US 20230303716A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2023/0303716 A1**

Lee et al. (43) **Pub. Date: Sep. 28, 2023**

(54) **ANTI-HER2 ANTIBODY OR ANTIGEN-BINDING FRAGMENT THEREOF, AND CHIMERIC ANTIGEN RECEPTOR COMPRISING SAME**

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(21) Appl. No.: **18/298,310**

(22) Filed: **Apr. 10, 2023**

Related U.S. Application Data

(60) Division of application No. 16/881,650, filed on May 22, 2020, now Pat. No. 11,649,294, which is a continuation-in-part of application No. 16/764,276, filed on Sep. 28, 2020, filed as application No. PCT/KR2018/013928 on Nov. 14, 2018.

(30) **Foreign Application Priority Data**

Nov. 14, 2017 (KR) 1020170151841

Publication Classification

(51) **Int. Cl.**

C07K 16/32	(2006.01)
C12N 5/0783	(2010.01)
A61K 38/17	(2006.01)
C07K 14/705	(2006.01)
A61P 35/00	(2006.01)

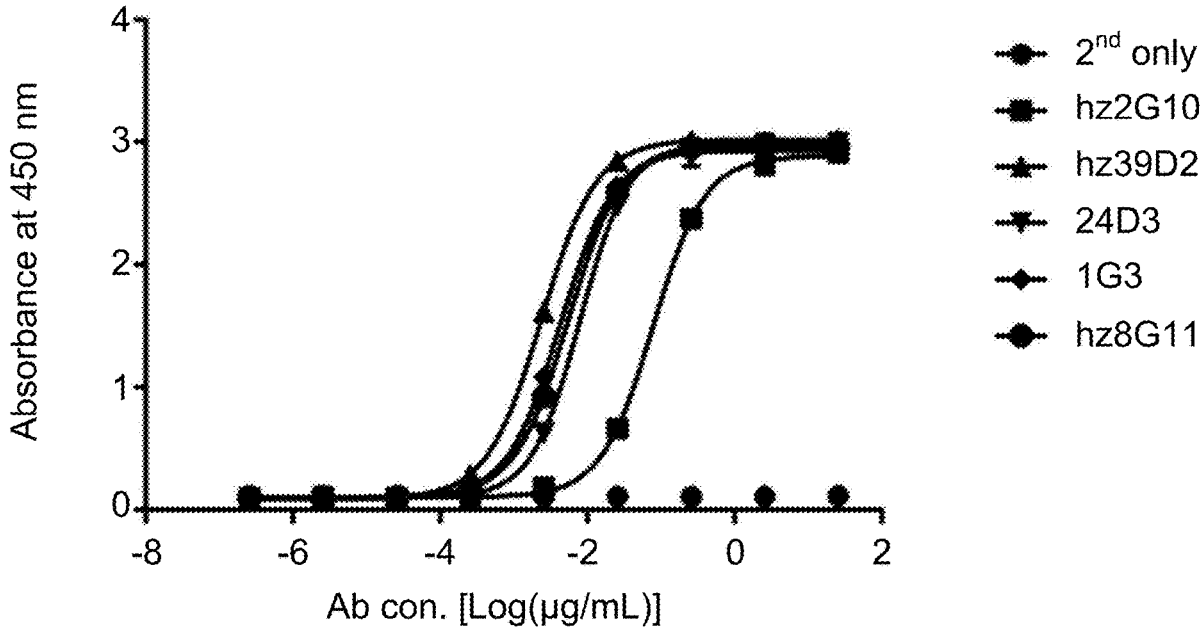
(52) **U.S. Cl.**

CPC ***C07K 16/32*** (2013.01); ***C12N 5/0646*** (2013.01); ***A61K 38/177*** (2013.01); ***C07K 14/705*** (2013.01); ***A61P 35/00*** (2018.01); *C07K 2317/565* (2013.01)

(57) **ABSTRACT**

The present disclosure relates to a novel anti-HER2 antibody or an antigen-binding fragment thereof used in the prevention or treatment of cancer, a chimeric antigen receptor including the same, and uses thereof. The antibody of the present disclosure is an antibody that specifically binds to HER2 which is highly expressed in cancer cells (particularly, breast cancer or gastric cancer cells), and binds to an epitope that is different from an epitope to which trastuzumab binds.

Specification includes a Sequence Listing.



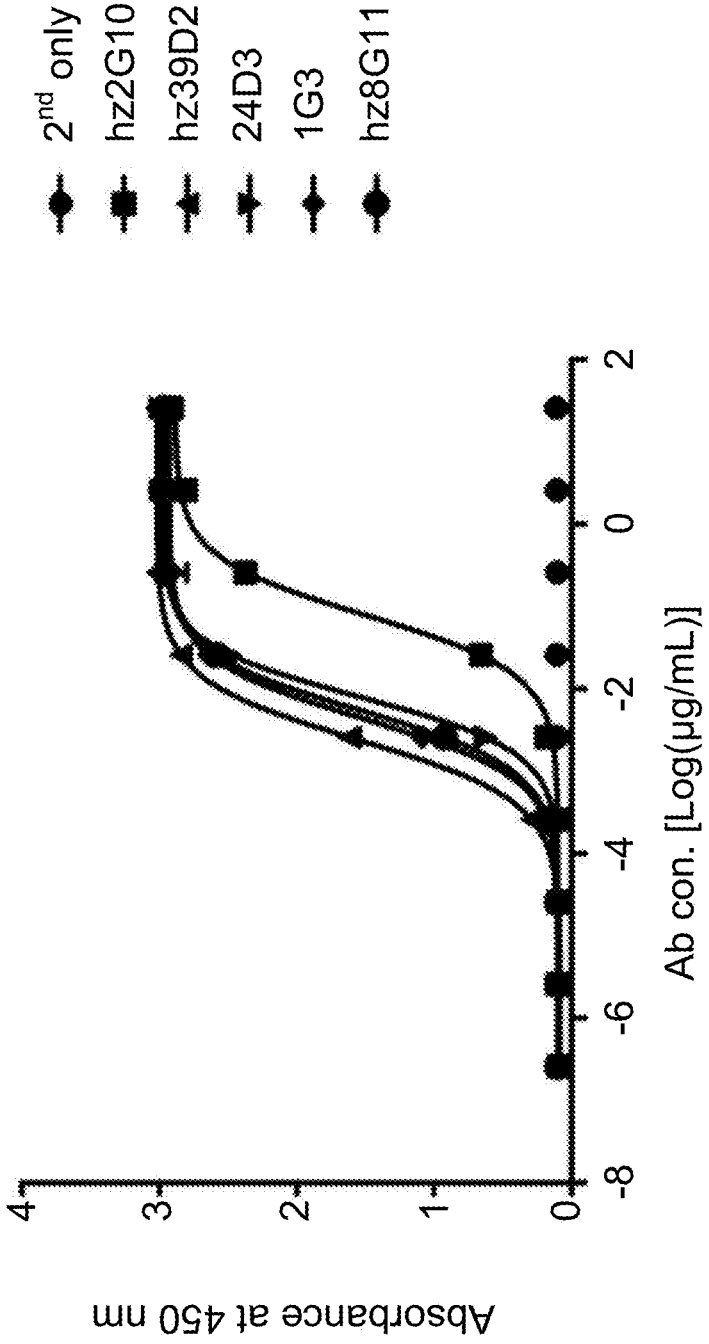

2nd only
hz2G10
hz39D2
24D3
1G3
hz8G11
Absorbance at 450 nm
0
1
2
3
4
-8
-6
-4
-2
0
2
Ab con. [Log(µg/mL)]

FIG. 1

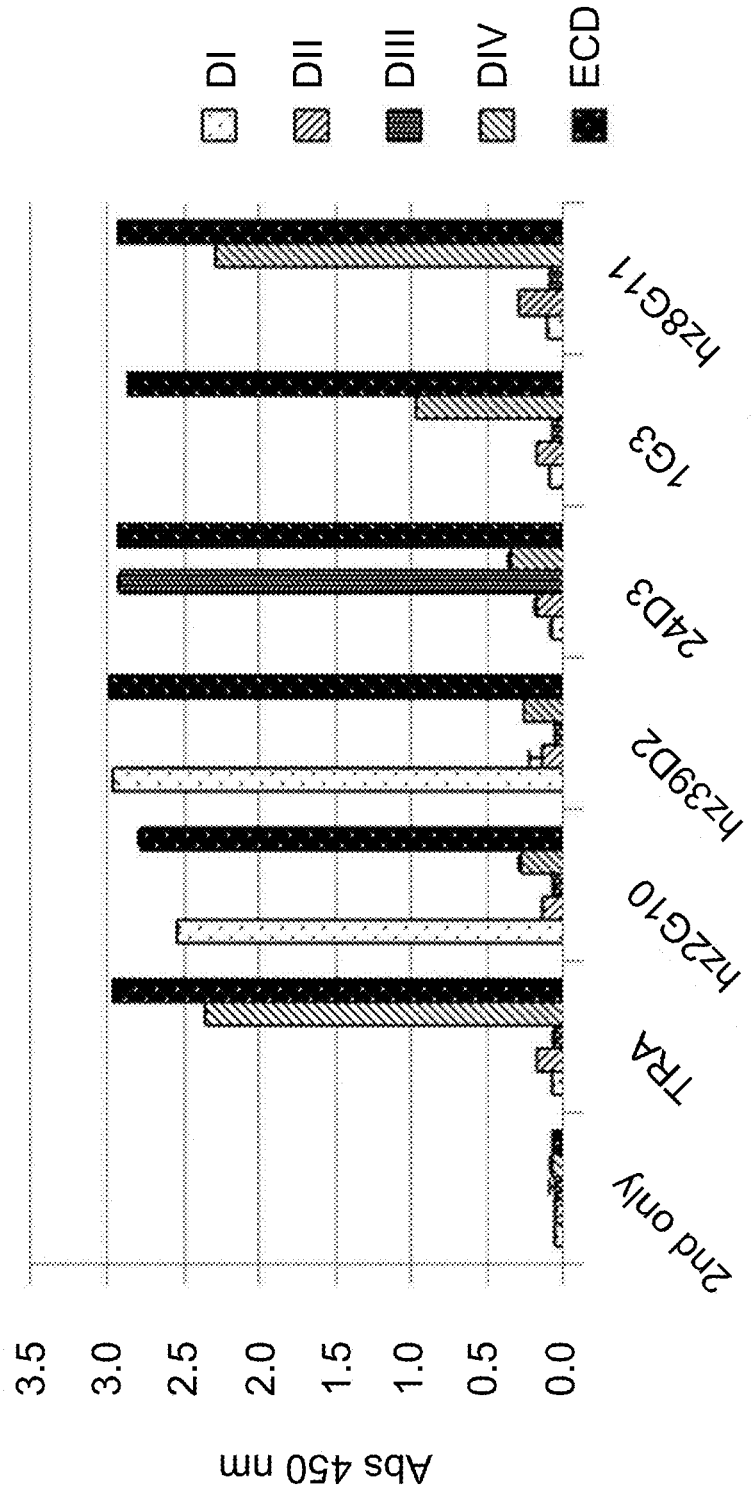

DI
DII
DIII
DIV
ECD
hz8G11
1G3
24D3
hz39D2
hz22G10
TRA
2nd only
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Abs 450 nm

FIG. 2

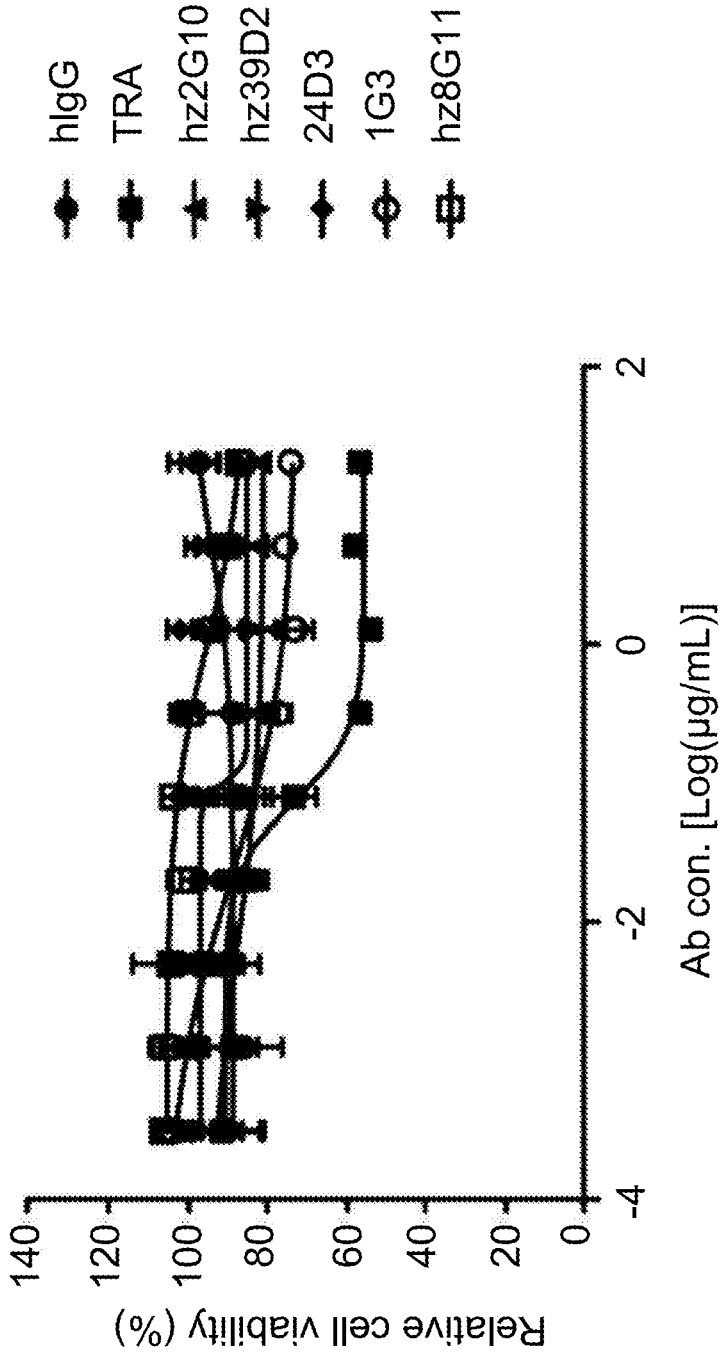

hIgG
TRA
hz2G10
hz39D2
24D3
1G3
hz8G11
Relative cell viability (%)
140
120
100
80
60
40
20
0
-4
-2
0
2
Ab con. [Log(μg/mL)]

FIG. 3A

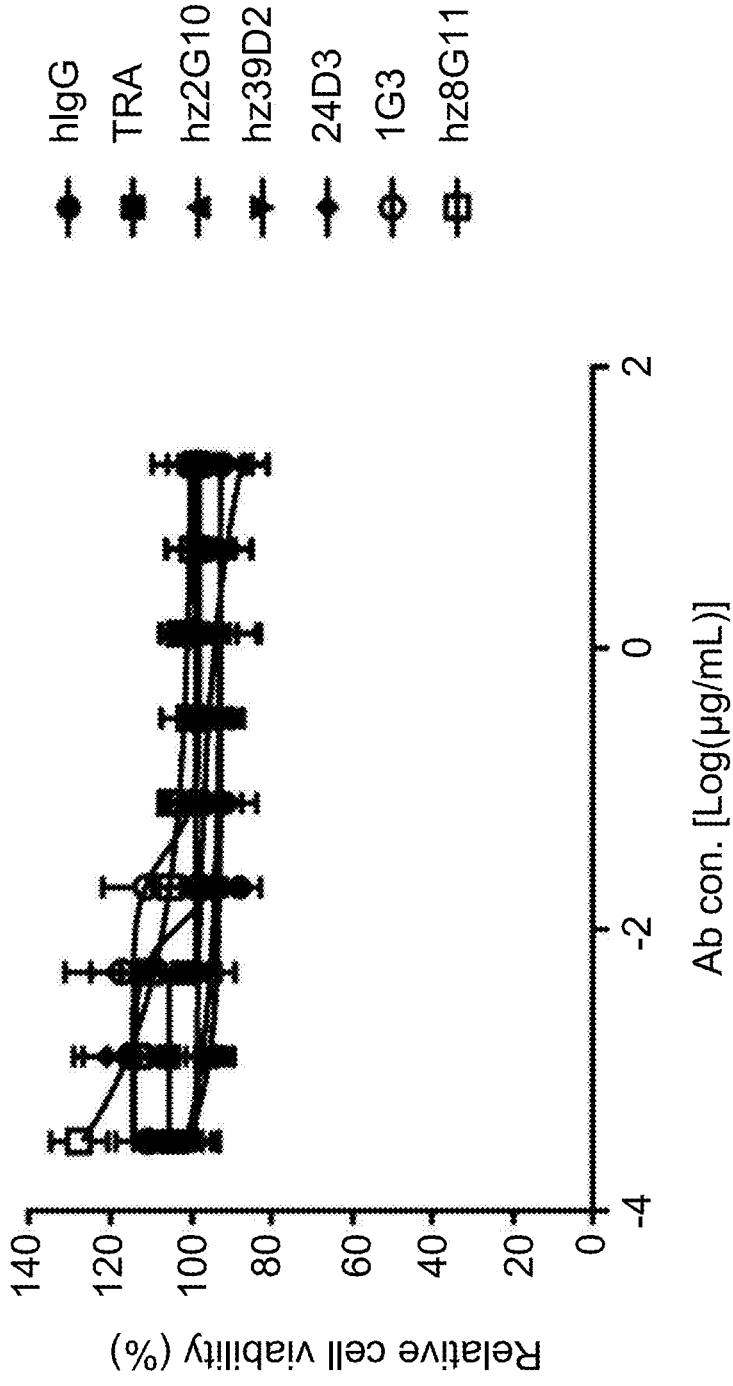

hIgG
TRA
hz2G10
hz39D2
24D3
1G3
hz8G11
Relative cell viability (%)
140
120
100
80
60
40
20
0
-4
-2
0
2
Ab con. [Log(μg/mL)]

FIG. 3B

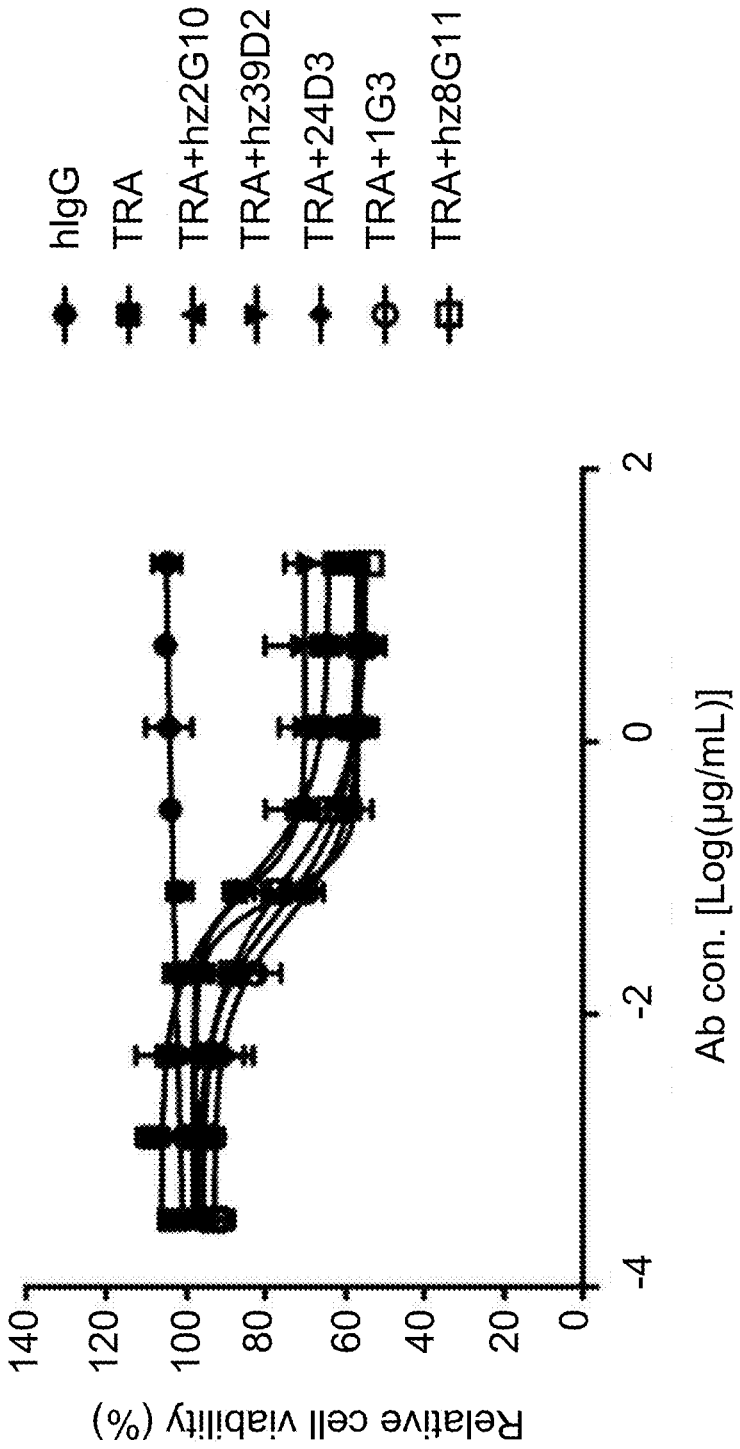

hIgG
TRA
TRA+hz2G10
TRA+hz39D2
TRA+24D3
TRA+1G3
TRA+hz8G11
Relative cell viability (%)
140
120
100
80
60
40
20
0
-4
-2
0
2
Ab con. [Log(μg/mL)]

FIG. 3C

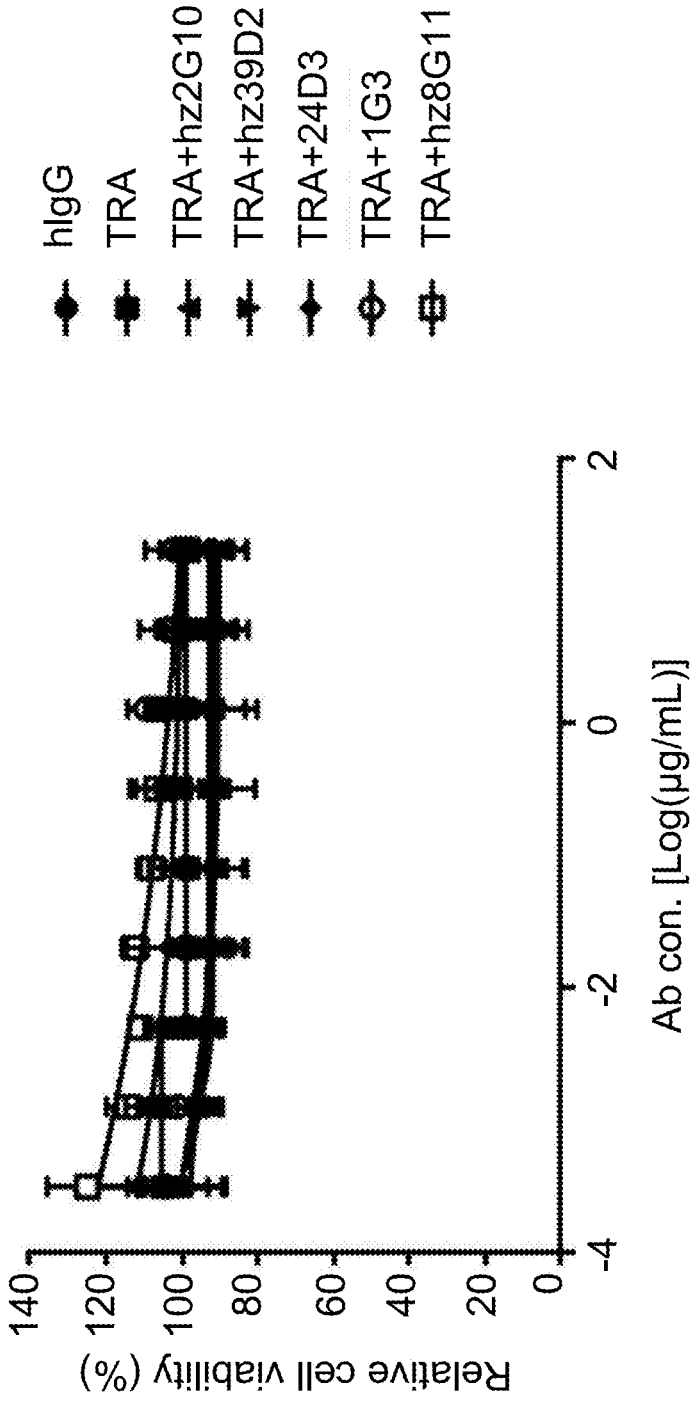

hIgG
TRA
TRA+hz2G10
TRA+hz39D2
TRA+24D3
TRA+1G3
TRA+hz8G11
Relative cell viability (%)
140
120
100
80
60
40
20
0
-4
-2
0
2
Ab con. [Log(μg/mL)]

FIG. 3D

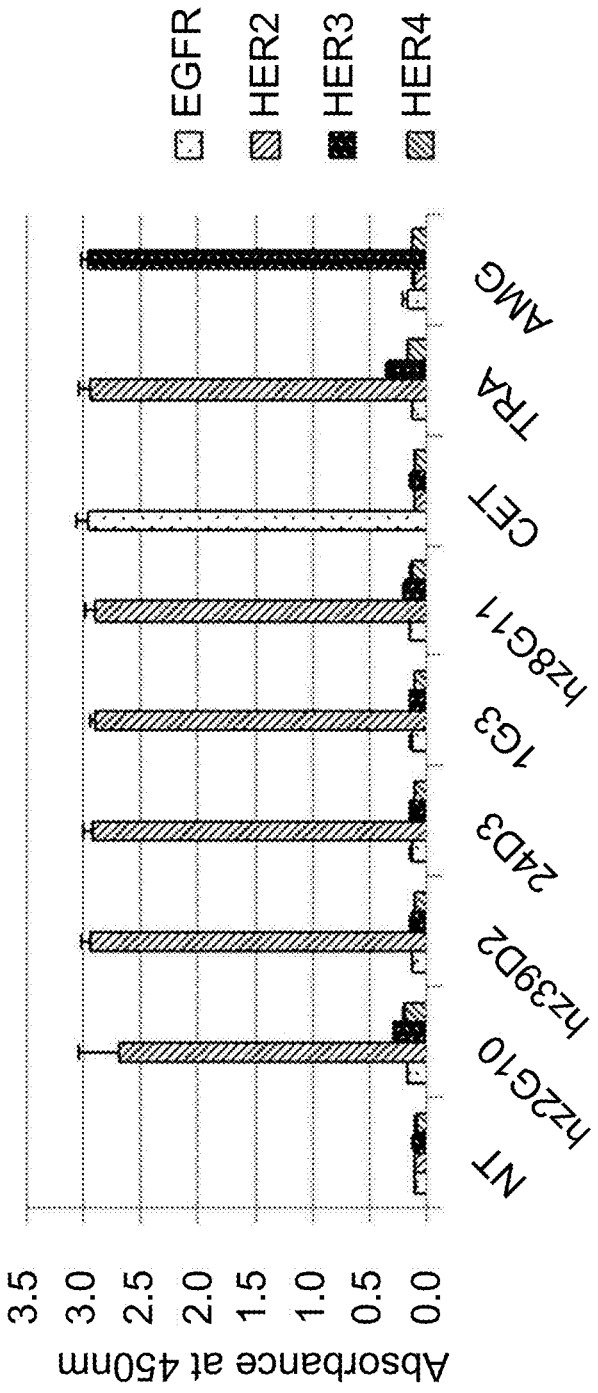

EGFR
HER2
HER3
HER4
Absorbance at 450nm
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
NT
hz22G10
hz39D2
24D3
1G3
hz8G11
CET
TRA
AMG

