomolgus macaque HER3 (Figure 6B).

Anti-HER3 antibody clone 4-35-B4 was found to bind to human HER2 and human EGFR (Figure 7A). Anti-HER3 antibody clone 4-35-B4 also displayed substantial cross-reactivity with mouse HER3, rat HER3 and cynomolgus macaque HER3 (Figure 7B).

All of the 10D1 variants were demonstrated to bind to human HER3 (Figures 33A and 33B).

3.3 Global affinity study using Octet QK384 system

The anti-HER3 antibody clones in IgG1 format were analysed for binding affinity to human HER3.

Bio-Layer Interferometry (BLI) experiments were performed using the Octet QK384 system (ForteBio). anti-Human IgG Capture (AHC) Octet sensor tips (Pall ForteBio, USA) were used to anti-HER3 antibodies (25 nM). All measurements were performed at 25°C with agitation at 1000 rpm. Kinetic measurements for antigen binding were performed by loading His-tagged human HER3 antigens at different concentrations for 120 s, followed by a 120 s dissociation time by transferring the biosensors into assay buffer containing wells. Sensograms were referenced for buffer effects and then fitted using the Octet QK384 user software (Pall ForteBio, USA). Kinetic responses were subjected to a global fitting using a one site binding model to obtain values for association (K_{on}), dissociation (K_{off}) rate constants and the equilibrium dissociation constant (K_D). Only curves that could be reliably fitted with the software (R^2>0.90) were included in the analysis.

A representative sensorgram for the analysis of clone 10D1 is shown in Figure 8. Clone 10D1 was found to bind to human HER3 with an affinity of K_D = 9.58 nM.

The humanised/optimized 10D1 variants bind to human HER3 with very high affinity. Representative sensorgrams are shown in Figures 36A to 36M.

The affinities determined for 10D1 clone variants are shown below:

Antibody clone	**Affinity (K_D)**
10D1_c89	72.6 pM
10D1_c90	< 1 pM
10D1_c91	176 pM
10D1_11B	0.41 nM

10D1_c85o	17.3 nM
10D1_c87	< 1 pM
10D1_c93	< 1 pM
10D1_c76	< 1 pM
10D1_c77	1.93 nM
10D1_c78	< 1 pM
10D1_c75	< 1 pM
10D1_c85	7.58 nM
10D1_c85o1	18.2 nM

Clone 4-35-B2 was found to bind to human HER3 with an affinity of K_D = 80.9 nM (Figure 9), and clone 4-35-B4 was found to bind to human HER3 with an affinity of K_D = 50.3 nM (Figure 10).

3.4 Analysis of thermostability by Differential Scanning Fluorimetry

Briefly, triplicate reaction mixes of antibodies at 0.2 mg/mL and SYPRO Orange dye (ThermoFisher) were prepared in 25 µL of PBS, transferred to wells of MicroAmp Optical 96-Well Reaction Plates (ThermoFisher), and sealed with MicroAmp Optical Adhesive Film (ThermoFisher). Melting curves were run in a 7500 fast Real-Time PCR system (Applied Biosystems) selecting TAMRA as reporter and ROX as passive reference. The thermal profile included an initial step of 2 min at 25°C and a final step of 2 min at 99°C, with a ramp rate of 1.2%. The first derivative of the raw data was plotted as a function of temperature to obtain the derivative melting curves. Melting temperatures (Tm) of the antibodies were extracted from the peaks of the derivative curves.

The first derivative of the raw data obtained for Differential Scanning Fluorimetry analysis of the thermostability of antibody clone 10D1 is shown in Figure 11. Three different samples of the antibody were analysed. The Tm was determined to be 70.3°C.

The analysis was also performed for the 10D1 variant clones and LJM716. The first derivative of the raw data and the determined Tms are shown in Figures 35A to 35C.

3.5 Analysis of anti-HER3 antibody 10D1 epitope

Anti-HER3 antibody 10D1 was analysed to determine whether it competes with anti-HER3 antibodies MM-121 and/or LJM-716 for binding to HER3. The epitope for MM-121 has been mapped in domain I of HER3; it blocks the NRG ligand binding site. The epitope for LJM-716 has been mapped to conformational epitope distributed across domains II and IV, and it locks HER3 in an inactive conformation.

Bio-Layer Interferometry (BLI) experiments were performed using the Octet QK384 system (ForteBio). anti-Penta-HIS (HIS1K) coated biosensor tips (ForteBio, USA) were used to capture His-tagged human HER3 (75 nM; 300 s). Binding by saturating antibody (400 nm; 600 s) was detected, followed by a dissociation step (120s), followed by detection of binding with competing antibody (300 nM; 300 s),

followed by a dissociation step (120s). The variable region of MM-121 antibody was cloned in the PDZ vector having human IgG2 and IgKappa Fc backbone. The variable region of LJM-716 antibody was cloned in the PDZ vector having human IgG1 and IgKappa Fc backbone.

The results of the analysis are shown in Figures 14A and 14B. Anti-HER3 antibody was found not to compete with MM-121 and/or LJM-716 for binding to HER3.

10D1 was found to bind a distinct and topologically distant epitope of HER3 than MM-121 and/or LJM-716.

The epitope for 10D1 was mapped using overlapping 15-mer amino acids to cover the entire HER3 extracellular domain. Each unique 15-mer was elongated by a GS linker at C and N-terminals, conjugated to a unique well in 384 well plates, and the plates were incubated with 0.1, 1, 10 and 100 ug/ml of 10D1 antibody for 16hrs at 4°C. The plates were washed and then incubated for 1hr at 20°C with POD-conjugated goat anti-human IgG. Finally POD substrate solution was added to the wells for 20 min. before binding was assessed by measurement of chemiluminescence at 425nm using a LI-COR Odyssey Imaging System, and quantification and analysis was performed using the PepSlide Analyzer software package. The experiment was performed in duplicate.

The 10D1 epitope was found not to be directly located at a β-hairpin structure of the HER3 dimerisation arm located at domain II, but instead at a dimerisation interface N-terminal to the β-hairpin.

The site of HER3 to which 10D1 and 10D1-derived clones was determined to bind corresponds to positions 218 to 235 of the amino acid sequence of human HER3 (as shown e.g. in SEQ ID NO:1); the amino acid sequence for this region of HER3 is shown in SEQ ID NO:229. Within this region, two consensus binding site motifs were identified, and are shown in SEQ ID NOs:230 and 231.

Binding to this location of HER3 acts to impede HER family heterodimerisation and consequent downstream signalling pathways (see Example 4). Binding is ligand (NRG) independent. The 10D1 binding site is solvent accessible in both the open and closed HER3 conformations, is not conserved between HER3 and other HER family members, and is 100% conserved between human, mouse, rat and monkey HER3 orthologs.

Example 4: Functional characterisation

4.1 Inhibition of dimerisation of HER2 and HER3

The anti-HER3 antibodies were analysed for their ability to inhibit heterodimerisation of HER3 and HER2.

Briefly, 96-well plates (Nunc, Denmark) were coated with 0.1 µg/ml His-tagged HER2 protein in PBS for 16 h at 4°C. After blocking for 1 h with 1 % BSA in PBS at room temperature, recombinant biotinylated human HER3 protein was added in the presence of different concentrations of anti-HER3 antibody clone 10D1, and pates were incubated for 1 h at room temperature. Plates were subsequently washed three times, and then incubated with HRP-conjugated secondary antibody for 1 h at room temperature. After

washing, plates were developed with colorimetric detection substrate 3,3′,5,5′-tetramethylbenzidine (Turbo-TMB; Pierce, USA) for 10 min. The reaction was stopped with 2M H_2SO_4, and OD was measured at 450 nM.

The results are shown in Figure 15. Anti-HER3 antibody clone 10D1 was found to inhibit interaction between HER2 and HER3 in a dose-dependent fashion.

In further experiments, inhibition of HER2:HER3 dimerisation was analysed

In further experiments, inhibition of HER2:HER3 dimerisation was evaluated using the PathHunter Pertuzumab Bioassay Kit (DiscoverX) according to the manufacturer's instructions.

Briefly, HER2 and HER3 overexpressing U2OS cells were thawed using 1 ml of pre-warmed CP5 media and 5,000 cells were seeded per well and cultured at 37°C in 5% CO_2 atmosphere for 4 hr. Cells were then treated with an 8-point serial dilution of 10D1F.FcA or Pertuzumab, starting from 25 µg/ml.

After 4 hr incubation, 30 ng/ml of heregulin-β2 was added to each well and the cells were incubated for a further 16 hr. 10 µL of PathHunter bioassay detection reagent 1 was added to wells, and incubated for 15 min at room temperature in the dark. This was followed by addition of 40 µL PathHunter bioassay detection reagent 2, and incubation for 60 min at room temperature in the dark. Plates were then read using Synergy4 Biotek with 1 second delay.

The results are shown in Figure 65. 10D1F.FcA was found inhibit HER2:HER3 dimerisation with greater efficiency than pertuzumab, as reflected by its lower IC50.

