*Atg*ctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgcctttctgctgatcccc
(GMCSFRss;SEQ ID NO:31)
Tacatccagatgacccagaccacctccagcctgagcgccagcctgggcgaccgggtgaccatcagctgc
cgggccagccaggacatcagcaagtacctgaactggtatcagcagaagcccgacggcaccgtcaagctg
ctgatctaccacaccagccggctgcacagcggcgtgcccagccggtttagcggcagcggctccggcaccg
actacagcctgaccatctccaacctggaacaggaagatatcgccacctacttttgccagcagggcaacaca
ctgccctacacctttggcggcggaacaaagctggaaatcaccggcagcacctccggcagcggcaagcct
ggcagcggcgagggcagcaccaagggcgaggtgaagctgcaggaaagcggccctggcctggtggccc
ccagccagagcctgagcgtgacctgcaccgtgagcggcgtgagcctgcccgactacggcgtgagctggat
ccggcagccccccaggaagggcctggaatggctgggcgtgatctggggcagcgagaccacctactacaa
cagcgccctgaagagccggctgaccatcatcaaggacaacagcaagagccaggtgttcctgaagatgaa
cagcctgcagaccgacgacaccgccatctactactgcgccaagcactactactacggcggcagctacgcc
atggactactggggccagggcaccagcgtgaccgtgagcagc (CD19scFv; SEQ ID NO:10)
Gaatctaagtacggaccgccctgccccccttgccct (IgG4hinge; SEQ ID NO:50)
Atgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagcctgctggtcaccgtggccttcatcatc
ttttgggtg (CD28tm; SEQ ID NO:12)
Aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaaga
ggaagatggctgtagctgccgatttccagaagaagaagaaggaggatgtgaactg (41BB; SEQ ID
NO:1)
Agggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacga
gctgaacctgggcagaagggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgg
gcggcaagcctcggcggaagaacccccaggaaggcctgtataacgaactgcagaaagacaagatggc
cgaggcctacagcgagatcggcatgaagggcgagcggaggcggggcaagggccacgacggcctgtat
cagggcctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggccctgcccccaagg
(CD3Zeta; SEQ ID NO:16)
Ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccct
agg (T2A; SEQ ID NO:88)
Atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgcaaagtg
tgtaacggaataggtattggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaa
ctgcacctccatcagtggcgatctccacatcctgccggtggcatttaggggtgactccttcacacatactcctc
ctctggatccacaggaactggatattctgaaaaccgtaaaggaaatcacagggtttttgctgattcaggcttgg
cctgaaaacaggacggacctccatgcctttgagaacctagaaatcatacgcggcaggaccaagcaacat
ggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgat
ggagatgtgataatttcaggaaacaaaaatttgtgctatgcaaatacaataaactggaaaaaactgtttggga
cctccggtcagaaaaccaaaattataagcaacagaggtgaaaacagctgcaaggccacaggccaggtct
gccatgccttgtgctcccccgagggctgctggggcccggagcccagggactgcgtctcttgccggaatgtca
gccgaggcagggaatgcgtggacaagtgcaaccttctggagggtgagccaagggagtttgtggagaactc
tgagtgcatacagtgccacccagagtgcctgcctcaggccatgaacatcacctgcacaggacggggacca
gacaactgtatccagtgtgcccactacattgacggcccccactgcgtcaagacctgcccggcaggagtcat
gggagaaaacaacaccctggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaac
tgcacctacggatgcactgggccaggtcttgaaggctgtccaacgaatgggcctaagatcccgtccatcgcc
actgggatggtgggggccctcctcttgctgctggtggtggccctggggatcggcctcttcatg*tga*
(EGFRt; SEQ ID NO:27)

FIG. 1

GMCSFRss

DNA: *ATG*CTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCC
AA: M L L L V T S L L L C E L P H P A