FIG. 4

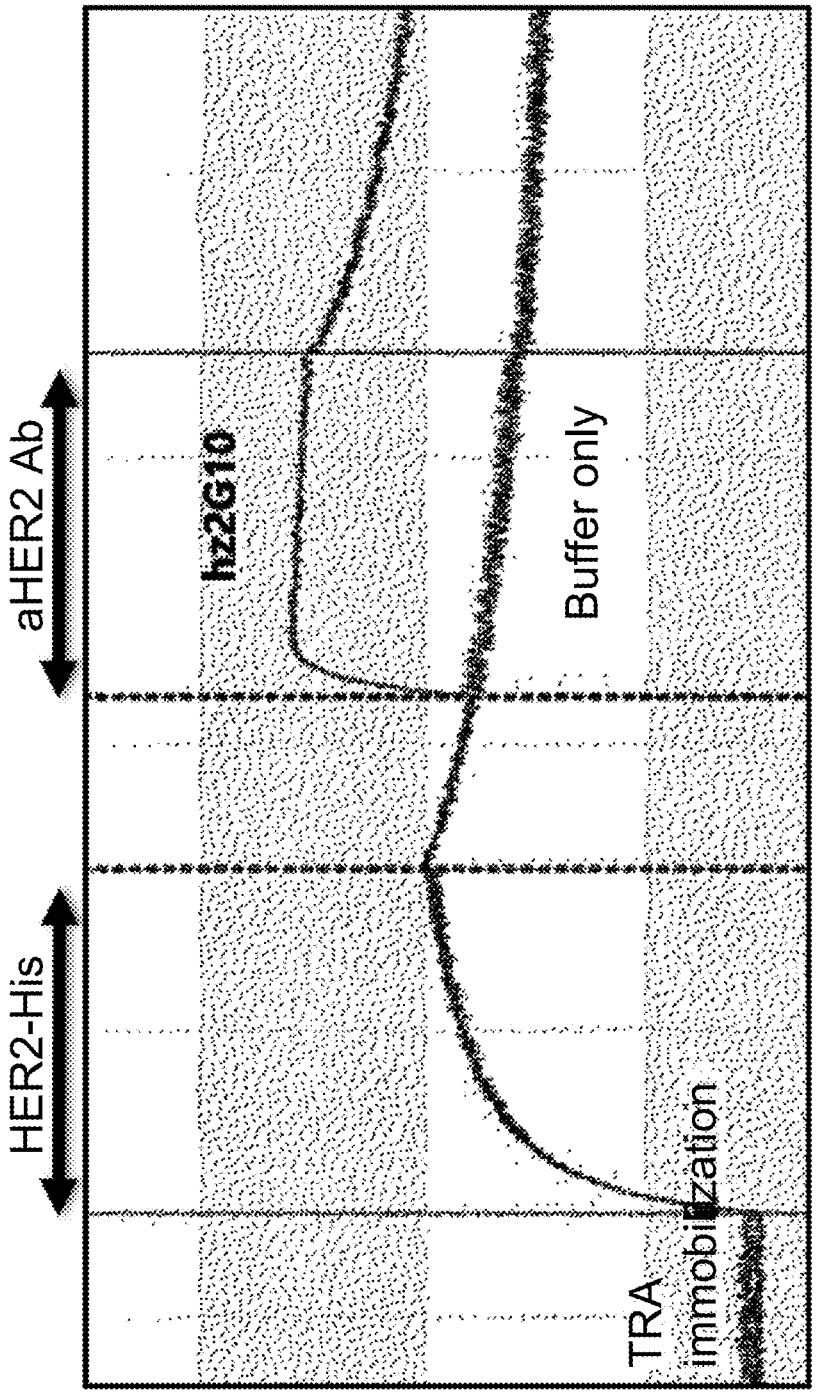


FIG. 5A

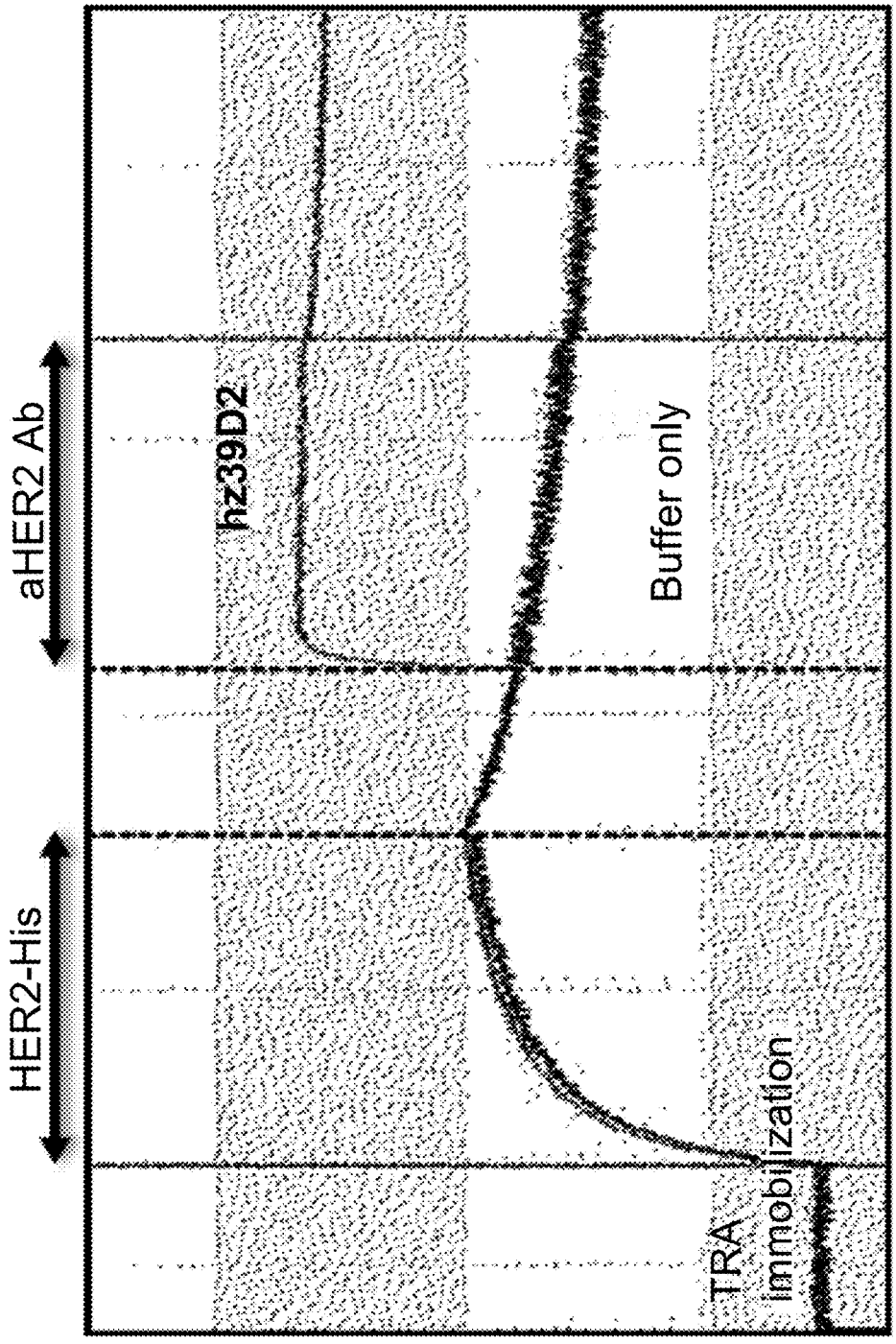


FIG. 5B

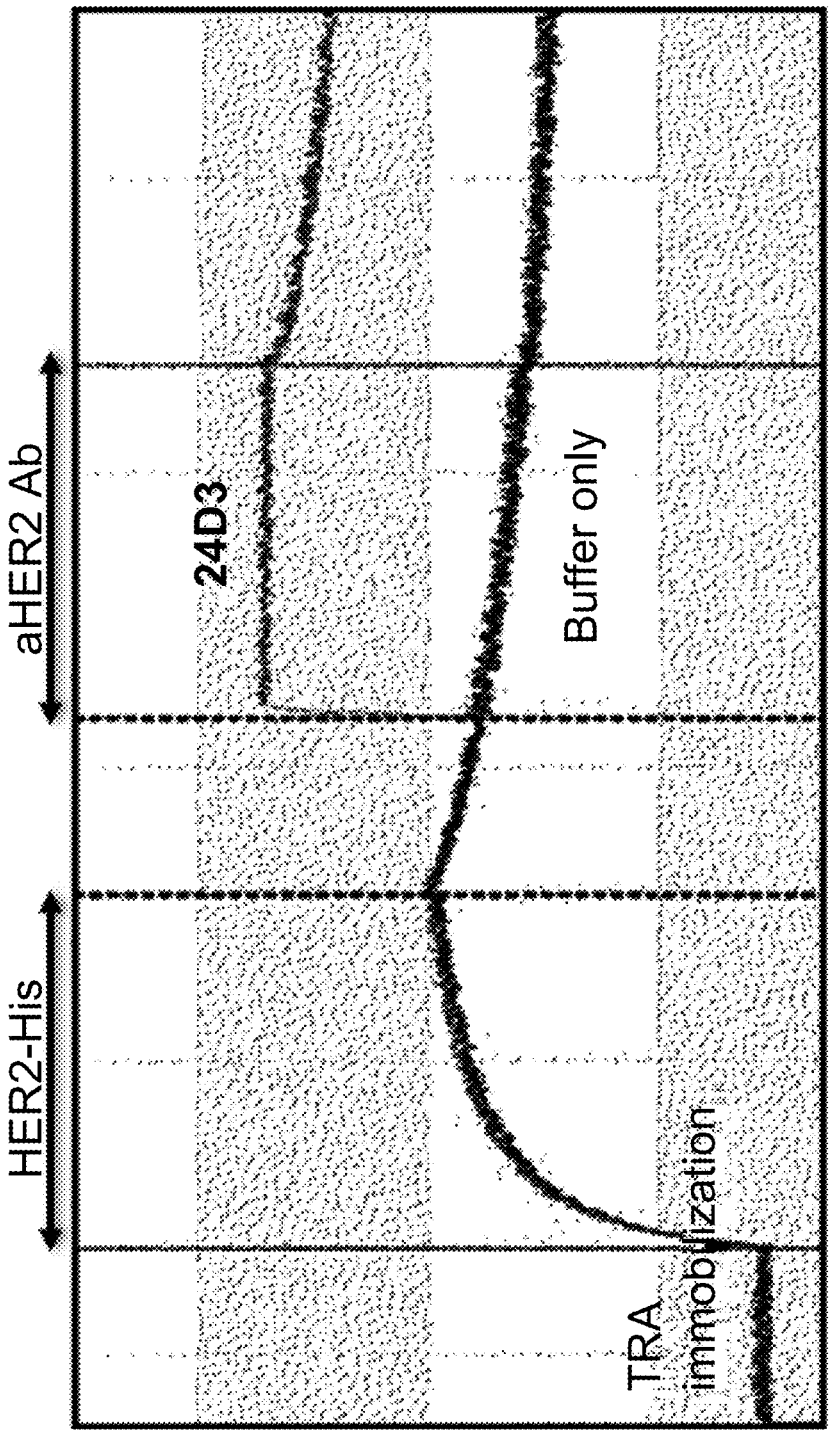


FIG. 5C

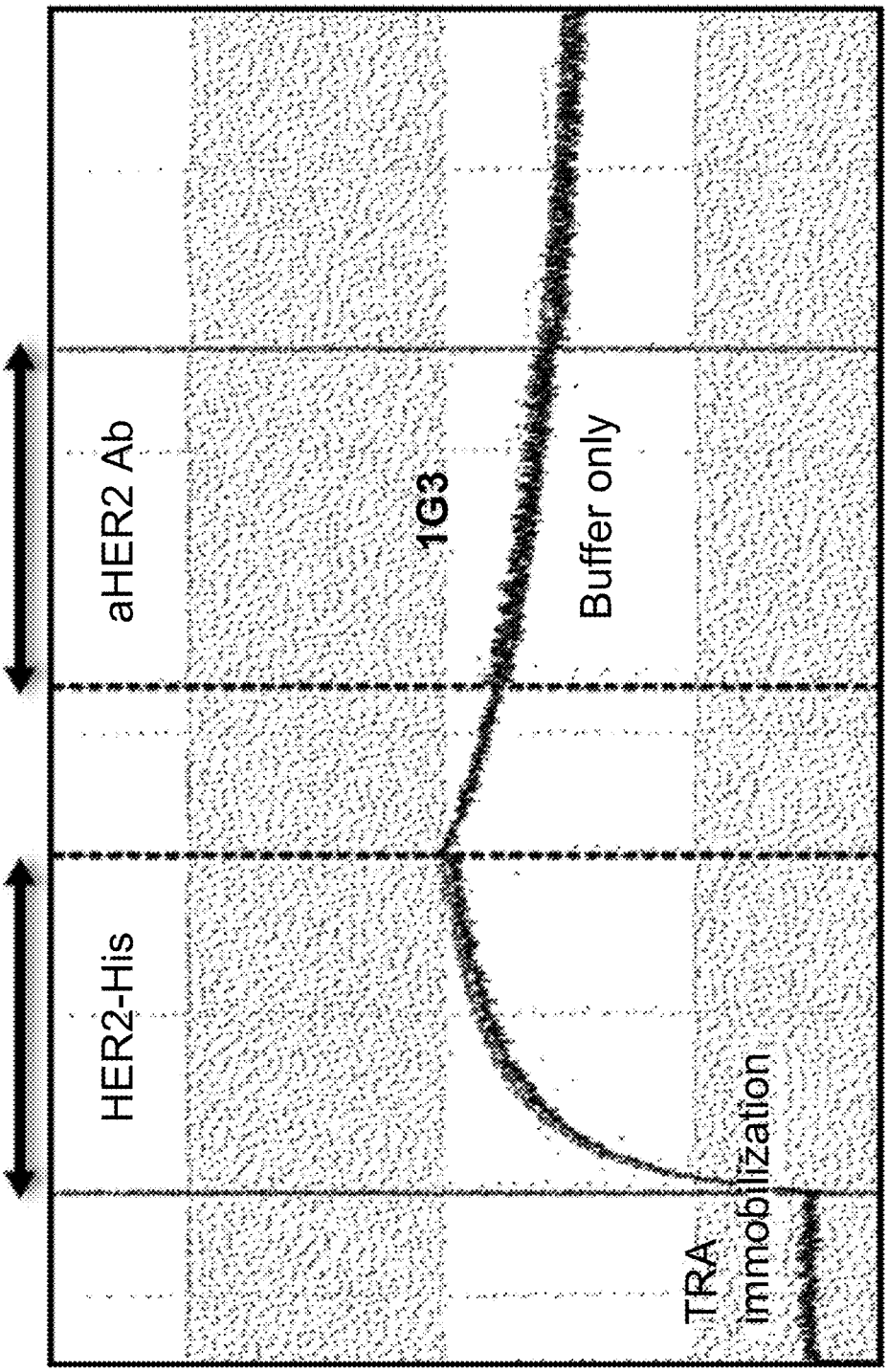


FIG. 5D

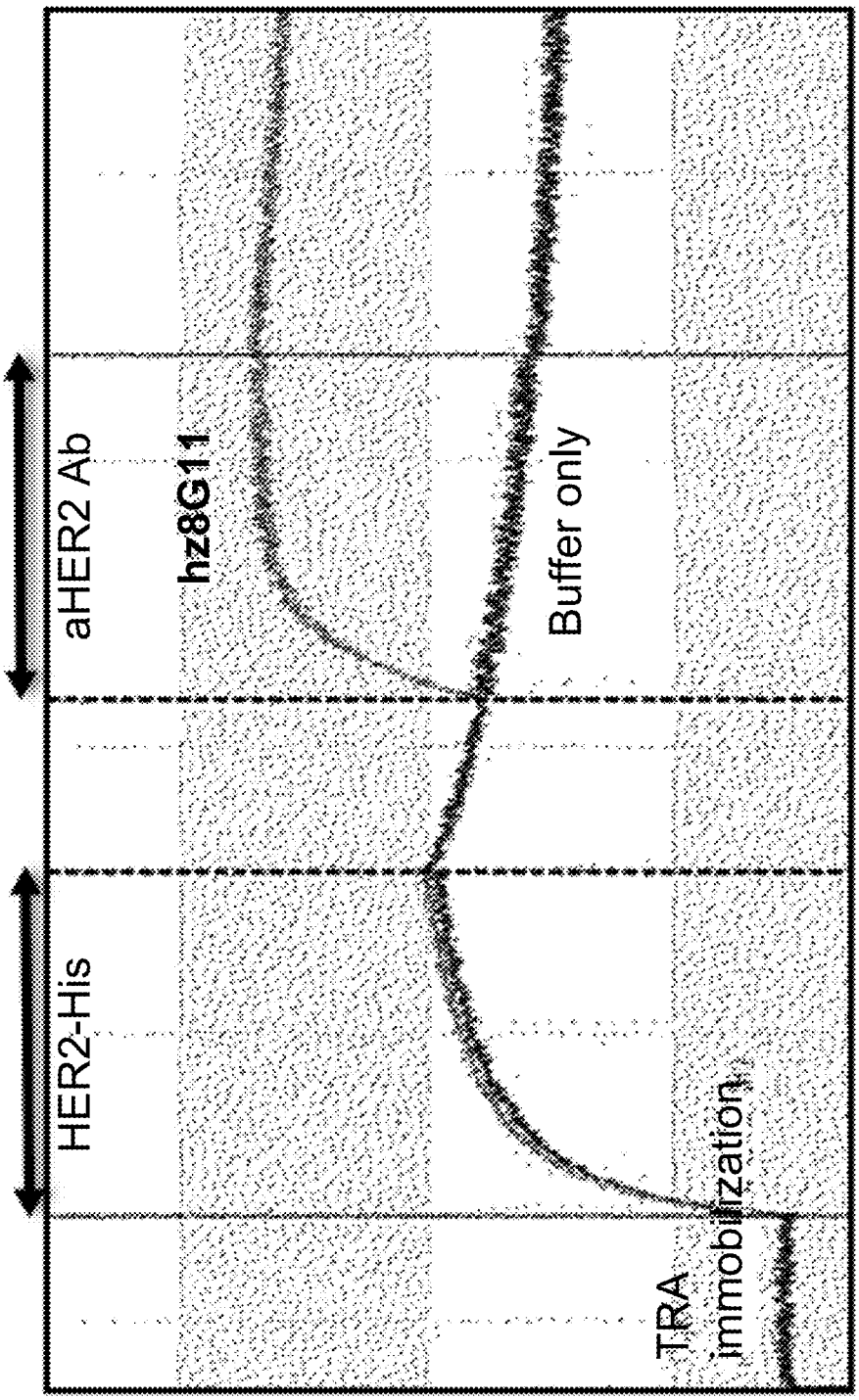


FIG. 5E

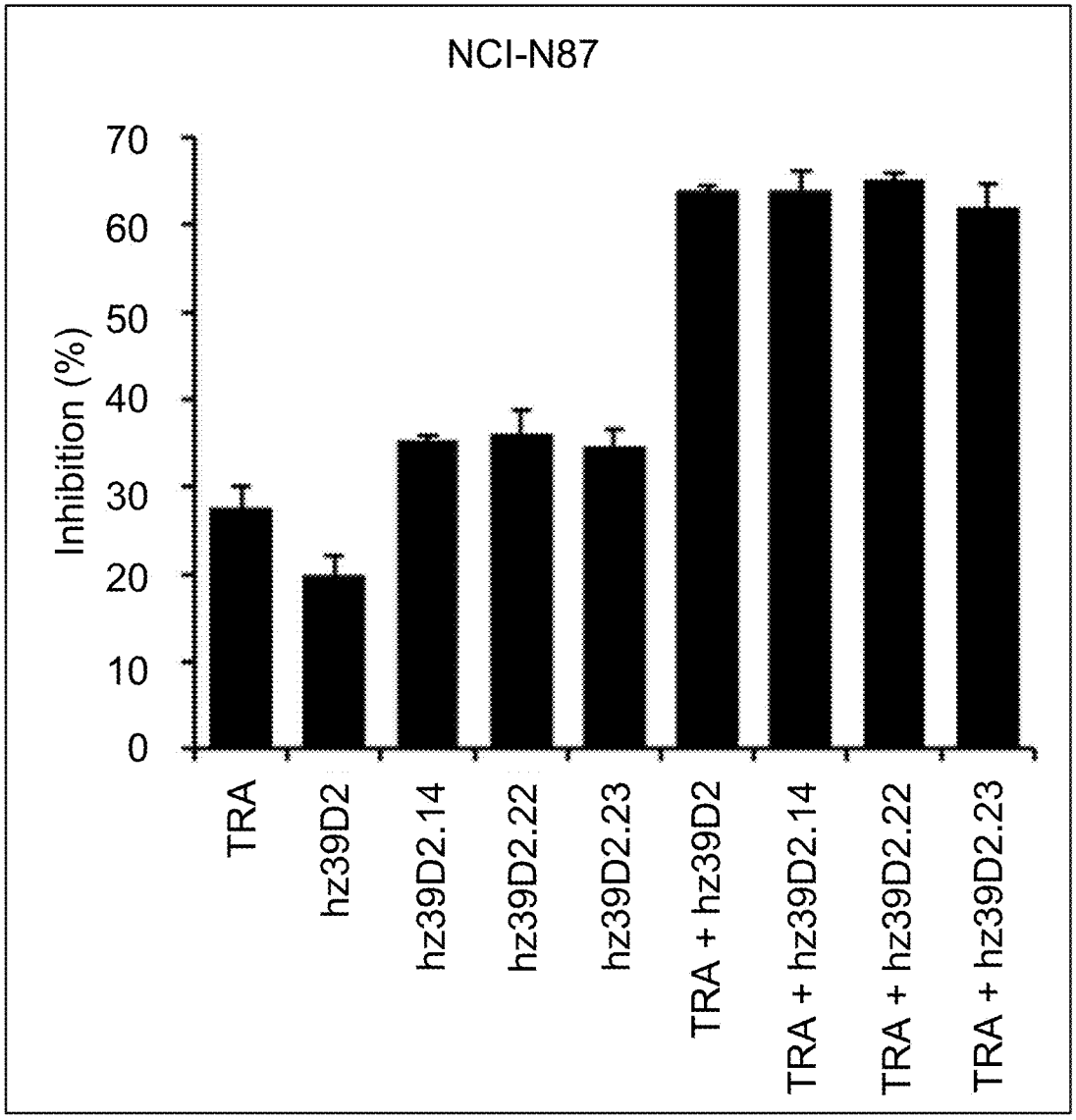

NCI-N87
Inhibition (%)
70
60
50
40
30
20
10
0
TRA
hz39D2
hz39D2.14
hz39D2.22
hz39D2.23
TRA + hz39D2
TRA + hz39D2.14
TRA + hz39D2.22
TRA + hz39D2.23

FIG. 6A

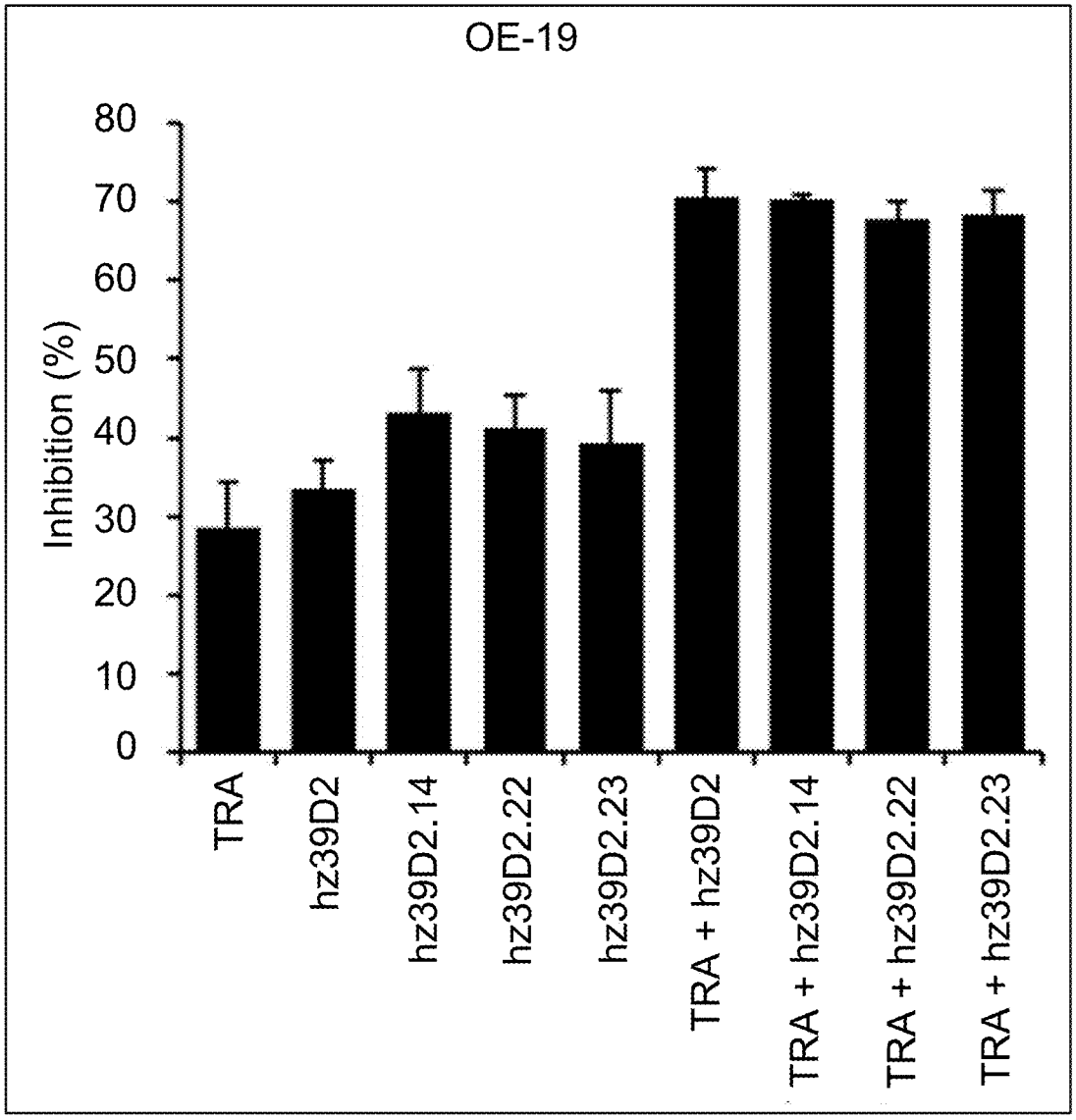

OE-19
Inhibition (%)
80
70
60
50
40
30
20
10
0
TRA
hz39D2
hz39D2.14
hz39D2.22
hz39D2.23
TRA + hz39D2
TRA + hz39D2.14
TRA + hz39D2.22
TRA + hz39D2.23

FIG. 6B

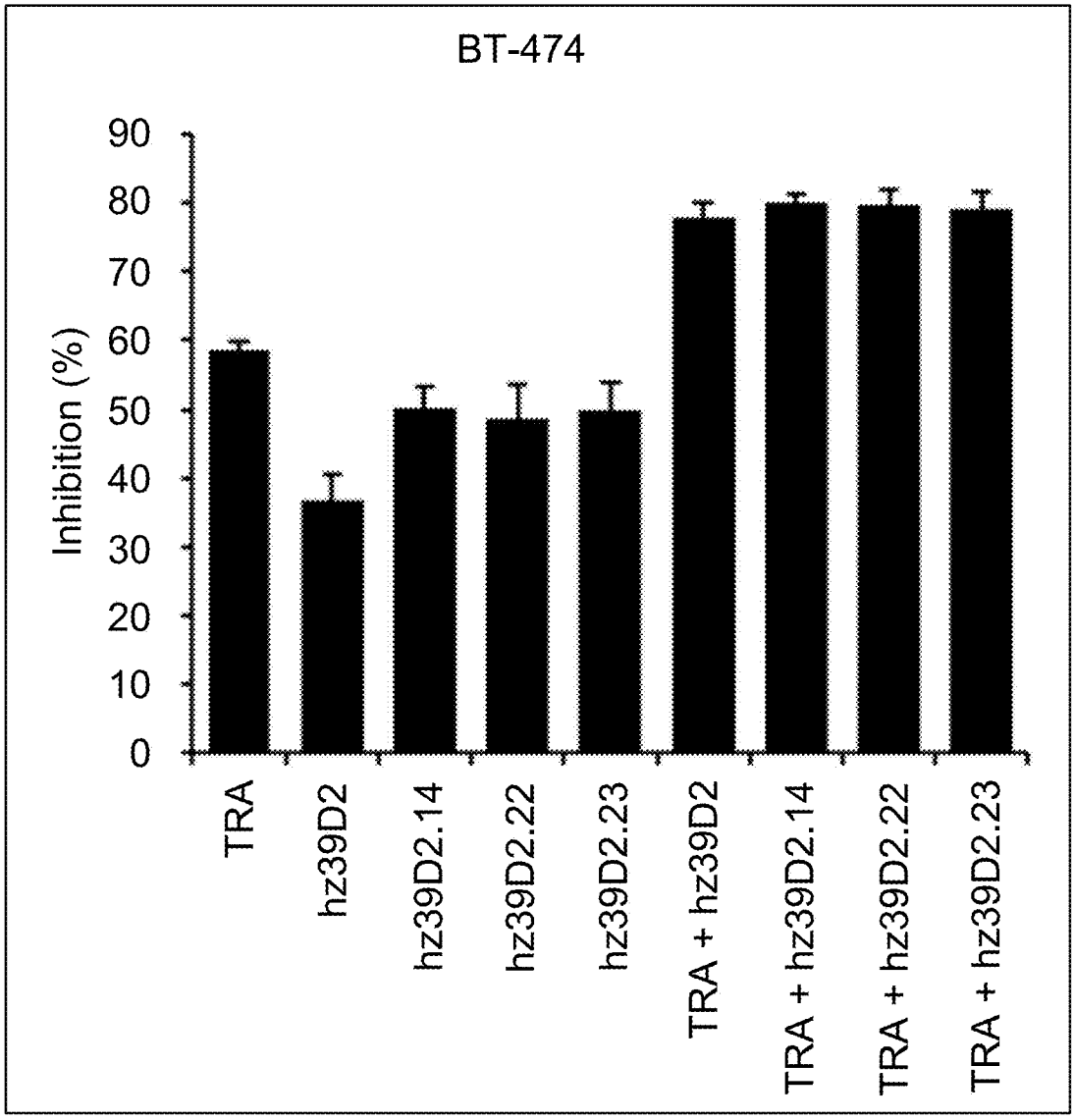

BT-474
Inhibition (%)
90
80
70
60
50
40
30
20
10
0
TRA
hz39D2
hz39D2.14
hz39D2.22
hz39D2.23
TRA + hz39D2
TRA + hz39D2.14
TRA + hz39D2.22
TRA + hz39D2.23

FIG. 6C

Clone #	Signal Peptide	Tumor Targeting Domain	Hinge	Transmembrane Domain	Stimulatory Signal 1	Stimulatory Signal 2	Stimulatory Signal 3
2	CD8a	hz39D2	CD8a	CD8a	CD3z		
3	CD8a	hz39D2	CD8a	CD8a	4-1BB	CD3z	
6	CD8a	hz39D2	CD8a	CD8a	CD28	CD3z	
14	CD8a	hz39D2	CD8a	CD28	CD28	OX40L	CD3z