4.2 Identification of cancer cell lines for analysis

The inventors characterised expression of EGFR protein family members by cancer cell lines to identify appropriate cells to investigate inhibition of HER3.

Figure 16A shows mRNA expression data EGFR family members and ligands by N87, SNU16, HT29, FaDu, A549, HCC95, OvCAR8 and AHCN cells according to the Cancer Cell Line Encyclopaedia (CCLE; Barretina et al., Nature (2012) 483: 603-607 and The Cancer Cell Line Encyclopedia Consortium & The Genomics of Drug Sensitivity in Cancer Consortium, Nature (2015) 528: 84-87). Figure 16A also shows protein expression data for EGFR, HER2 and HER3 as determined by FlowLogic.

Cell lines used in the experiments were purchased from ATCC and cultured as recommended. Briefly, cell lines maintained in the indicated cell culture medium, supplemented with 10% FBS and 1% Pen/Strep. Cells were cultured at 37°C, in 5% CO_2 incubators. Cultured cells were plated at the appropriate seeding density in a 96 well plate: HT29, HCC95, FADU and OvCar8 cells were seeded at 2000 cells/well, NCI-N87 cells were seeded at 5000 cells/well, SNU-16, ACHN and cells were seeded at 1500 cells/well, and A549 cells were seeded at 1200 cells/well.

Figure 16B shows surface expression of EGFR, HER2 and HER3 as determined by flow cytometry. Briefly, 500,000 cells were stained in staining buffer containing 0.5% BSA and 2mM EDTA with primary antibodies (20 µg/ml) for 1.5 h at 4°C. The secondary Antibody used was anti-human Alexafluor488 at 10 µg/ml for 20 min at 4°C.

4.3 Inhibition of HER3-mediated signalling

Anti-HER3 antibody 10D1 was analysed for its ability to inhibit HER-3 mediated signalling *in vitro*.

Briefly, N87 and FaDu cells were seeded in wells of a 6 well plate with 10% serum at 37°C, 5% CO_2. After 16 hrs, cells were starved by culture overnight in 1% FBS cell culture medium (to reduce signalling elicited by growth factors in the serum). On the following day cells were treated with 50 µg/ml anti-HER3 antibody 10D1 for 4 hrs, followed by 15 min stimulation with NRG (100 ng/ml). Proteins were then extracted, quantified using standard Bradford protein assay, fractionated by SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were then blocked and immunoblotted with the following antibodies overnight at 4°C anti-pHER3, anti-pAKT, pan anti-HER3, pan anti-AKT and anti-beta-actin. The blots were visualized via Bio-Rad Clarity Western ECL substrate, and bands were quantified using densiometric analysis; data were normalized to beta actin controls.

The results are shown in Figure 17. Anti-HER3 antibody 10D1 was found to inhibit HER3 phosphorylation and downstream signalling.

In further experiments the inventors investigated the intracellular signalling pathways affected by anti-HER3 antibody-mediated inhibition of HER3.

FaDu cells were seeded in wells of a 6 well plate with 10% serum at 37°C, 5% CO_2. After 16 hrs, cells were starved by culture overnight in 1% FBS cell culture medium. On the following day cells were treated with 50 µg/ml anti-HER3 antibody 10D1 for 4 hrs, followed by 15 min stimulation with NRG (100 ng/ml). Proteins were then extracted, quantified using standard Bradford protein assay, and incubated overnight with pre-blocked Phosphoprotein Antibody Array membrane (Ray Biotech) at 4°C. The membrane was then washed with washing buffer and incubated with detection antibody cocktail for 2 hrs at room temperature, followed by washing and incubation with HRP-Conjugated anti-IgG. After 2 hrs the membrane was washed and probed using the kit detection buffer. Images were captured with Syngene Gbox imaging system, the intensity of each dot/phosphoprotein was measured and percent inhibition was calculated by comparison with intensity measured for cells treated in the same way in the absence of the antibody.

The results are shown in Figure 18. Anti-HER3 antibody 10D1 was found to inhibit PI3K/AKT/mTOR and MAPK signalling.

In further experiments the inventors investigated the effect of treatment with anti-HER3 antibody 10D1 on proliferation of HER3-expressing cells.

Briefly, N87 and FaDu cells were treated with serially diluted concentrations of anti-HER3 antibody 10D1, starting from 100 µg/ml with a 9-point half log dilution. Cell proliferation was measuring using the CCK-8 proliferation assay (Dojindo, Japan) after a period of 5 days, in accordance with the manufacturer's instructions. Briefly 1x CCK-8 solution was added to each well followed by incubation for 2 h at 37°C. The OD was then measured at 450 nm.

Figures 19A and 19B shows the percent cell confluence relative to untreated control cells (data points are averages of three replicates).

Anti-HER3 antibody 10D1 displayed dose-dependent inhibition of cell proliferation by N87 and FaDu cells.

Example 5: Analysis *in vivo*

5.1 Pharmacokinetic analysis

Female NCr nude mice approximately 6-8 weeks old were housed under specific pathogen-free conditions and treated in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

500 µg anti-HER3 antibody was administered and blood was obtained from 3 mice by cardiac puncture at baseline (- 2 hr), 0.5 hr, 6 hr, 24 hr, 96 hr, 168 hr and 336 hr after administration. Antibody in the serum was quantified by ELISA.

The results are shown in Figure 50. Anti-HER3 antibody clone 10D1 was found to have a half-life of 16.3 days in NCr nude mice.

5.2 Safety immunotoxicity

Anti-HER3 antibody clone 10D1 was analysed *in silico* for safety and immunogenicity using IMGT DomainGapAlign (Ehrenmann et al., Nucleic Acids Res., 38, D301-307 (2010)) and IEDB deimmunization (Dhanda et al., Immunology. (2018) 153(1):118-132) tools.

Anti-HER3 antibody clone 10D1 had numbers of potential immunogenic peptides few enough to be considered safe, and did not possess any other properties that could cause potential developability issues.

The Table of Figure 37 provides an overview of the properties of the 10D1 variant clones relevant to safety and developability.

Mice treated with anti-HER3 antibodies in the experiments described in Example 5.3 were monitored for changes in weight and gross necroscopy. No differences were detected in these mice as compared to mice treated with vehicle only.

Hemotoxicity was investigated in an experiment in which 6-8 week old female BALB/c mice (20-25 g) were injected intraperitoneally with a single dose of 1000 µg anti-HER3 10D1 antibody or an equal

volume of PBS. Blood samples were obtained at 96 hours post injection and analysed for numbers of different types of white blood cells by flow cytometry and electrolyte indices for NA^+, K^+, and Cl^-.

Figures 51A and 51B show that the numbers of the different cell types and electrolyte indices were found to be within the Charles River reference range (3 mice) and did not differ significantly from the PBS-treated group (3 mice). Left bars represent vehicle, right bars represent 10D1 treatment, end points of the Charles River reference range indicated with dotted lines. No differences in clinical signs, gross necroscopy or weight were detected between the different groups.

Mice were also analysed for correlates hepatotoxicity, nephrotoxicity and pancreatic toxicity at 96 hours post injection. The levels of alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine (CREA), alkaline phosphatase (ALP), glucose (GLU), calcium (CAL), total bilirubin (BIL), total protein (TPR) and albumin (ALB) detected following administration of a single dose of 1000 µg anti-HER3 antibody were found to be within the Charles River reference range and do not differ significantly from the levels of these markers in the PBS-treated group. These are shown in Figures 51C to 51F. Left bars represent vehicle, right bars represent 10D1 treatment, end points of the Charles River reference range indicated with dotted lines. 10D1 treatment has no effect on the kidney, liver or pancreatic indices and thus does not affect normal kidney, liver or pancreatic functions.

5.3 Analysis of efficacy to treat cancer in vivo

Female NCr nude mice approximately 6-8 weeks old were purchased from InVivos (Singapore). Animals were housed under specific pathogen-free conditions and were treated in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

Cell lines used included N87 cells (gastric cancer), FaDu cells (head and neck cancer), OvCAR8 cells (ovarian cancer), SNU16 cells (gastric cancer), HT29 cells (colorectal cancer), A549 cells (lung cancer), HCC95 cells (lung cancer) and AHCN cells (kidney cancer).

Tumor volumes were measured 3 times a week using a digital caliper and calculated using the formula [L x W2/2]. Study End point was considered to have been reaches once the tumors of the control arm measured >1.5 cm in length.

5.3.1 N87 model

Figure 20 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in a N87 cell-line derived mouse gastric carcinoma model. The model was established by subcutaneous injection of 1×10^6 N87 cells into the right flank (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 10 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~76%.

Figure 21 shows the results obtained in a similar experiment in wherein anti-HER3 antibody clone 4-35-B4 was administered weekly IP at a dose of 11 mg/kg (total of 4 doses). Anti-HER3 antibody clone 4-35-B4 was similarly found to be highly potent in this model, and capable of inhibiting tumor growth by ~60%.