CD19scFv

DNA: TTTCTGCTGATCCCC:GACATCCAGATGACCCAGACCACCTCCAGCCTGAGC
AA: F L L I P D I Q M T Q T T S S L S

DNA: GCCAGCCTGGGCGACCGGGTGACCATCAGCTGCCGGGCCAGCCAGGACATC
AA: A S L G D R V T I S C R A S Q D I

DNA: AGCAAGTACCTGAACTGGTATCAGCAGAAGCCCGACGGCACCGTCAAGCTG
AA: S K Y L N W Y Q Q K P D G T V K L

DNA: CTGATCTACCACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTTAGC
AA: L I Y H T S R L H S G V P S R F S

DNA: GGCAGCGGCTCCGGCACCGACTACAGCCTGACCATCTCCAACCTGGAACAG
AA: G S G S G T D Y S L T I S N L E Q

DNA: GAAGATATCGCCACCTACTTTTGCCAGCAGGGCAACACACTGCCCTACACC
AA: E D I A T Y F C Q Q G N T L P Y T

DNA: TTTGGCGGCGGAACAAAGCTGGAAATCACCGGCAGCACCTCCGGCAGCGGC
AA: F G G G T K L E I T G S T S G S G

DNA: AAGCCTGGCAGCGGCGAGGGCAGCACCAAGGGCGAGGTGAAGCTGCAGGAA
AA: K P G S G E G S T K G E V K L Q E

DNA: AGCGGCCCTGGCCTGGTGGCCCCCAGCCAGAGCCTGAGCGTGACCTGCACC
AA: S G P G L V A P S Q S L S V T C T

DNA: GTGAGCGGCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCCGGCAGCCC
AA: V S G V S L P D Y G V S W I R Q P

DNA: CCCAGGAAGGGCCTGGAATGGCTGGGCGTGATCTGGGGCAGCGAGACCACC
AA: P R K G L E W L G V I W G S E T T

DNA: TACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGC
AA: Y Y N S A L K S R L T I I K D N S

DNA: AAGAGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCC
AA: K S Q V F L K M N S L Q T D D T A

DNA: ATCTACTACTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCCATGGAC
AA: I Y Y C A K H Y Y Y G G S Y A M D

IgG4hinge

DNA: TACTGGGGCCAGGGCACCAGCGTGACCGTGAGCAGC:GAGAGCAAGTACGGA
AA: Y W G Q G T S V T V S S E S K Y G

CD28tm

DNA: CCGCCCTGCCCCCCTTGCCCT:ATGTTCTGGGTGCTGGTGGTGGTCGGAGGC
AA: P P C P P C P M F W V L V V V G G

DNA: GTGCTGGCCTGCTACAGCCTGCTGGTCACCGTGGCCTTCATCATCTTTTGG
AA: V L A C Y S L L V T V A F I I F W

4-1BB

DNA: GTG:AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATG
AA: V K R G R K K L L Y I F K Q P F M

DNA: AGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCA
AA: R P V Q T T Q E E D G C S C R F P

FIG. 2

CD3**Zeta**

DNA: GAAGAAGAAGAAGGAGGATGTGAACTGCGGGTGAAG:TTCAGCAGAAGCGCC
AA: E E E E G G C E L R V K F S R S A

DNA: GACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAAC
AA: D A P A Y Q Q G Q N Q L Y N E L N

DNA: CTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCGGGAC
AA: L G R R E E Y D V L D K R R G R D

DNA: CCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTAT
AA: P E M G G K P R R K N P Q E G L Y

DNA: AACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATG
AA: N E L Q K D K M A E A Y S E I G M

DNA: AAGGGCGAGCGGAGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTG
AA: K G E R R R G K G H D G L Y Q G L

DNA: TCCACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGCCC
AA: S T A T K D T Y D A L H M Q A L P

T2A

DNA: CCAAGG:CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGT
AA: P R L E G G G E G R G S L L T C G

EGFRt

DNA: GACGTGGAGGAGAATCCCGGCCCTAGG:**ATGCTTCTCCTGGTGACAAGCCTT**
AA: D V E E N P G P R M L L L V T S L

DNA: **CTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATCCCACGCAAAGTG**
AA: L L C E L P H P A F L L I P R K V

DNA: **TGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCT**
AA: C N G I G I G E F K D S L S I N A

DNA: **ACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCAC**
AA: T N I K H F K N C T S I S G D L H

DNA: **ATCCTGCCGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTG**
AA: I L P V A F R G D S F T H T P P L

DNA: **GATCCACAGGAACTGGATATTCTGAAAACCGTAAAGGAAATCACAGGGTTT**
AA: D P Q E L D I L K T V K E I T G F

DNA: **TTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATGCCTTTGAG**
AA: L L I Q A W P E N R T D L H A F E

DNA: **AACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTCTT**
AA: N L E I I R G R T K Q H G Q F S L

DNA: **GCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAG**
AA: A V V S L N I T S L G L R S L K E

DNA: **ATAAGTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCA**
AA: I S D G D V I I S G N K N L C Y A

DNA: **AATACAATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAA**
AA: N T I N W K K L F G T S G Q K T K

DNA: **ATTATAAGCAACAGAGGTGAAAACAGCTGCAAGGCCACAGGCCAGGTCTGC**
AA: I I S N R G E N S C K A T G Q V C

FIG. 2 (Cont'd)

DNA: **CATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAGGGACTGC**
AA: H A L C S P E G C W G P E P R D C

DNA: **GTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCAAC**
AA: V S C R N V S R G R E C V D K C N

DNA: **CTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAG**
AA: L L E G E P R E F V E N S E C I Q

DNA: **TGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGG**
AA: C H P E C L P Q A M N I T C T G R

DNA: **GGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGC**
AA: G P D N C I Q C A H Y I D G P H C

DNA: **GTCAAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGG**
AA: V K T C P A G V M G E N N T L V W

DNA: **AAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAAACTGCACC**
AA: K Y A D A G H V C H L C H P N C T