FIG. 7

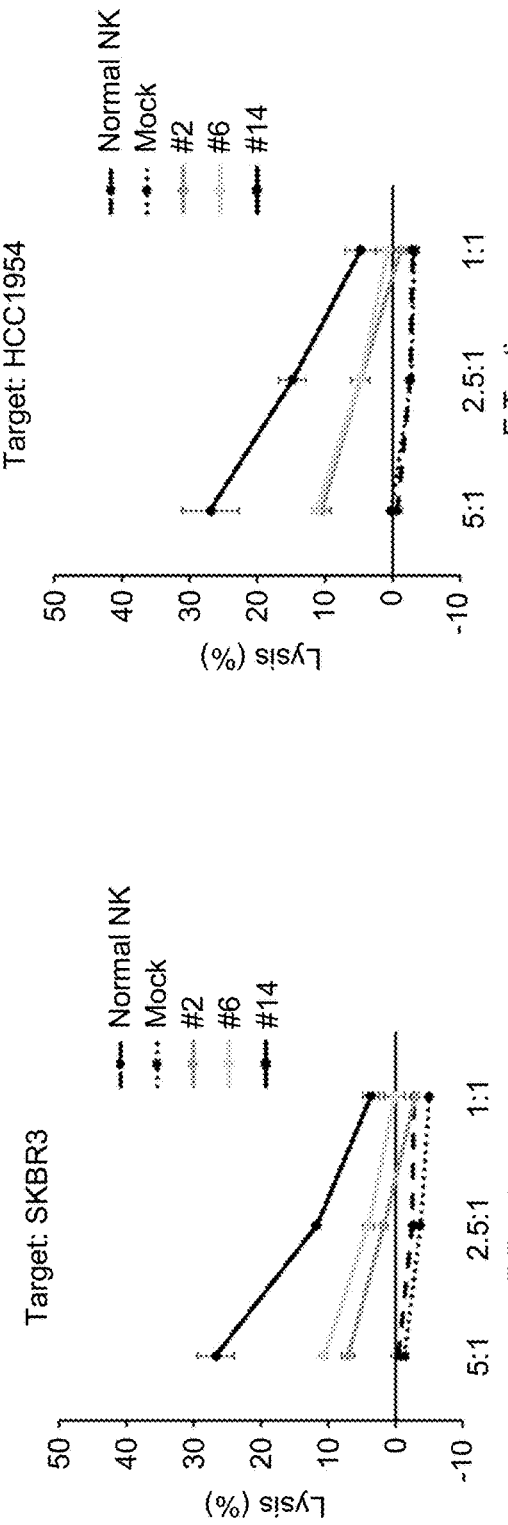


FIG. 8A

FIG. 8B

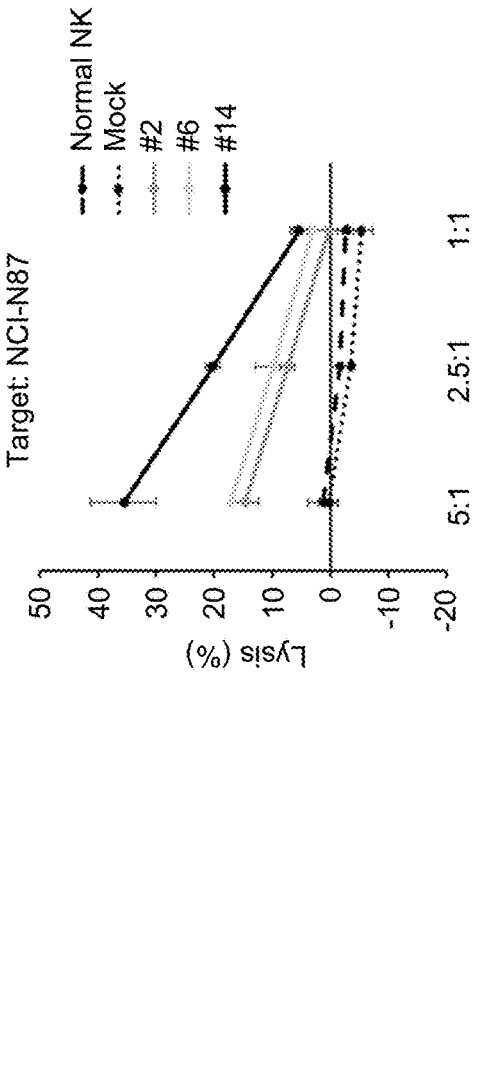


FIG. 8C

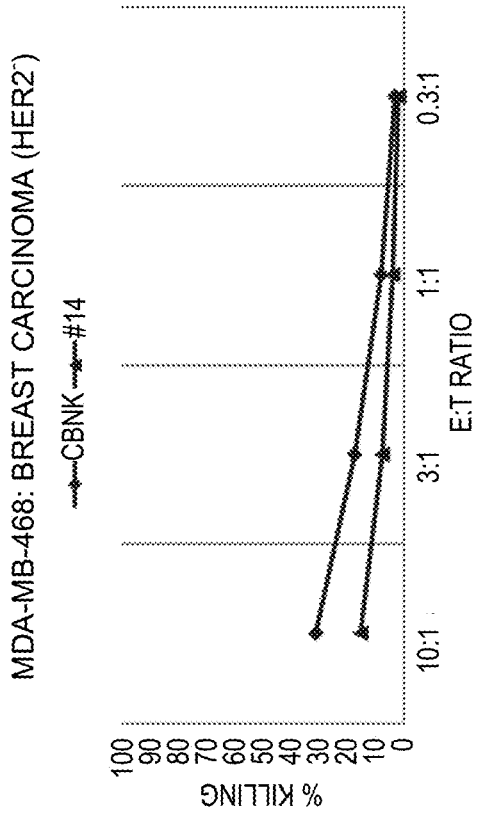

MDA-MB-468: BREAST CARCINOMA (HER2-)
CBNK
#14
% KILLING
100
90
80
70
60
50
40
30
20
10
0
10:1
3:1
1:1
0.3:1
E:T RATIO

FIG. 9B

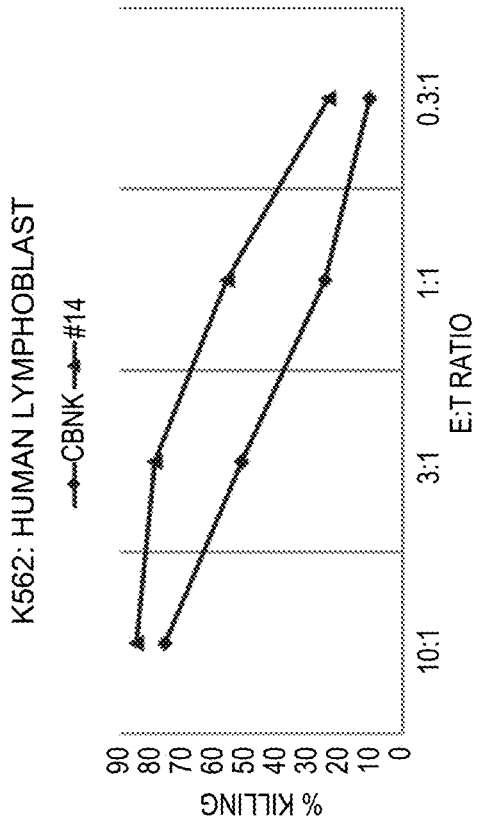

K562: HUMAN LYMPHOBLAST
CBNK
#14
% KILLING
90
80
70
60
50
40
30
20
10
0
10:1
3:1
1:1
0.3:1
E:T RATIO

FIG. 9A

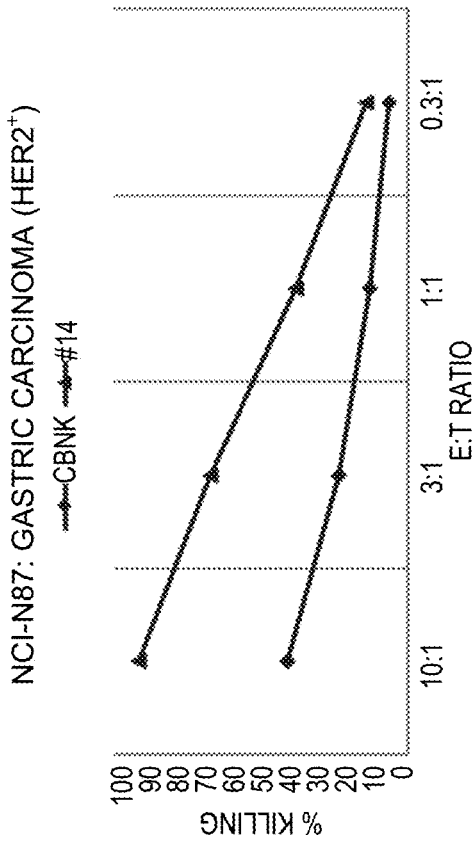


FIG. 9D

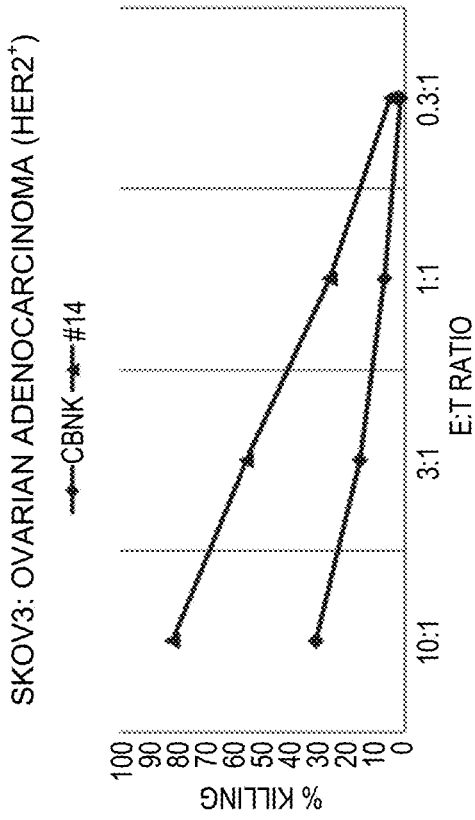


FIG. 9C

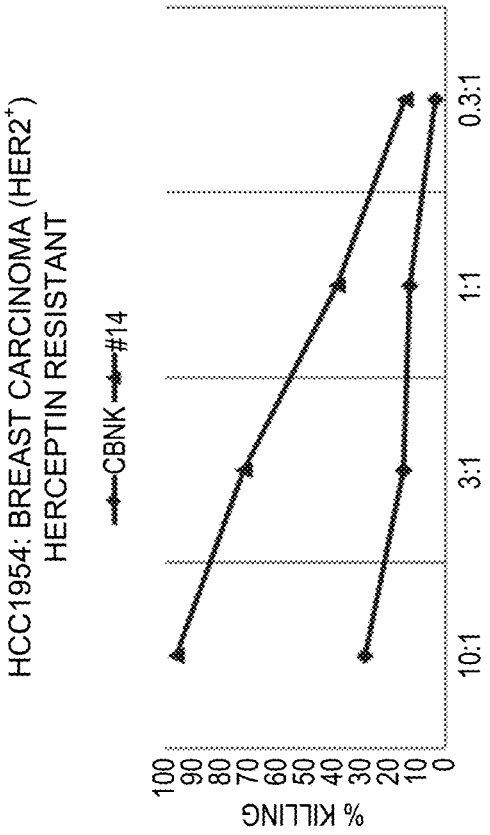


FIG. 9E

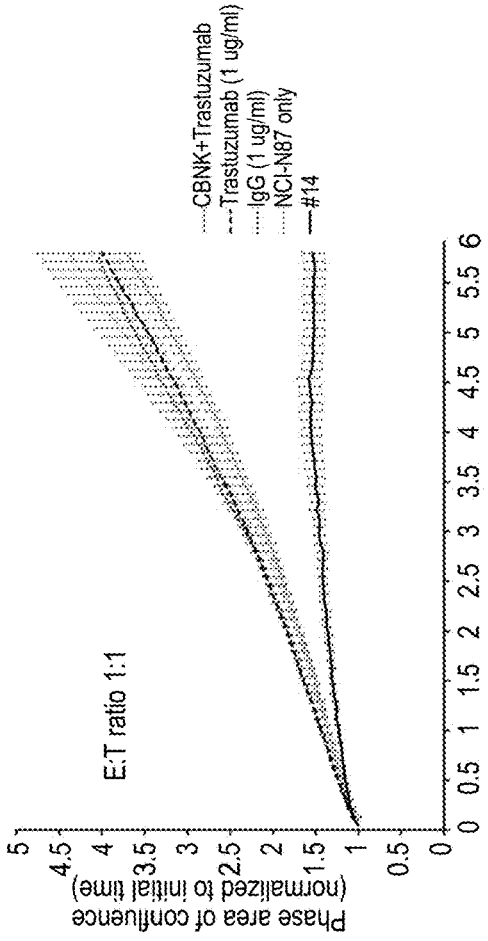


FIG. 10A

FIG. 10B

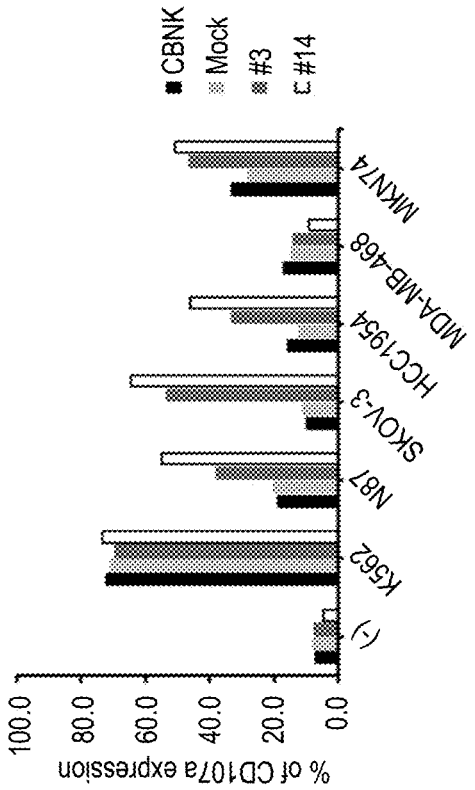


FIG. 11B

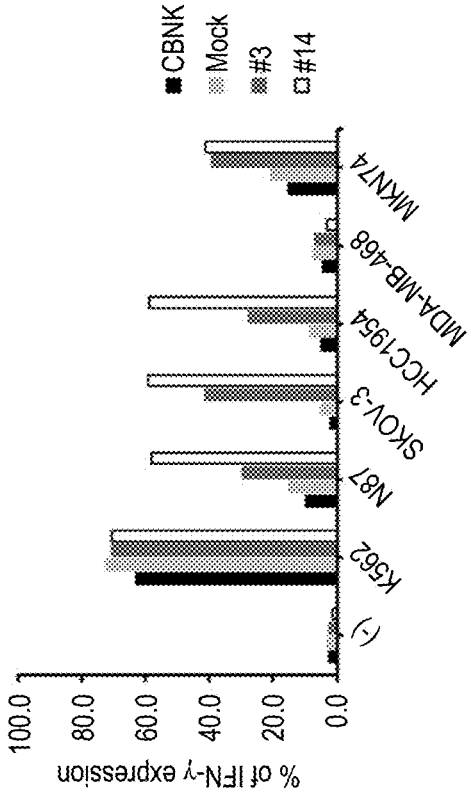


FIG. 11A

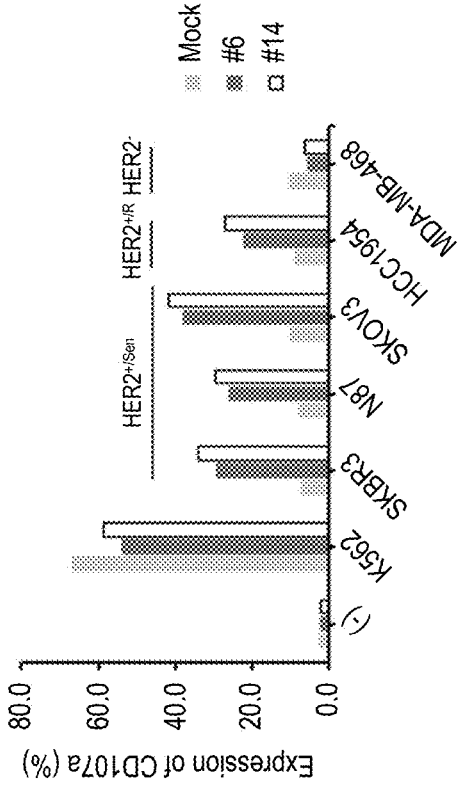


FIG. 12B

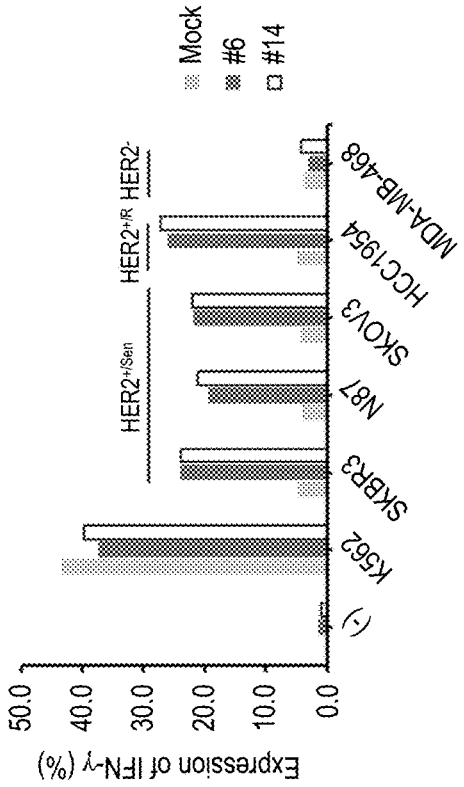


FIG. 12A

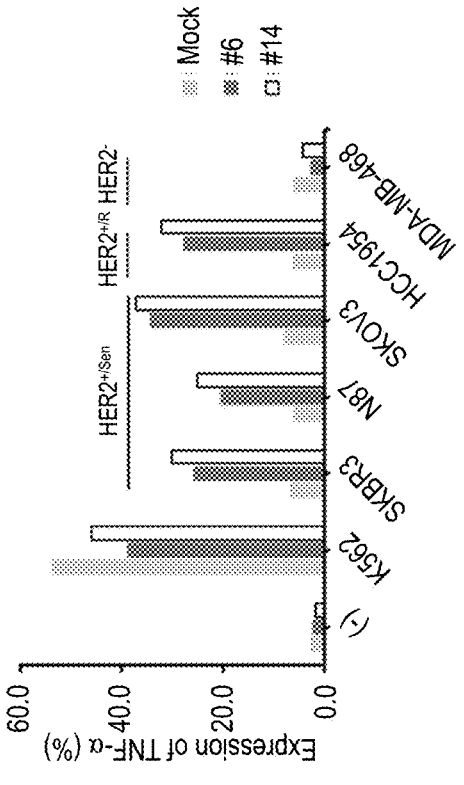


FIG. 12C

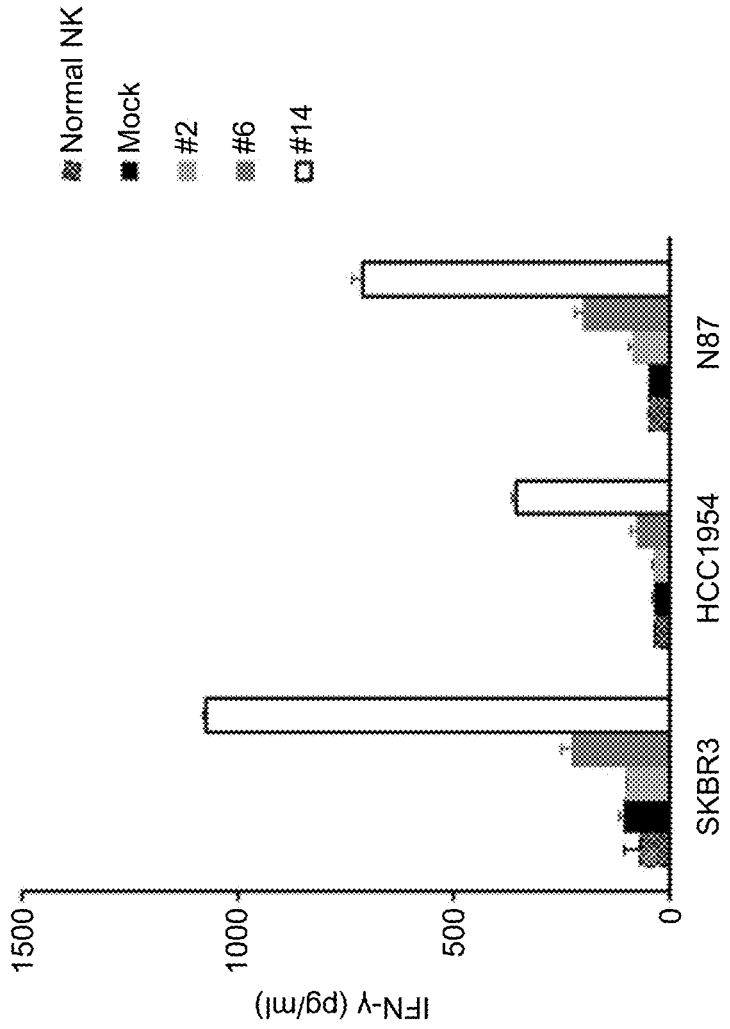

Normal NK
Mock
#2
#6
#14
IFN-γ (pg/ml)
1500
1000
500
0
SKBR3
HCC1954
N87

FIG. 13

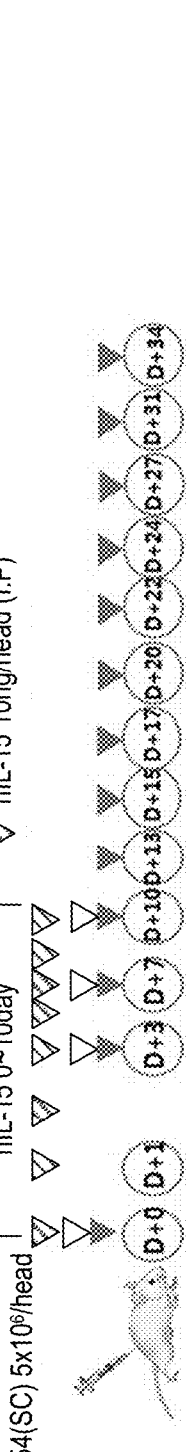


FIG. 14A

FIG. 14B

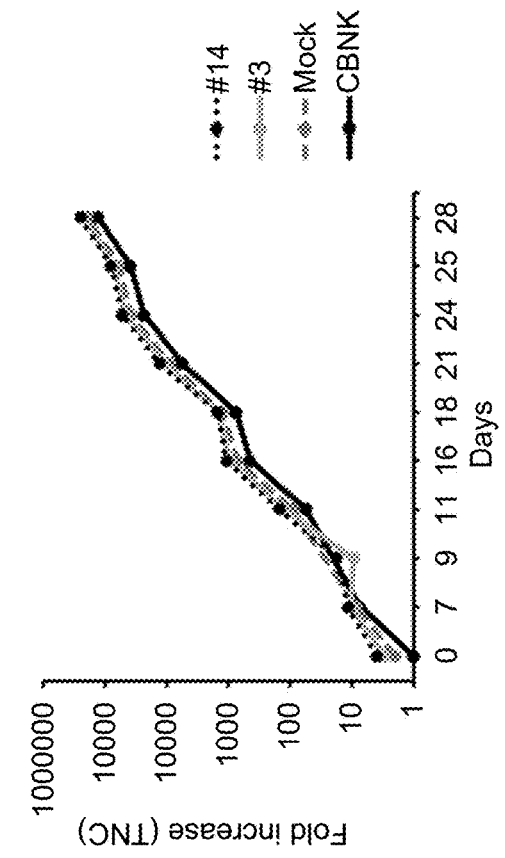


FIG. 15A

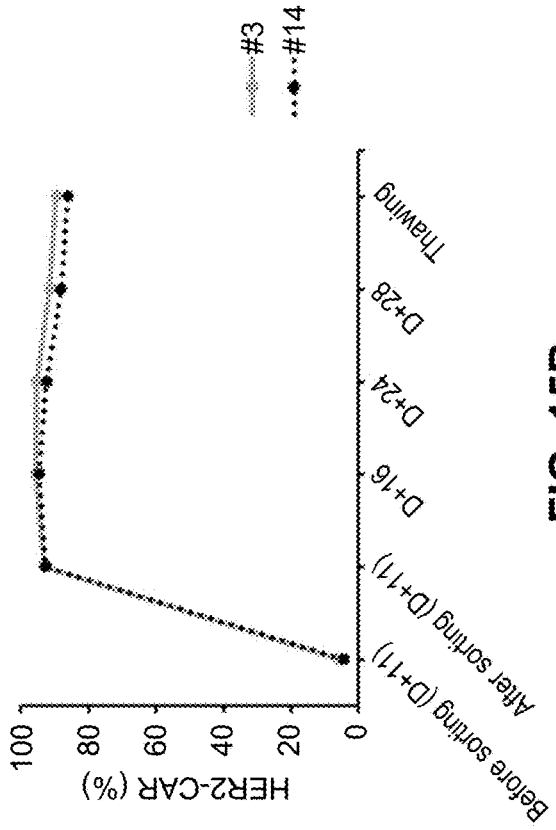


FIG. 15B

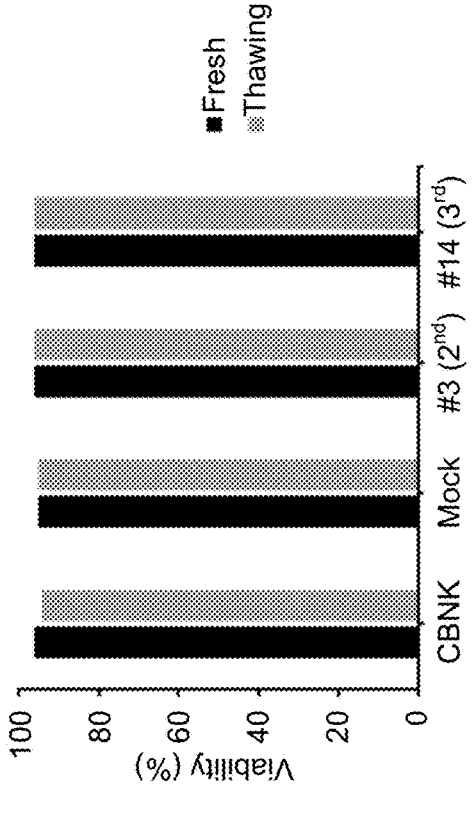


FIG. 15C

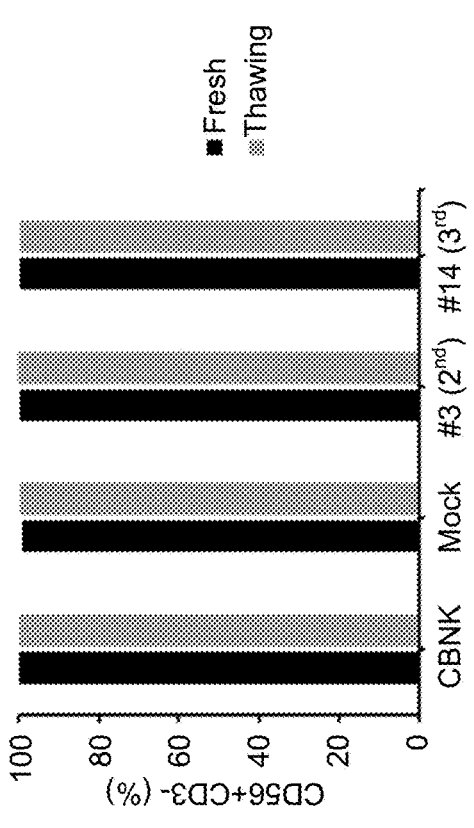


FIG. 15D

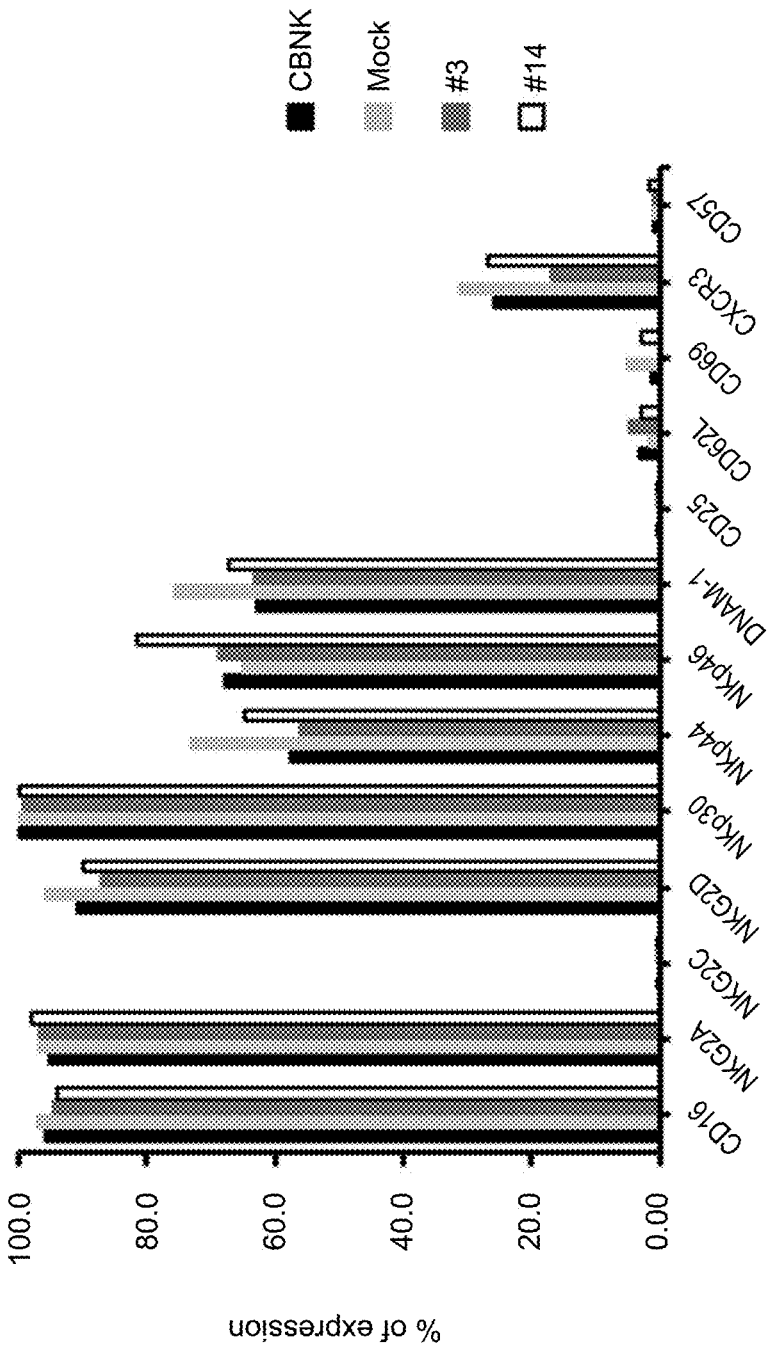

CBNK
Mock
#3
#14
CD57
CXCR3
CD69
CD62L
CD25
DNAM-1
NKp46
NKp44
NKp30
NKG2D
NKG2C
NKG2A
CD16
100.0
80.0
60.0
40.0
20.0
0.00
% of expression

FIG. 16

ANTI-HER2 ANTIBODY OR ANTIGEN-BINDING FRAGMENT THEREOF, AND CHIMERIC ANTIGEN RECEPTOR COMPRISING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of and claims the benefit of priority to U.S. application Ser. No. 16/881,650, filed on May 22, 2020, which is a continuation-in-part application of and claims the benefit of priority to U.S. application Ser. No. 16/764,276, filed on May 14, 2020, which is a National Stage of International Application No. PCT/KR2018/013928, filed on Nov. 14, 2018, claiming priority based on Korean Patent Application No. 10-2017-0151841, filed Nov. 14, 2017, the contents of which are hereby incorporated by reference.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] This application contains a Sequence Listing that has been submitted electronically as an XML file named 49755-0034002_SL_ST26.xml. The XML file, created on Apr. 6, 2023, is 214,314 bytes in size. The material in the XML file is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

[0003] The research was conducted under the support of the Ministry of Trade, Industry and Energy of Korea with the project number 1415118385. The R&D management agency of the project is the Korea Institute for Advancement of Technology, the R&D project title is "Global innovation technology alliance", and the research title is "Development of global antibody drug based on novel epitope screening platform technology". The research was conducted by AbClon Inc. from Nov. 1, 2011 until Oct. 31, 2014.

[0004] This application claims the priority of Korean Patent Application No. 10-2017-0151841 filed on Nov. 14, 2017 in the Korean Intellectual Property Office, the disclosure of which is incorporated herein by reference in its entirety.

[0005] The present disclosure relates to a novel anti-HER2 antibody or an antigen-binding fragment thereof, a chimeric antigen receptor including the same, and uses thereof.

BACKGROUND ART

[0006] The Her2/neu (ErbB2) gene encodes a 185-kDa transmembrane glycoprotein that belongs to the family of epidermal growth factor receptors (EGFRs). The Her2 protein is composed of an extracellular domain consisting of 620 amino acid residues, a transmembrane domain 23 amino acid residues, and an intracellular domain with tyrosine kinase activity, consisting of 490 amino acid residues (Akiyama T, et al., *Science,* 232 (4758): 1644-1646 (1986)).

[0007] Anti-HER2 antibodies with various characteristics have been described: Tagliabue et al., *Int. J. Cancer* 47: 933-937 (1991); McKenzie et al., *Oncogene* 4: 543-548 (1989); Maier et al., *Cancer Res.* 51: 5361-5369 (1991); Bacus et al., *Molecular Carcinogenesis* 3: 350-362 (1990); Stancovski et al., *PNAS USA* 88: 8691-8695 (1991); Bacus et al., *Cancer Research* 52: 2580-2589 (1992); Xu et al., *Int. J. Cancer* 53: 401-408 (1993); WO94/00136; Kasprzyk et al., *Cancer Research* 52: 2771-2776 (1992); Hancock et al., *Cancer Res.* 51: 4575-4580 (1991); Shawver et al., *Cancer Res.* 54: 1367-1373 (1994); Arteaga et al., *Cancer Res.* 54: 3758-3765 (1994); Harwerth et al., *J. Biol. Chem.* 267: 15160-15167 (1992); U.S. Pat. No. 5,783,186; Kao et al., US Patent Application Publication No. 2009/0285837 (2009); Ross et al., *The Oncologist* 8: 307-325 (2003); and Klapper et al., *Oncogene* 14: 2099-2109 (1997).

[0008] The most commercially successful anti-HER2 antibody is trastuzumab antibody (commercially available as Herceptin™, U.S. Pat. No. 5,821,337) and many researches have been conducted thereon: Sapino, A., et al., *Annals of Oncology* (2007) 18: 1963-1968; Bussolati, G, et al., *British Journal of Cancer* (2005) 92, 1261-1267; and Glazyrin A, et al., *J Histology & Cytochemistry* (2007) 55 (1): 25-33.

[0009] Although the trastuzumab antibody has been commercially successful, use of the trastuzumab antibody for therapeutic purposes is limited because there are various cancer cells which have non-reactivity (or resistance) to the antibody or have reduced sensitivity. Accordingly, there have been attempts to resolve the therapeutic problem of the antibody.

[0010] For example, U.S. Pat. No. 7,674,460 discloses a method for increasing the HER2 sensitivity of cancer cells using an HER2 antagonist such as the trastuzumab antibody and a PC cell-derived growth factor (PCDGF) antagonist. WO 2011/127297 discloses a method for inhibiting the proliferation of trastuzumab-resistant tumor cells using a combination of a FoxM1 inhibitor and the trastuzumab antibody.

[0011] US Patent Application Publication No. 2010-0183604 discloses a method for treating trastuzumab-resistant cancer using a cofilin inhibitor, a PAK1 inhibitor, a LIMK inhibitor, an RHO inhibitor, a ROCK1 inhibitor or a ROCK2 inhibitor.

DISCLOSURE

Technical Problem

[0012] The inventors of the present disclosure have made efforts to develop a novel antibody which is capable of preventing or treating cancer (particularly, breast cancer and gastric cancer), exhibits better killing ability (or proliferation-inhibiting ability) for cancer cells which have non-reactivity (or resistance) to the trastuzumab antibody or have reduced sensitivity, and is capable of preventing or treating cancer with improved anticancer activity when co-administered with the trastuzumab antibody as compared to single administration of trastuzumab. As a result, they have developed a novel antibody which exhibits better killing ability for HER2-overexpressed cancer cells on which the trastuzumab antibody hardly acts, or exhibits improved anticancer activity when co-administered with the trastuzumab antibody, and have completed the present disclosure.

SUMMARY OF THE INVENTION

[0013] The present disclosure provides an antibody or an antigen binding fragment thereof against HER2 (human epidermal growth factor receptor 2) comprising any one of: (a) a heavy chain variable region comprising a CDRH1 of SEQ ID NO 1, a CDRH2 of SEQ ID NO 2 and a CDRH3 of SEQ ID NO 3, and a light chain variable region comprising a CDRL1 of SEQ ID NO 4, a CDRL2 of SEQ ID NO

5 and a CDRL3 of SEQ ID NO 6; (b) a heavy chain variable region comprising a CDRH1 of SEQ ID NO 7, a CDRH2 of SEQ ID NO 8 and a CDRH3 of SEQ ID NO 9, 71 or 72, and a light chain variable region comprising a CDRL1 of SEQ ID NO 10, a CDRL2 of SEQ ID NO 11 and a CDRL3 of SEQ ID NO 12, 73 or 74; (c) a heavy chain variable region comprising a CDRH1 of SEQ ID NO 13, a CDRH2 of SEQ ID NO 14 and a CDRH3 of SEQ ID NO 15, and a light chain variable region comprising a CDRL1 of SEQ ID NO 16, a CDRL2 of SEQ ID NO 17 and a CDRL3 of SEQ ID NO 18; (d) a heavy chain variable region comprising a CDRH1 of SEQ ID NO 19, a CDRH2 of SEQ ID NO 20 and a CDRH3 of SEQ ID NO 21, and a light chain variable region comprising a CDRL1 of SEQ ID NO 22, a CDRL2 of SEQ ID NO 23 and a CDRL3 of SEQ ID NO 24; or (d) a heavy chain variable region comprising a CDRH1 of SEQ ID NO 25, a CDRH2 of SEQ ID NO 26 and a CDRH3 of SEQ ID NO 27, and a light chain variable region comprising a CDRL1 of SEQ ID NO 28, a CDRL2 of SEQ ID NO 29 and a CDRL3 of SEQ ID NO 30.

[0014] In some aspects of the disclosure, the heavy chain variable region of (a) comprises an amino acid sequence of SEQ ID NO 31 or 75; the heavy chain variable region of (b) comprises an amino acid sequence of SEQ ID NO 39, 83, 87, 95 or 103; the heavy chain variable region of (c) comprises an amino acid sequence of SEQ ID NO 47; the heavy chain variable region of (d) comprises an amino acid sequence of SEQ ID NO 55; and the heavy chain variable region of (e) comprises an amino acid sequence of SEQ ID NO 63 or 79.

[0015] In some aspects of the disclosure, the light chain variable region of (a) comprises an amino acid sequence of SEQ ID NO 35 or 77; the light chain variable region of (b) comprises an amino acid sequence of SEQ ID NO 43, 85, 91, 99 or 107; the light chain variable region of (c) comprises an amino acid sequence of SEQ ID NO 51; the light chain variable region of (d) comprises an amino acid sequence of SEQ ID NO 59; and the light chain variable region of (e) comprises an amino acid sequence of SEQ ID NO 67 or 81.

[0016] In some aspects of the disclosure, the antibody or the antigen-binding fragment thereof comprising (a) comprises a heavy chain comprising an amino acid sequence of SEQ ID NO 33; the antibody or the antigen-binding fragment thereof comprising (b) comprises a heavy chain comprising an amino acid sequence of SEQ ID NO 41, 89, 97 or 105; the antibody or the antigen-binding fragment thereof comprising (c) comprises a heavy chain comprising an amino acid sequence of SEQ ID NO 49; the antibody or the antigen-binding fragment thereof comprising (d) comprises a heavy chain comprising an amino acid sequence of SEQ ID NO 57; and the antibody or the antigen-binding fragment thereof comprising (e) comprises a heavy chain comprising an amino acid sequence of SEQ ID NO 65.

[0017] In some aspects of the disclosure, the antibody or the antigen-binding fragment thereof comprising (a) comprises a light chain comprising an amino acid sequence of SEQ ID NO 37; the antibody or the antigen-binding fragment thereof comprising (b) comprises a light chain comprising an amino acid sequence of SEQ ID NO 45, 93, 101 or 109; the antibody or the antigen-binding fragment thereof comprising (c) comprises a light chain comprising an amino acid sequence of SEQ ID NO 53; the antibody or the antigen-binding fragment thereof comprising (d) comprises a light chain comprising an amino acid sequence of SEQ ID NO 61; and the antibody or the antigen-binding fragment thereof comprising (e) comprises a light chain comprising an amino acid sequence of SEQ ID NO 69.

[0018] The present disclosure further provides a fusion protein comprising the antibody or the antigen-binding fragment as described herein.

[0019] The present disclosure further provides a chimeric antigen receptor polypeptide comprising an HER2-binding domain; a transmembrane domain (TM); a costimulatory domain; and an intracellular signaling domain (ICD).

[0020] In some aspects of the disclosure, the HER2-binding domain comprises the antibody or the antigen-binding fragment thereof as described herein.

[0021] In some aspects of the disclosure, the transmembrane domain is a transmembrane domain of a protein selected from a group consisting of T-cell receptor alpha, beta or zeta chain, CD28, CD3 epsilon, CD45, CD4, CDS, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

[0022] In some aspects of the disclosure, the costimulatory domain is a functional signaling domain obtained from a protein selected from a group consisting of MHC class I molecule, TNF receptor protein, immunoglobulin-like protein, cytokine receptor, integrin, signaling lymphocytic activation molecule (SLAM), activating NK cell receptor, BTLA (B- and T-lymphocyte attenuator), Toll-like ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11 a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8 beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand binding specifically to CD83.

[0023] In some aspects of the disclosure, the intracellular signaling domain comprises a functional signaling domain of 4-1BB, CD28, OX40 or CD3 zeta, or a combination thereof.

[0024] In some aspects of the disclosure, the intracellular signaling domain comprises a functional signaling domain of OX40 ligand.

[0025] The present disclosure further provides a nucleic acid molecule encoding the anti-HER2 antibody or the antigen-binding fragment thereof as described herein.

[0026] The present disclosure further provides a nucleic acid molecule encoding the chimeric antigen receptor polypeptide as described herein.

[0027] The present disclosure further provides a recombinant vector comprising the nucleic acid molecule encoding the anti-HER2 antibody or the antigen-binding fragment thereof as described herein or the chimeric antigen receptor polypeptide as described herein.

[0028] The present disclosure further provides a host cell transformed with the recombinant vector as described herein.

[0029] The present disclosure further provides an effector cell expressing the chimeric antigen receptor polypeptide as described herein.

[0030] In some aspects of the disclosure, the effector cell is selected from a group consisting of a dendritic cell, a killer dendritic cell, a mast cell, a natural killer cell, a B lymphocyte, a T lymphocyte, a macrophage and precursor cells thereof.

[0031] In some aspects of the disclosure, the T lymphocyte is selected from a group consisting of an inflammatory T lymphocyte, a cytotoxic T lymphocyte, a regulatory T lymphocyte or a helper T lymphocyte.

[0032] The present disclosure further provides a pharmaceutical composition for preventing or treating cancer, comprising a pharmaceutically effective amount of the anti-HER2 antibody or the antigen-binding fragment thereof as described herein a pharmaceutically acceptable carrier.

[0033] The present disclosure further provides a pharmaceutical composition for treating cancer, comprising the effector cell expressing the chimeric antigen receptor polypeptide as described herein.

[0034] In some aspects of the disclosure, the cancer is breast cancer, ovarian cancer, gastric cancer, lung cancer, liver cancer, bronchial cancer, nasopharyngeal cancer, laryngeal cancer, pancreatic cancer, bladder cancer, colorectal cancer, colon cancer, cervical cancer, brain cancer, prostate cancer, bone cancer, head and neck cancer, skin cancer, thyroid cancer, parathyroid cancer or ureteral cancer.

[0035] In some aspects of the disclosure, the pharmaceutical composition further comprises the trastuzumab antibody.

[0036] The present disclosure further provides a kit for diagnosing cancer, comprising the anti-HER2 antibody or the antigen-binding fragment thereof as described herein.

[0037] The present disclosure further provides a chimeric antigen receptor comprising an extracellular domain that binds Her2, wherein the extracellular domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 113 (hz39D2 (VL-GS linker-VH)).

[0038] In some aspects of the disclosure, the chimeric antigen receptor further comprises an extracellular signaling domain linked to the extracellular domain; a hinge domain linked to the extracellular domain; a transmembrane domain linked to the hinge domain; and

[0039] an intracellular stimulatory signal linked to the hinge domain.

[0040] In some aspects of the disclosure, the extracellular signaling domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90% at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 111 (CD8a signal peptide). In some aspects, the extracellular signaling domain comprises SEQ ID NO: 111 (CD8a signal peptide).

[0041] In some aspects of the disclosure, the hinge domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 115 (CD8a hinge). In some aspects of the disclosure, the hinge domain comprises SEQ ID NO: 115 (CD8a hinge).

[0042] In some aspects of the disclosure, the transmembrane domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 117 (CD8α TM) or SEQ ID NO: 119 (CD28 TM). In some aspects of the disclosure, the transmembrane domain comprises SEQ ID NO: 117 CD8a TM) or SEQ ID NO: 119 (CD28 TM).

[0043] In some aspects of the disclosure, the intracellular stimulatory signal comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 121 (CD3-ζ). In some aspects, the intracellular stimulatory signal comprises SEQ ID NO: 121 (CD3-ζ).

[0044] In some aspects of the disclosure, the chimeric antigen receptor further comprises a second intracellular stimulatory signal, wherein the second intracellular stimulatory signal comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 123 (4-1BB) or SEQ ID NO: 125 (CD28). In some aspects of the disclosure, the second intracellular stimulatory signal comprises SEQ ID NO: 123 (4-1BB) or SEQ ID NO: 125 (CD28).

[0045] In some aspects of the disclosure, the chimeric antigen receptor further comprises a third intracellular stimulatory signal, wherein the third intracellular stimulatory signal comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 127 (OX40L). In some aspects of the disclosure, wherein the third intracellular stimulatory signal comprises SEQ ID NO: 127 (OX40L).

[0046] In some aspects of the disclosure, the chimeric antigen receptor comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 129 (Clone 2), SEQ ID NO: 131 (Clone 3), SEQ ID NO: 133 (Clone 6), or SEQ ID NO: 135 (Clone 14). In some aspects of the disclosure, the chimeric antigen receptor comprises SEQ ID NO: 129 (Clone 2), SEQ ID NO: 131 (Clone 3), SEQ ID NO: 133 (Clone 6), or SEQ ID NO: 135 (Clone 14).

[0047] The present disclosure further provides a nucleic acid molecule encoding the chimeric antigen receptor having an extracellular domain comprising an amino acid sequence with at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 113 (hz39D2 (VL-GS linker-VH)) as described herein.

[0048] The present disclosure further provides a vector comprising the nucleic acid molecule encoding the chimeric antigen receptor having an extracellular domain comprising an amino acid sequence with at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%,

or at least 99% sequence identity to SEQ ID NO: 113 (hz39D2 (VL-GS linker-VH)) as described herein.

[0049] The present disclosure further provides an immune cell expressing the chimeric antigen having an extracellular domain comprising an amino acid sequence with at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 113 (hz39D2 (VL-GS linker-VH)) as described herein.

[0050] In some aspects of the disclosure, the immune cell is a natural killer cell.

[0051] The present disclosure further provides a pharmaceutical composition comprising the immune cell as described herein and a pharmaceutically acceptable carrier.

[0052] The present disclosure further provides a method for treating cancer comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition as described herein.

[0053] In some aspects of the disclosure, the cancer is selected from the group consisting of breast cancer, ovarian cancer, gastric cancer, lung cancer, liver cancer, bronchial cancer, nasopharyngeal cancer, laryngeal cancer, pancreatic cancer, bladder cancer, colorectal cancer, colon cancer, cervical cancer, brain cancer, prostate cancer, bone cancer, head and neck cancer, skin cancer, thyroid cancer, parathyroid cancer and ureteral cancer.