5.3.2 SNU16 model

Figure 22 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in a SNU16 cell-line derived mouse gastric carcinoma model. The model was established by subcutaneous injection of 1 x 10^6 SNU16 cells into the right flank (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 9 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~68%.

5.3.3 FaDu model

Figure 23 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in a FaDu cell-line derived mouse model of head and neck squamous cell carcinoma. The model was established by subcutaneous injection of 1 x 10^6 FaDu cells into the right flanks of female NPG mice (NOD scid gamma phenotype; n = 6 mice per treatment group).

10D1 was administered IP, weekly at 500 µg per dose (for a total of 4 doses). Control treatment groups received an equal volume of PBS, or the same dose of an isotype control antibody.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~85%.

Figure 24 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in a FaDu cell-line derived mouse model of head and neck squamous cell carcinoma. The model was established by subcutaneous injection of 1 x 10^6 FaDu cells into the right flanks of female NCr nude mice (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 8 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~86%.

5.3.4 OvCAR8 model

Figure 25 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in an OvCAR8 cell-line derived mouse model of ovarian carcinoma. The model was established by subcutaneous injection of 1 x 10^6 OvCAR8 cells into the right flanks of female NCr nude mice (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 9 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~74%.

5.3.5 HCC-95 model

Figure 26 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in a HCC-95 cell-line derived mouse model of squamous cell lung carcinoma. The model was established by subcutaneous injection of 1 x 10^6 HCC-95 cells into the right flanks of female NCr nude mice (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 4 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~90%.

5.3.6 A549 model

Figure 27 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in an A549 cell-line derived mouse model of lung adenocarcinoma. The model was established by subcutaneous injection of 1 x 10^6 A549 cells into the right flanks of female NCr nude mice (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 10 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~91%.

Figure 28 shows the results obtained in a similar experiment in wherein the anti-cancer effect of anti-HER3 antibody clone 4-35-B2 was investigated in an A549 cell-line derived model established by injection of 1 x 10^6 A549 cells into the right flanks of female NPG mice (NOD scid gamma phenotype). Anti-HER3 antibody clone 4-35-B2 was administered IP weekly at a dose of 500 µg per dose (total of 4 doses). A control treatment group received an equal volume of PBS (6 mice per treatment group).

Anti-HER3 antibody clone 4-35-B2 was similarly found to be highly potent in this model, and capable of inhibiting tumor growth by ~63%.

5.3.6 ACHN model

Figure 29 shows the results obtained in an experiment wherein the anti-cancer effect of anti-HER3 antibody 10D1 ([1] of Example 2.2) was investigated in an ACHN cell-line derived mouse model of renal cell carcinoma. The model was established by subcutaneous injection of 1 x 10^6 ACHN cells into the right flanks of female NCr nude mice (n = 6 mice per treatment group).

10D1 was administered IP, biweekly at 500 µg per dose (for a total of 7 doses); a control treatment group received an equal volume of PBS.

Anti-HER3 antibody clone 10D1 was found to be highly potent in this model, and capable of inhibiting tumor growth by ~61%.

5.4 Treatment of Gastric Carcinoma

First in human

Patients with HER2+ advanced gastric cancer who have failed or cannot receive trastuzumab are treated by intravenous injection of anti-HER3 antibody selected from: 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92 and 10D1_c93, at a dose calculated in accordance with safety-adjusted 'Minimal Anticipated Biological Effect Level' (MABEL) approach. Patients are monitored for 28 days post-administration.

The patients are then evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE), to determine the safety and tolerability of the treatment, and to determine the pharmacokinetics of the molecules.

Treatment with the anti-HER3 antibodies is found to be safe and tolerable.

Dose escalation – monotherapy

12-48 patients with HER2+ advanced gastric cancer who have failed or cannot receive trastuzumab are treated by intravenous injection of anti-HER3 antibody selected from: 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92 and 10D1_c93 (e.g. 10D1_c89, 10D1_c90 or 10D1_c91; e.g. 10D1_c89), in accordance with a 3+3 model based escalation with overdose control (EWOC) dose escalation.

The patients are then evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) to determine the safety and tolerability of the treatment, and the pharmacokinetics of the

molecules and efficacy of the treatment is evaluated. The maximum tolerated dose (MTD) and maximum administered dose (MAD) are also determined.

Dose escalation – combination therapy

9-18 patients with HER2+ advanced gastric cancer who have failed or trastuzumab are treated by intravenous injection of anti-HER3 antibody selected from: 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92 and 10D1_c93 (e.g. 10D1_c89, 10D1_c90 or 10D1_c91; e.g. 10D1_c89) in combination with trastuzumab, in accordance with a 3+3 model based escalation with anti-PD-L1 antibody (3 mg/kg).

The patients are then evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) to determine the safety and tolerability of the treatment, and the pharmacokinetics of the molecules and efficacy of the treatment is evaluated.

Dose expansion

Patients with HER2+ advanced gastric cancer who have recently failed trastuzumab, and whose tumours have been well-characterised genetically and histologically are treated with anti-HER3 antibody selected from: 10D1, 10D1_c75, 10D1_c76, 10D1_c77, 10D1_c78v1, 10D1_c78v2, 10D1_11B, 10D1_c85v1, 10D1_c85v2, 10D1_c85o1, 10D1_c85o2, 10D1_c87, 10D1_c89, 10D1_c90, 10D1_c91, 10D1_c92, 10D1_c93 (e.g. 10D1_c89, 10D1_c90 or 10D1_c91; e.g. 10D1_c89) in combination with trastuzumab, cisplatin, and either 5-FU or capecitabine

The anti-HER3 antibodies are found to be safe and tolerable, to be able to reduce the number/proportion of cancer cells, reduce tumor cell marker expression, increase progression-free survival and increase overall survival.

Example 6: Affinity matured and humanised clones

Humanization of the variable regions of the parental mouse antibody 10D1P was done by CDR grafting. Human framework sequences for grafting were identified by blasting the parental amino acid sequence against the human V domain database and the genes with highest identity to the parental sequence were selected. Upon grafting the mouse CDRs into the selected human frameworks, residues in canonical positions of the framework were back mutated to the parental mouse sequence to preserve antigen binding. A total of 9 humanized variants of 10D1P were designed.

Affinity against human HER3 was increased by two rounds of affinity maturation using yeast display. In the first round, a mixed library of the 9 designed variants was constructed by random mutagenesis and screened by flow cytometry using biotinylated antigen. In the second round, one heavy chain and one light chain clones isolated in the first round were used as template to generate and screen a second library. A total of 10 humanized and affinity matured clones were isolated.

Potential liabilities (immunogenicity, glycosylation sites, exposed reactive residues, aggregation potential) in the variable regions of the designed and isolated humanized variants of 10D1P was assessed using in silico prediction tools. The sequences were deimmunised using IEDB deimmunisation tool. The final sequence of 10D1F was selected among the optimized variants based on its developability characteristics as well as in vitro physicochemical and functional properties.

Clone 10D1F comprises VH of SEQ ID NO:36 and VL of SEQ ID NO:83. 10D1F displays 89.9% homology with human heavy chain and 85.3% homology with human light chain.

The antigen-binding molecule comprising 10D1F variable regions and human IgG1 constant regions, and which is comprised of the polypeptides of SEQ ID NOs: 206 and 207, is designated 10D1F.FcA (also sometimes referred to herein as "10D1F.A" or "anti-HER3 clone 10D1_c89 IgG1" – see e.g. [16] of Example 2.2).

Example 7: Fc engineering

10D1 and 10D1 variants were engineered to comprise mutations in CH2 and/or CH3 regions to increase the potency of the antibodies, e.g. optimise Fc effector function, enhance antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular phagocytosis (ADCP), and improve half-life.

The Fc regions of clones 10D1 and 10D1F.FcA were modified to include modifications 'GASDALIE' (G236A, S239D, A330L, I332E) and 'LCKC' (L242C, K334C) in the CH2 region. The GASDALIE substitutions were found to increase affinity for the FcγRIIa (GA) and FcγRIIIa (SDALIE) receptors and enhance ADCP and NK-mediated ADCC (see Example 8.8), whilst decreasing affinity for C1q (AL) and reducing CDC. The LCKC substitutions were found to increase thermal stability of the Fc region by creating a new intramolecular disulphide bridge.

The modified version of 10D1F.FcA heavy chain polypeptide comprising the GASDALIE and LCKC mutations is shown in SEQ ID NO:225. The antigen-binding molecule comprised of the polypeptides of SEQ ID NOs: 225 and 207 is designated 10D1F.FcB (also sometimes referred to herein as "10D1F.B").

A modified version of 10D1 comprising the GASDALIE and LCKC substitutions in CH2 region was prepared and its ability to bind Fc receptor FcγRIIIa was analysed by Bio-Layer Interferometry. The sequence for 10D1 VH-CH1-CH2-CH3 comprising substitutions GASDALIE and LCKC corresponding to G236A, S239D, A330L, I332E and L242C, K334C is shown in SEQ ID NO:227.