DNA: **TACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTAAG**
AA: Y G C T G P G L E G C P T N G P K

DNA: **ATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTG**
AA: I P S I A T G M V G A L L L L L V

DNA: **GTGGCCCTGGGGATCGGCCTCTTCATG*TGA*** (SEQ ID NO:33)
AA: V A L G I G L F M * (SEQ ID NO:34)

FIG. 2 (Cont'd)

ZXR-014 Map of Sections

GMCSFRss: nt2084-2149

CD19scFv: nt2150-2884

IgG4Hinge: nt2885-2920

CD28tm: nt2921-3004

4-1BB: nt3005-3130

Zeta: nt3131-3466

T2A: nt3467-3538

EGFRt: nt3539-4612

FIG. 3A

Oligo name	Sequence	Region (SEQ ID NO.)
oJ02649	ATCAAAAGAATAGACCGAGATAGGGT	pre-U5 (SEQ ID NO:71)
oJ02648	CCGTACCTTTAAGACCAATGACTTAC	delU3 (SEQ ID NO:25)
oJ02650	TTGAGAGTTTTCGCCCCG	mid-Ampr (SEQ ID NO:64)
oJ02651	AATAGACAGATCGCTGAGATAGGT	post-Ampr (SEQ ID NO:70)
oJ02652	CAGGTATCCGGTAAGCGG	CoE1 ori (SEQ ID NO:24)
oJ02653	CGACCAGCAACCATAGTCC	SV40 (SEQ ID NO:87)
oJ02654	TAGCGGTTTGACTCACGG	CMV (SEQ ID NO:23)
oJ02655	GCAGGGAGCTAGAACGATTC	psi (SEQ ID NO:73)
oJ02656	ATTGTCTGGTATAGTGCAGCAG	RRE (SEQ ID NO:85)
oJ02657	TCGCAACGGGTTTGCC	EF1p (SEQ ID NO:26)
oJ02658	AGGAAGATATCGCCACCTACT	CD19Rop (SEQ ID NO:8)
oJ02601	CGGGTGAAGTTCAGCAGAAG	Zeta (SEQ ID NO:99)
oJ02735	ACTGTGTTTGCTGACGCAAC	WPRE (SEQ ID NO:96)
oJ02715	ATGCTTCTCCTGGTGACAAG	EGFRt (SEQ ID NO:29)

FIG. 3B

Uniprot P0861 IgG4-Fc (SEQ ID NO:92)

```
        10         20         30         40         50         60
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

        70         80         90        100        110        120
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV

       130        140        150        160        170        180
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY

       190        200        210        220        230        240
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK

       250        260        270        280        290        300
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG

       310        320
NVFSCSVMHE ALHNHYTQKS LSLSLGK
```

1-98 CH1

99-110 Hinge

111-220 CH2

221-327 CH3

Position 108 S→P

FIG. 4

Uniprot P10747 CD28 (SEQ ID NO:93)

```
        10         20         30         40         50         60
MLRLLLALNL FPSIQVTGNK ILVKQSPMLV AYDNAVNLSC KYSYNLFSRE FRASLHKGLD

        70         80         90        100        110        120
SAVEVCVVYG NYSQQLQVYS KTGFNCDGKL GNESVTFYLQ NLYVNQTDIY FCKIEVMYPP

       130        140        150        160        170        180
PYLDNEKSNG TIIHVKGKHL CPSPLFPGPS KPFWVLVVVG GVLACYSLLV TVAFIIFWVR

       190        200        210        220
SKRSRLLHSD YMNMTPRRPG PTRKHYQPYA PPRDFAAYRS
```

1-18 signal peptide

19-152 extracellular domain

153-179 transmembrane domain

180-220 intracellular domain

Position 186-187 LL→GG

FIG. 5

Uniprot Q07011 4-1BB (SEQ ID NO:95)

```
        10         20         30         40         50         60
MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR

        70         80         90        100        110        120
TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC

       130        140        150        160        170        180
CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE

       190        200        210        220        230        240
PGHSPQIISF FLALTSTALL FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG

       250
CSCRFPEEEE GGCEL
```

1-23 signal peptide

24-186 extracellular domain

187-213 transmembrane domain

214-255 intracellular domain

FIG. 6

Uniprot P20963 human CD3ζ isoform 3 (SEQ ID NO:94)

```
        10         20         30         40         50         60
MKWKALFTAA ILQAQLPITE AQSFGLLDPK LCYLLDGILF IYGVILTALF LRVKFSRSAD

        70         80         90        100        110        120
APAYQQGQNQ LYNELNLGRR EEYDVLDKRR GRDPEMGGKP QRRKNPQEGL YNELQKDKMA

       130        140        150        160
EAYSEIGMKG ERRRGKGHDG LYQGLSTATK DTYDALHMQA LPPR
```