BRIEF DESCRIPTION OF DRAWINGS

[0054] FIG. **1** is a line graph showing the a results of analyzing the affinity of hz2G10, hz39D2, 24D3, 1G3 and hz8G11 clones for the HER2-ECD-Fc antigen by ELISA.

[0055] FIG. **2** is a bar graph showing the a results of investigating the extracellular domain of HER2 to which hz2G10, hz39D2, 24D3, 1G3 and hz8G11 clones bind.

[0056] FIG. **3**A and FIG. **3**B are line graphs showing the results of analyzing the effect of single administration of five antibodies of the present disclosure (hz2G10, hz39D2, 24D3, 1G3 and hz8G11) on the inhibition of the growth of HER2-overexpressed breast cancer cells (SKBR3) and HER2-unexpressed breast cancer cells (MCF-7).

[0057] FIG. **3**C and FIG. **3**D are line graphs showing the results of analyzing the effect of co-administration of five antibodies of the present disclosure (hz2G10, hz39D2, 24D3, 1G3 and hz8G11) and the trastuzumab (TRA) antibody on the inhibition of the growth of HER2-overexpressed breast cancer cells (SKBR3) and HER2-unexpressed breast cancer cells (MCF-7).

[0058] FIG. **4** is a bar graph showing the results of investigating the specificity of antibodies developed by expressing the ErbB family. Cetuximab (CET), trastuzumab (TRA) and patritumab (AMG888, AMG) were used as control groups binding to EGFR, HER2 and HER3, respectively.

[0059] FIGS. **5**A-**5**E are line graphs showing the results of comparing the epitopes of developed antibodies with trastuzumab. For comparison with the epitope of trastuzumab, trastuzumab and HER2-His were immobilized on a sensor chip and then the binding with five antibodies of the present disclosure was analyzed.

[0060] FIGS. **6**A-**6**C are bar graphs showing the results of analyzing the effect of single administration of hz39D2 and affinity-improved clones thereof (hz39D2.14, hz39D2.22 and hz39D2.23) or co-administration with the trastuzumab antibody on the inhibition of the growth of HER2-overexpressed gastric cancer and breast cancer cells.

[0061] FIG. **7** is a table providing a summary of HER2-CAR constructs according to the present disclosure.

[0062] FIGS. **8**A-**8**C are line graphs showing the results of a cell killing assay (Calcein releasing cytotoxicity assay) assessing the cytotoxicity of cord-blood derived NK cells (CBNKs) expressing HER2-CAR constructs against HER2 positive target cancer cell lines.

[0063] FIGS. **9**A-**9**E are line graphs showing the results of a cell killing assay (Calcein releasing cytotoxicity assay) assessing the cytotoxicity of cord-blood derived NK cells expressing HER2-CAR construct clone #14 against HER2 positive target cancer cell lines.

[0064] FIGS. **10**A-**10**B are line graphs showing the long-term serial killing activity of HER2-CAR clones #6 and #14 assessed using the Incucyte live cell imaging system.

[0065] FIGS. **11**A-**11**B are bar graphs showing the NK cell degranulation activity and cytotoxic cytokine expression with the various HER2-CAR constructs evaluated by comparing intercellular expression level of CD107a and IFN-γ.

[0066] FIGS. **12**A-**12**C are bar graphs showing NK cell degranulation activity and cytotoxic cytokine expression of HER2-CAR clones #6 and #14 evaluated by comparing intercellular expression level of CD107a, IFN-γ, and TNF-α.

[0067] FIG. **13** is a bar graph showing the secretion of IFN-γ from anti-HER2-CAR-CBNKs (clones #2, #6, #14) when co-cultured with target cells.

[0068] FIGS. **14**A-**14**B are a scheme and line graph, respectively, showing the in vivo efficacy of anti-Her2-CAR clones #3 and #14 Xenograft models in NSG mice were generated by injecting 5×10^6 HCC1954 cells/mouse (HER-2 positive, trastuzumab resistant cells) subcutaneously. Tumor volume was assessed every 3-4 days after injection.

[0069] FIG. **15**A is a line graph showing the proliferation of HER2-CAR-NK cells. FIGS. **15**B-**15**D are line graphs (FIG. **15**B) and bar graphs (FIGS. **15**C and **15**D) showing the CAR expression levels, viability, and purity of HER2-CAR-NK cells during expansion, culture and cryopreservation.

[0070] FIG. **16** is a bar graph showing the phenotypic cell surface marker expression of

[0071] HER2-CAR-NK cells.

DETAILED DESCRIPTION OF THE INVENTION

[0072] The present disclosure is directed to providing an antibody (anti-HER2 antibody) against HER2 (human epidermal growth factor receptor 2) or an antigen-binding fragment thereof.

[0073] The present disclosure is also directed to providing a fusion protein including the anti-HER2 antibody or an antigen-binding fragment thereof.

[0074] The present disclosure is also directed to providing a chimeric antigen receptor (CAR) including the anti-HER2 antibody or an antigen-binding fragment thereof and an effector cell expressing the same.

[0075] The present disclosure is also directed to providing a nucleic acid molecule encoding the anti-HER2 antibody or an antigen-binding fragment thereof; or the chimeric antigen receptor.

[0076] The present disclosure is also directed to providing a recombinant vector including the nucleic acid molecule.

[0077] The present disclosure is also directed to providing a host cell transformed with the recombinant vector.

[0078] The present disclosure is also directed to providing a pharmaceutical composition for preventing or treating cancer, which contains the anti-HER2 antibody or an antigen-binding fragment thereof.

[0079] The present disclosure is also directed to providing a kit for diagnosing cancer, which includes the anti-HER2 antibody or an antigen-binding fragment thereof.

[0080] The present disclosure is also directed to providing a method for preventing or treating cancer by administering a composition containing the anti-HER2 antibody or an antigen-binding fragment thereof to a subject.

[0081] The present disclosure is also directed to providing a method for treating a disease related with HER2 overexpression (e.g., cancer) by administering an effector cell expressing the chimeric antigen receptor to a subject.

Technical Solution

[0082] The present disclosure provides an antibody binding specifically to HER2 (human epidermal growth factor receptor 2) and modified antibodies thereof that have undergone affinity maturation.

[0083] A first aspect of the present disclosure provides an antibody against HER2 (human epidermal growth factor receptor 2) including the followings or an antigen-binding fragment thereof:

[0084] (a) a heavy chain variable region including the following heavy chain CDR (complementarity-determining region) amino acid sequences:

[0085] CDRH1 of SEQ ID NO 1, CDRH2 of SEQ ID NO 2 and CDRH3 of SEQ ID NO 3; and

[0086] (b) a light chain variable region including the following light chain CDR amino acid sequences:

[0087] CDRL1 of SEQ ID NO 4, CDRL2 of SEQ ID NO 5 and CDRL3 of SEQ ID NO 6.

[0088] A second aspect of the present disclosure provides an antibody against HER2 (human epidermal growth factor receptor 2) including the followings or an antigen-binding fragment thereof:

[0089] (a) a heavy chain variable region including the following heavy chain CDR (complementarity-determining region) amino acid sequences:

[0090] CDRH1 of SEQ ID NO 7, CDRH2 of SEQ ID NO 8 and CDRH3 of SEQ ID NO 9, 71 or 72; and

[0091] (b) a light chain variable region including the following light chain CDR amino acid sequences:

[0092] CDRL1 of SEQ ID NO 10, CDRL2 of SEQ ID NO 11 and CDRL3 of SEQ ID NO 12, 73 or 74.

[0093] A third aspect of the present disclosure provides an antibody against HER2 (human epidermal growth factor receptor 2) including the following or an antigen-binding fragment thereof:

[0094] (a) a heavy chain variable region including the following heavy chain CDR (complementarity-determining region) amino acid sequences:

[0095] CDRH1 of SEQ ID NO 13, CDRH2 of SEQ ID NO 14 and CDRH3 of SEQ ID NO 15; and

[0096] (b) a light chain variable region including the following light chain CDR amino acid sequences:

[0097] CDRL1 of SEQ ID NO 16, CDRL2 of SEQ ID NO 17 and CDRL3 of SEQ ID NO 18.

[0098] A fourth aspect of the present disclosure provides an antibody against HER2 (human epidermal growth factor receptor 2) including the following or an antigen-binding fragment thereof:

[0099] (a) a heavy chain variable region including the following heavy chain CDR (complementarity-determining region) amino acid sequences:

[0100] CDRH1 of SEQ ID NO 19, CDRH2 of SEQ ID NO 20 and CDRH3 of SEQ ID NO 21; and

[0101] (b) a light chain variable region including the following light chain CDR amino acid sequences:

[0102] CDRL1 of SEQ ID NO 22, CDRL2 of SEQ ID NO 23 and CDRL3 of SEQ ID NO 24.

[0103] A fifth aspect of the present disclosure provides an antibody against HER2 (human epidermal growth factor receptor 2) including the following or an antigen-binding fragment thereof:

[0104] (a) a heavy chain variable region including the following heavy chain CDR (complementarity-determining region) amino acid sequences:

[0105] CDRH1 of SEQ ID NO 25, CDRH2 of SEQ ID NO 26 and CDRH3 of SEQ ID NO 27; and

[0106] (b) a light chain variable region including the following light chain CDR amino acid sequences:

[0107] CDRL1 of SEQ ID NO 28, CDRL2 of SEQ ID NO 29 and CDRL3 of SEQ ID NO 30.

[0108] The antibody of the first aspect, the antibody of the second aspect, the antibody of the third aspect, the antibody of the fourth aspect and the antibody of the fifth aspect are referred to, respectively, as 2G10, 39D2, 24D3, 1G3 and 8G11 antibodies. They are mouse antibodies or chimeric antibodies. Among them, the humanized antibodies are expressed with the prefix hz, e.g., as hz2G10, hz39D2 and hz8G11 antibodies.

[0109] The inventors of the present disclosure have made efforts to develop a novel antibody which is capable of preventing or treating cancer (particularly, breast cancer and gastric cancer), exhibits better killing ability (or proliferation-inhibiting ability) for cancer cells which have non-reactivity (or resistance) to the trastuzumab antibody or have reduced sensitivity, and is capable of preventing or treating cancer with improved anticancer activity when co-administered with the trastuzumab antibody as compared to single administration of trastuzumab. As a result, they have developed a novel antibody which exhibits better killing ability for HER2-overexpressed cancer cells on which the trastuzumab antibody hardly acts, or exhibits improved anticancer activity when co-administered with the trastuzumab antibody, and have completed the present disclosure.

[0110] The antibody of the present disclosure or an antigen-binding fragment thereof has a specific binding ability for HER2. In particular, among the antibodies of the present disclosure, hz2G10 and hz39D2 bind to an epitope in domain 1 of domains 1-4 of HER2, 24D3 binds to an epitope in domain 3, and 1G3 and hz8G11 bind to an epitope in domain 4, like trastuzumab, which is different from the epitope to which trastuzumab binds.

[0111] In the present disclosure, the term "trastuzumab" refers to an antibody disclosed in U.S. Pat. No. 5,821,337.

[0112] The antibody of the present disclosure has superior killing ability or proliferation-inhibiting ability for cancer cells which have non-reactivity (or resistance) to the trastuzumab antibody or have reduced sensitivity, when used

either alone or in combination with trastuzumab. In the present disclosure, the terms "killing", "proliferation-inhibiting" or "growth-inhibiting" are used interchangeable with the same meaning with regard to cancer cells.

[0113] In the present disclosure, the term "antibody" refers to an antibody specific for

[0114] HER2, and includes not only the whole antibody but also an antigen-binding fragment of the antibody molecule.

[0115] A whole antibody has two full-length light chains and two full-length heavy chains. The light chains and heavy chains are connected by disulfide bonds. The constant region of the heavy chain has gamma (γ), mu (μ), alpha (α), delta (δ) and epsilon (ε) types, and has subclasses gamma1 (γ1), gamma2 (γ2), gamma3 (γ3), gamma4 (γ4), alpha1 (α1) and alpha2 (α2). The constant region of the light chain has kappa (κ) and lambda (λ) types.

[0116] In the present disclosure, the term "antigen-binding fragment" refers to a fragment having antigen-binding ability, and includes Fab, F(ab'), $F(ab')_2$, Fv, etc. Among the antibody fragments, Fab (fragment antigen-binding) has a structure having a variable region of the light and heavy chains, a constant region of the light chain and the first constant region (C_{H1}) of the heavy chain and has one antigen-binding site. Fab' differs from Fab in that it has a hinge region including at least one cysteine residue at the C-terminus of the heavy chain CH1 domain. In the $F(ab')_2$ antibody, a cysteine residue in the hinge region of Fab' forms a disulfide bond. Recombinant techniques for generating Fv fragments with minimal antibody fragments in which Fv has only the heavy chain variable region and the light chain variable region are known in the related art. A double-chain variable fragment (dcFv) is linked to a heavy chain variable region and a light chain variable region via a non-covalent bond, and a single-chin variable fragment (scFv) is generally linked to covalently to the variable region of a heavy chain via a peptide linker, or to the C-terminus, to form a dimer such as the double-chain Fv. These antibody fragments can be obtained using proteases (for example, Fab can be obtained by cleaving a whole antibody with papain, and the F(ab')2 fragment can be obtained by cleaving with pepsin), or can be prepared using genetic recombination techniques.

[0117] Specifically, in the present disclosure, the antibody includes a monoclonal antibody, a multispecific antibody, a human antibody, a humanized antibody, a chimeric antibody, a single-chain Fv (scFv), a single-chain antibody, an Fab fragment, an F(ab') fragment, a disulfide-linked Fv (dsFv), an anti-idiotypic (anti-Id) antibody, and epitope-binding fragments of these antibodies, although not being limited thereto.

[0118] In the present disclosure, the term "heavy chain" encompasses a full-length heavy chain including a variable region domain V_H and three constant region domains C_{H1}, C_{H2} and C_{H3}, including an amino acid sequence having a variable region sequence sufficient for conferring specificity to an antigen, and fragments thereof. Also, in the present disclosure, the term "light chain" encompasses a full-length light chain including a variable region domain V_L and a constant region domain C_L, including an amino acid sequence having a variable region sequence sufficient for conferring specificity to an antigen, and fragments thereof.

[0119] In the present disclosure, the term "variable region" or "variable domain" refers to a domain of an antibody heavy chain or light chain associated with binding of an antibody to an antigen. In general, the variable domains of a heavy chain and a light chain (V_H and V_L, respectively) of a native antibody have similar structures, and each domain includes four conserved framework region (FRs) and three hypervariable regions (HVRs) (Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007)).

[0120] In the present disclosure, the term "CDR (complementarity-determining region)" refers to the amino acid sequence of the hypervariable region of a heavy chain and a light chain of an immunoglobulin (Kabat et al., Sequences of Proteins of Immunological Interest, 4th ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)). Each of the heavy chain (CDRH1, CDRH2 and CDRH3) and the light chain (CDRL1, CDRL2 and CDRL3) includes three CDRs. The CDR provides major contact residue for binding of an antibody to an antigen or an epitope.

[0121] In the present disclosure, the term "framework region" or "FR" refers to a variable domain residue other than a hypervariable region (HVR) residue. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3 and FR4. Accordingly, the HVR and FR sequences generally appear in the following order in V_H:

[0122] FRH1 (framework region 1 of heavy chain)-CDRH1 (complementarity-determining region 1 of heavy chain)-FRH2-CDRH2-FRH3-CDRH3-FRH4.

[0123] And, the HVR and FR sequences generally appear in the following order in V_L (or V_k):

[0124] FRL1 (framework region 1 of light chain)-CDRL1 (complementarity-determining region 1 of light chain)-FRL2-CDRL2-FRL3-CDRL3-FRL4.

[0125] In the present disclosure, the term "specific binding" means that an antibody or an antigen-binding fragment thereof, or another construct such as scFv forms a relatively stable complex with an antigen under physiological conditions. The specific binding may be characterized by an equilibrium dissociation constant of about 1×10^{-6} M or smaller (e.g., the smaller the K_d, the tighter the binding). Methods for determining if two molecules bind specifically are well known in the art, for example, equilibrium dialysis, surface plasmon resonance, etc.

[0126] In the present disclosure, the term "affinity" refers to the strength of the sum of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless specified otherwise, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between the members of a binding pair (e.g., an antibody and an antigen). The affinity of a molecule X for its partner Y may generally be represented by a dissociation constant (K_d). The affinity can be measured by common methods known in the art, including those described in the present disclosure.

[0127] In the present disclosure, the "human antibody" or "humanized antibody" possesses an amino acid sequence which corresponds to an antibody produced by human or a human cell, or an antibody derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences.

[0128] In the present disclosure, the term "chimeric antibody" refers to an antibody in which a portion of the heavy chain and/or light chain is derived from a particular source or species while the remainder of the heavy chain and/or light chain is derived from a different source or species.

[0129] The anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof may include variants of the amino acid sequences described in the attached sequence listings within the scope of specifically recognizing HER2. For example, the amino acid sequence of an antibody may be modified to improve the binding affinity and/or other biological properties of the antibody. Such modification includes, for example, deletion, insertion and/or substitution of the amino acid sequence residue of the antibody.

[0130] Such amino acid variation is made based on the relative similarity of amino acid side chain substituents, such as hydrophobicity, hydrophilicity, charge, size, etc. From analysis of the size, shape and type of amino acid side chain substituents, it is recognized that arginine, lysine and histidine are positively charged residues; alanine, glycine and serine have similar sizes; and phenylalanine, tryptophan and tyrosine have similar shapes. Based on these considerations, it is thus recognized that arginine, lysine and histidine; alanine, glycine and serine; and phenylalanine, tryptophan and tyrosine are biologically functional equivalents.

[0131] For introduction of mutation, the hydropathy indices of amino acids may be considered. Each amino acid is assigned a hydropathy index according to its hydrophobicity and charge: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophan (−0.9); tyrosine (−1.3); proline (−1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5).

[0132] The hydropathy indices of amino acids are very important in imparting the interactive biological function of proteins. It is well known that similar biological activity can be retained when substitution is made with an amino acid having a similar hydropathy index. In this regard, when mutation is introduced, substitution is made between amino acids showing difference in the hydropathy index preferably within ±2, more preferably within ±1, even more preferably within ±0.5.

[0133] Meanwhile, it is also well known that substitution between amino acids having similar hydrophilicity values leads to proteins with equivalent biological activity. As disclosed in U.S. Pat. No. 4,554,101, the following hydrophilicity values are assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ±1); glutamate (+3.0 ±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (−0.4); proline (−0.5 ±1); alanine (−0.5); histidine (−0.5); cysteine (−1.0); methionine (−1.3); valine (−1.5); leucine (−1.8); isoleucine (−1.8); tyrosine (−2.3); phenylalanine (−2.5); tryptophan (−3.4).

[0134] In this regard, when mutation is introduced, substitution is made between amino acids showing difference in the hydrophilicity value preferably within ±2, more preferably within ±1, even more preferably within ±0.5.

[0135] Amino acid substitutions in proteins that do not entirely alter the activity of the molecules are known in the art (H. Neurath, R. L. Hill, The Proteins, Academic Press, New York, 1979). The most commonly occurring substitutions are substitutions between the following amino acid residues: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Thy/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu and Asp/Gly.

[0136] In an exemplary embodiment of the present disclosure, the heavy chain variable region of the hz2G10 antibody and the 2G10 antibody respectively includes amino acid sequences of SEQ ID NO 31 and 32.

[0137] In an exemplary embodiment of the present disclosure, the light chain variable region of the hz2G10 antibody and the 2G10 antibody respectively includes amino acid sequences of SEQ ID NO 35 and 77.

[0138] In an exemplary embodiment of the present disclosure, the heavy chain variable region of the hz39D2 antibody and the 39D2 antibody respectively includes amino acid sequences of SEQ ID NO 39 and 83.

[0139] In an exemplary embodiment of the present disclosure, the light chain variable region of the hz39D2 antibody and the 39D2 antibody respectively includes amino acid sequences of SEQ ID NO 43 and 85.

[0140] In an exemplary embodiment of the present disclosure, the heavy chain variable region of the 24D3 antibody includes an amino acid sequence of SEQ ID NO 47.

[0141] In an exemplary embodiment of the present disclosure, the light chain variable region of the 24D3 antibody includes an amino acid sequence of SEQ ID NO 51.

[0142] In an exemplary embodiment of the present disclosure, the heavy chain variable region of the 1G3 antibody includes an amino acid sequence of SEQ ID NO 55.

[0143] In an exemplary embodiment of the present disclosure, the light chain variable region of the 1G3 antibody includes an amino acid sequence of SEQ ID NO 59.

[0144] In an exemplary embodiment of the present disclosure, the heavy chain variable region of the hz8G11 antibody and the 8G11 antibody respectively includes amino acid sequences of SEQ ID NO 63 and 79.

[0145] In an exemplary embodiment of the present disclosure, the light chain variable region of the hz8G11 antibody and the 8G11 antibody respectively includes amino acid sequences of SEQ ID NO 67 and 81.

[0146] The antibody of the present disclosure includes a monoclonal antibody, a multispecific antibody, a human antibody, a humanized antibody, a chimeric antibody, a single-chain Fv (scFv), a single-chain antibody, an Fab fragment, an F(ab') fragment, a disulfide-linked Fv (dsFv), an anti-idiotypic (anti-Id) antibody, and epitope-binding fragments of these antibodies, although not being limited thereto.

[0147] Meanwhile, the antibody of the present disclosure is unique in that its CDR sequence has very low homology (similarity) to the CDR sequences of existing anti-HER2 antibodies. For example, as a result of BLAST search for hz2G10 from among the antibodies of the present disclosure, the highest CDR sequence homology of the antibody of the present disclosure to the antibodies disclosed in U.S. Pat. Nos. 8,314,213 and 8,404,811 was less than 50%. In addition, the antibodies disclosed in U.S. Pat. Nos. 8,314,213 and 8,404,811 bind to CD25 and EGFL7, respectively, and are different from the antibody of the present disclosure in their targets.

[0148] In addition, the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof encompasses an anti-HER2 antibody including a slight change in the amino acid sequence described above, including the modification that hardly affects the tertiary structure and function of the antibody, or an antigen-binding fragment thereof. Accordingly, in some exemplary embodiments, the

antibody may have an amino acid sequence with at least 90%, 93%, 95% or 98% similarity to the above-described sequence.

[0149] Also, in the present disclosure, the heavy chain variable region and the light chain variable region of the antibody or an antigen-binding fragment thereof may be linked by a linker composed of an amino acid sequence represented by the general formula $(G_nS_m)_p$ or $(S_mG_n)_p$.

[0150] In the formula, n, m and p satisfy the followings:

[0151] n is an integer from 1 to 7;

[0152] m is an integer from 0 to 7;

[0153] n+m is an integer which is 8 or smaller; and

[0154] p is an integer from 1 to 7.

[0155] In a specific exemplary embodiment of the present disclosure, n=1-5 and m=0-5. In a more specific exemplary embodiment, n=4 and m=1. In a further more specific exemplary embodiment, the linker is $(G_4S)_3$ or $(S_4G)_3$.

[0156] In another exemplary embodiment, the linker is VDGS. In another exemplary embodiment, the linker is ASGS.

[0157] In addition, the light chain variable region and the heavy chain variable region of the antibody according to the present disclosure or an antigen-binding fragment may in the following orientations: light chain variable region-linker-heavy chain variable region; or heavy chain variable region-linker-light chain variable region.

[0158] Another aspect of the present disclosure provides a fusion protein including an anti-HER2 antibody or an antigen-binding fragment thereof.

[0159] In the present disclosure, the fusion protein is prepared for the productivity purification efficiency, improved biological activity, increased stability, improved folding and/or binding to a functional moiety for additional function of the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof. The fusion protein may be formed as two or more polypeptide chains are linked by a covalent bond, or may be in the form of a conjugate wherein two or more polypeptide chains are linked by chemical conjugation.

[0160] Another aspect of the present disclosure provides a chimeric antigen receptor polypeptide including the followings:

[0161] (a) an HER2-binding domain;

[0162] (b) a transmembrane domain (TM);

[0163] (c) a costimulatory domain(domain); and

[0164] (d) an intracellular signaling domain (ICD).

[0165] In the present disclosure, the term "chimeric antigen receptor (CAR)" refers to an artificially constructed hybrid protein (fusion protein) or polypeptide containing a target-binding domain (e.g. single-chain variable fragment (scFv)) linked to an effector cell-signaling or effector cell-activating domain (e.g. T-cell signaling or T-cell activating domain). In general, the chimeric antigen receptor has the ability of redirecting T-cell specificity and reactivity toward a selected target in a non-MHC restricted manner by taking advantage of the antigen-binding property of a monoclonal antibody. The non-MHC-restricted antigen recognition confers the ability to recognize an antigen on T-cells expressing CAR, thus bypassing the major mechanism of tumor escape. Moreover, when expressed in T-cells, the CAR advantageously does not dimerize with the endogenous T-cell receptor (TCR) alpha and beta chains.

[0166] In an exemplary embodiment of the present disclosure, the chimeric antigen receptor of the present disclosure recognizes the HER2 antigen and is expressed on the cell surface since it includes the HER2-binding domain including the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof.

[0167] The chimeric antigen receptor of the present disclosure includes a transmembrane domain because it is expressed on the cell surface. The transmembrane domain may be a transmembrane domain of a protein selected from a group consisting of the T-cell receptor alpha, beta or zeta chain, CD28, CD3 epsilon, CD45, CD4, CDS, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, although not being limited thereto.

[0168] In a specific exemplary embodiment of the present disclosure, the transmembrane domain may be a transmembrane domain of CD8 or CD28.

[0169] The costimulatory domain of the chimeric antigen receptor of the present disclosure may be a functional signaling domain obtained from a protein selected from a group consisting of MHC class I molecule, TNF receptor protein, immunoglobulin-like protein, cytokine receptor, integrin, signaling lymphocytic activation molecule (SLAM), activating NK cell receptor, BTLA (B- and T-lymphocyte attenuator), Toll-like ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8 beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand binding specifically to CD83, although not being limited thereto.

[0170] In a specific exemplary embodiment of the present disclosure, the costimulatory domain may be a functional signaling domain obtained from a protein selected from a group consisting of CD28, OX40, 4-1BB (CD137) and/or ICOS (CD278), more specifically a functional signaling domain of CD28 and/or OX40.

[0171] In another exemplary embodiment of the present disclosure, the intracellular signaling domain is a functional signaling domain of 4-1BB, CD28, OX40 or CD3 zeta, or a combination thereof. Most specifically, the intracellular signaling domain is a functional signaling domain of CD3 zeta.

[0172] In another embodiment of the present disclosure, the intracellular signaling domain may be a functional signaling domain of OX40 ligand (OX40L). In another embodiment, the intracellular signaling domain is OX40 ligand.

[0173] The HER2-binding domain of the chimeric antigen receptor of the present disclosure is linked to the transmembrane domain by a hinge domain.

[0174] In another exemplary embodiment of the present disclosure, the hinge domain may be IgG4 hinge, CD8 hinge or IgD hinge.

[0175] Another aspect of the present disclosure provides a nucleic acid molecule encoding the anti-HER2 antibody or

an antigen-binding fragment thereof, or the chimeric antigen receptor polypeptide described above.

[0176] In the present disclosure, the term "nucleic acid molecule"encompasses DNA (gDNA and cDNA) and RNA molecules, and the nucleotides that are the basic building blocks of the nucleic acid molecule include not only natural nucleotides but also analogues having modified sugar or base moieties (Scheit, *Nucleotide Analogs,* John Wiley, New York (1980); Uhlman and Peyman, *Chemical Reviews,* 90: 543-584 (1990)).

[0177] A nucleotide sequence encoding the antibody of the present disclosure or an antigen-binding fragment thereof, or the chimeric antigen receptor polypeptide is not limited to a specific nucleotide sequence as long as it is a nucleotide sequence encoding the amino acid sequences constituting the chimeric antigen receptor molecule.

[0178] This is because the variation in nucleotide sequences may not lead to change in protein sequences through expression. This is called codon degeneracy. Accordingly, the nucleotide sequence includes a nucleotide sequence including functionally equivalent codons, or codons encoding the same amino acid (for example, six codons encode arginine or serine due to codon degeneracy) or codons encoding a biologically equivalent amino acid.

[0179] In a specific exemplary embodiment of the present disclosure, the nucleotide sequence encoding the polypeptide constituting the heavy chain CDR, light chain CDR, heavy chain variable region, light chain variable region, heavy chain or light chain of the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof is described in the attached sequence listings.

[0180] The nucleic acid molecule of the present disclosure, which encodes the anti-HER2 antibody or an antigen-binding fragment thereof, or the chimeric antigen receptor polypeptide, is understood to encompass a nucleotide sequence exhibiting substantial identity for the nucleotide sequence. The substantial identity means that, when the nucleotide sequence of the present disclosure is aligned to another sequence correspond to each other as much as possible and the aligned sequences are analyzed using an algorithm commonly used in the art, the nucleotide sequences exhibit at least 80% homology, more specifically at least 90% homology, most specifically at least 95% homology.

[0181] When considering the variation of biologically equivalent activity, it is understood that the nucleic acid molecule encoding the antibody of the present disclosure or an antigen-binding fragment; or the chimeric antigen receptor polypeptide encompasses a sequence exhibiting substantial identity to the sequences described in the sequence listings. The substantial identity means that, when the sequence of the present disclosure and another sequence are aligned to correspond to each other as much as possible and the aligned sequences are analyzed using an algorithm commonly used in the art, the sequences have at least 61% homology, more specifically 70% homology, further more specifically 80% homology, most specifically 90% homology. Methods of the alignment for sequence comparison are known in the art. Various methods and algorithms for the alignment are disclosed in Smith and Waterman, *Adv. Appl. Math.* 2: 482 (1981); Needleman and Wunsch, *J. Mol. Bio.* 48: 443 (1970); Pearson and Lipman, *Methods in Mot Biol.* 24: 307-31 (1988); Higgins and Sharp, *Gene* 73: 237-44 (1988); Higgins and Sharp, *CABIOS* 5: 151-3 (1989); Corpet et al., *Nuc. Acids Res.* 16: 10881-90 (1988); Huang et al., *Comp. Appl. BioSci.* 8: 155-65 (1992) and Pearson et al., *Meth. Mol. Biol.* 24: 307-31 (1994). The NCBI's basic local alignment search tool (BLAST) (Altschul et al., *J. Mol. Biol.* 215: 403-10 (1990)) is accessible from the NBCI (National Center for Biotechnology Information) and on the Internet and may be used in connection with sequence analysis programs such as blastp, blastn, blastx, tblastn and tblastx. BLAST may be accessed through the BLAST webpage of the NCBI's website. The method for comparing sequence homology using such a program is available from the BLAST help page of the NCBI's website.

[0182] In connection with the claims of the application/patent, sequence identity is determined according to the Needleman and Wunsch algorithm.

[0183] Another aspect of the present disclosure provides a recombinant vector including the nucleic acid molecule.

[0184] In the present disclosure, the term "vector" includes a delivery vector and an expression vector.

[0185] In the present disclosure, the term "delivery vector" refers to a composition of a material which contains an isolated nucleic acid and can be used to deliver the isolated nucleic acid into a cell. It includes a linear polynucleotide, a polynucleotide associated with an ionic or amphiphilic compound, a plasmid and a virus, although not being limited thereto. More specifically, the delivery vector includes a self-replicating plasmid or virus. The term is also construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acids into cells, such as, for example, polylysine compounds, liposomes, etc. Examples of the viral delivery vector include an adenoviral vector, an adeno-associated viral vector, a retroviral vector and a lentiviral vector, although not being limited thereto.

[0186] In the present disclosure, the term "expression vector" refers to a vector including a recombinant nucleotide including an expression control sequence operably linked to a nucleotide sequence to be expressed for expression of a target gene in a host cell. The expression vector includes a cis-acting element sufficient for expression and other elements for expression can be provided by a host cell or an in-vitro expression system. The expression vector includes a plasmid vector including a recombinant polynucleotide; a cosmid vector; and a viral vector such as a bacteriophage vector, an adenoviral vector, a lentiviral vector, a retroviral vector and an adeno-associated viral vector. In a specific exemplary embodiment of the present disclosure, a nucleic acid molecule encoding a switch molecule is operatively linked to a promoter of the vector of the present disclosure. In the present disclosure, the term "operatively linked" refers to functional linkage between a nucleic acid expression control sequence (e.g., a promoter, a signal sequence, or an array of a transcription factor binding site) and another nucleic acid sequence, wherein the control sequence affects the transcription and/or translation of the another nucleic acid sequence.

[0187] The recombinant vector system of the present disclosure may be constructed according to various methods known in the art. Specific methods are described in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (2001), which is incorporated into the present disclosure by reference.

[0188] The vector of the present disclosure may be constructed as a vector for gene cloning, a vector for protein expression, or a vector for gene delivery. In addition, the

vector of the present disclosure may be constructed by using a prokaryotic cell or a eukaryotic cell as a host cell.

[0189] For example, when the vector of the present disclosure is an expression vector and a eukaryotic cell is used as a host cell, a promoter derived from the genome of a mammalian cell (e.g., metallothionein promoter, β-actin promoter, human hemoglobin promoter and human muscle creatine promoter) or a promoter derived from a mammalian virus (e.g., adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus promoter, tk promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, Moloney virus promoter, Epstein-Barr virus (EBV) promoter and Rous sarcoma virus (RSV) promoter) may be used, and they generally have a polyadenylation sequence as a transcription termination sequence.

[0190] In an exemplary embodiment of the present disclosure, when the vector is a delivery vector, it may be a "retroviral vector". Retrovirus provides a convenient platform for a gene delivery system. A gene selected for gene delivery may be inserted in the retroviral vector and may be packaged within a retroviral particle. Then, the recombinant retrovirus may be delivered to a target host cell in vivo or in vitro. Many retroviral vectors are known in the art. In a specific exemplary embodiment of the present disclosure, the retroviral vector may be a pMT retroviral vector which is an MLV-based retroviral vector, although not being limited thereto.

[0191] In another exemplary embodiment of the present disclosure, the vector is a lentiviral vector or an adenoviral vector.

[0192] The vector of the present disclosure may be fused with other sequences for easy purification of the polypeptide or protein expressed thereby. For example, the fused sequence may be glutathione S-transferase (Pharmacia, USA), maltose-binding protein (NEB, USA), FLAG (IBI, USA), 6x His (hexahistidine; Quiagen, USA), etc. Meanwhile, the expression vector of the present disclosure may include a selectable marker gene and/or a reporter gene for evaluating the expression of the antibody of the present disclosure or an antigen-binding fragment thereof, or a CAR polypeptide including the same. The selectable marker gene includes an antibiotic-resistant gene commonly used in the art, e.g., genes resistant to ampicillin, gentamicin, carbenicillin, chloramphenicol, streptomycin, kanamycin, geneticin, neomycin and tetracycline. The reporter gene includes luciferase, beta-galactosidase, chloramphenicol, acetyltransferase or green fluorescent protein gene.

[0193] Methods for introducing the recombinant vector of the present disclosure into a cell and expressing the same are well known in the related art. The vector may be easily introduced into a host cell, e.g., a mammalian cell, a bacterial cell, a yeast cell or an insect cell according to methods known in the art. For example, the vector may be delivered into a host cell by physical, chemical or biological means. The physical means includes calcium phosphate coprecipitation, lipofection, particle bombardment, microinjection, electroporation, etc. The chemical means includes a colloidal dispersion system, e.g., a macromolecular complex, a nanocapsule, a microsphere, a bead, and a lipid-based system including an oil-in-water emulsion, a micelle, a mixed micelle and a liposome. And, the biological means includes use of a DNA or RNA vector such as a lentiviral vector, a retroviral vector, etc. as described above.

[0194] Another aspect of the present disclosure provides a host cell transformed with the recombinant vector.

[0195] The host cell capable of cloning and expressing the vector of the present disclosure stably and continuously may be any host cell known in the art. For example, a eukaryotic host cell suitable for the vector includes a monkey kidney cell 7 (COST), an NSO cell, an SP2/0 cell, a Chinese hamster ovary (CHO) cell, a W138 cell, a baby hamster kidney (BHK) cell, a MDCK cell, a myeloma cell, a HuT 78 cell and an HEK-293 cell, although not being limited thereto.