Briefly, anti-Penta-HIS (HIS1K) coated biosensor tips (Pall ForteBio, USA) were used to capture His-tagged FcγRIIIa (V158) (270 nM) for 120 s. All measurements were performed at 25°C with agitation at 1000 rpm. Association kinetic measurements for antigen binding were performed by incubating anti-HER3 antibodies at different concentrations (500 nM to 15.6 nM) for 60 s, followed by a 120 s dissociation time by transferring the biosensors into assay buffer (pH 7.2) containing wells. Sensograms were referenced for buffer effects and then fitted using the Octet QK384 user software (Pall ForteBio, USA). Kinetic responses were subjected to a global fitting using a one site binding model to obtain values for

association (K_{on}), dissociation (K_{off}) rate constants and the equilibrium dissociation constant (K_D). Only curves that could be reliably fitted with the software (R^2>0.90) were included in the analysis.

The thermostability of the variant was also analysed by Differential Scanning Fluorimetry analysis as described in Example 3.4.

Figures 38A and 38B show the BLI analysis and thermostability analysis, respectively, for 10D1 comprising GASDALIE and LCKC Fc substitutions. The Fc engineered 10D1 variant showed significantly improved binding to FcγRIIIa (~9 fold increase in affinity) compared to non-Fc-engineered 10D1 (see Figure 39A) with thermal stability maintained above 60°C.

A construct for 10D1 comprising the GASD substitutions in CH2 region was also prepared; a sequence of 10D1 VH-CH1-CH2-CH3 comprising substitutions corresponding to G236A and S239D is shown in SEQ ID NO:228.

The affinity of anti-HER3 antibody clone 10D1 ([1] of Example 2.2) and the GASD variant thereof were analysed by Bio-Layer Interferometry for affinity of binding to FcγRIIIa. BLI was performed as described above.

Figures 39A and 39B show representative sensorgrams, K_{on}, K_{off} and K_D values. As expected, the 10D1 GASD variant (39B) displayed dramatically increased affinity for FcγRIIIa compared to 10D1 (39A).

The thermostability of the 10D1 GASD variant was also analysed by Differential Scanning Fluorimetry analysis as described in Example 3.4. The results are shown in Figure 40.

Further 10D1F Fc variant

Another antibody variant was created comprising an N297Q substitution in the CH2 region. A representative sequence for 10D1F VH-CH1-CH2-CH3 comprising the N297Q substitution is shown in SEQ ID NO:226. This 'silent form' prevents both N-linked glycosylation of the Fc region and Fc binding to Fcγ receptors and is used as a negative control.

Figures 41A and 41B show the binding affinity of 10D1F hIgG1 Fc variants 10D1F.FcA and 10D1F.FcB to human and mouse Fc receptors, determined as described above. 10D1F.FcB was found to show significantly improved binding to human and mouse Fcγ and FcRn receptors compared to non-modified 10D1F.FcA or commercially available antibodies. ND = K_D Not Determined due to low binding affinity.

Example 8: Characterisation of humanised and modified clones

8.1 Analysis of cell surface antigen-binding by flow cytometry

Wildtype (WT) HEK293 cells (which do not express high levels of HER3) and HEK293 cells transfected with vector encoding human HER3 (i.e. HEK293 HER O/E cells) were incubated with 10 µg/ml of humanised anti-HER3 antibody 10D1F.FcA (10D1F), anti-HER3 antibody 10D1 (10D1P) or isotype

control antibody at 4°C for 1.5 hr. The anti-HER3 antibody clone LJM716 (described e.g. in Garner et al., Cancer Res (2013) 73: 6024–6035, and Example 3.5) was included in the analysis as a positive control.

The cells were washed with buffer (PBS with 2mM EDTA and 0.5% BSA) and resuspended in FITC-conjugated anti-FC antibody (Invitrogen, USA) at 10 µg/ml for 20 min at 4°C. Cells were washed again and resuspended in 200 µL of FACS flow buffer (PBS with 5mM EDTA) for flow cytometric analysis using MACSQuant 10 (Miltenyi Biotec, Germany). Unstained WT and transfected HEK293 cells were included in the analysis as negative controls. After acquisition, all raw data were analyzed using Flowlogic software. Cells were gated using forward and side scatter profile percentage of positive cells was determined for native and overexpressing cell populations.

The results are shown in Figures 42A and 42B. Anti-HER3 antibody 10D1F.FcA was shown to bind to human HER3 with high specificity (42A). 10D1F.FcA, 10D1P and LJM716 were shown to bind to human HER3-expressing cells to a similar extent (42B).

8.2 ELISAs for determining antibody specificity and cross-reactivity

ELISAs were used to confirm the binding specificity of the 10D1F.FcA antibody. The antibodies were analysed for their ability to bind to human HER3 polypeptide as well as human HER1 (EGFR) and human HER2 (Sino Biological Inc., China). Human IgG isotype and an irrelevant antigen were included as negative controls.

ELISAs were carried out according to standard protocols. Plates were coated with 0.1 µg/ml of target polypeptide in phosphate-buffered saline (PBS) for 16 h at 4°C. After blocking for 1 h with 1 % BSA in Tris buffer saline (TBS) at room temperature, the anti-HER3 antibody was serially diluted with the highest concentration being 10 µg/ml, and added to the plate. Post 1 h incubation at room temperature, plates were washed three times with TBS containing 0.05% Tween 20 (TBS-T) and were then incubated with a HRP-conjugated anti-His antibody (Life Technologies, Inc., USA) for 1 h at room temperature. After washing, plates were developed with colorimetric detection substrate 3,3′,5,5′-tetramethylbenzidine (Turbo-TMB; Pierce, USA) for 10 min. The reaction was stopped with 2M H_2SO_4, and OD was measured at 450 nM.

The results are shown in Figure 43. Anti-HER3 antibody 10D1F.FcA was found not to bind to human HER2 or human HER1 (EGFR) even at high concentrations of the antibody.

The ability of 10D1F.FcA to bind HER4 was analysed using flow cytometry. Wildtype (WT) HEK293 cells (which do not express high levels of HER4) and HEK293 cells transfected with vector encoding human HER4 (i.e. HEK293 HER O/E cells) were incubated with 10 µg/ml of anti-HER3 antibody 10D1F.FcA (10D1F) or isotype control antibody (negative control) at 4°C for 1.5 hr. The anti-HER3 antibody clones LJM716 (described e.g. in Garner et al., Cancer Res (2013) 73: 6024–6035) and MM-121 (seribantumab), as described in Example 3.5, were included in the analysis as positive control. Also included was a commercial anti-HER4 antibody (Novus, Cat: FAB11311P). Unstained HEK293 cells were included in the analysis as negative controls.

HEK293 cells were incubated with 10 µg/ml of each antibody for 1 hour at 4°C. Flow cytometry was performed as described above. Cells were contacted with FITC-conjugated anti-FC antibody (Invitrogen, USA) at for 30 min at 4°C.

The results are shown in Figure 44. Anti-HER3 antibody 10D1F.FcA was found not to bind to cell-surface expressed HER4.

In addition, antibody 10D1F.FcA was analysed for its ability to bind to HER3 polypeptide homologues from mouse, rat and monkey (Sino Biological Inc., China). *M. musculus, R. norvegicus* and *M. cynomolgus* HER3 homologues share 91.1, 91.0 and 98.9% sequence identity respectively with human HER3 and the HER3 signalling pathways are conserved between the four species.

ELISAs were performed as above.

The results are shown in Figure 45. 10D1F.FcA antibody was found to bind with high affinity to HER3 cyno, mouse, rat and human orthologs, thus displaying substantial cross-reactivity between species.

8.3 Global affinity study using Octet QK384 system

The anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB were analysed for binding affinity to human HER3.

Bio-Layer Interferometry (BLI) experiments were performed using the Octet QK384 system (ForteBio). Antibodies (25 nM) were coated onto anti-Human IgG Capture (AHC) Octet sensor tips (Pall ForteBio, USA). Binding was detected using titrated HIS-tagged human HER3 in steps of baseline (60 s), loading (120 s), baseline2 (60 s), association (120 s), dissociation (FcA 120 s, FcB 600 s) and regeneration (15 s). Antigen concentrations are shown in the table in Figures 46A and 46B. Sensorgrams were analysed as described in Example 3.3. Values were obtained for association (K_{on}), dissociation (K_{off}) rate constants and the equilibrium dissociation constant (K_D).

Representative sensorgrams for the analysis of clones 10D1F.FcA and 10D1F.FcB are shown in Figures 46A and 46B. 10D1F.FcA binds to human HER3 with a high affinity of K_D = 72.6 pM (46A). 10D1F.FcB binds to human HER3 with a high affinity of K_D = 22.2 pM (46B).