1-21 signal peptide

22-30 extracellular

31-51 transmembrane

52-164 intracellular domain

61-89 ITAM1

100-128 ITAM2

131-159 ITAM3

FIG. 7

Human IgG1	EPKSCDKTHTCPPCP (SEQ ID NO:44)
Human IgG2	ERKCCVECPPCP (SEQ ID NO:48)
Human IgG3	ELKTPLGDTHTCPRCP (SEQ ID NO:45)
	$(EPKSCDTPPPCPRCP)_3$ (SEQ ID NO:46)
Human IgG4	ESKYGPPCPSCP (SEQ ID NO:47)
Modified Human IgG4	ESKYGPPCPPCP (SEQ ID NO:68)
Modified Human IgG4	YGPPCPPCP (SEQ ID NO:67)
Modified Human IgG4	KYGPPCPPCP (SEQ ID NO:66)
Modified Human IgG4	EVVKYGPPCPPCP (SEQ ID NO:65)

FIG. 8

R12 long spacer CAR: PJ_R12-CH2-CH3-41BB-Z-T2A-tEGFR (SEQ ID NO:80)

GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC
AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACT
AGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGG
ACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCG
CGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAG
GCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATG
GGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGC
AAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTA
GACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTAT
ATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAG
CTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCT
GACACAGGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCA
AATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAA
GGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAAGA
TTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGAC
CATCAATGAGGAAGCTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGG
AATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAA
TGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGC
TGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTC
CAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGG
TTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCAGTATTCATC
CACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACAT
AATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGG
GTTTATTACAGGGACAGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGG
TTAGGCGTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGA
GCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCC
TAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCC
CGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG
GGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGC
CGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTG
GTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTT
TGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCT
GCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGA
CCGGCGCCTACGGCTAGCGAATTCCTCGAGGCCACCATGCTGCTGCTGGTGACAAGCCTGC
TGCTGTGCGAGCTGCCCCACCCCGCCTTTCTGCTGATCCCCCAGGAACAGCTCGTCGAAAGC
GGCGGCAGACTGGTGACACCTGGCGGCAGCCTGACCCTGAGCTGCAAGGCCAGCGGCTTCG
ACTTCAGCGCCTACTACATGAGCTGGGTCCGCCAGGCCCCTGGCAAGGGACTGGAATGGAT
CGCCACCATCTACCCCAGCAGCGGCAAGACCTACTACGCCACCTGGGTGAACGGACGGTTC
ACCATCTCCAGCGACAACGCCCAGAACACCGTGGACCTGCAGATGAACAGCCTGACAGCCG
CCGACCGGGCCACCTACTTTTGCGCCAGAGACAGCTACGCCGACGACGGCGCCCTGTTCAA

FIG. 9

CATCTGGGGCCCTGGCACCCTGGTGACAATCTCTAGCGGCGGAGGCGGATCTGGTGGCGGA
GGAAGTGGCGGCGGAGGATCTGAGCTGGTGCTGACCCAGAGCCCCTCTGTGTCTGCTGCCC
TGGGAAGCCCTGCCAAGATCACCTGTACCCTGAGCAGCGCCCACAAGACCGACACCATCGA
CTGGTATCAGCAGCTGCAGGGCGAGGCCCCCAGATACCTGATGCAGGTGCAGAGCGACGGC
AGCTACACCAAGAGGCCAGGCGTGCCCGACCGGTTCAGCGGATCTAGCTCTGGCGCCGACC
GCTACCTGATCATCCCCAGCGTGCAGGCCGATGACGAGGCCGATTACTACTGTGGCGCCGA
CTACATCGGCGGCTACGTGTTCGGCGGAGGCACCCAGCTGACCGTGACCGGCGAGTCTAAG

IgG4 spacer

TACGGACCGCCCTGCCCCCCTTGCCCT

CH2

GCCCCCGAGTTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCC
TGATGATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCAGGAAGATCC
CGAGGTCCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCC
AGAGAGGAACAGTTCAACAGCACCTACCGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGA
CTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCG
AAAAGACCATCAGCAAGGCCAAG

CH3

GGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGATGACCAAGA
ACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTG
GGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCGAC
GGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACG
TCTTTAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGC
CTGTCCCTGGGCAAG

4-1BB

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGACAG
TGGCCTTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC
CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAG
AAGAAGAAGGAGGATGTGAACTG

CD3ζ

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTGT
ACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCG
GGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATAACGAA
CTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGAGGC
GGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGGATACCTACGA
CGCCCTGCACATGCAGGCCCTGCCCCCAAGG

T2A

CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATC
CCGGCCCTAGG

tEGFR

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATC
CCACGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTA
CGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGG
CATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTCTGA

FIG. 9 (Cont'd)

AAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGG
ACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGT
TTTCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGA
TAAGTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAA
CTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGA
AAACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCT
GGGGCCCGGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATG
CGTGGACAAGTGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGT
GCATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGG
GGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGAC
CTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCC
GGCCATGTGTGCCACCTGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGTCT
TGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGG
CCCTCCTCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATGTGAGCGGCCGC
TCTAGACCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAACCTCTGGATTAC
AAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATA
CGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCT
TGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTG
GCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACC
TGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATC
GCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCG
TGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGA
TTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTT
CCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGAC
GAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCGTCGACTAGCCGTACCTT
TAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGG
GACTGGAAGGGCTAATTCACTCCCAAAGAAGACAAGATCTGCTTTTTGCCTGTACTGGGT
CTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTG
CTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGT
GACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGA
ATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCGGTACCCAATTCG
CCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA
AACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGT
AATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGA
ATGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTC
ATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAG
ATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCC
AACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACC
CTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAG
CCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAG
AAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAA
CCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCG
GGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCG
CTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTA
TTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCT
CACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG
TTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACG
TTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGAC
GCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTA
CTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGC

FIG. 9 (Cont'd)

TGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCG
AAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGG
AACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAA
TGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACA
ATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCC
GGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCAT
TGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAG
TCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAG
CATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTT
AATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGT
GAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGT
TTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG
CAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGT
AGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGA
TAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTC
GGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAAC
TGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGG
ACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGG
GGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGAT
TTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTT
TACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGAT
TCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACG
ACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC
TCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAA
AGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGC
TTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACA
CAGGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACA
AAAGCTGGAGCTCCACCGCGGTGGCGGCCTCGAGGTCGAGATCCGGTCGACCAGCAACC
ATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCT
CCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTG
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTCG
ACGGTATCGATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTA
TTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACAT
AACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAA
TAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGA
GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCC
CCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTAT
GGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCG
GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC
CACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAAT
GTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGAATTCGGAGT
GGCGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTG

FIG. 9 (Cont'd)

Leader _R12- Hinge-CH2-CH3- CD28tm/41BB-Z-T2A-tEGFR (SEQ ID NO:58)

Leader

MLLLVTSLLLCELPHPAFLLIP

R12 scFv

QEQLVESGGRLVTPGGSLTLSCKASGFDFSAYYMSWVRQAPGKGLEWIATIYPSSGKTYYATWVNG
RFTISSDNAQNTVDLQMNSLTAADRATYFCARDSYADDGALFNIWGPGTLVTISSGGGGSGGGGSGG
GGSELVLTQSPSVSAALGSPAKITCTLSSAHKTDTIDWYQQLQGEAPRYLMQVQSDGSYTKRPGVPD
RFSGSSSGADRYLIIPSVQADDEADYYCGADYIGGYVFGGGTQLTVTG

Hinge Spacer

ESKYGPPCPPCP

CH2

APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK

CH3

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

CD28

MFWVLVVVGGVLACYSLLVTVAFIIFWV

4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD3 zeta

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

T2A

LEGGGEGRGSLLTCGDVEENPGPR

tEGFR

MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTH
TPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLK
EISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRD
CVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGP
HCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALL
LLLVVALGIGLFM

FIG. 10

R12 intermediate spacer CAR: PJ_R12-CH3-41BB-Z-T2A-tEGFR

(SEQ ID NO:79)

GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCA
ATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAG
AGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACT
TGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGC
ACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTA
GAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATGGGAA
AAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCA
GGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAA
TACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATAC
AGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGAC
AAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGA
CACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAATGGTACAT
CAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCC
CAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCAT
GCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGA
AGCTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTG
TTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTA
CAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAG
GCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTG
GCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTC
ATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAA
GGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAA
ACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAG
AGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTC
GCGAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCC
CGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTA
AACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTAT
ATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG
AAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCC
ACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCG
TCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTAC
CTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTC
GTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACGGCTAGCGAATTCCT
CGAGGCC

R12 ScFv

ACCATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCCTTTCTGCT
GATCCCCCAGGAACAGCTCGTCGAAAGCGGCGGCAGACTGGTGACACCTGGCGGCAGCCTGA
CCCTGAGCTGCAAGGCCAGCGGCTTCGACTTCAGCGCCTACTACATGAGCTGGGTCCGCCAG
GCCCCTGGCAAGGGACTGGAATGGATCGCCACCATCTACCCCAGCAGCGGCAAGACCTACTA
CGCCACCTGGGTGAACGGACGGTTCACCATCTCCAGCGACAACGCCCAGAACACCGTGGACC
TGCAGATGAACAGCCTGACAGCCGCCGACCGGGCCACCTACTTTTGCGCCAGAGACAGCTAC
GCCGACGACGGCGCCCTGTTCAACATCTGGGGCCCTGGCACCCTGGTGACAATCTCTAGCGG
CGGAGGCGGATCTGGTGGCGGAGGAAGTGGCGGCGGAGGATCTGAGCTGGTGCTGACCCAG