[0196] Another aspect of the present disclosure provides an effector cell expressing the chimeric antigen receptor (CAR) polypeptide.

[0197] In an exemplary embodiment of the present disclosure, the effector cell is selected from a group consisting of a dendritic cell, a killer dendritic cell, a mast cell, a natural killer cell, a B lymphocyte, a T lymphocyte, a macrophage and precursor cells thereof, although not being limited thereto. The T lymphocyte cell is selected from a group consisting of an inflammatory T lymphocyte, a cytotoxic T lymphocyte, a regulatory T lymphocyte or a helper T lymphocyte.

[0198] In the present disclosure, the effector cell includes a group of autologous cells or allogenic cells. That is to say, the effector cell includes a group of autologous cells or allogenic cells expressing the HER2-specific CAR polypeptide.

[0199] In another exemplary embodiment of the present disclosure, the effector cell includes a group of cells transfected or transduced with a vector including a nucleic acid molecule encoding the HER2-specific CAR polypeptide. The transfection or transduction may be achieved by various means known in the art without limitation.

[0200] Accordingly, in a specific exemplary embodiment of the present disclosure the present disclosure, the HER2-specific CAR-encoding nucleic acid molecule is delivered into an effector cell, e.g., a T lymphocyte or a natural killer cell, and transcribed into mRNA. The HER2-specific CAR polypeptide is translated from the mRNA and expressed on the surface of the effector cell.

[0201] Another aspect of the present disclosure provides a pharmaceutical composition for preventing or treating cancer, which contains: (a) a pharmaceutically effective amount of the anti-HER2 antibody of the present disclosure or the antigen-binding fragment thereof described above; and (b) a pharmaceutically acceptable carrier.

[0202] Another aspect of the present disclosure provides a pharmaceutical composition for treating cancer or an inflammatory disease, which contains an effector cell expressing the chimeric antigen receptor polypeptide described above.

[0203] The pharmaceutical composition is a pharmaceutical composition for immunotherapy, which contains an effector cell expressing the anti-HER2 antibody or an antigen-binding fragment thereof; or the chimeric antigen receptor polypeptide.

[0204] In the present disclosure, "immunotherapy" refers to treatment of cancer by activating the immune system. Immunotherapy is classified into active immunotherapy and passive immunotherapy. Active immunotherapy includes i) cancer vaccine therapy of activating the immune system by injecting cancer cells or substances produced by cancer cells into human body, and ii) immunomodulatory therapy of activating specific leukocytes by administering immuno-

modulatory agents such as cytokines (interferons, interleukins, etc.), growth factors, etc. Passive immunotherapy includes antibody therapy and immune cell therapy. Specifically, immune cell therapy includes dendritic cell vaccine therapy, chimeric antigen receptor T (CAR-T) cell therapy, natural killer (NK) cell therapy, cytotoxic T lymphocyte (CTL) therapy, adoptive cell transfer, etc., although not being limited thereto. In the present disclosure, the immunotherapy mainly refers to antibody therapy using the anti-HER2 antibody and immune cell therapy using the HER2-specific CAR.

[0205] The pharmaceutical composition of the present disclosure contains an effector cell expressing the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof; the chimeric antigen receptor polypeptide; or the chimeric antigen receptor as an active ingredient. Therefore, description of the details described above will be omitted to avoid redundancy.

[0206] As demonstrated in the following examples, the anti-HER2 antibody of the present disclosure exhibits better killing ability for MCF-7 cells on which the trastuzumab antibody hardly acts. In addition, the anti-HER2 antibody of the present disclosure exhibits improved killing ability for SKBR3 breast cancer cells when co-administered with trastuzumab. Accordingly, the composition of the present disclosure is very effective for combined administration with the trastuzumab antibody for treatment of cancer and for treatment of cancer not treated with trastuzumab.

[0207] The cancer that can be prevented or treated by the composition of the present disclosure includes various cancers known in the art. For example, it includes breast cancer, ovarian cancer, gastric cancer, lung cancer, liver cancer, bile duct cancer, bronchial cancer, nasopharyngeal cancer, laryngeal cancer, pancreatic cancer, bladder cancer, kidney cancer, colorectal cancer, colon cancer, cervical cancer, brain cancer, prostate cancer, bone cancer, head and neck cancer, skin cancer, thyroid cancer, parathyroid cancer or ureteral cancer.

[0208] Specifically, the cancer that can be prevented or treated by the composition of the present disclosure is HER2-expressing cancer, more specifically HER2-expressing breast cancer or gastric cancer.

[0209] The pharmaceutically acceptable carrier contained in the pharmaceutical composition of the present disclosure is one commonly used in preparation, and includes lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia gum, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, mineral oil, etc., although not being limited thereto. The pharmaceutical composition of the present disclosure may further contain a lubricant, a wetting agent, a sweetener, a flavor, an emulsifier, a suspending agent, a preservative, etc. in addition to the above-described ingredients. Suitable pharmaceutically acceptable carriers and preparations are described in detail in Remington's Pharmaceutical Sciences (19th ed., 1995).

[0210] The pharmaceutical composition of the present disclosure may be administered orally or parenterally. For example, it may be administered intravenously, subcutaneously, intramuscularly, intraperitoneally, topically, intranasally, intrapulmonarily, intrathecally, ocularly, intradermally, transdermally, etc.

[0211] An administration dosage of the pharmaceutical composition of the present disclosure varies depending on such factors as formulation method, administration method, the age, body weight, sex of a patient, pathological condition, food, administration time, administration route, excretion rate and responsiveness. A normally trained physician can easily determine and prescribe an administration dosage for effective treatment or prevention. In a specific exemplary embodiment of the present disclosure, a daily administration dosage of the pharmaceutical composition of the present disclosure is 0.0001-100 mg/kg. In the present disclosure, the term "pharmaceutically effective amount" refers to an amount sufficient for preventing or treating cancer.

[0212] The pharmaceutical composition of the present disclosure may be formulated into a unit-dosage form or a multiple-dosage form using a pharmaceutically acceptable carrier and/or excipient according to a method that can be easily employed by those of ordinary skill in the art to which the present disclosure belongs. The formulation may be in the form of a solution in an oily or aqueous medium, a suspension, an emulsion, an extract, a pulvis, a suppository, a powder, a granule, a tablet or a capsule, and may further contain a dispersant or a stabilizer.

[0213] In an exemplary embodiment of the present disclosure, the pharmaceutical composition of the present disclosure may further contain the trastuzumab antibody.

[0214] The pharmaceutical composition of the present disclosure may further contain, in addition to the active ingredient derived above, another pharmaceutically active medication or drug, e.g., a chemotherapy agent such as asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc., a targeted therapy agent such bevacizumab, olaparib, etc., or an immune checkpoint inhibitor such as nivolumab or pembrolizumab, or may be co-administered with them.

[0215] Another aspect of the present disclosure provides a method for treating cancer, which includes a step of administering a composition containing an effector cell expressing the anti-HER2 antibody or an antigen-binding fragment thereof; or the HER2-specific chimeric antigen receptor to a subject in need of treatment.

[0216] The cancer to be treated by the therapeutic method of the present disclosure is the same as defined above with regard to the pharmaceutical composition.

[0217] In an exemplary embodiment of the present disclosure, the subject may be a mammal or human.

[0218] Since the method for treating cancer or an inflammatory disease of the present disclosure uses an effector cell expressing the antibody or an antigen-binding fragment; or the chimeric antigen receptor described above as an active ingredient, description of the details described above will be omitted to avoid redundancy.

[0219] The anti-HER2 antibody or an antigen-binding fragment thereof described above may be used for diagnosis, e.g., diagnosis of cancer.

[0220] Accordingly, another aspect of the present disclosure provides a kit for diagnosing cancer, which includes the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof.

[0221] Since the diagnostic kit of the present disclosure includes the anti-HER2 antibody of the present disclosure or an antigen-binding fragment thereof described above and diagnoses the same disease as described above with regard

to the pharmaceutical composition of the present disclosure, description of the details described above will be omitted to avoid redundancy.

[0222] Since the kit includes an antibody, it can be prepared to be suitable for various immunoassay or immunostaining applications. The immunoassay or immunostaining includes radioimmunoassay, radioimmunoprecipitation, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), capture ELISA, inhibition or competition assay, sandwich assay, flow cytometry, immunofluorescence staining and immunoaffinity purification, although not being limited thereto. Methods for the immunoassay or immunostaining are described in Enzyme Immunoassay, E. T. Maggio, ed., CRC Press, Boca Raton, Florida, 1980; Gaastra, W., Enzyme-linked immunosorbent assay (ELISA), in Methods in Molecular Biology, Vol. 1, Walker, J. M. ed., Humana Press, NJ, 1984; and Ed Harlow and David Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999, which are incorporated in the present disclosure by reference.

[0223] For example, when the method of the present disclosure is carried out by radioimmunoassay, an antibody labeled with a radioisotope (e.g., C^{14}, I^{125}, P^{32} or S^{35}) may be used to detect the HER2 protein. When the method of the present disclosure is carried out by ELISA, a specific exemplary embodiment of the present disclosure includes: (i) a step of coating a sample to be analyzed on the surface of a solid substrate; (ii) a step of reacting the sample with the anti-HER2 antibody of the present disclosure as a primary antibody; (iii) a step of reacting the resultant of the step (ii) with a secondary antibody coupled with an enzyme; and (iv) a step of measuring the activity of the enzyme.

[0224] Appropriate examples of the solid substrate are a hydrocarbon polymer (e.g., polystyrene or polypropylene), glass, a metal or a gel, most specifically, a microtiter plate.

[0225] The enzyme coupled with the secondary antibody may include an enzyme that catalyzes chromogenic reaction, fluorescence reaction, luminescent reaction or infrared reaction, although not being limited thereto. For example, it includes alkaline phosphatase, 3-galactosidase, horseradish peroxidase, luciferase and cytochrome P450. When alkaline phosphatase is used as the enzyme coupled with the secondary antibody, a chromogenic substrate such as bromochloroindolyl phosphate (BCIP), nitro blue tetrazolium (NBT), naphthol-AS-B 1-phosphate and enhanced chemifluorescence (ECF) may be used as the substrate. When horseradish peroxidase is used, a substrate such as chloronaphthol, aminoethylcarbazole, diaminobenzidine, D-luciferin, lucigenin (bis-N-methylacridinium nitrate), resorufin benzyl ether, luminol, Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine), HYR (p-phenylenediamine-HCl and pyrocatechol), tetramethylbenzidine (TMB), 2,2-azino-di[3-ethylbenzthiazoline sulfonate] (ABTS), o-phenylenediamine (OPD), naphthol/pyronin, glucose oxidase, t-NBT (nitro blue tetrazolium) and m-PMS (phenzaine methosulfate) may be used.

[0226] When the method of the present disclosure is carried out by capture ELISA, the method includes: (i) a step of coating the HER2 antibody as a capture antibody on the surface of a solid substrate; (ii) a step of reacting the capture antibody with a sample; (iii) a step of reacting the resultant of the step (ii) with an HER2 detection antibody conjugated with a label; and (iv) a step of measuring a signal generated from the label.

[0227] The anti-HER2 antibody of the present disclosure has a label that generates a signal that can be detected by the detection antibody. The label includes a chemical substance (e.g., biotin), an enzyme (alkaline phosphatase, β-galactosidase, horseradish peroxidase or cytochrome P450), a radioactive substance (e.g., C^{14}, I^{125}, P^{32} and S^{35}), a fluorescent material (e.g., fluorescein), a light-emitting material, a chemiluminescent material and a FRET (fluorescence resonance energy transfer) material, although not being limited thereto. Various labels and labeling method are described in Ed Harlow and David Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999.

[0228] In the ELISA method and the capture ELISA, the measurement of the enzyme activity or the measurement of the signal may be carried out according to various methods known in the art. The signal may be detected easily by using streptavidin when biotin is used as the label, and using luciferin when luciferase is used.

[0229] The sample to which the kit of the present disclosure can be applied includes a cell, a tissue, a tissue-derived extract, a lysate, a purification product, a blood, a plasma, a serum, a lymph or ascites, although not being limited thereto.

[0230] The antibody of the present disclosure may be used in in-vivo or in-vitro imaging.

[0231] Another aspect of the present disclosure provides a composition for imaging, which contains a conjugate in which the antibody of the present disclosure is conjugated to a label generating a detectable signal.

[0232] The label generating a detectable signal includes a T1 contrast agent (e.g., a Gd chelate compound), a T2 contrast agent (e.g., a superparamagnetic material (e.g., magnetite, Fe_3O_4,γ-Fe_2O_3, manganese ferrite, cobalt ferrite and nickel ferrite)), a radioiosotope (e.g. ^{11}C, ^{15}O, ^{13}N, P^{32}, S^{35}, ^{44}Sc, ^{45}Ti, ^{118}I, ^{136}La, ^{198}Tl, ^{200}Tl, ^{205}Bi and ^{206}Bi), a fluorescent material (fluorescein, phycoerythrin, rhodamine, lissamine, Cy3 and Cy5), a chemiluminescent material, a magnetic particle, a mass label or an electron-dense particle, although not being limited thereto.

Advantageous Effects

[0233] The features and advantages of the present disclosure may be summarized as follows:

[0234] (a) The antibody of the present disclosure or an antigen-binding fragment is an antibody that specifically binds to HER2 which is highly expressed in cancer cells (particularly, breast cancer or gastric cancer cells), and binds to an epitope that is different from an epitope to which trastuzumab binds. The present disclosure provides the antibody or the antigen-binding fragment, a chimeric antigen receptor including the same, and uses thereof.

[0235] (b) The antibody of the present disclosure or an antigen-binding fragment is unique in that its CDR sequence has very low homology to the CDR sequences of existing HER2-targeting antibodies.

[0236] (c) When compared with trastuzumab, the antibody of the present disclosure exhibits better killing ability for HER2-unexpressed cancer cells which have non-reactivity (or resistance) to the trastuzumab antibody or have reduced sensitivity. In addition, when the anti-HER2 antibody of the present disclosure is administered in combination with trastuzumab, a synergistic

killing ability is achieved for cancer cells on which the trastuzumab antibody acts. Therefore, a composition of the present disclosure can be used for combined administration with the trastuzumab antibody for the treatment of cancer, or for the treatment of cancer not treated with trastuzumab. In particular, when expressed in effector cells such as T lymphocytes, etc., the chimeric antigen receptor including the anti-HER2 antibody of the present disclosure or an antigen-binding fragment may be used for immune cell therapy of various HER2-related cancers.

[0237] (d) Without wishing to be bound by theory, it is considered that the antibody of the present disclosure exhibits the above-described effects since it binds to an epitope that is different from an epitope to which trastuzumab binds and inhibits HER2 in a different manner from that of trastuzumab.

BEST MODE FOR CARRYING OUT INVENTION

[0238] Hereinafter, the present disclosure will be described in detail through examples. However, the following examples are for illustrative purposes only and it will be apparent to those of ordinary skill in the art that the scope of the present disclosure is not limited by the examples.

EXAMPLES

Example 1

Development of Anti-HER2 Antibody

[0239] For development of antibodies, the extracellular domain (ECD) of the HER2 protein was produced using animal cells. The DNA in which a hinge and an Fc region (CH_2-CH_3) of human IgG1 were bound to the C-terminus of ECD was cloned into pCEP4 (Invitrogen, Cat. No. V044-50) using HindIII and BamHI restriction enzymes. Then, the cloned vector was transiently transformed into FreeStyle 293F (Invitrogen, Cat. No. R790-07) cells using polyethyleneimine (Polyscience Inc., Cat. No. 23966) and then purified from the cell culture using a Protein-A Ceramic HyperD F resin (PALL, Cat No. 20078-028). The purified protein was quantitated using a protein assay dye (Bio-Rad, Cat. No. 500-0006) and its concentration and purity were investigated via Coomassie Blue staining following SDS-PAGE. 100 μg of the protein antigen was mixed with a Freund's adjuvant (Sigma, Cat. No. F5506) and then injected intraperitoneally into BALB/c mouse (Dae Han Bio). 2 weeks later, 100 μg of the antigen diluted in PBS was injected further. 3 days later, the spleen of the mouse was taken out and lymphocytes were isolated. The isolated lymphocytes were mixed with SP2/0-Ag14 myeloma cells (ATCC, Cat. No. CRL-1581) at a ratio of 5:1 and then fused using PEG-1500 (Roche, Cat. No. 783641). The fused cells (hybridoma) were selectively sorted out and cultured in a medium containing a HAT supplement (Sigma, Cat. No. H0262).

[0240] The obtained hybridoma cells were examined via ELISA to determine whether they were the cells producing an antibody that bind to the antigen. HER2-ECD-Fc or ChromPure human IgG (hIgG, Jackson Immunoresearch Lab. Inc., Cat. No. 009-000-003) was immobilized at room temperature onto a Costar 96-well plate (Corning, Cat. No. 3590) at a concentration of 1μg/mL for 1 hour. The resultant was washed 3 times with TBS-T (0.05% Triton X-100) and then blocked at room temperature for 30 minutes with 300 μL of TBS-T/SM (2% skim milk). After washing the blocked plate 3 times and adding the hybridoma culture, the antibody was allowed to bind at 37° C. for 1 hour. After washing 3 times and then adding anti-mIgG-HRP (Pierce, Cat. No. 31439) diluted to 1:5,000 in TBS-T/SM, as a secondary antibody, the antibody was allowed to bind at 37° C. for 1 hour. After washing the resultant 3 times and adding TMB (SurModics, Cat. No. TMBC-1000-01), the mixture was allowed to develop color at room temperature for 5 minutes. Then, the color development was stopped by adding 1 N sulfuric acid (DukSan, Cat. No. 254). Absorbance was measured at 450 nm using Victor X3 (PerkinElmer, Cat. No. 2030-0030) and the antibody binding specifically to HER2-ECD-Fc was selected.

[0241] Since HER2 is a protein expressed on cell surface, it was investigated whether the developed antibody was bound to HER2-overexpressing cells via cell-based ELISA. HER2-overexpressing SKOV-3 ovary cancer cells (Korean Cell Line Bank, Cat. No. 30077) were aliquoted onto a Costar 96-well cell culture plate (Corning, Cat. No. 3595) at 10,000 cell/well and then cultured for 24 hours. On the following day, after removing the cell culture supernatant, the resultant was washed 3 times with PBS and cultured further at 37° C. for 2 hours after adding the hybridoma cell culture. After washing 3 times with TBS-T and adding goat anti-mIgG-HRP diluted in PBS/FBS (3% FBS) to 1:5,000, as a secondary antibody, the resultant was treated at room temperature for 1 hour. After washing 3 times with TBS-T, it was allowed to develop color using TMB. 61 clones showing higher absorbance than that of the SP2/0 cell culture as a negative control group were selected.

[0242] The five antibodies (hz2G10, hz39D2, 24D3, 1G3, hz8G11) finally selected from the monoclonal antibodies binding specifically to HER2 were modified to chimeric antibodies or humanized antibodies (hz). The amino acid sequences of the chimeric antibodies or humanized antibodies are described in the attached sequence listings.

[0243] The absorbance of the finally selected five antibodies (hz2G10, hz39D2, 24D3, 1G3, hz8G11) is shown in FIG. **1** and Table 1.

Verification of Binding of HER2 Proteins of Five Selected Antibodies to Extracellular Domain (ECD)

[0244]

TABLE 1

	Concentration (ug/mL)								
Antibodies	5×10^{-7}	5×10^{-6}	5×10^{-5}	5×10^{-4}	5×10^{-3}	5×10^{-2}	5×10^{-1}	5	50
PBS	0.13	0.13	0.14	0.14	0.14	0.13	0.16	0.15	0.14
hz2G10	0.12	0.12	0.12	0.12	0.22	1.16	2.69	2.79	2.81
hz39D2	0.12	0.12	0.15	0.47	2.29	2.92	2.78	2.90	2.83
24D3	0.11	0.11	0.12	0.22	1.13	2.76	2.90	2.92	2.75
1G3	0.11	0.11	0.14	0.35	1.77	2.79	2.78	2.81	2.76
hz8G11	0.12	0.12	0.14	0.34	1.67	2.72	2.94	2.90	2.74

Example 2

Verification of Binding Site of Developed Antibody for HER2 Protein

[0245] The binding site of the selected five antibodies (hz2G10, hz39D2, 24D3, 1G3, hz8G11) for the extracellular domain (ECD) of the HER2 protein was verified by ELISA. For ELISA, the extracellular domain (ECD) of the ERBB family protein was produced using animal cells and was used as an antigen. Specifically, the DNA in which a hinge and an Fc region (CH_2-CH_3) of human IgG1 were bound to the C-terminus of ECD was cloned into pCEP4 (Invitrogen, Cat. No. V044-50) using HindIII and BamHI restriction enzymes. Then, the cloned vector was transiently transformed into FreeStyle 293F (Invitrogen, Cat. No. R790-07) cells using polyethyleneimine (Polyscience Inc., Cat. No. 23966) and then HER2-ECD DI Fc, HER2-ECD DII Fc, HER2-ECD DIII Fc, HER2-ECD DIV Fc and HER2-ECD Fc fusion proteins were purified from the cell culture using a Protein-A Ceramic HyperD F resin (PALL, Cat No. 20078-028). The purified protein was quantitated using a protein assay dye (Bio-Rad, Cat. No. 500-0006) and its concentration and purity were investigated via Coomassie Blue staining following SDS-PAGE.

[0246] The HER2-ECD DI Fc, HER2-ECD DII Fc, HER2-ECD DIII Fc, HER2-ECD

[0247] DIV Fc and HER2-ECD Fc fusion proteins were immobilized at 4° C. overnight onto a Costar 96-well plate (Corning, Cat. No. 3590) at a concentration of 1μg/mL for 1 hour. The resultant was washed 3 times with TBS-T (0.05% Triton X-100) and then blocked at room temperature for 1 hour with 100 μL of TBS-T/BSA (5% BSA). After washing the blocked plate 3 times and adding the anti-HER2 antibody, the antibody was allowed to bind at room temperature for 1 hour. After washing 3 times and then adding anti-human IgG-HRP diluted to 1:3,000 in TBS-T/BSA, as a secondary antibody, the antibody was allowed to bind at room temperature for 1 hour. After washing the resultant 3 times and adding TMB (SurModics, Cat. No. TMBC-1000-01), the mixture was allowed to develop color at room temperature for 5 minutes. Then, the color development was stopped by adding 1 N sulfuric acid (DukSan, Cat. No. 254). Absorbance was measured at 450 nm using Victor X3 (PerkinElmer, Cat. No. 2030-0030).

[0248] The result is shown in FIG. **2**.

[0249] As show in FIG. **2**, among the antibodies developed in the present disclosure, hz2G10 and hz39D2 were bound to the domain 1 of the extracellular domains of the HER2 protein, 24D3 was bound to the domain 3, and 1G3 and hz8G11 were bound to the domain 4.

[0250] From this result, it can be seen that the five antibodies of the present disclosure can inhibit the growth of the HER2-overexpressed cancer cells by binding to the HER domain which is different from the extracellular domain 4 of the HER2 protein to which trastuzumab (TRA) binds (hz2G10, hz39D2, 24D3), or can exhibit remarkably superior effect of inhibiting cellular growth when used alone or co-administered with trastuzumab. Therefore, they can be usefully used for prevention or treatment of cancer related with the expression of the HER2 proteins either alone or together with trastuzumab.

Example 3

Comparison of Inhibitory Effect of Developed Antibody Against Growth of Breast Cancer Cells

[0251] Cell viability was analyzed by treating HER2-overexpressed SKBR3 breast cancer cells or HER2-unexpressed breast cancer cells with MCF-7 either alone or together with trastuzumab. For co-administration, the developed antibody and trastuzumab were mixed at a weight ratio of 1:1. SKBR3 cells (Korean Cell Line Bank, Cat. No. 30030, 5,000 cells/well) and MCF-7 cells (ATCC, Cat. No. HTB22, 5,000 cells/well) were aliquoted onto a 96-well plate and cultured for 24 hours. The cells were cultured further for 4 days after treating with the purified antibody at a final concentration of 20 μg/mL. For measurement of cell viability, CCK-8 (Dojindo, Cat. No. CK-04-13) was added to a final concentration of 10% and absorbance was measured after treating at 37° C. for 3 hours. Relative cell viability was calculated with respect to the absorbance of the antibody-untreated well as 100%.

[0252] The result is shown in FIGS. **3***a*-**3***d* and Table 2.

Relative Cell Viability of HER2-Positive SKBR3 Breast Cancer Cells and HER2-Negative MCF-7 Breast Cancer Cells Treated with Antibody (Single Treatment)

[0253]

TABLE 2

	Relative cell viability at 20 μg/mL (%)	
Clones	SKBR3	MCF-7
hIgG	94.68	92.11
TRA (trastuzumab) alone	63.68	98.22
hz2G10	89.06	97.43
hz39D2	96.46	91.42

TABLE 2-continued

Relative cell viability at 20 μg/mL (%)		
Clones	SKBR3	MCF-7
24D3	93.97	87.33
1G3	74.81	98.66
hz8G11	74.02	98.95

[0254] In the above table, hIgG stands for human IgG. As seen from FIG. **3**A, FIG. **3**B and Table 2, the five antibodies of present disclosure showed the effect of inhibiting the proliferation of SKBR3 breast cancer cells when treated alone. They showed comparable or better effect of inhibiting cellular growth as compared to the positive control trastuzumab at different concentrations (FIG. **3**A). However, the five antibodies of present disclosure did not show significant effect of inhibiting cell proliferation of the HER2-negative MCF-7 cells like the positive control trastuzumab (FIG. **3**B).

Relative Cell Viability of HER2-Positive SKBR3 Breast Cancer Cells and HER2-Negative MCF-7 Breast Cancer Cells Treated with Antibody (Co-Treatment)

[0255]

TABLE 3

Relative cell viability at 20 μg/mL (%)		
Clones	SKBR3	MCF-7
hIgG	94.68	92.11
TRA (trastuzumab) alone	63.68	98.22
TRA + hz2G10	68.98	90.56
TRA + hz39D2	77.29	90.62
TRA + 24D3	63.75	97.21
TRA + 1G3	62.16	102.33
TRA + hz8G11	52.62	98.41

[0256] In the above table, hIgG denotes the test group treated with human IgG, and TRA+ denotes the test groups co-administered with trastuzumab and the antibody of the present disclosure. As seen from FIG. **3**C, FIG. **3**D and Table 3, all of the five antibodies of present disclosure (hz2G10, hz39D2, 24D3, 1G3, hz8G11) showed comparable or better effect of inhibiting cellular growth when treated to the SKBR3 breast cancer cells together with trastuzumab as compared to single treatment with trastuzumab (FIG. **3**C).

[0257] Without wishing to be bound by theory, it is considered that the antibody of the present disclosure exhibits the above-described effect since it binds to an epitope on HER2 that is different from an epitope to which trastuzumab binds and inhibits HER2 in a different manner from that of trastuzumab.

Example 4

Antibody Sequence Analysis

[0258] For analysis of the antibody sequence, a phage Fab antibody library was constructed using the respective hybridoma RNAs and a 3-step panning was conducted to obtain a phage that binds to HER2-ECD-Fc (Phage display: a laboratory manual, Carlos Barbas III, et al., Cold Spring Harbor Laboratory Press). After culturing the hybridoma, RNA was isolated using the SV total RNA isolation system (Promega, Cat. No. Z3100) and cDNA was synthesized therefrom. Using a known primer set (see Phage display: a laboratory manual, Carlos Barbas III, et al., Cold Spring Harbor Laboratory Press), the variable region of the antibody was amplified and cloned into the pComb3X vector (Barbas Laboratory, Scripps Research Institute) using a Sfi-I restriction enzyme after ligating to human Ck (kappa chain) and CH_1, and then transformed into ER2537 bacteria (New England Biolabs, Cat. No. 801-N). The transformed bacteria were transfected with the VCSM13 helper phage (Stratagene, Cat. No. 200251) and a clone which binds to HER2-ECD-Fc was obtained using an immunotube to which HER2-ECD-Fc was immobilized.

[0259] From the colonies of the antibodies, the antibody that binds to HER2-ECD-Fc was confirmed via ELISA. The colonies of the transformed bacteria were cultured at 37° C. until the absorbance at 600 nm reached 0.5, treated with IPTG at a final concentration of 1 mM, and allowed to express antibodies in the form of Fab while culturing overnight at 30° C. After collecting cells by centrifuging 5 mL of the culture, the cells were suspended in 0.4 mL of 1× TES (50 mM Tris, 1 mM EDTA, 20% (v/v) sucrose, pH 8.0) and then treated at 4° C. for 10 minutes. After adding 0.6 mL of 0.2× TES thereto and treating further at 4° C. for 30 minutes, the resultant was centrifuged and a supernatant was taken. After washing a Costar 96-well half area plate (Corning Inc., Cat. No. 3690) coated with HER2-ECD-Fc at a concentration of 1 μg/mL 3 times with TBS-T, it was blocked with TBS-T/SM (3% non-fat skim milk, 0.05% Triton X-100) at room temperature for 1 hour. The culture broth or periplasmic extract (periplasm) of each colony was diluted at a ratio of 1:3 using TBS-T/SM and allowed to bind at room temperature for 1 hour. After washing 3 times and diluting to 1:5000 with anti-HA-HRP (Roche, Cat. No. 120-138-190-01) as a secondary antibody, the resultant was allowed to bind at room temperature for 1 hour. After washing 3 times, the resultant was allowed to develop color using TMB.

[0260] Most colonies showed absorbance of 0.2 or higher in the cell culture or periplasmic extract, and the base sequence of the antibody was analyzed for these clones. The base sequence analysis revealed that the colonies derived from the single hybridoma had the same sequences.

[0261] The CDR sequence of the antibody produced from each clone is summarized in

[0262] Table 4 and Table 5.

TABLE 4

Clones	CDRH1	CDRH2	CDRH3
hz2G10	DYYMY (SEQ ID NO 1)	YINSGGG STYYPDT VKG (SEQ ID NO 2)	EALYDYD YAMDY (SEQ ID NO 3)
hz39D2	NYGVN (SEQ ID NO 7)	WINTHTGEP TYAEEPKG (SEQ ID NO 8)	DDYYVRV DY (SEQ ID NO 9)

TABLE 4-continued

Clones	CDRH1	CDRH2	CDRH3
24D3	SCAMS (SEQ ID NO 13)	TISGGGS YTYYPDS VKG (SEQ ID NO 14)	HGGYESW FPY (SEQ ID NO 15)
1G3	DTYMH (SEQ ID NO 19)	RID PANGYTR YDPNFQG (SEQ ID NO 20)	YYYGFYA MDY (SEQ ID NO 21)
hz8G11	GYYMH (SEQ ID NO 25)	HINPNNG GTSYNQK FKG (SEQ ID NO 26)	EEAFAY (SEQ ID NO 27)

TABLE 5

Clones	CDRL1	CDRL2	CDRL3
hz2G10	KSSQSLL YSNGKTY LN (SEQ ID NO 4)	LVSKLDS (SEQ ID NO 5)	VQGTHFP LT (SEQ ID NO 6)
hz39D2	KASQDIN SYLS (SEQ ID NO 10)	RANRLVD (SEQ ID NO 11)	LQYDEFP WT (SEQ ID NO 12)
24D3	RSSQSLV HSNGNTY LH (SEQ ID NO 16)	KVSNRFS (SEQ ID NO 17)	SQSTHVP PWT (SEQ ID NO 18)
1G3	KASQDVS TAVA (SEQ ID NO 22)	SASYRYT (SEQ ID NO 23)	QQHYSTP PT (SEQ ID NO 24)
hz8G11	RASQDIS NYLN (SEQ ID NO 28)	YTSRLHS (SEQ ID NO 29)	QQGITPP WT (SEQ ID NO 30)

[0263] Tables 4 and 5 show the amino acid sequences of the heavy chain CDR (CDRH) and the light chain CDR (CDRL) of the developed antibodies.

Example 5

Specificity of Developed Antibody for HER2

[0264] It was investigated whether the developed five antibodies of the present disclosure specifically bind to HER2 belonging to the ErbB family proteins by ELISA. In order to confirm whether the developed antibody binds specifically to HER2 belonging to the ErbB family proteins, the extracellular domains of EGFR, HER2, HER3 and HER4 belonging to the ErbB family were examined via ELISA. The extracellular domain of EGFR (EGFR-ECD-Fc) was produced in the same manner as the HER2-ECD-Fc described above in Example 2, and the HER3 (R&D Systems, #348-RB-050) and HER4 (R&D Systems, #1131-ER-050) proteins were purchased. Cetuximab (CET), trastuzumab (TRA) and patritumab (AMG888, AMG) were used as control group antibodies binding to EGFR, HER2 and HER3, respectively.

[0265] The result is shown in FIG. **4**.

[0266] As seen from FIG. **4**, it was confirmed that the five antibodies of the present disclosure bind specifically to HER2 among the human ErbB family proteins.

Example 6

Comparison of Epitopes of Developed Antibody and Trastuzumab

[0267] It is known that the anti-HER2 antibody trastuzumab binds to the domain 4 among the four domains of HER2 ECD. In order to investigate whether the developed antibodies and trastuzumab share the epitope for HER2, epitope binning was conducted using Octet (Pall ForteBio). Trastuzumab was immobilized onto an AR2G sensor chip (ForteBio, Cat. Nos. 18-5092 (tray), 18-5093 (pack), 18-5094 (case)) at a concentration of 10 μg/mL by amine coupling using ECD/NHS. After allowing the HER2-ECD-His protein to bind to the trastuzumab-immobilized sensor chip at a concentration of 10 μg/mL for 10 minutes, the binding between trastuzumab and HER2-ECD was stabilized for 5 minutes. Then, the five antibodies of the present disclosure were bound at a concentration of 10 μg/mL for 10 minutes and the binding between the antigen and the antibodies was stabilized for 10 minutes. After the immobilization of trastuzumab, all the antibodies and antigen were diluted using a kinetics buffer (ForteBio, Cat No. 18-1092). The same buffer was used during the stabilization. If the additionally added antibody binds to the trastuzumab-bound HER2-ECD protein, it can be interpreted that the antibody does not share the epitope with trastuzumab.

[0268] The result is shown in FIGS. **5***a*-**5***e*.

[0269] As seen from FIGS. **5***a*-**5***e*, it was confirmed that the developed antibodies hz2G10, hz39D2, 24D3 and hz8G11 had different epitopes from that of trastuzumab because they were bound to the trastuzumab-bound HER2-ECD. In contrast, 1G3 did not bind to the trastuzumab-bound HER2-ECD, suggesting that it shares the epitope with trastuzumab.

Example 7

Development of hz39D2 Antibody with Increased Affinity

[0270] In order to develop antibodies with improved affinity based on the humanized 39D2 antibody (hz39D2), the inventors of the present disclosure have developed a phage antibody library with CDR3 of the light chain or heavy chain randomized (Phage display: a laboratory manual, Carlos Barbas III, et al., Cold Spring Harbor Laboratory Press). D and Y corresponding to D101 and Y102 of the CDR3 of the heavy chain according to Kabat numbering and P, W and T corresponding to P95, W96, T97 of the CDR3 of the light chain according to Kabat numbering were excluded from the randomization because they are commonly observed amino acids in human antibodies. Primers were synthesized such that adenine (A), cytosine (C), guanine (G) and thymine (T) were inserted randomly into the first and second positions of the codon corresponding to the amino acid to be randomized, with the same ratio, and guanine (G) or cytosine (C)

was inserted into the third position at the same ratio. From the developed library, the clones with improved affinity were selected through biopanning using the HER2-ECD-His protein. The CDR sequence data of the finally selected three antibodies, hz39D2.14, hz39D2.22 and hz39D2.23, are summarized in Table 6 and Table 7. The amino acid residues modified to improve affinity are underlined.