8.4 Analysis of thermostability by Differential Scanning Fluorimetry

Differential Scanning Fluorimetry was performed for antibodies 10D1F.FcA and 10D1F.FcB as described in Example 3.4.

The first derivative of the raw data obtained for Differential Scanning Fluorimetry analysis of the thermostability of antibody clone 10D1F.FcA is shown in Figure 47A. Three different samples of the antibody were analysed and the Tm was determined to be 70.0°C.

The first derivative of the raw data obtained for Differential Scanning Fluorimetry analysis of the thermostability of antibody clone 10D1F.FcB is shown in Figure 47B. Three different samples of the antibody were analysed and the Tm was determined to be 62.7°C.

8.5 Antibody purity analysis

The purity of antibodies 10D1F.FcA and 10D1F.FcB was analysed by size exclusion chromatography (SEC). 150 µg of 10D1F.FcA in 500 µl PBS pH 7.2 or 150 µg of 10D1F.FcB in 500 µl PBS pH 7.45 was injected on a Superdex 200 10/30 GL column in PBS running buffer at a flow rate of 0.75 min/ml or 0.5 min/ml, respectively, at room temperature and the A280 of flow through was recorded.

The results are shown in Figures 48A (10D1F.FcA) and 48B (10D1F.FcB).

8.6 Analysis of anti-HER3 antibody 10D1F.FcA epitope

Anti-HER3 antibody 10D1F.FcA was analysed to determine whether it competes with anti-HER3 antibodies M-05-74 or M-08-11 (Roche) for binding to HER3. Epitopes of M-05-74 and M-08-11 were both mapped to the β-hairpin structure of the HER3 dimerisation arm located at domain II. M-08-11 does not bind to HER4 whereas M-05-74 recognises the HER4 dimerisation arm. Binding of M-05-74 and M-08-11 to HER3 is ligand (NRG) independent.

BLI experiments were performed as described in Example 3.5 with one alteration: 400 nM of competing antibodies were used. The variable regions of M-05-74 and M-08-11 antibodies were cloned in the PDZ vector having human IgG1 and IgKappa Fc backbone.

The results of the analysis are shown in Figures 49A and 49B. Anti-HER3 10D1F.FcA antibody was found not to compete with M-05-74 or M-08-11 for binding to HER3. 10D1F.FcA was found to bind a distinct and topologically distant epitope of HER3 compared to M-05-74 and M-08-11. Binding of 10D1F.FcA to HER3 is ligand (NRG) independent.

Conclusions:

- Binding of 10D1F.FcA to human HER3 can be achieved in a ligand-independent manner.
- 10D1F.FcA binding epitope is distinct and topologically distant from that of M-05-74 and M-08-11.

8.7 Inhibition of dimerisation of HER2-HER3 and EGFR-HER3

Anti-HER3 antibody 10D1F.FcA was analysed for its ability to inhibit heterodimerisation of HER3 and HER2.

Plate-based ELISA dimerisation assays were carried out according to standard protocols. Plates were coated with 1 µg/ml HER2-Fc protein. After blocking and washing, the plate was incubated with different concentrations of candidate antibodies 10D1F.FcA, MM-121, LJM716, Pertuzumab, Roche M05, Roche M08 or isotype control and constant HER3 His 2 µg/ml and NRG 0.1 µg/ml for 1 hour. Plates were then washed and incubated for 1 hour with secondary anti-HIS HRP antibody. Plates were washed, treated

with TMB for 10 mins and the reaction was stopped using 2M H_2SO_4 stop solution. The absorbance was read at 450 nm.

The results are shown in Figure 52. Anti-HER3 antibody clone 10D1F.FcA was found to directly inhibit interaction between HER2 and HER3 in a dose dependent fashion.

In another assay, inhibition of dimerization was detected using PathHunter® Pertuzumab Bioassay Kit (DiscoverX, San Francisco, USA). HER2 and HER3 overexpressing U20S cells were thawed using 1 ml of pre-warmed CP5 media and 5K cells were seeded per at 37°C, 5% CO_2 for 4 hrs. Cells were treated with serially diluted concentrations of 10D1F.FcA, Seribantumab, or Pertuzumab starting from 25 µg/ml with an 8-point serial dilution. After 4 hrs incubation, 30 ng/ml of heregulin-β2 was added to each well and the plates were further incubated for 16 hrs. Following incubation, 10 µL PathHunter bioassay detection reagent 1 was added and incubated for 15 mins at room temperature in the dark, followed by addition of 40 µL PathHunter bioassay detection reagent 2 which was then incubated for 60 mins at room temperature in the dark. Plates were read using Synergy4 Biotek with 1 second delay.

10D1F.FcA was found to have an EC_{50} value of 3.715e-11 for inhibition of HER2-HER3 heterodimerisation. In the same assay, the comparative EC_{50} value for Seribantumab/MM-121 was found to be 6.788e-10 and the comparative EC_{50} value for Pertuzumab was found to be 2.481e-10.

Anti-HER3 antibody 10D1F.FcA was analysed for its ability to inhibit heterodimerisation of EGFR and HER3.

Plate-based ELISA dimerisation assays were performed according to standard protocols. Plate was coated with 1 µg/ml human EGFR-His. After blocking and washing, the plate was incubated with different concentrations of candidate antibodies 10D1F.FcA, MM-121, LJM716, Pertuzumab, or isotype control with constant HER3-biotin 4 µg/ml and NRG 0.1 µg/ml for 1 hour. Plates were then washed and incubated for 1 hour with secondary anti-avidin HRP antibody. Plates were washed, treated with TMB for 10 mins and the reaction was stopped using 2M H_2SO_4 stop solution. The absorbance was read at 450 nm.

The results are shown in Figure 53. Anti-HER3 antibody clone 10D1F.FcA was found to directly inhibit interaction between EGFR and HER3 in a dose dependent fashion.

8.8 Analysis of ability to induce ADCC

Anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB were analysed for their ability to induce antibody-dependent cell-mediated cytotoxicity (ADCC).

Target cells (HEK293 overexpressing HER3) were plated in U-bottom 96-well plates at a density of 20,000 cells/well. Cells were treated with a dilution series (50,000 ng/ml – 0.18 ng/ml) of one of 10D1F.FcA, 10D1F.FcB, 10D1F.FcA_N297Q (silent form), LJM-716, Seribantumab (MM-121) or left untreated, and incubated at 37°C and 5% CO_2 for 30 min. Effector cells (Human Natural Killer Cell Line

No-GFP-CD16.NK-92; 176V) were added to the plate containing target cells at a density of 60,000 cells/well.

The following controls were included: Target cell maximal LDH release (target cells only), spontaneous release (target cells and effector cells without antibody), background (culture media only). Plates were spun down and incubated at 37°C and 5% CO_2 for 21 hrs.

LDH release assay (Pierce LDH Cytotoxicity Assay Kit): before the assay, 10 µl of Lysis Buffer (10X) were added to target cell maximal LDH release controls and incubated at 37°C and 5% CO_2 for 20 min. After incubation, plates were spun down and 50 µL of the supernatant were transferred to clear flat-bottom 96-well plates. Reactions were started by addition of 50 µl of LDH assay mix containing substrate to the supernatants and incubated at 37°C for 30 min. Reactions were stopped by addition of 50 µl of stop solution and absorbance was recorded at 490 nm and 680 nm with a BioTek Synergy HT microplate reader.

For data analysis, absorbance from test samples was corrected to background and spontaneous release from target cells and effector cells. Percent cytotoxicity of test samples was calculated relative to target cell maximal LDH release controls and plot as a function of antibody concentration.

The results are shown in Figure 54. Anti-HER3 antibody 10D1F.FcB was found to induce potent ADCC activity against HER3 overexpressing cells in a dose dependent fashion.

8.9 Inhibition of HER3-mediated signalling

Anti-HER3 antibody 10D1F.FcA was analysed for its ability to inhibit HER-3 mediated signalling *in vitro* in cancer cell lines.

N87, FaDu or OvCAR8 cells were seeded in wells in a 6 well plate with 10% serum overnight at 37°C with 5% CO_2. Cells were starved with 0.2% FBS culture medium for 16 hrs, and were then treated for 0.5 hours with different antibodies at IC50 corresponding to the cell line. Antibodies tested were: 10D1F.FcA (10D1), Seribantumab (SBT), Elegemtumab (LJM), Pertuzumab (PTM), Cetuximab (CTX), and Trastuzumab (TZ).

Before harvesting, cells were stimulated with 100 ng/ml of NRG1. Protein extracted from cell lines were quantified using standard Bradford protein assay. Protein samples (50 µg) were fractionated by SDS-PAGE and transferred to nitrocellulose membrane. Membranes were then blocked and immunoblotted with the indicated antibodies. The results were visualized via Bio-Rad Clarity Western ECL substrate. The blots were quantified using densiometric analysis and data was normalised to beta actin.

The results are shown in Figures 55A to 55C. Anti-HER3 antibody 10D1F.FcA was found to inhibit HER3 phosphorylation and downstream signalling in N87 (55A), FaDu (55B), OvCar8 (55C) and A549 (55D) cell lines.