FIG. 11

AGCCCCTCTGTGTCTGCTGCCCTGGGAAGCCCTGCCAAGATCACCTGTACCCTGAGCAGCG
CCCACAAGACCGACACCATCGACTGGTATCAGCAGCTGCAGGGCGAGGCCCCCAGATACCT
GATGCAGGTGCAGAGCGACGGCAGCTACACCAAGAGGCCAGGCGTGCCCGACCGGTTCAG
CGGATCTAGCTCTGGCGCCGACCGCTACCTGATCATCCCCAGCGTGCAGGCCGATGACGAG
GCCGATTACTACTGTGGCGCCGACTACATCGGCGGCTACGTGTTCGGCGGAGGCACCCAGC
TGACCGTGACCGGCGAGTCTAAG

Hinge Spacer

TACGGACCG CCCTGCCCCCCTTGCCCT

CH3

GGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGATGACCAAG
AACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGT
GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCG
ACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCA
ACGTCTTTAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCT
GAGCCTGTCCCTGGGCAAG

4-1BB

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGACA
GTGGCCTTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAA
CCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGA
AGAAGAAGAAGGAGGATGTGAACTG

CD3ζ

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTG
TACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGC
CGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATAAC
GAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGG
AGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGGATACC
TACGACGCCCTGCACATGCAGGCCCTGCCCCCAAGG

T2A

CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTAGG

tEGFR

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGAT
CCCACGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGC
TACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGT
GGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTC
TGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACG
GACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTT
TTCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAA
GTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGA
AAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAAAACAGC
TGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCG
GAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAG
TGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCC
ACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTG

FIG. 11 (Cont'd)

TATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAGT
CATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACCT
GTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAAT
GGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTG
GTGGCCCTGGGGATCGGCCTCTTCATGTGAGCGGCCGCTCTAGACCCGGGCTGCAGGAAT
TCGATATCAAGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTA
TTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG
CTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTT
ATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACG
CAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTT
CCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGG
GGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCT
TGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTT
CGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTC
CGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATC
GATACCGTCGACTAGCCGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGC
CACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGAAGACAAGATC
TGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGG
CTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGT
GTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTG
GAAAATCTCTAGCAGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCC
CGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGT
CGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCG
CCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCC
TGAATGGCGAATGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTA
AATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATA
GACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTG
GACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCAT
CACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGG
GAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGA
AGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTA
ACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGG
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTC
ATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAA
CATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCA
GAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCG
AACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATG
ATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAG
AGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCAC
AGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATG
AGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCG
CTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAA

FIG. 11 (Cont'd)

TGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTG
CGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGAT
GGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT
GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCA
GATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATG
AACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGAC
CAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGT
GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGC
GTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCT
GCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCT
ACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTC
TAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGG
ACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCA
CACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATG
AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGT
CGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCC
TGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTT
TGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGA
GTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGA
AGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGC
AGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTG
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTG
TGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGC
TCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCTCGA
GGTCGAGATCCGGTCGACCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCC
TAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAG
AGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGG
CCTAGGCTTTTGCAAAAAGCTTCGACGGTATCGATTGGCTCATGTCCAACATTACCGCCATG
TTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC
ATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC
GACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTT
CCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT
ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGC
TATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCAC
GGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAA
CGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTG
TACGGAATTCGGAGTGGCGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTAC
TGGGTCTCTCTG

FIG. 11 (Cont'd)

Leader _R12- Hinge- CH3- CD28tm/41BB-Z-T2A-tEGFR (SEQ ID NO:57)

Leader

MLLLVTSLLLCELPHPAFLLIP

R12 scFV

QEQLVESGGRLVTPGGSLTLSCKASGFDFSAYYMSWVRQAPGKGLEWIATIYPSSGKTYYA
TWVNGRFTISSDNAQNTVDLQMNSLTAADRATYFCARDSYADDGALFNIWGPGTLVTISSGG
GGSGGGGSGGGGSELVLTQSPSVSAALGSPAKITCTLSSAHKTDTIDWYQQLQGEAPRYLM
QVQSDGSYTKRPGVPDRFSGSSSGADRYLIIPSVQADDEADYYCGADYIGGYVFGGGTQLT
VTG

Hinge Spacer

ESKYGPPCPPCP

CH3

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

CD28tm

MFWVLVVVGGVLACYSLLVTVAFIIFWV

4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD3ζ

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY
NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

T2A

LEGGGEGRGSLLTCGDVEENPGPR

tEGFR

MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFR
GDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVV
SLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVC
HALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQA
MNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTY
GCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

FIG. 12

R12 short spacer CAR: PJ_R12-Hinge-41BB-Z-T2A-tEGFR (SEQ ID NO:83)

GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAAT
AAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGAT
CCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAG
CGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAG
AGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGA
GATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAA
GGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGA
TTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAA
CCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTG
TGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAAC
AAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAA
TTACCCTATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAA
TGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATT
ATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAG
CCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGGCAAAGAGAAGAGTGGTGCAGAG
AGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTA
TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAG
CAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCAT
CAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGA
TTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCAGTATT
CATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACA
TAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTT
TATTACAGGGACAGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTTAGG
CGTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACA
TCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGT
GGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGG
AGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAA
CACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCG
CCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCC
GCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCT
ACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTC
GTTTTCTGTTCTGCGCCGTTACAGATC