TABLE 6

Clones	CDRH1	CDRH2	CDRH3
hz39D2	NYGVN (SEQ ID NO 7)	WINTHTGEP TYAEEFKG (SEQ ID NO 8)	DDYYVRV DY (SEQ ID NO 9)
hz39D2.14	NYGVN (SEQ ID NO 7)	WINTHTGEP TYAEEFKG (SEQ ID NO 8)	D**E**YYVRT DY (SEQ ID NO 71)
hz39D2.22	NYGVN (SEQ ID NO 7)	WINTHTGEP TYAEEFKG (SEQ ID NO 8)	D**E**YYVRV DY (SEQ ID NO 72)
hz39D2.23	NYGVN (SEQ ID NO 7)	WINTHTGEP TYAEEFKG (SEQ ID NO 8)	D**E**YYVRV DY (SEQ ID NO 73)

TABLE 7

Clones	CDRL1	CDRL2	CDRL3
hz39D2	KASQDIN SYLS (SEQ ID NO 10)	RANR LVD (SEQ ID NO 11)	LQYDEFP WT (SEQ ID NO 12)
hz39D2.14	KASQDIN SYLS (SEQ ID NO 10)	RANR LVD (SEQ ID NO 11)	LQYDEFP WT (SEQ ID NO 12)
hz39D2.22	KASQDIN SYLS (SEQ ID NO 10)	RANR LVD (SEQ ID NO 11)	L**EL**DEFP WT (SEQ ID NO 73)
hz39D2.23	KASQDIN SYLS (SEQ ID NO 10)	RANR LVD (SEQ ID NO 11)	LQ**L**DEFP WT (SEQ ID NO 74)

[0271] IgG antibodies were produced to verify the increased affinity of the three selected antibodies (hz39D2.14, hz39D2.22 and hz39D2.23). 2000 RU of goat anti-human IgG (Invitrogen, #H10500) was immobilized onto a CM5 sensor chip by ECD/NHS. Then, the antibodies were allowed to bind at a rate of 50 μL/min for 5 minutes and then stabilized for 5 minutes by flowing a buffer. After stabilizing the antibodies, the HER2-ECD-His protein was allowed to bind at a rate of 50 μL/min for 4 minutes and then separated by flowing a buffer for 15 minutes. After analyzing the concentration, the resultant was recycled using 10 mM glycine (pH 1.5) and then subjected to the subsequent assay. The affinity of the antibodies was analyzed using the BIAevaluation software. The analysis result is summarized in Table 8. As seen from Table 8, all of the three selected antibodies (hz39D2.14, hz39D2.22 and hz39D2.23) showed improved affinity as compared to hz39D2.

TABLE 8

Clones	K_a (1/Ms)	K_d (1/s)	K_D (M)
hz39D2	6.8E+04	2.5E−03	3.7E−08
hz39D2.14	3.7E+04	3.0E−04	8.0E−09
hz39D2.22	8.1E+04	1.6E−04	2.0E−09
hz39D2.23	7.1E+04	2.0E−04	2.8E−09

[0272] The anticancer effect of the three antibodies (hz39D2.14, hz39D2.22 and hz39D2.23) with improved affinity was analyzed using HER2-overexpressed NCI-N87 and 0E-19 gastric cancer cells and BT-474 breast cancer cells. After treating the cells with each antibody at a concentration of 5μg/mL either alone or in combination with trastuzumab, the viability of the cancer cells was analyzed (FIGS. **6***a*-**6***c*). As seen from FIGS. **6***a*-**6***c*, it was confirmed that the hz39D2.14, hz39D2.22, and hz39D2.23 antibodies with improved affinity showed improved effect of inhibiting cancer cell proliferation when treated alone.

Example 8

Construction of Chimeric Antigen Receptors (CAR) Targeting Human HER2

[0273] Various CAR domain structures were constructed, cloned into lentivral expression systems, expressed in Umbilical Cord Blood-derived Natural Killer cells and tested in assays of tumor cell engagement and cytotoxic killing. The Anti-Her2 antibody disclosed herein was constructed as a scFv structure and used in combination with various CAR costimulatory domains as described herein.

[0274] Plasmid Construction: A signal sequence comprising the scFv of Her2 specific hz39D2 (VL-GS linker-VH), a hinge and transmembrane domain of CD8a, and intracellular domains of 4-1BB, OX40, OX40 ligand, and CD3t were each independently synthesized. These molecules were assembled in various combinations using splicing by overlap extension PCR (SOE-PCR). The sequences of the PCR products were confirmed by direct sequencing. Each PCR product was cut into Nhe1 and EcoRI, and then inserted into Nhe1 and EcoRI sites of a 3rd generation self-inactivating lentiviral expression vector such as MSCV-EF1α-GFP vector or EF1a-MCS vector.

[0275] Her2-Z CAR (Clone #2) (SEQ ID NO: 129) was produced by connecting: the signal sequence domain of CD8a (nucleotides 890-952, GenBank NM 001768.6, SEQ ID NO: 112); the extracellular domain of Her2 specific hz39D2 scFv (VL-GS linker-VH) (SEQ ID NO: 114); human CD8a-derived hinge and transmembrane domains (nucleotides 1292-1507, GenBank NM 001768.6, SEQ ID NO: 116 and SEQ ID NO: 118); CD3ζ-derived intracellular signaling domain (nucleotides 299-634, GenBank NM 000734.3, SEQ ID NO: 122); and stop codon TGA.

[0276] Her2-BBZ CAR (Clone #3) (SEQ ID NO: 131) was produced by connecting: the signal sequence domain of CD8a (nucleotides 890-952, GenBank NM 001768.6, SEQ ID NO: 112); the extracellular domain of Her2 specific hz39D2 scFv (VL-GS linker-VH) (SEQ ID NO: 114); human CD8a-derived hinge and transmembrane domains (nucleotides 1292-1507, GenBank NM 001768.6, SEQ ID NO: 116 and SEQ ID NO: 118); CD137 (4-1BB)-derived

intracellular signaling domain (nucleotides 901-1026, GenBank NM 001561.5, SEQ ID NO: 124); CD3-derived intracellular signaling domain (nucleotides 299-634, GenBank NM 000734.3, SEQ ID NO: 122; and stop codon TGA.

[0277] Her2-28Z CAR (Clone #6) (SEQ ID NO: 133) was produced by connecting the signal sequence domain of CD8a (nucleotides 890-952, GenBank NM 001768.6, SEQ ID NO: 112); the extracellular domain of Her2 specific hz39D2 scFv (VL-GS linker-VH) (SEQ ID NO: 114); human CD8a-derived hinge domain (nucleotides 1292-1435, GenBank NM 001768.6, SEQ ID NO: 116; CD28-derived transmembrane and intracellular signaling domains (nucleotides 679-882, GenBank NM 006139.3, SEQ ID NO: 120 and SEQ ID NO: 126); CD3-derived intracellular signaling domain (nucleotides 299-634, GenBank NM 000734.3, SEQ ID NO: 122); and stop codon TGA.

[0278] Her2-28OX40LZ CAR (Clone #14) (SEQ ID NO: 135) was produced by connecting: the signal sequence domain of CD8a (nucleotides 890-952, GenBank NM 001768.6, SEQ ID NO: 112); the extracellular domain of Her2 specific hz39D2 scFv (VL-GS linker-VH) (SEQ ID NO: 114); CD8a-derived hinge domain (nucleotides 1292-1435, GenBank NM 001768.6, SEQ ID NO: 116); CD28-derived transmembrane and intracellular signaling domains (nucleotides 679-882, GenBank NM 006139.3, SEQ ID NO: 120 and SEQ ID NO: 126); CD252 (OX40 ligand)-derived intracellular signaling domain (nucleotides 141-206, GenBank NM 003326.4, SEQ ID NO: 128); CD3-derived intracellular signaling domain (nucleotides 299-634, GenBank NM 000734.3, SEQ ID NO: 122); and stop codon TGA.

[0279] The structures of the HER2 CAR constructs as disclosed herein are summarized in FIG. **7**. The domains of the CARs disclosed herein were linked in series (in tandem) to one another and linked in frame. Virus Production and Gene Transfer: To prepare VSVG-pseudotyped lentivirus, 293T cells cultured in a DMEM medium were co-transfected with various types of vectors such as PCDH1-MSCV-Her2 specific hz39D2 scFv-construct-EF1-copGFP vector, EF1a-Her2 specific hz39D2 scFv-construct vector, PCDH1-MSCV-EF1-copGFP control vector, or EF1a-GFP control vector (for production of Mock infection virus using empty vector) together with HIV-based pPACKH1 lentivirus Package Kit (System Biosciences). For this purpose, Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) was used. Each lentivirus was prepared by transfection of 80% dense HEK293T cells in a flask with the various Her2 specific hz39D2 scFv construct expression vectors or a control plasmid together with pPACKH1 lentivirus packaging plasmids. After 6 hours, the medium was replaced by a DMEM medium containing 10% FBS. The conditioned medium containing lentivirus was collected after 48 hours of transfection, followed by filtering with a 0.45 μm filter unit (Milliopore, Billerica, MA, USA) in order to remove cell debris. A viral supernatant containing the virus was concentrated about 50 times by centrifugation at 3000 rpm and 4° C. for 20 minutes using Amicon Filter (Millipore). The concentrated virus was stored at −80° C.

[0280] For the lentiviral infection, PBMC derived NK cells or cord blood derived NK cells in an exponential growth phase were adjusted to a concentration of 1×10^6 cells/ml using Cellgro (Cellgenix) including 1% human plasma and 500 IU/mL interleukin-2, and then a lentiviral supernatant in 10 to 50 MOI was added in the presence of 4 μM BX795, 1:400 Lentiboost and 20 ng/ml IL-21 followed by centrifugation at 1000 g for 60 minutes. After centrifugation, the cells were left in a humidified incubator at 37° C. and 5% CO_2 conditions. After 24 hours of transduction, the culture medium was replaced: plate was centrifuged at 400 g for 5 minutes and transduction medium was aspirated. Equal volume fresh Cellgro (Cellgenix) including 1% human plasma and 500 IU/mL interleukin-2 was added for future use. Control cells were transduced with a vector only.

[0281] Expression Analysis of Anti-HER2-CAR (hz39D2 scFv): Her2 specific hz39D2 scFv CAR-transduced NK cells, the control vector-transduced NK (NK-Mock) or NK parent cells were washed twice with FACS buffer, and the washed cells were stained using 7-AAD (Beckman coulter), anti-CD3, anti-CD56, and recombinant histidine tagged human HER2 proteins (R&D systems) with PE-conjugated anti-histidne (Abcam) mAbs. An expression ratio and a mean fluorescence intensity (MFI) of the stained cells were measured using a BD LSRFortessa.

[0282] First, NK cells were gated in regard to singlet, and then gated in regard to 7AAD-and CD3-CD56+. The transduction efficiency of the Her2 specific hz39D2 scFv CAR constructs was determined by flow cytometric analysis of cells expressing CAR among CD3-CD56+ cells.

Example 9

Cytotoxic Activity of Anti-Her2 CAR Constructs in NK cells

[0283] Cells: The human breast cancer cell line HCC1954, SKBR3 and MDA-MB 468, ovarian cancer cell line SKOV-3, gastric cancer cell line N87 and human erythroleukemic cell line K562 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). HCC1954, N87, SKOV-3 and K562 were maintained in RPMI-1640 (ATCC) (Manassas, VA) with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA). SKBR3 and MDA-MB 468 were maintained in DMEM (Gibco, Grand Island, NY, USA) with 10% FBS. PBMC or cord blood derived NK cells and transduced NK cells were maintained in CellGro® serum-free media +1% human plasma+500 IU/mL interleukin-2. Human embryonic kidney fibroblast 293T cells were obtained from the ATCC and maintained in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% FBS % (Gibco, Grand Island, NY, USA).

[0284] Calcein Releasing Cytotoxicity Assay: Target cells were labeled at 37° C. for 1 hour with 30 μM calcein-acetoxymethyl ester (Calcein-AM; Molecular probes). After washing, the labeled target cells were dispensed to 1×10^4 cells per well in 96-well plates. Control or CAR transduced NK cells were harvested, washed, and then were added at different E/T (effector-to-target) ratios. After 2 hours, the plates were centrifuged at 2000 rpm for 3 minutes, and a supernatant of 100 μL was collected and subjected to measurement of calcein release using a fluorescence microplate reader (Victor3, PerkinElmer) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Specific calcein release amount was calculated by the following equation: percent specific lysis =(test release-spontaneous release)×100/(maximal release−spontaneous release). For maximal lysis, a 1% Triton X-100 solution was used.

[0285] Comparison of CAR Constructs in vitro Her2+Cell Killing: The cell killing activities of clone #2, #6 and #14 were compared against various target cells. Cord-blood derived NK (CBNK) cells were transduced with each construct using lentiviral vector system at 40 multiplicity of infection (MOI) at day 7 after culture, followed on day 11 by a positive magnetic activated cell sorting (MACS) process for HER-2-CAR-NK expressing cells. As controls, we used unmodified CBNK and mock-transduced CBNK that was transduced with lentivirus vector harboring GFP gene instead of the CAR genes. Killing assay (Calcein releasing cytotoxicity assay) was performed using HER2 positive target cancer cell lines: SKBR3 (breast cancer), HCC1954 (breast cancer, trastuzumab resistant), NCI-N87 (gastric cancer) at different ratios of effector to target cells (5:1, 2.5:1, 1:1). All of the HER-2-CAR-NKs showed higher cytotoxicity to HER-2 expressing target cells than non-transduced or mock vector transduced NK cells. Clone #14 showed unexpectedly the highest cytotoxicity to all three target cells (FIGS. **8**A-8C).

[0286] The killing activity of clone #14 was further assessed by testing its cytotoxicity to various cancer cell lines: K562 (lymphoblast, HER-2 negative, but NK susceptible control), MDA-MB-468 (breast cancer, HER-2 negative), SKOV3 (ovarian cancer, HER-2 positive), NCI-N87 (gastric cancer, HER-2 positive), and trastuzumab-resistant cell line HCC1954 (breast cancer, HER-2 positive). The cytotoxicity of clone #14 was compared with unmodified CBNK at various ratios of effector to target cells (10:1, 3:1, 1:1 & 0.3:1). Clone #14 showed significantly higher and unexpected cytotoxicity against HER2 positive cancer cell lines than unmodified CBNKs. HER2-CAR-NK was also active on HCC1954 (breast cancer, HER-2 positive), which has previously seen to be resistant to trastuzumab mAb treatment (FIGS. **9**A-**9**E).

[0287] In vitro Long-Term Her2+Cell Killing: The long-term serial killing activity of clones #6 & #14 was assessed using the Incucyte live cell imaging system. NCI-N87 Her2+gastric carcinoma target cells were grown and monitored in the Incucyte system for 6 days in the presence of: control, non-specific IgG, trastuzumab (anti-Her2 monoclonal antibody), CBNK cells, CBNK cells in combination with trastuzumab, or CBNK transduced with clone #14 CAR construct. The experiment was completed at effector to the target (E:T) cellular ratios of 1:1 and 0.3:1. Unexpectedly, clone #14 killed significantly more Her2+target cells than either CBNKs or CBNK in combination with tastuzumab for both the E:T 1:1 and 0.3:1 conditions (FIGS. **10**A and **10**B).

Example 10

NK Cell Activation by Anti-Her2-CAR Constructs in Response to Target Cells

[0288] Intercellular Cytokine Staining (ICS) Assay for CD107a, IFN-γ, and TNF-α: To measure intracellular cytokines and CD107a of NK cells, NK cells were co-cultured with tumor targets at 1:1 ratio for 4 h in the presence of anti-CD107a-APC (H4A3; BD Biosciences, USA), GolgiStop™ and GolgiPlug™ (BD Bioscience, USA). After 4 hours, cells were washed with BD FACS flow buffer and stained with anti-CD3-FITC, anti-CD56-APC-eFluor®780, and 7-AAD permeabilized by BD CytoFix/CytoPerm™ and then stained with anti-IFN-γ-PE (B27; BD Biosciences) and anti-TNF-α-PE-Cy7 (Mab 11; eBioscience). Stained cells were acquired on LSR Fortessa and data analysis was conducted using FlowJo software (TreeStar Inc., OR).

[0289] NK cell degranulation activity and cytotoxic cytokine expression with the various

[0290] CAR constructs was evaluated by comparing intercellular expression level of CD107a & IFN-γ. Umbilical Cord blood-derived NK (CBNK) cells transduced with clone #3, clone #14 lentivirus were compared with control CBNK cells and CBNK cells with mock lentiviral transduction. NK cells expressing clone #3, clone #14 and control cells were co-cultured with target cancer cells: K562 (HER2-negative, but susceptible to NK cell), NCI-N87, SKOV3, HCC1954, MKN74 (HER-2 negative), and MDA-MB-468 (HER-2 negative) at 1:1 ratio of effector to target cells for 2hrs, followed by FACS analysis gating CD56+/CD107a or CD56+/IFN-γ. Both clones #3 & #14 showed an increase in intercellular expression of CD107a & IFN-γ in response to HER-2 positive target cells. Unexpectedly, clone #14 showed consistently greater degranulation activity and IFN-γ expression than clone #3 (FIG. **11**A and FIG. **11**B).

[0291] A similar assay was performed comparing Clone #6 and Clone #14 transduced CBNK cells with mock transduced CBNK cells. Again, degranulation and IFN-γ expression was unexpectedly greater in the anti-Her2 CAR transduced CBNK cells compared with control cells in response to Her2+target cells. Further, intracellular expression of TNF-a was unexpectedly greater in the anti-Her2 CAR transduced CBNK cells compared with Control cells in response to Her2+target cells (FIG. **12**A, FIG. **12**B, FIG. **12**C).

[0292] IFN-γ Secretion: The secretion of IFN-γ from anti-HER2-CAR-CBNKs (clones #2, #6, #14) was compared by co-culturing with target cells. Cell culture supernatants from the 4 hr killing assay (FIG. **8**A-**8**C) were assayed for secreted IFN-γ using an ELISA assay. CBNK cells expressing Clone #14 unexpectedly secreted significantly more IFN-γ when co-cultured with target cancer cells than all other clones and controls. Clone #6 consistently secreted more IFN-γ in response to target cancer cells in comparison with control cells and Clone #2 (FIG. **13**).

Example 11

In Vivo Anti-Tumor Activity of Anti-Her2-CAR-NK Cells

[0293] To evaluate in vivo efficacy of anti-Her2-CAR clones #3 and #14, Xenograft models in NSG mice were generated by injecting 5×10^6 HCC1954 cells/mouse (HER-2 positive, trastuzumab resistant cells) subcutaneously at Day 0. Subsequently, 2×10^7 cells of clone #3, #14, or mock-transduced CBNK were administered to the mice subcutaneously at day 0, 3, 7, 10. Additionally, Human IL-15 (10 ng/head) was intraperitoneally injected 8 times every 1~2 days up to day 10 and human IL-2 (1 x 104 IU/head) was intraperitoneally injected 13 times every 2-4 days up to day 34. Tumor volume was assessed every 3-4 days after injection. Both clone #3 & #14 significantly suppressed tumor growth in mice on comparison with control, Mock-transduced CBNKs. Unexpectedly, clone #14 showed significantly better suppression of tumor growth than clone #3 (FIG. **14**A and FIG. **14**B).

Example 12

Culturing and Characterization of Anti-Her2-CAR-NK Cells

[0294] NK Cell Isolation From Umbilical Cord Blood or Peripheral Blood: CD3+cells were removed by magnetic sorting system VarioMACS (Miltenyi Biotec, Germany) for NK cell enrichment from healthy donor derived UCB or PBMCs.

[0295] Generation of Feeder Cell Lines: HuT 78 cells were transduced with 4-1BBL, mTNF-α, or mblL-21 in combination. The 4-1BBL insert was prepared from 4-1BB expressing vector (Origene, USA) by PCR. OX40L was synthesized from Bioneer (South Korea). mblL-21 was synthesized with the sequence of IL-21 active protein, CD8 signal peptide, CD8 hinge, and CD8 transmembrane and further codon optimized. cDNA of mTNF-a was prepared by reverse transcription-polymerase chain reaction (RT-PCR) from PBMCs. TNF-a converting enzyme (TACE) recognition site mutation was introduced by replacing Ala-Val (A-V) with Pro-Val (P-V) by site directed mutagenesis kit (Agilent Technologies, USA). Inserted genes and lentiviral vectors (SBI, USA) were digested by EcoRI and BamHI (New England BioLabs, USA) and ligated by In-Fusion HD cloning kit (Clontech, USA). Lentiviral concentrate was produced in 293T by lipofectamine 2000 (Thermofisher Scientific, USA) and concentrated by Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membrane (Merckmillipore, USA). 0.5×10^6 cells/mL HuT 78 cells were suspended in 1 mL OPTU-MEM containing 50 uL lentiviral concentrate and 10 μg/mL polybrene (Santa Cruz Biotechnology, USA) and spinoculated at 1800 g, 32° for 90 minutes. HuT 78 cells transduced with lentiviral system were selected with antibiotics. 4-1BBL/mTNF-α mblL-21 positive HuT 78 cells were isolated by flow cytometry-guided sorting (FACSMelody™ Cell Sorter, BD bioscience, USA).

[0296] Ex vivo Expansion and Cryopreservation of NK Cells: CD3+ depleted cells (1×10^6 cells/mL) were seeded in CellGro SCGM medium (CellGenix, Germany) containing 1%~2% donor-plasma, γ-irradiated (2,000 rad) eHuT 78 (2.5×10^6 cells/mL) in CellGro SCGM medium containing 2% donor plasma, 1000 IU/mL IL-2 and 10 ng/mL anti-CD3 monoclonal antibody OKT3. Depending on culture duration, eHuT 78 cells were stimulated every 2 weeks. Cultured cells were fed with Cellgro SCGM containing 1% donor-plasma and 1000 IU/mL IL-2 (culture medium) to maintain cell concentration of $1\text{-}2\times10^6$ cells/mL for 14 days or 28 days. For cryopreservation of NK cells, harvested cells were suspended in freezing media and stored in a liquid nitrogen tank.

[0297] Immunostaining and Flow Cytometric Analysis: The following monoclonal antibodies were used to stain NK cells: anti-CD56-APC-eFluor®780 (CMSSB), and anti-CD62L-PE (DREG-56) (eBioscience, USA), anti-CD3-FITC (UCHT1), anti-CD14-FITC (M5E2) anti-CD16-PE (3G8), anti-DNAM-1-PE (DX11), anti-CD25-PE (M-A251), anti-CD44-PE (515), anti-CD56-PE-Cy5 (B159), anti-CXCR3-PE(1C6/CXCR3), anti-NKp3O-PE (P30-15), anti-NKp44-PE (P44-8.1), anti-NKp46-PE (9E2/NKp46) antiOX40L-PE (ik-1), anti-4-1BBL-PE (C65-485), anti-4-1BB-PE (C65-485), anti-OX40-PE (ACT35), anti-CD27-PE (MT-271), anti-CD27L-PE (Ki-24), anti-CD30-PE (BerH8), anti-CD3OL-PE, anti-CD3-PE-cy5.5 (SP34-2), anti-CD4-FITC (RPA-T4) (BD Biosciences, USA), anti-NKG2A-PE (131411), anti-NKG2C-PE (134591), anti-NKG2D-PE (149810), anti-TNF-a (membrane)-PE (6401) anti-TNF-a (membrane)-PE (6401), anti-TNFRII-PE (22235) (R&D systems, USA), anti-CD3OL-PE (RM153) (Biolegend, USA). Live cells were gated with 7-AAD (Beckman-Coulter, USA). T cells and HuT 78 cells were stained with anti-TcRα/β-FITC (WT31), anti-CD2-PE (RPA-2.10), anti-CD7-FITC (4H9), anti-CD11a-FITC (G43-258), anti-CD25-PE (M-A251), anti-CD28-FITC (CD28.2), anti-CD44-PE (515) and anti-CD49d-PE (9F10) (BD Bioscience, USA). e-HuT 78 cells were stained with anti-TNF-a (membrane)-PE (6401), (R&D systems, USA), antiOX40L-PE (ik-1), anti-4-1BBL-PE (C65-485) (BD bioscience, USA), and anti-IL21-PE (3A3-N2) (eBioscience, USA). Stained cells were acquired on LSR Fortessa and data were analyzed using FlowJo software (TreeStar Inc., OR).

[0298] Comparison of Proliferation of HER2-CAR-NK Cells: The growth of CBNK cells, mock-transduced CBNK cells, or HER-2-CAR clones #6 and #14 was compared in culture for 28 days. During the culture period, cells were stimulated with irradiated feeder cells (eHut 78) twice at day 0 & day 14. Both clone #6 and #14 showed comparable growth pattern to unmodified and mock-transduced CBNKs (FIG. **15**A).

[0299] Assessment of CAR Expression During Expansion, Culture, and Cryopreservation: To assess the CAR expression level in Lentiviral transduced CBNK cells, the HER-2 CAR population was analyzed by flow cytometry at day 11, day 16, day 24, day 28 and after freezing and thawing. Both clone #6 and #14 maintained more than 80% of CAR expression up to 28 day and through freezing and thawing (FIG. **15**B).

[0300] Viability of HER2-CAR-NK Cells During Expansion, Culture, and Cryopreservation: The cell viability for HER-2CAR-NK was analyzed after 28 day-culture and freezing and thawing using propidium iodide staining-based live cell counting. Both clone #6 and #14 showed more than 95% of cell viability and no viability changes were observed in the process of freezing and thawing at 28 days after culture (FIG. **15**C and FIG. **15**D).

[0301] Purity of HER2-CAR-NK Cells During Expansion Culture and Cryopreservation: The cell purity for HER-2CAR-NK was analysed after expansion culture and the freezing and thawing process using flow cytometry analysis. Counts for CD56+/CD3− cells were assessed. Both clone #6 and #14 maintained near 100% of CD56+/CD3− cell population at 28 days after culture and post the process of freezing and thawing process (FIG. **15**D).

[0302] Phenotypic Cell Surface Marker Expression Analysis of HER2-CAR-NK Cells: The expression NK cell surface markers on unmodified, mock-transduced CBNK and clone #6 and #14 was assessed by flow cytometry analysis (CD16, NKG2A, NKG2C, NKG2D, NKp30, NKp44, NKp46, CD25, CD62L, CD69, CXCR3, CD57). The expression levels of all markers tested in both HER2-CAR-NK cells expressing clones #6 and #14 were comparable to those of control NK cells (FIG. **16**).

SEQUENCE LISTING

Sequence total quantity: 136

SEQ ID NO: 1 moltype = AA length = 5
FEATURE Location/Qualifiers
REGION 1..5
note = CDRH1 of hz2G10 antibody
source 1..5
mol_type = protein
organism = synthetic construct
SEQUENCE: 1
DYYMY 5

SEQ ID NO: 2 moltype = AA length = 17
FEATURE Location/Qualifiers
REGION 1..17
note = CDRH2 of hz2G10 antibody
source 1..17
mol_type = protein
organism = synthetic construct
SEQUENCE: 2
YINSGGGSTY YPDTVKG 17

SEQ ID NO: 3 moltype = AA length = 12
FEATURE Location/Qualifiers
REGION 1..12
note = CDRH3 of hz2G10 antibody
source 1..12
mol_type = protein
organism = synthetic construct
SEQUENCE: 3
EALYDYDYAM DY 12

SEQ ID NO: 4 moltype = AA length = 16
FEATURE Location/Qualifiers
REGION 1..16
note = CDRL1 of hz2G10 antibody
source 1..16
mol_type = protein
organism = synthetic construct
SEQUENCE: 4
KSSQSLLYSN GKTYLN 16

SEQ ID NO: 5 moltype = AA length = 7
FEATURE Location/Qualifiers
REGION 1..7
note = CDRL2 of hz2G10 antibody
source 1..7
mol_type = protein
organism = synthetic construct
SEQUENCE: 5
LVSKLDS 7

SEQ ID NO: 6 moltype = AA length = 9
FEATURE Location/Qualifiers
REGION 1..9
note = CDRL3 of hz2G10 antibody
source 1..9
mol_type = protein
organism = synthetic construct
SEQUENCE: 6
VQGTHFPLT 9

SEQ ID NO: 7 moltype = AA length = 5
FEATURE Location/Qualifiers
REGION 1..5
note = CDRH1 of hz39D2 antibody
source 1..5
mol_type = protein
organism = synthetic construct
SEQUENCE: 7
NYGVN 5

SEQ ID NO: 8 moltype = AA length = 17
FEATURE Location/Qualifiers
REGION 1..17
note = CDRH2 of hz39D2 antibody

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```
source                  1..17
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 8
WINTHTGEPT YAEEFKG                                          17

SEQ ID NO: 9            moltype = AA  length = 9
FEATURE                 Location/Qualifiers
REGION                  1..9
                        note = CDRH3 of hz39D2 antibody
source                  1..9
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 9
DDYYVRVDY                                                   9

SEQ ID NO: 10           moltype = AA  length = 11
FEATURE                 Location/Qualifiers
REGION                  1..11
                        note = CDRL1 of hz39D2 antibody
source                  1..11
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 10
KASQDINSYL S                                                11

SEQ ID NO: 11           moltype = AA  length = 7
FEATURE                 Location/Qualifiers
REGION                  1..7
                        note = CDRL2 of hz39D2 antibody
source                  1..7
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 11
RANRLVD                                                     7

SEQ ID NO: 12           moltype = AA  length = 9
FEATURE                 Location/Qualifiers
REGION                  1..9
                        note = CDRL3 of hz39D2 antibody
source                  1..9
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 12
LQYDEFPWT                                                   9

SEQ ID NO: 13           moltype = AA  length = 5
FEATURE                 Location/Qualifiers
REGION                  1..5
                        note = CDRH1 of 24D3 antibody
source                  1..5
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 13
SCAMS                                                       5

SEQ ID NO: 14           moltype = AA  length = 17
FEATURE                 Location/Qualifiers
REGION                  1..17
                        note = CDRH2 of 24D3 antibody
source                  1..17
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 14
TISGGGSYTY YPDSVKG                                          17

SEQ ID NO: 15           moltype = AA  length = 10
FEATURE                 Location/Qualifiers
REGION                  1..10
                        note = CDRH3 of 24D3 antibody
source                  1..10
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 15
HGGYESWFPY                                                  10
```

-continued

```
SEQ ID NO: 16          moltype = AA  length = 16
FEATURE                Location/Qualifiers
REGION                 1..16
                       note = CDRL1 of 24D3 antibody
source                 1..16
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 16
RSSQSLVHSN GNTYLH                                          16

SEQ ID NO: 17          moltype = AA  length = 7
FEATURE                Location/Qualifiers
REGION                 1..7
                       note = CDRL2 of 24D3 antibody
source                 1..7
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 17
KVSNRFS                                                    7

SEQ ID NO: 18          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = CDRL3 of 24D3 antibody
source                 1..10
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 18
SQSTHVPPWT                                                 10

SEQ ID NO: 19          moltype = AA  length = 5
FEATURE                Location/Qualifiers
REGION                 1..5
                       note = CDRH1 of 1G3 antibody
source                 1..5
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 19
DTYMH                                                      5

SEQ ID NO: 20          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = CDRH2 of 1G3 antibody
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 20
RIDPANGYTR YDPNFQG                                         17

SEQ ID NO: 21          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = CDRH3 of 1G3 antibody
source                 1..10
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 21
YYYGFYAMDY                                                 10

SEQ ID NO: 22          moltype = AA  length = 11
FEATURE                Location/Qualifiers
REGION                 1..11
                       note = CDRL1 of 1G3 antibody
source                 1..11
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 22
KASQDVSTAV A                                               11

SEQ ID NO: 23          moltype = AA  length = 7
FEATURE                Location/Qualifiers
REGION                 1..7
                       note = CDRL2 of 1G3 antibody
source                 1..7
                       mol_type = protein
```

-continued

```
                    organism = synthetic construct
SEQUENCE: 23
SASYRYT                                                          7

SEQ ID NO: 24       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = CDRL3 of 1G3 antibody
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 24
QQHYSTPPT                                                        9

SEQ ID NO: 25       moltype = AA  length = 5
FEATURE             Location/Qualifiers
REGION              1..5
                    note = CDRH1 of hz8G11 antibody
source              1..5
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 25
GYYMH                                                            5

SEQ ID NO: 26       moltype = AA  length = 17
FEATURE             Location/Qualifiers
REGION              1..17
                    note = CDRH2 of hz8G11 antibody
source              1..17
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 26
HINPNNGGTS YNQKFKG                                               17

SEQ ID NO: 27       moltype = AA  length = 6
FEATURE             Location/Qualifiers
REGION              1..6
                    note = CDRH3 of hz8G11 antibody
source              1..6
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 27
EEAFAY                                                           6

SEQ ID NO: 28       moltype = AA  length = 11
FEATURE             Location/Qualifiers
REGION              1..11
                    note = CDRL1 of hz8G11 antibody
source              1..11
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 28
RASQDISNYL N                                                     11

SEQ ID NO: 29       moltype = AA  length = 7
FEATURE             Location/Qualifiers
REGION              1..7
                    note = CDRL2 of hz8G11 antibody
source              1..7
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 29
YTSRLHS                                                          7

SEQ ID NO: 30       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = CDRL3 of hz8G11 antibody
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 30
QQGITPPWT                                                        9

SEQ ID NO: 31       moltype = AA  length = 121
FEATURE             Location/Qualifiers
```

-continued

```
REGION                  1..121
                        note = Amino acid sequence of hz2G10 heavy chain variable
                         region
source                  1..121
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 31
EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYYMYWVRQA PGKGLEWVAY INSGGGSTYY  60
PDTVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAREA LYDYDYAMDY WGQGTTVTVS  120
S                                                                  121

SEQ ID NO: 32           moltype = DNA  length = 363
FEATURE                 Location/Qualifiers
misc_feature            1..363
                        note = Nucleic acid sequence of hz2G10 heavy chain variable
                         region
source                  1..363
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 32
gaggtgcagt tggtcgagtc tggaggaggt ctggtacagc caggggaag tctgagactg  60
agctgcgccg cttctggttt tacctttagc gattactata tgtattgggt aagacaggca  120
cctggtaaag gtttggaatg ggtggcctac ataaactcgg gcgggggcag cacctactac  180
ccggataccg tgaagggccg cttcaccatc tcccgagaca acgcgaaaaa ttcattgtat  240
ctgcaaatga actcacttag agctgaagat actgccgttt actactgcgc cagagaagca  300
ctctatgact atgattacgc tatggattac tgggggcagg gcacaaccgt cactgtttct  360
agt                                                                363

SEQ ID NO: 33           moltype = AA  length = 451
FEATURE                 Location/Qualifiers
REGION                  1..451
                        note = Amino acid sequence of hz2G10 heavy chain
source                  1..451
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 33
EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYYMYWVRQA PGKGLEWVAY INSGGGSTYY  60
PDTVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAREA LYDYDYAMDY WGQGTTVTVS  120
SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS  180
SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKVE PKSCDKTHTC PPCPAPELLG  240
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY  300
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD  360
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR  420
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K                                 451