For the experiments using N87 cells, A549 cells, OvCar8 cells and FaDu cells total RNA was extracted at 16 hrs post antibody treatment was analysed to determine pathway activation based on the level of expression of key signal transduction pathway proteins by gene set enrichment analysis.
The results of the analysis are shown in Figures 63A to 63D. 10D1F.FcA was the most effective inhibitor of downstream signalling.

In further experiments using A549 cells, *in vitro* phosphorylation assays were performed as above except that the cells were treated for 0.5 hours or 4 hours with the different antibodies. The results are shown in Figure 64.

8.10 Analysis of binding to HER3 provided in 'open' and 'closed' conformations

In further experiments, anti-HER3 antibodies were analysed in order to determine the affinity of binding to human HER3 in the context of a human HER3:human NRG1 complex (that is, HER3 provided in the ligand-bound, 'open' conformation), as compared to the affinity of binding to human HER3 in the absence of NRG1 complex (that is, HER3 provided in the unbound, 'closed' conformation).

Binding of anti-HER3 antibodies to human HER3 was evaluated by BLI. Briefly, anti-Human IgG Capture (AHC) sensors (ForteBio) were loaded with anti-HER3 IgG antibodies (25 nM). Kinetic measurements were performed in absence or presence of NRG1. NRG1 was used at 1:1 molar ratio with HER3 wherein the complex was allowed to form at RT for 2 h. His-tagged human HER3 or HER3-NRG1 complexes were loaded to antibody coated AHC sensors at different concentrations for 120 s, followed by a 120 s dissociation time. All measurements were performed at 25°C with agitation at 1000 rpm. Sensorgrams were referenced for buffer effects and then analyzed using the Octet QK384 -software (ForteBio). Kinetic responses were globally fitted using a one-site binding model to obtain values for association (K_{on}), dissociation (K_{off}) rate constants and the equilibrium dissociation constant (K_D).

The following anti-HER3 antibodies were analysed in the experiments: 10D1F.A ([16] of Example 2.2), MM-121 and LJM-716.

The results are shown in Figures 78A to 78F. 10D1F.A was found to bind to human HER3 with sub-picomolar affinity in the presence or absence of NRG1 (Figures 78A and 78B). MM-121 bound to human HER3 with nanomolar in the presence or absence of NRG1 (Figures 78C and 78D). LJM-716 bound to human HER3 with subpicomolar affinity in the absence of NRG1 (Figure 78E). However, in the presence of NRG1, binding to HER3 was dramatically reduced (Figure 78F).

8.11 Conclusions

Taken together, the results identify 10D1F as an anti-HER3 antibody which binds to an epitope of HER3 providing it with the unique combination of properties that (i) it competitively inhibits heterodimerisation of HER3 with EGFR or HER2 (see Example 8.7 and Figures 52 and 53), and (ii) binds HER3 similarly well in the presence or absence of NRG1 (Figures 78A and 78B).

Example 9: Analysis of humanised and modified clones *in vitro* and *in vivo*

9.1 Pharmacokinetic analysis

Mice

Female NCr nude mice approximately 6-8 weeks old were housed under specific pathogen-free conditions and treated in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

500 µg anti-HER3 antibody 10D1F.FcA or 10D1F.FcB was administered and blood was obtained from 4 mice by cardiac puncture at baseline (- 2 hr), 6 hr, 24 hr, 96 hr, 168 hr and 336 hr after administration. Antibody in the serum was quantified by ELISA.

The parameters for the pharmacokinetic analysis were derived from a non-compartmental model: maximum concentration (C_{max}), AUC (0-336hr), AUC (0-infinity), Half-life ($t_{½}$), Clearance (CL), Volume of distribution at steady state (V_{ss}).

The results are shown in Figures 56A and 56B. Anti-HER3 antibody clone 10D1F.FcA was found to have a half-life of 253 hours (56A) and anti-HER3 antibody clone 10D1F.FcB was found to have a half-life of 273 hours (56B) in NCr nude mice.

Rats

10D1F variants were analysed to determine a single dose pharmacokinetic profile in female Sprague Dawley rats with mean weight of 320 g.

Antibody clones 10D1F.FcA and 10D1F.FcB were administered in a single dose of 4 mg (~10 mg/kg), 10 mg (~25 mg/kg), 40 mg (~100 mg/kg) or 100 mg (~250 mg/kg) via tail vein slow i.v. injection. Vehicle was administered as a negative control. Blood from 2 rats per treatment was obtained at baseline (- 24 hr), 6 hr, 24 hr, 96 hr, 168 hr and 336 hr after administration. Antibody in the serum was quantified by ELISA.

The parameters for the pharmacokinetic analysis were derived from a non-compartmental model: maximum concentration (C_{max}), AUC (0-336hr), AUC (0-infinity), Half-life ($t_{½}$), Clearance (CL), Volume of distribution at steady state (V_d).

The results are shown in Figures 57A (10 mg/kg), 57B (25 mg/kg), 57C (100 mg/kg) and 57D (250 mg/kg).

9.2 Safety immunotoxicity

The toxicological effects of 10D1F.FcA and 10D1F.FcB were analysed.

Mice

6-8 week old female BALB/c mice (20-25 g) were injected intraperitoneally with a single dose of either 10D1F.FcA and 10D1F.FcB at one of doses: 200 ug (~10 mg/kg), 500 ug (~25 mg/kg), 2 mg (~100

mg/kg), or 5 mg (~250 mg/kg), or an equal volume of PBS. 3 mice were injected with each treatment, 4 mice were injected with PBS control. Blood samples were obtained at 96 hours post injection and analysed for RBC indices (total RBC count, haematocrit, haemoglobin, platelet count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration) and WBC indices (total WBC count, lymphocyte count, neutrophil count, monocyte count). Analysis was performed using a HM5 Hematology Analyser.

The results are shown in Figures 58A, 58B (RBC indices) and 58C (WBC indices). Anti-HER3 antibodies 10D1F.FcA and 10D1F.FcB had no effect on the RBC indices, but were found to have an effect on the WBC indices at higher doses (10D1F.FcA 250 mg/kg, 10D1F.FcB 100 mg/kg, 10D1F.FcB 250 mg/kg).

Hepatotoxicity, nephrotoxicity and pancreatic toxicity were also analysed 96 hours post injection. 10D1F.FcA and 10D1F.FcB had no effect on the levels of alanine aminotransferase, alkaline phosphatase, albumin, total protein (liver indices; Figure 58D), creatine, blood urea nitrogen, glucose or amylase (kidney and pancreatic indices; Figure 58E). Nor did 10D1F.FcA and 10D1F.FcB have an effect on electrolyte indices sodium, potassium, calcium or phosphate (Figure 58F).

Mice treated with 10D1F.FcA or 10D1F.FcB showed no abnormalities after 96 hours in weight, behaviour, skin condition, oral examination, stool and urine examination or eye examination. Enlarged spleen (splenomegaly) approximately 1.5 times the normal size was observed in mice treated with higher doses: 10D1F.FcA 250 mg/kg, 10D1F.FcB 100 mg/kg, 10D1F.FcB 250 mg/kg.

A further study was performed in the BALB/c mice to assess the toxicological effects of repeat doses of 500 µg (~25 mg/kg) 10D1F.FcA or 10D1F.FcB. Antibody was administered once a week for four weeks. Blood was obtained 28 days after the first administration. There was no effect observed on RBC, liver, kidney, pancreatic or electrolyte indices for either antibody, no signs of clinical abnormalities and no differences detected in gross necroscopy. Total WBC count, lymphocyte count and neutrophil count was observed to be decreased in mice treated with 10D1F.FcA or 10D1F.FcB but this was not considered to be toxic.

In another study, BALB/c mice were administered with a single dose of 10D1F.FcA or an equal volume of PBS (vehicle control), and analysed after 96 hours (vehicle) or 336 hours (10D1F.FcA). Representative results are shown in Figures 69A to 69C.

Rats

6-8 week old female Sprague Dawley rats (400-450 g) were injected intraperitoneally with a single dose of either 10D1F.FcA or 10D1F.FcB antibody at one of doses: 4 mg (~10 mg/kg), 10 mg (~25 mg/kg), 40 mg (~100 mg/kg), 100 mg (~ 250 mg/kg). Blood was obtained at -24 hours, 6 hours, 24 hours, 96 hours, 168 hours and 336 hours. Up to 366 hours post injection there was no effect on RBC indices, no toxic effect on WBC indices, and no effect on liver, kidney, pancreatic or electrolyte indices. There were no signs of clinical abnormalities and no differences detected in gross necroscopy.

Representative results obtained from rats administered with 250 mg/kg 10D1F.FcA are shown in Figures 70A to 70C.

The absence of toxicity signals in rodent toxicology models indicates superior clinical safety for 10D1 and variants.