R12 scFV

ACCATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCCTTTCTGCTGA
TCCCCCAGGAACAGCTCGTCGAAAGCGGCGGCAGACTGGTGACACCTGGCGGCAGCCTGACCCT
GAGCTGCAAGGCCAGCGGCTTCGACTTCAGCGCCTACTACATGAGCTGGGTCCGCCAGGCCCCT
GGCAAGGGACTGGAATGGATCGCCACCATCTACCCCAGCAGCGGCAAGACCTACTACGCCACCT
GGGTGAACGGACGGTTCACCATCTCCAGCGACAACGCCCAGAACACCGTGGACCTGCAGATGAA
CAGCCTGACAGCCGCCGACCGGGCCACCTACTTTTGCGCCAGAGACAGCTACGCCGACGACGGC
GCCCTGTTCAACATCTGGGGCCCTGGCACCCTGGTGACAATCTCTAGCGGCGGAGGCGGATCTG
GTGGCGGAGGAAGTGGCGGCGGAGGATCTGAGCTGGTGCTGACCCAGAGCCCCTCTGTGTCTG
CTGCCCTGGGAAGCCCTGCCAAGATCACCTGTACCCTGAGCAGCGCCCACAAGACCGACACCAT
CGACTGGTATCAGCAGCTGCAGGGCGAGGCCCCCAGATACCTGATGCAGGTGCAGAGCGACGG
CAGCTACACCAAGAGGCCAGGCGTGCCCGACCGGTTCAGCGGATCTAGCTCTGGCGCCGACCGC
TACCTGATCATCCCCAGCGTGCAGGCCG

FIG. 13

ATGACGAGGCCGATTACTACTGTGGCGCCGACTACATCGGCGGCTACGTGTTCGGCGGAGG
CACCCAGCTGACCGTGACCGGCGAGTCTAAG

Hinge/Spacer

TACGGACCGCCCTGCCCCCCTTGCCCT

4-1BB

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGACAG
TGGCCTTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC
CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAA
GAAGAAGAAGGAGGATGTGAACTGCAAGCTGTGACCGGCGCCTACGGCTAGF

CD3ζ

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTGT
ACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCG
GGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATAACGAA
CTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGAGG
CGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGGATACCTAC
GACGCCCTGCACATGCAGGCCCTGCCCCCAAGG

T2A

CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTAGG

tEGFR

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATC
CCACGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCT
ACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTG
GCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTCT
GAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGG
ACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTT
TCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGT
GATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGAAAA
AACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAAAACAGCTGC
AAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAG
CCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCA
ACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCACCCA
GAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTGTATCC
AGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAGTCATGGG
AGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCAT
CCAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTA
AGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTGGTGGCCCT
GGGGATCGGCCTCTTCATGTGAGCGGCCGCTCTAGACCCGGGCTGCAGGAATTCGATATCA
AGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTA
TGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC
CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGT
GGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGG
TTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTG
CCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGG
GCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGT
GTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGC
GGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGC
CCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCGTCGACTAGCCG
TACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGG

FIG. 13 (Cont'd)

GGGGACTGGAAGGGCTAATTCACTCCCAAAGAAGACAAGATCTGCTTTTTGCCTGTACT
GGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCC
ACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTT
GTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAG
CAGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCGGTACCCA
ATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACT
GGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGC
TGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGA
ATGGCGAATGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAA
TCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATA
GACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGT
GGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAA
CCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCT
AAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGG
AAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGC
TGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGG
CACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAAT
ATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAG
AGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTC
CTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGT
GCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCG
CCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATT
ATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATG
ACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGA
GAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACA
ACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC
TCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA
CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTT
ACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACC
ACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTG
AGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT
CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCG
CTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATA
TACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTT
GATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC
GTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTG
CAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAAC
TCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGT
GTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTC
TGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGT
GCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGC

FIG. 13 (Cont'd)

GGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCT
GGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT
GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCT
TTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTA
TCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTC
GCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA
GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAG
CTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTA
ATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGC
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTAT
GACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTG
GAGCTCCACCGCGGTGGCGGCCTCGAGGTCGAGATCCGGTCGACCAGCAACC
ATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCC
CATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCG
CCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAG
GCTTTTGCAAAAAGCTTCGACGGTATCGATTGGCTCATGTCCAACATTACCGCCA
TGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGT
TCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCT
GGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCC
ATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT
AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT
TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTT
ATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG
GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGG
GGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAA
ATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGG
GCGGTAGGCGTGTACGGAATTCGGAGTGGCGAGCCCTCAGATCCTGCATATAAG
CAGCTGCTTTTTGCCTGTACTGGGTCTCTCTG