SEQ ID NO: 34           moltype = DNA  length = 1353
FEATURE                 Location/Qualifiers
misc_feature            1..1353
                        note = Nucleic acid sequence of hz2G10 heavy chain
source                  1..1353
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 34
gaggtgcagt tggtcgagtc tggaggaggt ctggtacagc caggggaag tctgagactg  60
agctgcgccg cttctggttt tacctttagc gattactata tgtattgggt aagacaggca  120
cctggtaaag gtttggaatg ggtggcctac ataaactcgg gcgggggcag cacctactac  180
ccggataccg tgaagggccg cttcaccatc tcccgagaca acgcgaaaaa ttcattgtat  240
ctgcaaatga actcacttag agctgaagat actgccgttt actactgcgc cagagaagca  300
ctctatgact atgattacgc tatggattac tgggggcagg gcacaaccgt cactgtttct  360
agtgcctcca ccaagggccc ctccgtgttc cctctggccc cctccagcaa gtccacctct  420
ggcggcacag ccgccctggg ctgcctggtg aaagactact tccccgagcc cgtgaccgtg  480
tcctggaact ctggcgccct gacctccggc gtgcacacct tccctgccgt gctgcagtcc  540
tccggcctgt actccctgtc ctccgtggtg accgtgccct ccagctctct gggcacccag  600
acctacatct gtaacgtgaa ccacaagccc tccaacacca aggtggacaa gaaggtggaa  660
cccaagtcct gcgacaagac ccacacctgt cccccctgcc ctgcccctga actgctgggc  720
ggaccttccg tgttcctgtt cccccaaag cccaaggaca ccctgatgat ctcccggacc  780
cccgaagtga cctgcgtggt ggtggacgtg tcccacgagg accctgaagt gaagttcaat  840
tggtacgtgg acggcgtgga agtgcacaat gccaagacca agcccagaga ggaacagtac  900
aactccacct accgggtggt gtctgtgctg accgtgctgc accaggactg gctgaacggc  960
aaagaataca agtgcaaagt ctccaacaag gccctgcctg cccccatcga aaagaccatc  1020
tccaaggcca agggccagcc ccgcgagccc caggtgtaca ccctgccccc tagccgggac  1080
gagctgacca agaaccaggt gtccctgacc tgtctggtga aaggcttcta cccctccgac  1140
attgccgtgg aatgggagtc caacggccag cccgagaaca actacaagac cacccccct  1200
gtgctggact ccgacggctc attcttcctg tactccaagc tgaccgtgga caagtcccgg  1260
tggcagcagg gcaacgtgtt ctcctgctcc gtgatgcacg aggccctgca caaccactac  1320
acccagaagt ccctgtccct gagccccggc aag                                1353
```

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```
SEQ ID NO: 35          moltype = AA  length = 112
FEATURE                Location/Qualifiers
REGION                 1..112
                       note = Amino acid sequence of hz2G10 light chain variable
                        region
source                 1..112
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 35
DIVMTQSPLS LSVTPGQPAS ISCKSSQSLL YSNGKTYLNW LLQKPGQSPQ RLIYLVSKLD  60
SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCVQGTHFP LTFGGGTKVE IK         112

SEQ ID NO: 36          moltype = DNA  length = 336
FEATURE                Location/Qualifiers
misc_feature           1..336
                       note = Nucleic acid sequence of hz2G10 light chain variable
                        region
source                 1..336
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 36
gacattgtca tgacgcagag ccccctttca ctcagcgtga ctcccggtca gcccgccagc  60
atttcctgta aaagctctca gtcgctcctg tacagcaatg gcaagactta tctgaattgg  120
ctgttacaga aaccaggcca aagccctcaa aggcttatct acctggtgag taagttagac  180
agcggggtgc ctgacagatt tagcggatct ggaagcggga ccgatttcac actaaaaatc  240
agcagggttg aggcagagga cgtgggcgtg tattattgtg tgcagggcac acacttccca  300
ctcacattcg ggggaggcac aaaggtggaa atcaag                            336

SEQ ID NO: 37          moltype = AA  length = 219
FEATURE                Location/Qualifiers
REGION                 1..219
                       note = Amino acid sequence of hz2G10 light chain
source                 1..219
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 37
DIVMTQSPLS LSVTPGQPAS ISCKSSQSLL YSNGKTYLNW LLQKPGQSPQ RLIYLVSKLD  60
SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCVQGTHFP LTFGGGTKVE IKRTVAAPSV  120
FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL  180
SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC                         219

SEQ ID NO: 38          moltype = DNA  length = 657
FEATURE                Location/Qualifiers
misc_feature           1..657
                       note = Nucleic acid sequence of hz2G10 light chain
source                 1..657
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 38
gacattgtca tgacgcagag ccccctttca ctcagcgtga ctcccggtca gcccgccagc  60
atttcctgta aaagctctca gtcgctcctg tacagcaatg gcaagactta tctgaattgg  120
ctgttacaga aaccaggcca aagccctcaa aggcttatct acctggtgag taagttagac  180
agcggggtgc ctgacagatt tagcggatct ggaagcggga ccgatttcac actaaaaatc  240
agcagggttg aggcagagga cgtgggcgtg tattattgtg tgcagggcac acacttccca  300
ctcacattcg ggggaggcac aaaggtggaa atcaagcgga ccgtggccgc tccctccgtg  360
ttcatcttcc caccctccga cgagcagctg aagtccggca ccgccagcgt ggtctgcctg  420
ctgaacaact tctacccccg cgaggccaag gtgcagtgga aggtggacaa cgccctgcag  480
tccggcaact cccaggaatc cgtcaccgag caggactcca aggacagcac ctactccctg  540
tcctccaccc tgaccctgtc caaggccgac tacgagaagc acaaggtgta cgcctgcgaa  600
gtgacccacc agggcctgtc cagccccgtg accaagtcct tcaaccgggg cgagtgc      657

SEQ ID NO: 39          moltype = AA  length = 118
FEATURE                Location/Qualifiers
REGION                 1..118
                       note = Amino acid sequence of hz39D2 heavy chain variable
                        region
source                 1..118
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 39
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY  60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDD YYVRVDYWGQ GTTVTVSS    118

SEQ ID NO: 40          moltype = DNA  length = 354
FEATURE                Location/Qualifiers
```

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misc_feature 1..354
note = Nucleic acid sequence of hz39D2 heavy chain variable region
source 1..354
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 40
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt 60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca 120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat 180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac 240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgat 300
tactatgtga gggtggatta ctgggggcag gggaccaccg tgacagtctc aagt 354

SEQ ID NO: 41 moltype = AA length = 448
FEATURE Location/Qualifiers
REGION 1..448
note = Amino acid sequence of hz39D2 heavy chain
source 1..448
mol_type = protein
organism = synthetic construct
SEQUENCE: 41
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY 60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDD YYVRVDYWGQ GTTVTVSSAS 120
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 180
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS 240
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST 300
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT 360
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ 420
GNVFSCSVMH EALHNHYTQK SLSLSPGK 448

SEQ ID NO: 42 moltype = DNA length = 1344
FEATURE Location/Qualifiers
misc_feature 1..1344
note = Nucleic acid sequence of hz39D2 heavy chain
source 1..1344
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 42
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt 60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca 120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat 180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac 240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgat 300
tactatgtga gggtggatta ctgggggcag gggaccaccg tgacagtctc aagtgcctcc 360
accaagggcc cctccgtgtt ccctctggcc ccctccagca agtccacctc tggcggcaca 420
gccgccctgg gctgcctggt gaaagactac ttccccgagc ccgtgaccgt gtcctggaac 480
tctggcgccc tgacctccgg cgtgcacacc ttccctgccg tgctgcagtc ctccggcctg 540
tactccctgt cctccgtggt gaccgtgccc tccagctctc tgggcaccca gacctacatc 600
tgtaacgtga accacaagcc ctccaacacc aaggtggaca agaaggtgga acccaagtcc 660
tgcgacaaga cccacacctg tccccctgc cctgcccctg aactgctggg cggaccttcc 720
gtgttcctgt tccccccaaa gcccaaggac accctgatga tctcccggac ccccgaagtg 780
acctgcgtgg tggtggacgt gtcccacgag gaccctgaag tgaagttcaa ttggtacgtg 840
gacggcgtgg aagtgcacaa tgccaagacc aagcccagag aggaacagta caactccacc 900
taccgggtgg tgtctgtgct gaccgtgctg caccaggact ggctgaacgg caaagaatac 960
aagtgcaaag tctccaacaa ggccctgcct gcccccatcg aaaagaccat ctccaaggcc 1020
aagggccagc cccgcgagcc ccaggtgtac accctgcccc ctagccggga cgagctgacc 1080
aagaaccagg tgtccctgac ctgtctggtg aaaggcttct accctccga cattgccgtg 1140
gaatgggagt ccaacggcca gcccgagaac aactacaaga ccacccccc tgtgctggac 1200
tccgacggct cattcttcct gtactccaag ctgaccgtgg acaagtcccg gtggcagcag 1260
ggcaacgtgt tctcctgctc cgtgatgcac gaggccctgc acaaccacta cacccagaag 1320
tccctgtccc tgagccccgg caag 1344

SEQ ID NO: 43 moltype = AA length = 107
FEATURE Location/Qualifiers
REGION 1..107
note = Amino acid sequence of hz39D2 light chain variable region
source 1..107
mol_type = protein
organism = synthetic construct
SEQUENCE: 43
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS 60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ YDEFPWTFGQ GTKVEIK 107

SEQ ID NO: 44 moltype = DNA length = 321

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FEATURE                 Location/Qualifiers
misc_feature            1..321
                        note = Nucleic acid sequence of hz39D2 light chain variable
                         region
source                  1..321
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 44
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc  60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca  120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc  180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct  240
gaagactttg ccacttacta ctgtctgcaa tacgatgagt tcccatggac cttcggccag  300
ggcaccaagg tggagattaa a                                            321

SEQ ID NO: 45           moltype = AA  length = 214
FEATURE                 Location/Qualifiers
REGION                  1..214
                        note = Amino acid sequence of hz39D2 light chain
source                  1..214
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 45
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ YDEFPWTFGQ GTKVEIKRTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                              214

SEQ ID NO: 46           moltype = DNA  length = 642
FEATURE                 Location/Qualifiers
misc_feature            1..642
                        note = Nucleic acid sequence of hz39D2 light chain
source                  1..642
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 46
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc  60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca  120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc  180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct  240
gaagactttg ccacttacta ctgtctgcaa tacgatgagt tcccatggac cttcggccag  300
ggcaccaagg tggagattaa acggaccgtg gccgctccct ccgtgttcat cttcccaccc  360
tccgacgagc agctgaagtc cggcaccgcc agcgtggtct gcctgctgaa caacttctac  420
ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag  480
gaatccgtca ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc  540
ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc  600
ctgtccagcc ccgtgaccaa gtccttcaac cggggcgagt gc                      642

SEQ ID NO: 47           moltype = AA  length = 119
FEATURE                 Location/Qualifiers
REGION                  1..119
                        note = Amino acid sequence of 24D3 heavy chain variable
                         region
source                  1..119
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 47
EVKLVESGGG LVKPGGSLKL SCAASGFTFS SCAMSWVRQT PEKRLEWVAT ISGGGSYTYY  60
PDSVKGRFTI SRDNAKNTLY LQMSSLRSED TAMYYCARHG GYESWFPYWG QGTLVTVSA   119

SEQ ID NO: 48           moltype = DNA  length = 357
FEATURE                 Location/Qualifiers
misc_feature            1..357
                        note = Nucleic acid sequence of 24D3 heavy chain variable
                         region
source                  1..357
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 48
gaggtgaagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc  60
tcctgtgcag cctctggatt cactttcagt agctgtgcca tgtcttgggt ccgccagact  120
ccggagaaga ggctggagtg ggtcgcaacc attagtggtg gtggtagtta cacctactat  180
ccagacagtg tgaaggggcg attcaccatc tccagagaca atgccaagaa caccctgtac  240
ctgcaaatga gcagtctgag gtctgaggac acggccatgt attactgtgc aagacatggc  300
gggtacgagt cctggtttcc ttactggggc caagggactc tggtcactgt ctctgca     357
```

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SEQ ID NO: 49 moltype = AA length = 449
FEATURE Location/Qualifiers
REGION 1..449
note = Amino acid sequence of 24D3 heavy chain
source 1..449
mol_type = protein
organism = synthetic construct
SEQUENCE: 49
EVKLVESGGG LVKPGGSLKL SCAASGFTFS SCAMSWVRQT PEKRLEWVAT ISGGGSYTYY 60
PDSVKGRFTI SRDNAKNTLY LQMSSLRSED TAMYYCARHG GYESWFPYWG QGTLVTVSAA 120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG 180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP 240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL 360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ 420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK 449

SEQ ID NO: 50 moltype = DNA length = 1347
FEATURE Location/Qualifiers
misc_feature 1..1347
note = Nucleic acid sequence of 24D3 heavy chain
source 1..1347
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 50
gaggtgaagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60
tcctgtgcag cctctggatt cactttcagt agctgtgcca tgtcttgggt ccgccagact 120
ccggagaaga ggctggagtg ggtcgcaacc attagtggtg gtggtagtta cacctactat 180
ccagacagtg tgaaggggcg attcaccatc tccagagaca atgccaagaa caccctgtac 240
ctgcaaatga gcagtctgag gtctgaggac acggccatgt attactgtgc aagacatggc 300
gggtacgagt cctggtttcc ttactggggc caagggactc tggtcactgt ctctgcagcc 360
tccaccaagg gcccctccgt gttcctctg gccccctcca gcaagtccac ctctggcggc 420
acagccgccc tgggctgcct ggtgaaagac tacttccccg agcccgtgac cgtgtcctgg 480
aactctggcg ccctgacctc cggcgtgcac accttccctg ccgtgctgca gtcctccggc 540
ctgtactccc tgtcctccgt ggtgaccgtg ccctccagct ctctgggcac ccagacctac 600
atctgtaacg tgaaccacaa gccctccaac accaaggtgg acaagaaggt ggaacccaag 660
tcctgcgaca agacccacac ctgtcccccc tgccctgccc ctgaactgct gggcggacct 720
tccgtgttcc tgttcccccc aaagcccaag gacaccctga tgatctcccg gacccccgaa 780
gtgacctgcg tggtggtgga cgtgtcccac gaggaccctg aagtgaagtt caattggtac 840
gtggacggcg tggaagtgca caatgccaag accaagccca gagaggaaca gtacaactcc 900
acctaccggg tggtgtctgt gctgaccgtg ctgcaccagg actggctgaa cggcaaagaa 960
tacaagtgca aagtctccaa caaggccctg cctgccccca tcgaaaagac catctccaag 1020
gccaagggcc agccccgcga gccccaggtg tacaccctgc cccctagccg ggacgagctg 1080
accaagaacc aggtgtccct gacctgtctg gtgaaaggct tctaccctc cgacattgcc 1140
gtggaatggg agtccaacgg ccagcccgag aacaactaca agaccacccc ccctgtgctg 1200
gactccgacg gctcattctt cctgtactcc aagctgaccg tggacaagtc ccggtggcag 1260
cagggcaacg tgttctcctg ctccgtgatg cacgaggccc tgcacaacca ctacacccag 1320
aagtccctgt ccctgagccc cggcaag 1347

SEQ ID NO: 51 moltype = AA length = 113
FEATURE Location/Qualifiers
REGION 1..113
note = Amino acid sequence of 24D3 light chain variable region
source 1..113
mol_type = protein
organism = synthetic construct
SEQUENCE: 51
DIVMTQSPLS LPVSLGDQAS ISCRSSQSLV HSNGNTYLHW YLQKPGQSPK LLIYKVSNRF 60
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP PWTFGGGTKL EIK 113

SEQ ID NO: 52 moltype = DNA length = 339
FEATURE Location/Qualifiers
misc_feature 1..339
note = Nucleic acid sequence of 24D3 light chain variable region
source 1..339
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 52
gatattgtga tgacccagtc tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60
atctcttgca gatctagtca gagccttgta cacagtaatg gaaacaccta tttacattgg 120
tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccgattt 180
tctggggttc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240
agcagagtgg aggctgagga tctgggagtt tatttctgct ctcaaagtac acatgttcct 300
ccgtggacgt tcggtggagg gaccaagctg gaaatcaaa 339

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SEQ ID NO: 53          moltype = AA  length = 220
FEATURE                Location/Qualifiers
REGION                 1..220
                       note = Amino acid sequence of 24D3 light chain
source                 1..220
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 53
DIVMTQSPLS LPVSLGDQAS ISCRSSQSLV HSNGNTYLHW YLQKPGQSPK LLIYKVSNRF  60
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP PWTFGGGTKL EIKRTVAAPS  120
VFIFPPSDEQ LKSGTASVVC LLNNFYPREA KVQWKVDNAL QSGNSQESVT EQDSKDSTYS  180
LSSTLTLSKA DYEKHKVYAC EVTHQGLSSP VTKSFNRGEC                        220

SEQ ID NO: 54          moltype = DNA  length = 660
FEATURE                Location/Qualifiers
misc_feature           1..660
                       note = Nucleic acid sequence of 24D3 light chain
source                 1..660
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 54
gatattgtga tgacccagtc tccactctcc ctgcctgtca gtcttggaga tcaagcctcc  60
atctcttgca gatctagtca gagccttgta cacagtaatg gaaacaccta tttacattgg  120
tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccgattt  180
tctggggttc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc  240
agcagagtgg aggctgagga tctgggagtt tatttctgct ctcaaagtac acatgttcct  300
ccgtggacgt tcggtggagg gaccaagctg gaaatcaaac ggaccgtggc cgctccctcc  360
gtgttcatct tcccaccctc cgacgagcag ctgaagtccg gcaccgccag cgtggtctgc  420
ctgctgaaca acttctaccc ccgcgaggcc aaggtgcagt ggaaggtgga caacgccctg  480
cagtccggca actcccagga atccgtcacc gagcaggact ccaaggacag cacctactcc  540
ctgtcctcca ccctgaccct gtccaaggcc gactacgaga agcacaaggt gtacgcctgc  600
gaagtgaccc accagggcct gtccagcccc gtgaccaagt ccttcaaccg gggcgagtgc  660

SEQ ID NO: 55          moltype = AA  length = 119
FEATURE                Location/Qualifiers
REGION                 1..119
                       note = Amino acid sequence of 1G3 heavy chain variable
                        region
source                 1..119
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 55
EVQLQQSGAE LVKPGASVKL SCTASDFNIV DTYMHWVKQR PEQGLEWIGR IDPANGYTRY  60
DPNFQGKATI TADTSSNTAY LQLSSLTSED TAVYYCARYY YGFYAMDYWG QGTTVTVSS   119

SEQ ID NO: 56          moltype = DNA  length = 357
FEATURE                Location/Qualifiers
misc_feature           1..357
                       note = Nucleic acid sequence of 1G3 heavy chain variable
                        region
source                 1..357
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 56
gaggttcagc tgcagcagtc tggggcagag cttgtgaagc caggggcctc agtcaagttg  60
tcctgcacag cttctgactt caacattgta gacacctata tgcactgggt gaagcagagg  120
cctgaacagg gcctggagtg gattggaagg attgatcctg cgaatggtta tactagatat  180
gacccgaact tccagggcaa ggccactata acagcagaca catcctccaa cacagcctac  240
ctgcagctca gcagcctgac atctgaggac actgccgtct attactgtgc ccgttattac  300
tacggcttct atgctatgga ctactggggt caaggaacca cggtcaccgt ctcctca     357

SEQ ID NO: 57          moltype = AA  length = 449
FEATURE                Location/Qualifiers
REGION                 1..449
                       note = Amino acid sequence of 1G3 heavy chain
source                 1..449
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 57
EVQLQQSGAE LVKPGASVKL SCTASDFNIV DTYMHWVKQR PEQGLEWIGR IDPANGYTRY  60
DPNFQGKATI TADTSSNTAY LQLSSLTSED TAVYYCARYY YGFYAMDYWG QGTTVTVSSA  120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG  180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP  240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS  300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL  360
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TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ  420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK                                   449

SEQ ID NO: 58          moltype = DNA  length = 1347
FEATURE                Location/Qualifiers
misc_feature           1..1347
                       note = Nucleic acid sequence of 1G3 heavy chain
source                 1..1347
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 58
gaggttcagc tgcagcagtc tggggcagag cttgtgaagc caggggcctc agtcaagttg  60
tcctgcacag cttctgactt caacattgta gacacctata tgcactgggt gaagcagagg  120
cctgaacagg gcctggagtg gattggaagg attgatcctg cgaatggtta tactagatat  180
gacccgaact tccagggcaa ggccactata acagcagaca catcctccaa cacagcctac  240
ctgcagctca gcagcctgac atctgaggac actgccgtct attactgtgc ccgttattac  300
tacggcttct atgctatgga ctactggggt caaggaacca cggtcaccgt ctcctcagcc  360
tccaccaagg gcccctccgt gttcccctctg gcccccctcca gcaagtccac ctctggcggc  420
acagccgccc tgggctgcct ggtgaaagac tacttccccg agcccgtgac cgtgtcctgg  480
aactctggcg ccctgacctc cggcgtgcac accttccctg ccgtgctgca gtcctccggc  540
ctgtactccc tgtcctccgt ggtgaccgtg ccctccagct ctctgggcac ccagacctac  600
atctgtaacg tgaaccacaa gccctccaac accaaggtgg acaagaaggt ggaacccaag  660
tcctgcgaca agacccacac ctgtccccct tgccctgccc ctgaactgct gggcggacct  720
tccgtgttcc tgttcccccc aaagcccaag gacaccctga tgatctcccg gacccccgaa  780
gtgacctgcg tggtggtgga cgtgtcccac gaggaccctg aagtgaagtt caattggtac  840
gtggacggcg tggaagtgca caatgccaag accaagccca gagaggaaca gtacaactcc  900
acctaccggg tggtgtctgt gctgaccgtg ctgcaccagg actggctgaa cggcaaagaa  960
tacaagtgca aagtctccaa caaggccctg cctgccccca tcgaaaagac catctccaag  1020
gccaagggcc agccccgcga gccccaggtg tacaccctgc cccctagccg ggacgagctg  1080
accaagaacc aggtgtccct gacctgtctg gtgaaaggct tctacccctc cgacattgcc  1140
gtggaatggg agtccaacgg ccagcccgag aacaactaca agaccacccc ccctgtgctg  1200
gactccgacg gctcattctt cctgtactcc aagctgaccg tggacaagtc ccggtggcag  1260
cagggcaacg tgttctcctg ctccgtgatg cacgaggccc tgcacaacca ctacacccag  1320
aagtccctgt ccctgagccc cggcaag                                      1347

SEQ ID NO: 59          moltype = AA  length = 107
FEATURE                Location/Qualifiers
REGION                 1..107
                       note = Amino acid sequence of 1G3 light chain variable
                        region
source                 1..107
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 59
DIVMTQSHKF MSTSVGDRVS ITCKASQDVS TAVAWYQQKP GQSPKLLIYS ASYRYTGVPD  60
RFTGSGSGTD FTFTISSVQA EDLAVYYCQQ HYSTPPTFGG GTKLELK               107

SEQ ID NO: 60          moltype = DNA  length = 321
FEATURE                Location/Qualifiers
misc_feature           1..321
                       note = Nucleic acid sequence of 1G3 light chain variable
                        region
source                 1..321
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 60
gatattgtga tgacgcagtc tcacaaattc atgtccacat cagtaggaga cagggtcagc  60
atcacctgca aggccagtca ggatgtgagt actgctgtag cctggtatca acagaaacca  120
ggacaatctc ctaaactact gatttactcg gcatcctacc ggtacactgg agtccctgat  180
cgcttcactg gcagtggatc tgggacggat ttcactttca ccatcagcag tgtgcaggct  240
gaagacctgg cagtttatta ctgtcagcaa cattatagta ctcctcccac gttcggaggg  300
gggaccaagc tggagctgaa a                                            321

SEQ ID NO: 61          moltype = AA  length = 214
FEATURE                Location/Qualifiers
REGION                 1..214
                       note = Amino acid sequence of 1G3 light chain
source                 1..214
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 61
DIVMTQSHKF MSTSVGDRVS ITCKASQDVS TAVAWYQQKP GQSPKLLIYS ASYRYTGVPD  60
RFTGSGSGTD FTFTISSVQA EDLAVYYCQQ HYSTPPTFGG GTKLELKRTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                              214
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SEQ ID NO: 62          moltype = DNA  length = 642
FEATURE                Location/Qualifiers
misc_feature           1..642
                       note = Nucleic acid sequence of 1G3 light chain
source                 1..642
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 62
gatattgtga tgacgcagtc tcacaaattc atgtccacat cagtaggaga cagggtcagc  60
atcacctgca aggccagtca ggatgtgagt actgctgtag cctggtatca acagaaacca  120
ggacaatctc ctaaactact gatttactcg gcatcctacc ggtacactgg agtccctgat  180
cgcttcactg gcagtggatc tgggacggat ttcactttca ccatcagcag tgtgcaggct  240
gaagacctgg cagtttatta ctgtcagcaa cattatagta ctcctcccac gttcggaggg  300
gggaccaagc tggagctgaa acggaccgtg gccgctccct ccgtgttcat cttcccaccc  360
tccgacgagc agctgaagtc cggcaccgcc agcgtggtct gcctgctgaa caacttctac  420
ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag  480
gaatccgtca ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc  540
ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc  600
ctgtccagcc ccgtgaccaa gtccttcaac cggggcgagt gc                      642

SEQ ID NO: 63          moltype = AA  length = 115
FEATURE                Location/Qualifiers
REGION                 1..115
                       note = Amino acid sequence of hz8G11 heavy chain variable
                        region
source                 1..115
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 63
QVQLVQSGQE VKKPGASVKV SCKASGYSFT GYYMHWVRQA PGQGLEWIGH INPNNGGTSY  60
NQKFKGRTTL TVDKSISTAY MELSRLRSDD TAVYYCAREE AFAYWGQGTL VTVSS       115

SEQ ID NO: 64          moltype = DNA  length = 345
FEATURE                Location/Qualifiers
misc_feature           1..345
                       note = Nucleic acid sequence of hz8G11 heavy chain variable
                        region
source                 1..345
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 64
caggtacagc tagtgcagag cggccaggaa gtaaagaagc caggcgcctc tgttaaggtg  60
tcatgtaagg ccagcggtta cagcttcact ggctattaca tgcactgggt ccggcaggca  120
cccggacaag ggctggaatg gataggtcac attaatccaa acaatggcgg taccagttat  180
aaccagaaat ttaaggggag gacaaccctg acagttgata aatccatcag tacagcatat  240
atggagctca gcagactgag aagcgacgat actgctgtgt actactgcgc gcgggaggag  300
gctttcgcct actggggcca agggacctta gtgactgtct catca                  345

SEQ ID NO: 65          moltype = AA  length = 445
FEATURE                Location/Qualifiers
REGION                 1..445
                       note = Amino acid sequence of hz8G11 heavy chain
source                 1..445
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 65
QVQLVQSGQE VKKPGASVKV SCKASGYSFT GYYMHWVRQA PGQGLEWIGH INPNNGGTSY  60
NQKFKGRTTL TVDKSISTAY MELSRLRSDD TAVYYCAREE AFAYWGQGTL VTVSSASTKG  120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP ELLGGPSVFL  240
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV  300
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ  360
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  420
FSCSVMHEAL HNHYTQKSLS LSPGK                                        445

SEQ ID NO: 66          moltype = DNA  length = 1335
FEATURE                Location/Qualifiers
misc_feature           1..1335
                       note = Nucleic acid sequence of hz8G11 heavy chain
source                 1..1335
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 66
caggtacagc tagtgcagag cggccaggaa gtaaagaagc caggcgcctc tgttaaggtg  60
tcatgtaagg ccagcggtta cagcttcact ggctattaca tgcactgggt ccggcaggca  120
cccggacaag ggctggaatg gataggtcac attaatccaa acaatggcgg taccagttat  180
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aaccagaaat ttaaggggag gacaaccctg acagttgata aatccatcag tacagcatat  240
atggagctca gcagactgag aagcgacgat actgctgtgt actactgcgc gcgggaggag  300
gctttcgcct actggggcca agggacctta gtgactgtct catcagcctc caccaagggc  360
ccctccgtgt tccctctggc cccctccagc aagtccacct ctggcggcac agccgccctg  420
ggctgcctgg tgaaagacta cttccccgag cccgtgaccg tgtcctggaa ctctggcgcc  480
ctgacctccg gcgtgcacac cttccctgcc gtgctgcagt cctccggcct gtactccctg  540
tcctccgtgg tgaccgtgcc ctccagctct ctgggcaccc agacctacat ctgtaacgtg  600
aaccacaagc cctccaacac caaggtggac aagaaggtgg aacccaagtc ctgcgacaag  660
acccacacct gtcccccctg ccctgccct gaactgctgg gcggaccttc cgtgttcctg  720
ttcccccaa agcccaagga caccctgatg atctcccgga cccccgaagt gacctgcgtg  780
gtggtggacg tgtcccacga ggaccctgaa gtgaagttca attggtacgt ggacggcgtg  840
gaagtgcaca atgccaagac caagcccaga gaggaacagt acaactccac ctaccgggtg  900
gtgtctgtgc tgaccgtgct gcaccaggac tggctgaacg gcaaagaata caagtgcaaa  960
gtctccaaca aggccctgcc tgcccccatc gaaaagacca tctccaaggc caagggccag  1020
ccccgcgagc cccaggtgta caccctgccc cctagccggg acgagctgac caagaaccag  1080
gtgtccctga cctgtctggt gaaaggcttc taccctccg acattgccgt ggaatgggag  1140
tccaacggcc agcccgagaa caactacaag accacccccc ctgtgctgga ctccgacggc  1200
tcattcttcc tgtactccaa gctgaccgtg gacaagtccc ggtggcagca gggcaacgtg  1260
ttctcctgct ccgtgatgca cgaggccctg cacaaccact acacccagaa gtccctgtcc  1320
ctgagccccg gcaag                                                   1335

SEQ ID NO: 67          moltype = AA  length = 107
FEATURE                Location/Qualifiers
REGION                 1..107
                       note = Amino acid sequence of hz8G11 light chain variable
                        region
source                 1..107
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 67
DIQMTQSPSS LSASVGDRVT ISCRASQDIS NYLNWYQQKP GKAPKLLIYY TSRLHSGVPS  60
RFSGSGSGTD FSLTISSLQP EDIATYYCQQ GITPPWTFGG GTKVEIK               107

SEQ ID NO: 68          moltype = DNA  length = 321
FEATURE                Location/Qualifiers
misc_feature           1..321
                       note = Nucleic acid sequence of hz8G11 light chain variable
                        region
source                 1..321
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 68
gacatacaga tgacgcagag ccctagttca ctgtctgcct ccgtcggcga cagagtgacg  60
atcagctgcc gagccagcca agatattagt aactacctca attggtacca gcagaaacct  120
ggaaaagcac ccaagctttt gatctattac accagcaggc tgcatagcgg agtgccgagc  180
agattttcgg gttctggcag cggcaccgat ttctctctga ctatcagtag cctgcaaccc  240
gaagacattg ctacatatta ttgtcagcag ggaatcaccc ctccatggac atttggaggg  300
ggaacaaagg tggagattaa a                                            321

SEQ ID NO: 69          moltype = AA  length = 214
FEATURE                Location/Qualifiers
REGION                 1..214
                       note = Amino acid sequence of hz8G11 light chain
source                 1..214
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 69
DIQMTQSPSS LSASVGDRVT ISCRASQDIS NYLNWYQQKP GKAPKLLIYY TSRLHSGVPS  60
RFSGSGSGTD FSLTISSLQP EDIATYYCQQ GITPPWTFGG GTKVEIKRTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                              214

SEQ ID NO: 70          moltype = DNA  length = 642
FEATURE                Location/Qualifiers
misc_feature           1..642
                       note = Nucleic acid sequence of hz8G11 light chain
source                 1..642
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 70
gacatacaga tgacgcagag ccctagttca ctgtctgcct ccgtcggcga cagagtgacg  60
atcagctgcc gagccagcca agatattagt aactacctca attggtacca gcagaaacct  120
ggaaaagcac ccaagctttt gatctattac accagcaggc tgcatagcgg agtgccgagc  180
agattttcgg gttctggcag cggcaccgat ttctctctga ctatcagtag cctgcaaccc  240
gaagacattg ctacatatta ttgtcagcag ggaatcaccc ctccatggac atttggaggg  300
ggaacaaagg tggagattaa acggaccgtg gccgctccct ccgtgttcat cttcccaccc  360
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tccgacgagc agctgaagtc cggcaccgcc agcgtggtct gcctgctgaa caacttctac  420
ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag  480
gaatccgtca ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc  540
ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc  600
ctgtccagcc ccgtgaccaa gtccttcaac cggggcgagt gc                      642

SEQ ID NO: 71          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = CDRH3 of hz39D2.14 antibody
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 71
DEYYVRTDY                                                          9

SEQ ID NO: 72          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = CDRH3 of hz39D2.22 and hz39D2.23 antibody
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 72
DEYYVRVDY                                                          9

SEQ ID NO: 73          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = CDRL3 of hz39D2.22 antibody
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 73
LELDEFPWT                                                          9

SEQ ID NO: 74          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = CDRL3 of hz39D2.23 antibody
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 74
LQLDEFPWT                                                          9

SEQ ID NO: 75          moltype = AA  length = 121
FEATURE                Location/Qualifiers
REGION                 1..121
                       note = Amino acid sequence of 2G10 heavy chain variable
                        region
source                 1..121
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 75
EVKLLESGGG LVQPGGSLKL SCATSGFTFS DYYMYWVRQT PEMRLEWVAY INSGGGSTYY  60
PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYYCAREA LYDYDYAMDY WGQGTTVTVS  120
S                                                                  121

SEQ ID NO: 76          moltype = DNA  length = 363
FEATURE                Location/Qualifiers
misc_feature           1..363
                       note = Nucleic acid sequence of 2G10 heavy chain variable
                        region
source                 1..363
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 76
gaggtgaagc ttctcgagtc tgggggaggc ttagtgcagc ctggagggtc cctgaaactc  60
tcctgtgcaa cctctggatt cactttcagt gactattaca tgtattgggt tcgccagact  120
ccagagatga ggctggagtg ggtcgcatat attaatagtg gtggtggtag cacctattat  180
ccagacactg taaagggccg attcaccatc tccagagaca atgccaagaa caccctgtac  240
ctgcaaatga gccgtctgaa gtctgaggac acagccatgt attactgtgc aagagaggcc  300
ctctatgatt acgactatgc tatggactac tggggtcaag gaaccacggt caccgtctcc  360
tca                                                                363
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SEQ ID NO: 77          moltype = AA  length = 112
FEATURE                Location/Qualifiers
REGION                 1..112
                       note = Amino acid sequence of 2G10 light chain variable
                        region
source                 1..112
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 77
DIVMTQSPLT LSVTIGQPAS ISCKSSQSLL YSNGKTYLNW LLQRPGQSPK RLIYLVSKLD  60
SGVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYCVQGTHFP LTFGAGTKLE LK         112

SEQ ID NO: 78          moltype = DNA  length = 336
FEATURE                Location/Qualifiers
misc_feature           1..336
                       note = Nucleic acid sequence of 2G10 light chain variable
                        region
source                 1..336
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 78
gatattgtga tgacccagtc tccactcact ttgtcggtta ccattggaca accagcctct  60
atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaattgg  120
ttattacaga ggccaggcca gtctccaaag cgcctaatct atctggtgtc taaactggac  180
tctggagtcc ctgacaggtt cactggcagt gggtcaggaa cagattttac actgaaaatc  240
agcagagtgg aggctgagga tttgggagtt tattactgcg tgcaaggtac acattttccg  300
ctcacgttcg gtgctgggac caagctggag ctgaaa                            336

SEQ ID NO: 79          moltype = AA  length = 115
FEATURE                Location/Qualifiers
REGION                 1..115
                       note = Amino acid sequence of 8G11 heavy chain variable
                        region
source                 1..115
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 79
EVQLQQSGPD LVKPGTSVKI SCKASGYSFT GYYMHWVKQS HGKSLEWIGH INPNNGGTSY  60
NQKFKGKTIL TVDKSSSTAF MELRSLTSED SAVYYCAREE AFAYWGQGTL VTVSA       115

SEQ ID NO: 80          moltype = DNA  length = 345
FEATURE                Location/Qualifiers
misc_feature           1..345
                       note = Nucleic acid sequence of 8G11 heavy chain variable
                        region
source                 1..345
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 80
gaggtgcaac ttcagcagtc tggacctgac ctggtgaagc ctgggacttc agtgaagata  60
tcctgcaagg cttctggtta ctcattcact ggctactaca tgcactgggt gaagcagagc  120
catggaaaga gccttgagtg gattggacat attaatccta acaatggtgg tactagctac  180
aaccagaagt tcaagggcaa gaccatatta actgtggaca agtcttccag cacagccttc  240
atggagctcc gcagcctgac atctgaggac tctgcggtct attactgtgc aagagaagaa  300
gcctttgctt actggggcca agggactctg gtcactgtct ctgca                  345

SEQ ID NO: 81          moltype = AA  length = 107
FEATURE                Location/Qualifiers
REGION                 1..107
                       note = Amino acid sequence of 8G11 light chain variable
                        region
source                 1..107
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 81
DIVMTQSTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY TSRLHSGVPS  60
RFSGSGSGTD FSLTISNVEQ EDIATYFCQQ GITPPWTFGG GTKLELK                107

SEQ ID NO: 82          moltype = DNA  length = 321
FEATURE                Location/Qualifiers
misc_feature           1..321
                       note = Nucleic acid sequence of 8G11 light chain variable
                        region
source                 1..321
                       mol_type = other DNA
                       organism = synthetic construct
```