9.3 Analysis of efficacy to treat cancer in vitro

Anti-HER3 antibody 10D1F.FcA was analysed for its ability to inhibit tumour growth *in vitro* in a number of tumour models: N87 cells (gastric cancer), HCC95 cells (lung cancer), FaDu cells (head and neck cancer), SNU-16 cells (gastric cancer), A549 cells (lung cancer), OvCar8 cells (ovarian cancer), ACHN cells (kidney cancer) and HT29 cells (colorectal cancer). 10D1F.FcA efficacy was compared to other anti-HER3 antibodies seribantumab (MM-121) and LJM-716, and other EGFR family therapies cetuximab, trastuzumab and pertuzumab.

Cells were treated with serially diluted concentrations of therapeutic antibodies, starting from 1500 ug/ml with a 9-point dilution. Cell viability was measured using CCK-8 cell proliferation assay, 3-5 days post treatment. The percentage of cell inhibition shown is relative to cells treated with only buffer (PBS). Data points indicates average of three replicates.

The results are shown in Figure 59A to 59D. Anti-HER3 antibody 10D1F.FcA demonstrates superior *in vitro* tumour inhibition in multiple tumour models compared to other HER3 antibodies (59A & 59B) and EGFR family therapies (59C & 59D).

Figures 77A and 77B show the ability of different anti-ErbB antibodies to inhibit proliferation of different cancer cell lines *in vitro*, at the C_{max} concentration they achieve in mice administered IP with 25 mg/kg of the relevant antibody. 10D1F.FcA displays outstanding ability to inhibit growth of a wide variety of different cancer cell types.

9.4 Analysis of efficacy to treat cancer in vivo

Anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB were assessed for their effect on tumour growth in *in vivo* cancer models.

9.4.1 A549 model

Tumour cells were inserted subcutaneously into the right flanks of female NCr nude mice. Antibodies (25 mg/kg 10D1F.FcA, 10D1F.FcB, Cetuximab, LJM-716 or MM-121; n=6 for each treatment) or vehicle (n=8) were administered biweekly for six weeks.

The results are shown in Figure 60. Anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB both displayed potent efficacy in the A549 model of lung carcinoma. 10D1F.FcB was found to be particularly potent and made tumours regress.

9.4.2 FaDu model

Tumour cells were inserted with matrigel subcutaneously into the right flanks of female NCr nude mice. Antibodies (10 and 25 mg/kg 10D1F.FcA and 10D1F.FcB, or 25 mg/kg of Cetuximab, Trastuzumab, Pertuzumab, LJM-716 or MM-121; n=6 for each treatment) or vehicle (n=6) were administered once a week for six weeks.

The results are shown in Figure 61. Anti-HER3 antibody clones 10D1F.FcA and 10D1F.FcB were both found to be effective to prevent tumour growth in the FaDu model of head and neck cancer.

9.4.3 OvCar8 model

Tumour cells were inserted with matrigel subcutaneously into the right flanks of female NCr nude mice. Antibodies (10 and 25 mg/kg 10D1F.FcA, or 25 mg/kg Cetuximab, LJM-716 or MM-121; n=6) or vehicle (n=6) were administered once a week for six weeks.

The results are shown in Figure 62. Anti-HER3 antibody clone 10D1F.FcA was found to be effective at reducing tumour volume at higher dose.

9.4.4 N87 model

Tumour cells are inserted with matrigel subcutaneously into the right flanks of female NCr nude mice. Antibodies (25 mg/kg 10D1F.FcA, or 50 mg/kg of Trastuzumab, LJM-716 or MM-121; n=6 for each treatment) or vehicle (n=6) were administered biweekly for six weeks.

The results are shown in Figure 74. Anti-HER3 antibody 10D1F.FcA and was found to be effective to prevent tumour growth in the N87 model of gastric cancer.

Example 10: Analysis of inhibition of proliferation of BRAFV600E mutant thyroid cancer cell lines

The following cell lines were investigated:

Cell Line	Type of Cancer	Mutation
SW1736	Anaplastic thyroid cancer	BRAF V600E
BHT101	Anaplastic thyroid cancer	BRAF V600E
BCPAP	Papillary thyroid cancer	BRAF V600E and p53 mutation

The cells were investigated for surface expression of EGFR family members by flow cytometry. Briefly, 300,000 cells were incubated with 20 µg/ml of 10D1F.FcA, cetuximab or trastuzumab for 1 hr at 4°C. Alexafluor 488-conjugated anti-human antibody was used at 10 µg/ml as a secondary antibody (40 min at 4°C).

The results are shown in Figure 66A to 66C. SW1736, BHT101 and BCPAP cells were shown to express EGFR, HER2 and HER3.

The inventors investigated the ability of different HER3-binding antibodies to inhibit *in vitro* proliferation of different thyroid cancer cell lines harbouring the V600E BRAF mutation.

Briefly, cells of the different cell lines were seeded at a density of 1.5 x 10^5 cells/well, and treated the next day with a 10 point serial dilution starting from 1000 µg/ml of 10D1F.FcA, seribantumab, LJM-716, pertuzumab or isotype control antibody. After 3 days, proliferation was measured using a CCK-8 cell proliferation assay. Percent inhibition of proliferation was calculated relative to cells treated with an equal volume of PBS instead of antibodies.

The results are shown in Figures 67A to 67C. 10D1F.FcA was found to be more effective at inhibiting proliferation of cell lines harbouring BRAF V600E mutation than any other of the anti-HER3 antibodies analysed.

In further experiments, the ability of a combination of 10D1F.FcA and vemurafenib to inhibit *in vitro* proliferation of different thyroid cancer cell lines harbouring the V600E BRAF mutation was investigated.

Cells were seeded at a density of 1.5 x 10^5 cells/well, and treated the next day with a 10 point serial dilution starting from 1000 µg/ml of 10D1F.FcA or isotype control antibody, in the presence or absence of 200 nM vemurafenib. After 3 days, proliferation was measured using a CCK-8 cell proliferation assay. Percent inhibition of proliferation was calculated relative to cells treated with an equal volume of PBS instead of antibodies.

The results are shown in Figures 68A to 68C. 10D1F.FcA was found to enhance the ability of vemurafenib to inhibit proliferation of SW1736 and BHT101 cells, which are susceptible to vemurafenib. 10D1F.FcA was also found to be a potent inhibitor of proliferation of vemurafenib-resistant BCPAP cells.

Example 11: Analysis of inhibition of HER3-mediated signalling *in vivo*

The inventors investigated the ability of 10D1F.FcA to inhibit HER3-mediated signalling *in vivo*.

1 x 10^6 FaDu or OvCar8 cells were introduced subcutaneously into NCr nude mice, to establish ectopic xenograft tumors.

Once tumors had reached a volume of greater than 100 mm^3, mice were with treated by biweekly intraperitoneal injection of 10D1F.FcA at a dose of 25 mg/kg, or an equal volume of vehicle (control). After 4 weeks, tumors were harvested. Protein extracts were prepared from the tumors and quantified via Bradford assay, 50 µg samples were fractionated by SDS-PAGE, and analysed by western blot using antibodies in order to determine in vivo phosphorylation of HER3 and AKT, as described in Example 4.3.

The results are shown in Figure 71. 10D1F.FcA was found to inhibit phosphorylation of HER3 and AKT in tumor cells *in vivo*.

Example 12: Analysis of internalisation of anti-HER3 antibodies

The inventors investigated internalisation of anti-HER3 antibodies by HER3-expressing cells.

Briefly, 100,000 HEK293 cells engineered to express HER3, HCC95, N87 or OVCAR8 cells were seeded in wells of 96-well tissue culture plates and cultured overnight at 37°C in 5% CO_2. Cells were then treated with 120 nM of 10D1F.FcA, LJM-716, seribantumab or trastuzumab, and 360 nM of pHrodo iFL Green reagent, and incubated at 37°C in 5% CO_2. The cells in culture were imaged every 30 min for 24 hours, in 4 different fields of each well. The maximum signal intensity in the FITC channel of each field was quantified at 24 hours.

The results are shown in Figure 72. Modest to moderate internalization of LJM-716 and seribantumab was observed in OvCar8 cells, whereas modest internalization of trastuzumab was observed in N87 cells.

No significant internalization of 10D1F.FcA was observed in HCC95, N87, or OvCar8 cells.

As expected, significant internalization of 10D1F.FcA, LJM-716, and seribantumab was observed in HEK293 cells overexpressing HER3.

In further experiments, antibody internalisation was investigated by flow cytometry.

N87 cells were seeded in wells of 96-well tissue culture plates at a density of 50,000 cells/well, and allowed to adhere overnight (37°C, 5% CO_2). 10D1F.FcA or trastuzumab were mixed with labelling reagent, and the labelled complexes were added to cells. Samples were harvested at 0 min, 10 min, 30 min, 1 hour, 2 hour and 4.5 hour time points, by aspiration of cell culture medium, washing with PBS and treatment with accutase. Accutase activity was neutralised, and cells were resuspended in FACs buffer and analysed by flow cytometry.