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SEQUENCE: 82
gatattgtga tgacccagtc tacatcctcc ctgtctgcct ctctgggaga cagagtcacc 60
atcagttgca gggcaagtca ggacattagc aattatttaa actggtatca gcagaaacca 120
gatggaactg ttaaactcct gatctactac acatcaagat tacactcagg agtcccatca 180
aggttcagtg gcagtgggtc tggaacagat ttttctctca ccattagcaa cgtggagcaa 240
gaagacattg ccacttactt ttgccaacag ggtattacgc ctccgtggac gttcggtgga 300
gggaccaagc tggagctgaa a 321

SEQ ID NO: 83 moltype = AA length = 118
FEATURE Location/Qualifiers
REGION 1..118
note = Amino acid sequence of 39D2 heavy chain variable region
source 1..118
mol_type = protein
organism = synthetic construct
SEQUENCE: 83
EVQLQQSGPE LKKPGETVKI SCKASGYTFT NYGVNWVKQA PGKGLKWMGW INTHTGEPTY 60
AEEFKGRFAF SLETSASTAY LQINNLKNED TATYFCARDD YYVRVDYWGQ GTTLTVSS 118

SEQ ID NO: 84 moltype = DNA length = 354
FEATURE Location/Qualifiers
misc_feature 1..354
note = Nucleic acid sequence of 39D2 heavy chain variable region
source 1..354
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 84
gaggttcagc tgcagcagtc tggacctgag ctgaagaagc ctggagagac agtcaagatc 60
tcctgcaagg cttctgggta taccttcaca aactatggag tgaattgggt gaagcaggct 120
ccaggaaagg gtttaaagtg gatgggctgg ataaacaccc acactggaga gccaacatat 180
gctgaagagt tcaagggacg gtttgccttc tctttggaaa cctctgccag cactgcctat 240
ttgcagatca acaacctcaa aaatgaggac acggctacat atttctgtgc aagagatgat 300
tactacgtaa gggtagacta ctggggccaa ggcaccactc tcacagtctc ctca 354

SEQ ID NO: 85 moltype = AA length = 107
FEATURE Location/Qualifiers
REGION 1..107
note = Amino acid sequence of 39D2 light chain variable region
source 1..107
mol_type = protein
organism = synthetic construct
SEQUENCE: 85
DIVMTQSPSS MYASLGERVT ITCKASQDIN SYLSWFQQKP GKSPKTLIYR ANRLVDGVPS 60
RFSGSGSGQD YSLTISSLEY EDMGIYYCLQ YDEFPWTFGG GTKLELK 107

SEQ ID NO: 86 moltype = DNA length = 321
FEATURE Location/Qualifiers
misc_feature 1..321
note = Nucleic acid sequence of 39D2 light chain variable region
source 1..321
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 86
gatattgtaa tgacccagtc tccatcttcc atgtatgcat ccctaggaga gagagtcact 60
atcacttgca aggcgagtca ggacattaat agctatttaa gctggttcca gcagaaacca 120
gggaaatctc ctaagaccct gatctatcgt gcaaacagat tggtagatgg ggtcccatca 180
aggttcagtg gcagtggatc tgggcaagat tattctctca ccatcagcag cctggagtat 240
gaagatatgg gaatttatta ttgtctacag tatgatgagt ttccgtggac gttcggtgga 300
gggaccaagc tggagctgaa a 321

SEQ ID NO: 87 moltype = AA length = 118
FEATURE Location/Qualifiers
REGION 1..118
note = Amino acid sequence of hz39D2.14 heavy chain variable region
source 1..118
mol_type = protein
organism = synthetic construct
SEQUENCE: 87
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY 60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDE YYVRTDYWGQ GTTVTVSS 118

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SEQ ID NO: 88          moltype = DNA  length = 354
FEATURE                Location/Qualifiers
misc_feature           1..354
                       note = Nucleic acid sequence of hz39D2.14 heavy chain
                        variable region
source                 1..354
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 88
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt  60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca  120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat  180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac  240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgag  300
tactatgtga ggaccgatta ctgggggcag gggaccaccg tgacagtctc aagt        354

SEQ ID NO: 89          moltype = AA  length = 448
FEATURE                Location/Qualifiers
REGION                 1..448
                       note = Amino acid sequence of hz39D2.14 heavy chain
source                 1..448
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 89
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY  60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDE YYVRTDYWGQ GTTVTVSSAS  120
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL  180
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS  240
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST  300
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  360
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ  420
GNVFSCSVMH EALHNHYTQK SLSLSPGK                                     448

SEQ ID NO: 90          moltype = DNA  length = 1344
FEATURE                Location/Qualifiers
misc_feature           1..1344
                       note = Nucleic acid sequence of hz39D2.14 heavy chain
source                 1..1344
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 90
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt  60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca  120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat  180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac  240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgag  300
tactatgtga ggaccgatta ctgggggcag gggaccaccg tgacagtctc aagtgcctcc  360
accaagggcc cctccgtgtt ccctctggcc ccctccagca agtccacctc tggcggcaca  420
gccgccctgg gctgcctggt gaaagactac ttccccgagc ccgtgaccgt gtcctggaac  480
tctggcgccc tgacctccgg cgtgcacacc ttccctgccg tgctgcagtc ctccggcctg  540
tactccctgt cctccgtggt gaccgtgccc tccagctctc tgggcaccca gacctacatc  600
tgtaacgtga accacaagcc ctccaacacc aaggtggaca agaaggtgga acccaagtcc  660
tgcgacaaga cccacacctg tccccctgc cctgcccctg aactgctggg cggaccttcc  720
gtgttcctgt tcccccccaa gcccaaggac accctgatga tctcccggac ccccgaagtg  780
acctgcgtgg tggtggacgt gtcccacgag gaccctgaag tgaagttcaa ttggtacgtg  840
gacggcgtgg aagtgcacaa tgccaagacc aagcccagag aggaacagta caactccacc  900
taccgggtgg tgtctgtgct gaccgtgctg caccaggact ggctgaacgg caaagaatac  960
aagtgcaaag tctccaacaa ggccctgcct gcccccatcg aaaagaccat ctccaaggcc  1020
aagggccagc cccgcgagcc ccaggtgtac accctgcccc ctagccggga cgagctgacc  1080
aagaaccagg tgtccctgac ctgtctggtg aaaggcttct acccctccga cattgccgtg  1140
gaatgggagt ccaacggcca gcccgagaac aactacaaga ccacccccc tgtgctggac  1200
tccgacggct cattcttcct gtactccaag ctgaccgtgg acaagtcccg gtggcagcag  1260
ggcaacgtgt tctcctgctc cgtgatgcac gaggccctgc acaaccacta cacccagaag  1320
tccctgtccc tgagccccgg caag                                         1344

SEQ ID NO: 91          moltype = AA  length = 107
FEATURE                Location/Qualifiers
REGION                 1..107
                       note = Amino acid sequence of hz39D2.14 light chain
                        variable region
source                 1..107
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 91
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ YDEFPWTFGQ GTKVEIK                107
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SEQ ID NO: 92 moltype = DNA length = 321
FEATURE Location/Qualifiers
misc_feature 1..321
note = Nucleic acid sequence of hz39D2.14 light chain variable region
source 1..321
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 92
cggaccgtgg ccgctccctc cgtgttcatc ttcccaccct ccgacgagca gctgaagtcc 60
ggcaccgcca gcgtggtctg cctgctgaac aacttctacc cccgcgaggc caaggtgcag 120
tggaaggtgg acaacgccct gcagtccggc aactcccagg aatccgtcac cgagcaggac 180
tccaaggaca gcacctactc cctgtcctcc accctgaccc tgtccaaggc cgactacgag 240
aagcacaagg tgtacgcctg cgaagtgacc caccagggcc tgtccagccc cgtgaccaag 300
tccttcaacc ggggcgagtg c 321

SEQ ID NO: 93 moltype = AA length = 214
FEATURE Location/Qualifiers
REGION 1..214
note = Amino acid sequence of hz39D2.14 light chain
source 1..214
mol_type = protein
organism = synthetic construct
SEQUENCE: 93
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS 60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ YDEFPWTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC 214

SEQ ID NO: 94 moltype = DNA length = 642
FEATURE Location/Qualifiers
misc_feature 1..642
note = Nucleic acid sequence of hz39D2.14 light chain
source 1..642
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 94
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc 60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca 120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc 180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct 240
gaagactttg ccacttacta ctgtctgcaa tacgatgagt tcccatggac cttcggccag 300
ggcaccaagg tggagattaa acggaccgtg gccgctccct ccgtgttcat cttcccaccc 360
tccgacgagc agctgaagtc cggcaccgcc agcgtggtct gcctgctgaa caacttctac 420
ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag 480
gaatccgtca ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc 540
ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc 600
ctgtccagcc ccgtgaccaa gtccttcaac cggggcgagt gc 642

SEQ ID NO: 95 moltype = AA length = 118
FEATURE Location/Qualifiers
REGION 1..118
note = Amino acid sequence of hz39D2.22 heavy chain variable region
source 1..118
mol_type = protein
organism = synthetic construct
SEQUENCE: 95
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY 60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDE YYVRVDYWGQ GTTVTVSS 118

SEQ ID NO: 96 moltype = DNA length = 354
FEATURE Location/Qualifiers
misc_feature 1..354
note = Nucleic acid sequence of hz39D2.22 heavy chain variable region
source 1..354
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 96
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt 60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca 120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat 180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac 240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgag 300

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tactatgtga gggtggatta ctgggggcag gggaccaccg tgacagtctc aagt        354

SEQ ID NO: 97          moltype = AA  length = 448
FEATURE                Location/Qualifiers
REGION                 1..448
                       note = Amino acid sequence of hz39D2.22 heavy chain
source                 1..448
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 97
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY  60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDE YYVRVDYWGQ GTTVTVSSAS  120
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL  180
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS  240
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST  300
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  360
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ  420
GNVFSCSVMH EALHNHYTQK SLSLSPGK                                     448

SEQ ID NO: 98          moltype = DNA  length = 1344
FEATURE                Location/Qualifiers
misc_feature           1..1344
                       note = Nucleic acid sequence of hz39D2.22 heavy chain
source                 1..1344
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 98
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt  60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca  120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat  180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac  240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgag  300
tactatgtga gggtggatta ctgggggcag gggaccaccg tgacagtctc aagtgcctcc  360
accaagggcc cctccgtgtt ccctctggcc ccctccagca agtccacctc tggcggcaca  420
gccgccctgg gctgcctggt gaaagactac ttccccgagc ccgtgaccgt gtcctggaac  480
tctggcgccc tgacctccgg cgtgcacacc ttccctgccg tgctgcagtc ctccggcctg  540
tactccctgt cctccgtggt gaccgtgccc tccagctctc tgggcaccca gacctacatc  600
tgtaacgtga accacaagcc ctccaacacc aaggtggaca agaaggtgga acccaagtcc  660
tgcgacaaga cccacacctg tccccctgc cctgcccctg aactgctggg cggaccttcc  720
gtgttcctgt tccccccaaa gcccaaggac accctgatga tctcccggac ccccgaagtg  780
acctgcgtgg tggtggacgt gtcccacgag gaccctgaag tgaagttcaa ttggtacgtg  840
gacggcgtgg aagtgcacaa tgccaagacc aagcccagag aggaacagta caactccacc  900
taccgggtgg tgtctgtgct gaccgtgctg caccaggact ggctgaacgg caaagaatac  960
aagtgcaaag tctccaacaa ggccctgcct gcccccatcg aaaagaccat ctccaaggcc  1020
aagggccagc cccgcgagcc ccaggtgtac accctgcccc ctagccggga cgagctgacc  1080
aagaaccagg tgtccctgac ctgtctggtg aaaggcttct accctccga cattgccgtg  1140
gaatgggagt ccaacggcca gcccgagaac aactacaaga ccacccccc tgtgctggac  1200
tccgacggct cattcttcct gtactccaag ctgaccgtgg acaagtcccg gtggcagcag  1260
ggcaacgtgt tctcctgctc cgtgatgcac gaggccctgc acaaccacta cacccagaag  1320
tccctgtccc tgagccccgg caag                                         1344

SEQ ID NO: 99          moltype = AA  length = 107
FEATURE                Location/Qualifiers
REGION                 1..107
                       note = Amino acid sequence of hz39D2.22 light chain
                        variable region
source                 1..107
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 99
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLE LDEFPWTFGQ GTKVEIK                107

SEQ ID NO: 100         moltype = DNA  length = 321
FEATURE                Location/Qualifiers
misc_feature           1..321
                       note = Nucleic acid sequence of hz39D2.22 light chain
                        variable region
source                 1..321
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 100
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc  60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca  120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc  180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct  240
```

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gaagactttg ccacttacta ctgtctggag ctcgatgagt tcccatggac cttcggccag  300
ggcaccaagg tggagattaa a                                            321

SEQ ID NO: 101         moltype = AA  length = 214
FEATURE                Location/Qualifiers
REGION                 1..214
                       note = Amino acid sequence of hz39D2.22 light chain
source                 1..214
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 101
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLE LDEFPWTFGQ GTKVEIKRTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                              214

SEQ ID NO: 102         moltype = DNA  length = 642
FEATURE                Location/Qualifiers
misc_feature           1..642
                       note = Nucleic acid sequence of hz39D2.22 light chain
source                 1..642
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 102
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc  60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca  120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc  180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct  240
gaagactttg ccacttacta ctgtctggag ctcgatgagt tcccatggac cttcggccag  300
ggcaccaagg tggagattaa acggaccgtg gccgctccct ccgtgttcat cttcccaccc  360
tccgacgagc agctgaagtc cggcaccgcc agcgtggtct gcctgctgaa caacttctac  420
ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag  480
gaatccgtca ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc  540
ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc  600
ctgtccagcc ccgtgaccaa gtccttcaac cggggcgagt gc                      642

SEQ ID NO: 103         moltype = AA  length = 118
FEATURE                Location/Qualifiers
REGION                 1..118
                       note = Amino acid sequence of hz39D2.23 heavy chain
                        variable region
source                 1..118
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 103
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY  60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDE YYVRVDYWGQ GTTVTVSS    118

SEQ ID NO: 104         moltype = DNA  length = 354
FEATURE                Location/Qualifiers
misc_feature           1..354
                       note = Nucleic acid sequence of hz39D2.23 heavy chain
                        variable region
source                 1..354
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 104
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt  60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca  120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat  180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac  240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgag  300
tactatgtga gggtggatta ctgggggcag gggaccaccg tgacagtctc aagt        354

SEQ ID NO: 105         moltype = AA  length = 448
FEATURE                Location/Qualifiers
REGION                 1..448
                       note = Amino acid sequence of hz39D2.23 heavy chain
source                 1..448
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 105
QVQLVQSGSE LKKPGASVKV SCKASGYTFT NYGVNWVRQA PGQGLEWMGW INTHTGEPTY  60
AEEFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCARDE YYVRVDYWGQ GTTVTVSSAS  120
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL  180
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS  240
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VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST  300
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  360
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ  420
GNVFSCSVMH EALHNHYTQK SLSLSPGK                                     448

SEQ ID NO: 106         moltype = DNA  length = 1344
FEATURE                Location/Qualifiers
misc_feature           1..1344
                       note = Nucleic acid sequence of hz39D2.23 heavy chain
source                 1..1344
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 106
caagtccaac tcgtgcagtc aggatctgaa ctgaagaaac ctggagcgag cgttaaggtt  60
tcctgcaagg ccagcggcta tacgttcact aactatggtg tcaactgggt gagacaggca  120
cccggccagg gcctggagtg gatgggttgg atcaatactc acacagggga accaacatat  180
gctgaggagt tcaaaggacg gtttgttttt agtctggaca cctccgtgtc taccgcctac  240
ctgcagattt ccagccttaa agcagaggac actgctgtat actactgtgc cagagacgag  300
tactatgtga gggtggatta ctgggggcag gggaccaccg tgacagtctc aagtgcctcc  360
accaagggcc cctccgtgtt ccctctggcc ccctccagca agtccacctc tggcggcaca  420
gccgccctgg gctgcctggt gaaagactac ttccccgagc ccgtgaccgt gtcctggaac  480
tctggcgccc tgacctccgg cgtgcacacc ttccctgccg tgctgcagtc ctccggcctg  540
tactccctgt cctccgtggt gaccgtgccc tccagctctc tgggcaccca gacctacatc  600
tgtaacgtga accacaagcc ctccaacacc aaggtggaca agaaggtgga acccaagtcc  660
tgcgacaaga cccacacctg tccccctgc cctgccctg aactgctggg cggaccttcc  720
gtgttcctgt tcccccccaaa gcccaaggac accctgatga tctcccggac ccccgaagtg  780
acctgcgtgg tggtggacgt gtcccacgag gaccctgaag tgaagttcaa ttggtacgtg  840
gacggcgtgg aagtgcacaa tgccaagacc aagcccagag aggaacagta caactccacc  900
taccgggtgg tgtctgtgct gaccgtgctg caccaggact ggctgaacgg caaagaatac  960
aagtgcaaag tctccaacaa ggccctgcct gcccccatcg aaaagaccat ctccaaggcc  1020
aagggccagc cccgcgagcc ccaggtgtac accctgcccc ctagccggga cgagctgacc  1080
aagaaccagg tgtccctgac ctgtctggtg aaaggcttct acccctccga cattgccgtg  1140
gaatgggagt ccaacggcca gcccgagaac aactacaaga ccacccccc tgtgctggac  1200
tccgacggct cattcttcct gtactccaag ctgaccgtgg acaagtcccg gtggcagcag  1260
ggcaacgtgt tctcctgctc cgtgatgcac gaggccctgc acaaccacta cacccagaag  1320
tccctgtccc tgagccccgg caag                                         1344

SEQ ID NO: 107         moltype = AA  length = 107
FEATURE                Location/Qualifiers
REGION                 1..107
                       note = Amino acid sequence of hz39D2.23 light chain
                        variable region
source                 1..107
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 107
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ LDEFPWTFGQ GTKVEIK                107

SEQ ID NO: 108         moltype = DNA  length = 321
FEATURE                Location/Qualifiers
misc_feature           1..321
                       note = Nucleic acid sequence of hz39D2.23 light chain
                        variable region
source                 1..321
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 108
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc  60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca  120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc  180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct  240
gaagactttg ccacttacta ctgtctgcaa ctcgatgagt tcccatggac cttcggccag  300
ggcaccaagg tggagattaa a                                            321

SEQ ID NO: 109         moltype = AA  length = 214
FEATURE                Location/Qualifiers
REGION                 1..214
                       note = Amino acid sequence of hz39D2.23 light chain
source                 1..214
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 109
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ LDEFPWTFGQ GTKVEIKRTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
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LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                                214

SEQ ID NO: 110          moltype = DNA  length = 642
FEATURE                 Location/Qualifiers
misc_feature            1..642
                        note = Nucleic acid sequence of hz39D2.23 light chain
source                  1..642
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 110
gacattcaaa tgacacagtc tcccagctcc cttagtgctt cggtgggcga tcgggtgacc  60
ataacatgca aggcctcaca ggacatcaac agctatctct catggtttca gcagaagcca  120
ggaaaagcac ctaaaacgtt gatctacagg gccaatcgcc tcgttgacgg agtcccctcc  180
agattcagcg ggagtgggtc tggtcaggat tatactctga ccatctcctc tctgcagcct  240
gaagactttg ccacttacta ctgtctctgcaa ctcgatgagt tcccatggac cttcggccag  300
ggcaccaagg tggagattaa acggaccgtg gccgctccct ccgtgttcat cttcccaccc  360
tccgacgagc agctgaagtc cggcaccgcc agcgtggtct gcctgctgaa caacttctac  420
ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag  480
gaatccgtca ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc  540
ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc  600
ctgtccagcc ccgtgaccaa gtccttcaac cggggcgagt gc                      642

SEQ ID NO: 111          moltype = AA  length = 21
FEATURE                 Location/Qualifiers
REGION                  1..21
                        note = Signal peptide (CD8alpha)
source                  1..21
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 111
MALPVTALLL PLALLLHAAR P                                              21

SEQ ID NO: 112          moltype = DNA  length = 63
FEATURE                 Location/Qualifiers
misc_feature            1..63
                        note = Signal peptide (CD8alpha)
source                  1..63
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 112
atggctctgc cagtgactgc actgctgctg ccactggccc tgctgctgca cgcagctcga  60
cct                                                                  63

SEQ ID NO: 113          moltype = AA  length = 240
FEATURE                 Location/Qualifiers
REGION                  1..240
                        note = Anti-HER2 scFv
source                  1..240
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 113
DIQMTQSPSS LSASVGDRVT ITCKASQDIN SYLSWFQQKP GKAPKTLIYR ANRLVDGVPS  60
RFSGSGSGQD YTLTISSLQP EDFATYYCLQ YDEFPWTFGQ GTKVEIKGGG GSGGGGSGGG  120
GSQVQLVQSG SELKKPGASV KVSCKASGYT FTNYGVNWVR QAPGQGLEWM GWINTHTGEP  180
TYAEEFKGRF VFSLDTSVST AYLQISSLKA EDTAVYYCAR DDYYVRVDYW GQGTTVTVSS  240

SEQ ID NO: 114          moltype = DNA  length = 720
FEATURE                 Location/Qualifiers
misc_feature            1..720
                        note = Anti-HER2 scFv
source                  1..720
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 114
gacatccaga tgacccagag ccccagcagc ctgagcgcca gcgtgggcga cagagtgacc  60
atcacctgca aggccagcca ggacatcaac agctacctga gctggttcca gcagaagccc  120
ggcaaggccc ccaagaccct gatctacaga gccaacagac tggtggacgg cgtgcccagc  180
agattcagcg gcagcggcag cggccaggac tacaccctga ccatcagcag cctgcagccc  240
gaggacttcg ccacctacta ctgcctgcag tacgacgagt tcccctggac cttcggccag  300
ggcaccaagg tggagatcaa gggtggcggt ggatcgggcg gtggtggatc tggaggaggt  360
ggctcccagg tgcagctggt gcagagcggc agcgagctga agaagcccgg cgccagcgtg  420
aaggtgagct gcaaggccag cggctacacc ttcaccaact acggcgtgaa ctgggtgaga  480
caggcccccg gccagggcct ggagtggatg ggctggatca acacccacac cggcgagccc  540
acctacgccg aggagttcaa gggcagattc gtgttcagcc tggacaccag cgtgagcacc  600
gcctacctgc agatcagcag cctgaaggcc gaggacaccg ccgtgtacta ctgcgccaga  660
gacgactact acgtgagagt ggactactgg ggccagggca ccaccgtgac cgtgagcagc  720
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SEQ ID NO: 115          moltype = AA  length = 48
FEATURE                 Location/Qualifiers
REGION                  1..48
                        note = Hinge (CD8alpha)
source                  1..48
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 115
AKPTTTPAPR PPTPAPTIAS QPLSLRPEAC RPAAGGAVHT RGLDFACD               48

SEQ ID NO: 116          moltype = DNA  length = 144
FEATURE                 Location/Qualifiers
misc_feature            1..144
                        note = Hinge (CD8alpha)
source                  1..144
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 116
gcaaaaccta ccacaactcc tgcaccacgc ccccctactc cagcacctac catcgcatct  60
cagccactga gtctgcgacc agaggcctgc cggcccgccg ccggcggggc cgtccatacc  120
agagggctgg actttgcctg cgat                                        144

SEQ ID NO: 117          moltype = AA  length = 24
FEATURE                 Location/Qualifiers
REGION                  1..24
                        note = Transmembrane (CD8alpha)
source                  1..24
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 117
IYIWAPLAGT CGVLLLSLVI TLYC                                         24

SEQ ID NO: 118          moltype = DNA  length = 72
FEATURE                 Location/Qualifiers
misc_feature            1..72
                        note = Transmembrane (CD8alpha)
source                  1..72
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 118
atctacattt gggcccctct ggctggaaca tgtggcgtgc tgctgctgtc cctggtcatt  60
actctgtatt gt                                                      72

SEQ ID NO: 119          moltype = AA  length = 27
FEATURE                 Location/Qualifiers
REGION                  1..27
                        note = Transmembrane (CD28)
source                  1..27
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 119
FWVLVVVGGV LACYSLLVTV AFIIFWV                                      27

SEQ ID NO: 120          moltype = DNA  length = 81
FEATURE                 Location/Qualifiers
misc_feature            1..81
                        note = Transmembrane (CD28)
source                  1..81
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 120
ttttgggtcc tggtggtcgt gggaggggtg ctggcatgtt actcactgct ggtcaccgtg  60
gccttcatca tcttctgggt g                                            81

SEQ ID NO: 121          moltype = AA  length = 112
FEATURE                 Location/Qualifiers
REGION                  1..112
                        note = Intracellular stimulatory Signal- (CD3-lambda)
source                  1..112
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 121
RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN  60
ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR          112
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SEQ ID NO: 122          moltype = DNA  length = 339
FEATURE                 Location/Qualifiers
misc_feature            1..339
                        note = Intracellular stimulatory Signal- (CD3-lambda)
source                  1..339
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 122
cgagtgaagt tcagcaggtc cgccgacgct cctgcatacc agcagggaca gaaccagctg  60
tataacgagc tgaatctggg ccggagagag gaatacgacg tgctggacaa aaggcggggc  120
cgggaccccg aaatgggagg gaagccacga cggaaaaacc cccaggaggg cctgtacaat  180
gagctgcaaa aggacaaaat ggccgaggct tattctgaaa tcgggatgaa gggagagaga  240
aggcgcggaa aaggccacga tggcctgtac caggggctga gcaccgctac aaaggacacc  300
tatgatgcac tgcacatgca ggccctgccc cctcggtga                          339

SEQ ID NO: 123          moltype = AA  length = 42
FEATURE                 Location/Qualifiers
REGION                  1..42
                        note = Intracellular stimulatory signal (4-1BB)
source                  1..42
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 123
KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEGGC EL                     42

SEQ ID NO: 124          moltype = DNA  length = 126
FEATURE                 Location/Qualifiers
misc_feature            1..126
                        note = Intracellular stimulatory signal (4-1BB)
source                  1..126
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 124
aagcggggaa gaaagaaact gctgtacatc ttcaaacagc cctttatgag gcctgtgcag  60
accacacagg aggaagacgg ctgctcctgc cggttccccg aggaagagga aggcgggtgc  120
gagctg                                                             126

SEQ ID NO: 125          moltype = AA  length = 41
FEATURE                 Location/Qualifiers
REGION                  1..41
                        note = Intracellular stimulatory signal (CD28)
source                  1..41
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 125
RSKRSRLLHS DYMNMTPRRP GPTRKHYQPY APPRDFAAYR S                      41

SEQ ID NO: 126          moltype = DNA  length = 123
FEATURE                 Location/Qualifiers
misc_feature            1..123
                        note = Intracellular stimulatory signal (CD28)
source                  1..123
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 126
cggagcaaga ggtcccgcct gctgcacagc gactatatga acatgacccc acggagaccc  60
ggccctacac ggaaacatta ccagccctat gctccacccc gggacttcgc agcttacaga  120
agt                                                                123

SEQ ID NO: 127          moltype = AA  length = 22
FEATURE                 Location/Qualifiers
REGION                  1..22
                        note = Intracellular stimulatory signal (OX40 ligand)
source                  1..22
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 127
ERVQPLEENV GNAARPRFER NK                                           22

SEQ ID NO: 128          moltype = DNA  length = 66
FEATURE                 Location/Qualifiers
misc_feature            1..66
                        note = Intracellular stimulatory signal (OX40 ligand)
source                  1..66
                        mol_type = other DNA
                        organism = synthetic construct
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SEQUENCE: 128
gaaagagtgc agcccctgga agagaatgtc gggaatgccg ctcgcccaag atttgaaagg 60
aacaaa 66

SEQ ID NO: 129 moltype = AA length = 445
FEATURE Location/Qualifiers
REGION 1..445
note = Her2-Z CAR (Clone #2)
source 1..445
mol_type = protein
organism = synthetic construct
SEQUENCE: 129
MALPVTALLL PLALLLHAAR PDIQMTQSPS SLSASVGDRV TITCKASQDI NSYLSWFQQK 60
PGKAPKTLIY RANRLVDGVP SRFSGSGSGQ DYTLTISSLQ PEDFATYYCL QYDEFPWTFG 120
QGTKVEIKGG GGSGGGGSGG GGSQVQLVQS GSELKKPGAS VKVSCKASGY TFTNYGVNWV 180
RQAPGQGLEW MGWINTHTGE PTYAEEFKGR FVFSLDTSVS TAYLQISSLK AEDTAVYYCA 240
RDDYYVRVDY WGQGTTVTVS SAKPTTTPAP RPPTPAPTIA SQPLSLRPEA CRPAAGGAVH 300
TRGLDFACDI YIWAPLAGTC GVLLLSLVIT LYCRVKFSRS ADAPAYQQGQ NQLYNELNLG 360
RREEYDVLDK RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD 420
GLYQGLSTAT KDTYDALHMQ ALPPR 445

SEQ ID NO: 130 moltype = DNA length = 1338
FEATURE Location/Qualifiers
misc_feature 1..1338
note = Her2-Z CAR (Clone #2)
source 1..1338
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 130
atggctctgc cagtgactgc actgctgctg ccactggccc tgctgctgca cgcagctcga 60
cctgacatcc agatgaccca gagccccagc agcctgagcg ccagcgtggg cgacagagtg 120
accatcacct gcaaggccag ccaggacatc aacagctacc tgagctggtt ccagcagaag 180
cccggcaagg cccccaagac cctgatctac agagccaaca gactggtgga cggcgtgccc 240
agcagattca gcggcagcgg cagcggccag gactacaccc tgaccatcag cagcctgcag 300
cccgaggact tcgccaccta ctactgcctg cagtacgacg agttcccctg gaccttcggc 360
cagggcacca aggtggagat caagggtggc ggtggatcgg gcggtggtgg atctggagga 420
ggtggctccc aggtgcagct ggtgcagagc ggcagcgagc tgaagaagcc cggcgccagc 480
gtgaaggtga gctgcaaggc cagcggctac accttcacca actacggcgt gaactgggtg 540
agacaggccc ccggccaggg cctggagtgg atgggctgga tcaacaccca caccggcgag 600
cccacctacg ccgaggagtt caagggcaga ttcgtgttca gcctggacac cagcgtgagc 660
accgcctacc tgcagatcag cagcctgaag gccgaggaca ccgccgtgta ctactgcgcc 720
agagacgact actacgtgag agtggactac tggggccagg gcaccaccgt gaccgtgagc 780
agcgcaaaac ctaccacaac tcctgcacca cgccccccta ctccagcacc taccatcgca 840
tctcagccac tgagtctgcg accagaggcc tgccggcccg ccgccggcgg ggccgtccat 900
accagagggc tggactttgc ctgcgatatc tacatttggg cccctctggc tggaacatgt 960
ggcgtgctgc tgctgtccct ggtcattact ctgtattgtc gagtgaagtt cagcaggtcc 1020
gccgacgctc ctgcatacca gcagggacag aaccagctgt ataacgagct gaatctgggc 1080
cggagagagg aatacgacgt gctggacaaa aggcggggcc gggacccga aatgggaggg 1140
aagccacgac ggaaaaaccc ccaggagggc ctgtacaatg agctgcaaaa ggacaaaatg 1200
gccgaggctt attctgaaat cgggatgaag ggagagagaa ggcgcggaaa aggccacgat 1260
ggcctgtacc aggggctgag caccgctaca aaggacacct atgatgcact gcacatgcag 1320
gccctgcccc ctcggtga 1338

SEQ ID NO: 131 moltype = AA length = 487
FEATURE Location/Qualifiers
REGION 1..487
note = Her2-BBZ CAR (Clone #3)
source 1..487
mol_type = protein
organism = synthetic construct
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RDDYYVRVDY WGQGTTVTVS SAKPTTTPAP RPPTPAPTIA SQPLSLRPEA CRPAAGGAVH 300
TRGLDFACDI YIWAPLAGTC GVLLLSLVIT LYCKRGRKKL LYIFKQPFMR PVQTTQEEDG 360
CSCRFPEEEE GGCELRVKFS RSADAPAYQQ GQNQLYNELN LGRREEYDVL DKRRGRDPEM 420
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MQALPPR 487

SEQ ID NO: 132 moltype = DNA length = 1464
FEATURE Location/Qualifiers
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note = Her2-BBZ CAR (Clone #3)
source 1..1464

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source                 1..489
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KHYQPYAPPR DFAAYRSRVK FSRSADAPAY QQGQNQLYNE LNLGRREEYD VLDKRRGRDP  420
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SEQ ID NO: 134         moltype = DNA  length = 1470
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source                 1..1470
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SEQ ID NO: 135         moltype = AA  length = 511
FEATURE                Location/Qualifiers
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source                 1..511
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                       mol_type = other DNA
                       organism = synthetic construct
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gatgcactgc acatgcaggc cctgcccccc cggtga                             1536
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1.-**52**. (canceled)

53. A chimeric antigen receptor targeting human HER2, the chimeric antigen receptor comprising an amino acid sequence comprising, from N- to C-terminus:

an extracellular antigen binding domain comprising a heavy chain variable region comprising the amino acid sequences of SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12;

a CD8a hinge domain;

a CD28 transmembrane domain; and

an intracellular signaling domain comprising:

(i) a CD28 intracellular signaling domain,

(ii) a OX40L intracellular signaling domain, and

(iii) a CD3z intracellular signaling domain.

54. The chimeric antigen receptor of claim **53**, wherein the OX40L intracellular signaling domain comprises an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 127.

55. The chimeric antigen receptor of claim **54**, wherein the OX40L intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 127.

56. The chimeric antigen receptor of claim **53**, wherein the CD3z intracellular signaling domain comprises an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 121.

57. The chimeric antigen receptor of claim **53**, wherein the CD3z intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 121.

58. The chimeric antigen receptor of claim **56**, wherein the CD28 intracellular signaling domain comprises an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 125.

59. The chimeric antigen receptor of claim **56**, wherein the CD28 intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 125.

60. The chimeric antigen receptor of claim **58**, wherein the CD28 transmembrane domain comprises an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 119.

61. The chimeric antigen receptor of claim **60**, wherein the CD28 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 119.

62. The chimeric antigen receptor of claim **60**, wherein the CD8a hinge domain comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 115.

63. The chimeric antigen receptor of claim **62**, wherein the CD8a hinge domain comprises the amino acid sequence of SEQ ID NO: 115.

64. The chimeric antigen receptor of claim **62**, wherein the chimeric antigen receptor further comprises a CD8a extracellular signaling domain.

65. The chimeric antigen receptor of claim **53**, wherein the CD8a extracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 111.

66. The chimeric antigen receptor of claim **66**, wherein the extracellular antigen binding domain comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 113.

67. The chimeric antigen receptor of claim **66**, wherein the extracellular antigen binding domain comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 113.

68. The chimeric antigen receptor of claim **67**, wherein the extracellular antigen binding domain comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 113.

69. The chimeric antigen receptor of claim **68**, wherein the extracellular antigen binding domain comprises the amino acid sequence of SEQ ID NO: 113.

70. The chimeric antigen receptor of claim **65**, wherein the chimeric antigen receptor comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 135.

71. The chimeric antigen receptor of claim **66**, wherein the chimeric antigen receptor comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 135.

72. The chimeric antigen receptor of claim **67**, wherein the chimeric antigen receptor comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 135.

73. The chimeric antigen receptor of claim **68**, wherein the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NO: 135.

* * * * *