The results are shown in Figure 73A and 73B. The cells displayed minimal internalisation of 10D1F.FcA. By contrast, substantial internalisation of anti-HER2 antibody trastuzumab was observed.

Example 13: Use of HER3-binding antibodies in immunohistochemistry

Anti-HER3 antibody 10D1F in mIgG2a format was evaluated for its ability to be used in immunohistochemistry for the detection of human HER3 protein.

Processing of sections was performed using Bond reagents (Leica Biosystems). Arrays of commercially-available frozen tissue sections were obtained. Slides were dried in a desiccator for 10 min and then subjected to the following treatments, with water washes and/or TBS-T rinses between steps: (i) fixation by treatment with 100% acetone for 10 min at room temperature; (ii) endogenous peroxidase blocking by treatment with 3% (v/v) H_2O_2 for 15 min at room temperature; (iii) blocking by treatment with 10% goat serum for 30 min at room temperature, (iv) incubation with 10D1F-mIgG2a at 1:250 dilution of a 6.2 mg/ml solution overnight at 4°C, (v) incubation with HRP-polymer conjugated goat anti-mouse antibody

for 30 min at room temperature, and (vi) development with Bond Mixed DAB Refine for 5 min at room temperature, followed by rinsing with deionised water and 1x Bond Wash to stop the reaction.

Slides were then dehydrated, mounted in synthetic mounting media and scanned with high resolution.

The results are shown in Figures 75A and 75B. 10D1F preferentially stained malignant human tissue sections, with low cross-reactivity to normal tissue.

In further experiments, an A549 xenograft tumor was harvested in cold PBS, embedded in OCT cryoembedding medium, frozen in dry-ice and stored at -80 ℃. 10 µm sections were obtained using a cryostat.

Slides were dried in a desiccator for 10 min and then subjected to the following treatments, with water washes and/or TBS-T rinses between steps: (i) fixation by treatment with 100% acetone for 10 min at room temperature; (ii) endogenous peroxidase blocking by treatment with 3% (v/v) H_2O_2 for 15 min at room temperature; (iii) blocking by treatment with 10% goat serum for 30 min at room temperature, (iv) incubation with 10D1F.FcA at 1:50 dilution of 8.8 mg/ml solution, or with 1:200 dilution of Sino Biological rabbit anti-HER3 (Cat. No. 10201-T24) overnight at 4°C, (v) incubation with Invitrogen F(ab')2-Goat anti-Human IgG (H+L) HRP (A24470) (1:500), or HRP-polymer conjugated goat anti-rabbit antibody for 30 min at room temperature, and (vi) development with Bond Mixed DAB Refine for 5 min at room temperature, followed by rinsing with deionised water and 1x Bond Wash to stop the reaction.

Slides were then counterstained with haematoxylin, dehydrated, mounted in synthetic mounting media and scanned with high resolution.

The results are shown in Figures 76. 10D1F.FcA displayed specific membrane and cytoplasmic staining of A549 tumor xenograft cryosections.

Example 14: Therapeutic efficacy of HER3-binding antibodies in a patient-derived xenograft model of ovarian cancer comprising *CLU-NRG1* fusion

The inventors investigated the therapeutic efficacy of 10D1F in a patient-derived xenograft model of ovarian cancer comprising *CLU-NRG1* fusion.

Female BALB/c nude mice approximately 5-7 weeks old were housed under specific pathogen-free conditions and were treated in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

A model of ovarian cancer comprising *CLU-NRG1* fusion was established by inoculating mice subcutaneously at the right flank with four pellets of a patient-derived xenograft designated OV6308 (Crown Bioscience Inc.). OV6308 is an ovarian high-grade serous adenocarcinoma derived from a 51-year female patient, and comprises the *CLU-NRG1* gene fusion.

Tumor volumes were measured 3 times a week using a digital caliper and calculated using the formula [L x W2/2]. Study End point was considered to have been reached once the tumors of the control arm measured >1.5 cm in length.

Mice were administered biweekly by intraperitoneal injection with (i) 25 mg/kg of 10D1F.FcA (i.e. [16] of Example 2.2), or (ii) 25 mg/kg of hIgG1 isotype control antibody (n = 10 for each treatment group).

The results are shown in Figure 79. Treatment with 10D1F.FcA was found to be extremely potent, inhibiting tumor growth by 111% relative to the isotype control-treated group.

Claims:

[Claim 1]. Use of an antigen-binding molecule which is capable of binding to HER3 in the manufacture of a medicament for treating or preventing a cancer in a subject, wherein the cancer comprises cells having an NRG gene fusion mutation resulting in increased expression of a ligand for HER3, and wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:41

HC-CDR2 having the amino acid sequence of SEQ ID NO:45

HC-CDR3 having the amino acid sequence of SEQ ID NO:48; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:88

LC-CDR2 having the amino acid sequence of SEQ ID NO:92

LC-CDR3 having the amino acid sequence of SEQ ID NO:95.

[Claim 2]. The use according to claim 1, wherein the ligand for HER3 comprises an amino acid sequence having at least 60% sequence identity to the EGF-like domain of an NRG.

[Claim 3]. The use according to claim 1 or claim 2, wherein the NRG gene fusion is selected from *CLU-NRG1, CD74-NRG1, DOC4-NRG1, SLC3A2-NRG1, RBPMS-NRG1, WRN-NRG1, SDC4-NRG1, RAB2IL1-NRG1, VAMP2-NRG1, KIF13B-NRG1, THAP7-NRG1, SMAD4-NRG1, MDK-NRG1, TNC-NRG1, DIP2B-NRG1, MRPL13-NRG1, PARP8-NRG1, ROCK1-NRG1, DPYSL2-NRG1, ATP1B1-NRG1, CDH6-NRG1, APP-NRG1, AKAP13-NRG1, THBS1-NRG1, FOXA1-NRG1, PDE7A- NRG1, RAB3IL1-NRG1, CDK1-NRG1, BMPRIB-NRG1, TNFRSF10B-NRG1, MCPH1-NRG1 and SLC12A2-NRG2.*

[Claim 4]. The use according to any one of claims 1 to 3, wherein the NRG gene fusion is selected from *CLU-NRG1, CD74-NRG1, SLC3A2-NRG1 or VAMP2-NRG1.*

[Claim 5]. The use according to any one of claims 1 to 4, wherein the cancer comprises cells expressing HER3.

[Claim 6]. The use according to any one of claims 1 to 5, wherein the cancer derives from the lung, breast, head, neck, kidney, ovary, pancreas, prostate, uterus, gallbladder, colon, rectum, bladder, soft tissue or nasopharynx.

[Claim 7]. The use according to any one of claims 1 to 6, wherein the cancer is selected from lung cancer, non-small cell lung cancer, lung adenocarcinoma, invasive mucinous lung adenocarcinoma, lung squamous cell carcinoma, breast cancer, breast carcinoma, breast invasive carcinoma, head and neck cancer, head and neck squamous cell carcinoma, renal cancer, renal clear cell carcinoma, ovarian cancer, ovarian serous cystadenocarcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic

ductal adenocarcinoma, prostate cancer, prostate adenocarcinoma, endometrial cancer, uterine carcinosarcoma, gallbladder cancer, cholangiocarcinoma, colorectal cancer, bladder cancer, urothelial bladder cancer, sarcoma, soft tissue sarcoma, neuroendocrine tumor and neuroendocrine tumor of the nasopharynx.

[Claim 8]. The use according to any one of claims 1 to 7, wherein the cancer is selected from lung cancer, non-small cell lung cancer, lung adenocarcinoma, invasive mucinous lung adenocarcinoma and lung squamous cell carcinoma.

[Claim 9]. The use according to any one of claims 1 to 8, wherein the antigen-binding molecule comprises:

a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:36; and

a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:83.

[Claim 10]. The use according to any one of claims 1 to 9, wherein the antigen-binding molecule comprises:

a VH region incorporating the following framework regions (FRs):

HC-FR1 having the amino acid sequence of SEQ ID NO:53

HC-FR2 having the amino acid sequence of SEQ ID NO:59

HC-FR3 having the amino acid sequence of SEQ ID NO:66

HC-FR4 having the amino acid sequence of SEQ ID NO:71.

[Claim 11]. The use according to any one of claims 1 to 10, wherein the antigen-binding molecule comprises:

a VL region incorporating the following framework regions (FRs):

LC-FR1 having the amino acid sequence of SEQ ID NO:104

LC-FR2 having the amino acid sequence of SEQ ID NO:110

LC-FR3 having the amino acid sequence of SEQ ID NO:120

LC-FR4 having the amino acid sequence of SEQ ID NO:125.

[Claim 12]. The use according to any one of claims 1 to 11, wherein the antigen-binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171.

[Claim 13]. The use according to any one of claims 1 to 12, wherein the antigen-binding molecule comprises a light chain comprising the amino acid sequence of SEQ ID NO:177.