FIG. 13 (Cont'd)

Leader _R12 - CD28tm/41BB-Z-T2A-tEGFR (SEQ ID NO:56)

Leader

MLLLVTSLLLCELPHPAFLLIP

scFv R12

QEQLVESGGRLVTPGGSLTLSCKASGFDFSAYYMSWVRQAPGKGLEWIATIYPSSGKT
YYATWVNGRFTISSDNAQNTVDLQMNSLTAADRATYFCARDSYADDGALFNIWGPGTL
VTISSGGGGSGGGGSGGGGSELVLTQSPSVSAALGSPAKITCTLSSAHKTDTIDWYQQ
LQGEAPRYLMQVQSDGSYTKRPGVPDRFSGSSSGADRYLIIPSVQADDEADYYCGAD
YIGGYVFGGGTQLTVTG

Hinge/spacer

ESKYGPPCPPCP

CD28tm

MFWVLVVVGGVLACYSLLVTVAFIIFWV

4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD3ζ

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

T2A

LEGGGEGRGSLLTCGDVEENPGPR

tEGFR

MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPV
AFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHG
QFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRG
ENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVE
NSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWK
YADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

FIG. 14

R11 long spacer CAR: PJ_R11-CH2-CH3-41BB-Z-T2A-tEGFR (SEQ ID NO:75)

GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGC
CTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTG
GTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCC
GAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGG
CTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAA
TTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGG
GGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAA
ATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCT
GTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGAC
AGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAA
AGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAA
AGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCA
AAATTACCCTATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCCATATCACCTAG
AACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACC
CATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACA
GTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGC
AGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTC
CTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGT
ACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTAT
TGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAA
GAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCT
CTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCAGTATTCATCCA
CAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACA
TAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTT
CGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGC
TTAGGGTTAGGCGTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTCA
GTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAAT
TGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTG
GCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGA
ACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGC
ATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCG
CGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTT
TAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTC
AGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTT
CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACGGCTAGC

scFv R12

GAATTCGCCACCATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCCCACCC
CGCCTTTCTGCTGATCCCCCAGAGCGTGAAAGAGTCCGAGGGCGACCTGGTCACACCAG
CCGGCAACCTGACCCTGACCTGTACCGCCAGCGGCAGCGACATCAACGACTACCCCATC
TCTTGGGTCCGCCAGGCTCCTGGCAAGGGACTGGAATGGATCGGCTTCATCAACAGCGG
CGGCAGCACTTGGTACGCCAGCTGGGTCAAAGGCCGGTTCACCATCAGCCGGACCAGC
ACCACCGTGGACCTGAAGATGACAAGCCTGACCACCGACGACACCGCCACCTACTTTTG

FIG. 15

CGCCAGAGGCTACAGCACCTACTACGGCGACTTCAACATCTGGGGCCCTGGCACCCTG
GTCACAATCTCTAGCGGCGGAGGCGGCAGCGGAGGTGGAGGAAGTGGCGGCGGAGGA
TCCGAGCTGGTCATGACCCAGACCCCCAGCAGCACATCTGGCGCCGTGGGCGGCACCG
TGACCATCAATTGCCAGGCCAGCCAGAGCATCGACAGCAACCTGGCCTGGTTCCAGCAG
AAGCCCGGCCAGCCCCCCACCCTGCTGATCTACAGAGCCTCCAACCTGGCCAGCGGCG
TGCCAAGCAGATTCAGCGGCAGCAGATCTGGCACCGAGTACACCCTGACCATCTCCGG
CGTGCAGAGAGAGGACGCCGCTACCTATTACTGCCTGGGCGGCGTGGGCAACGTGTCC
TACAGAACCAGCTTCGGCGGAGGTACTGAGGTGGTCGTCAAA

Hinge/Spacer

TACGGACCGCCCTGCCCCCCTTGCCCT

CH2

GCCCCCGAGTTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACA
CCCTGATGATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCAGGA
AGATCCCGAGGTCCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGA
CCAAGCCCAGAGAGGAACAGTTCAACAGCACCTACCGGGTGGTGTCTGTGCTGACCGT
GCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGGC
CTGCCCAGCAGCATCGAAAAGACCATCAGCAAGGCCAAG

CH3

GGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGATGACCA
AGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGT
GGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTG
GACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAAGGCAACGTCTTTAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACC
CAGAAGAGCCTGAGCCTGTCCCTGGGCAAG

4-1BB

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGA
CAGTGGCCTTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTCA
AACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGAT
TTCCAGAAGAAGAAGAAGGAGGATGTGAACTG

CD3ζ

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGC
TGTACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAG
AGGCCGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCT
GTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAG
GGCGAGCGGAGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCC
ACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCAAGG

T2A

CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG
AATCCCGGCCCTAGG

tEGFR

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTG
ATCCCACGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATA
AATGCTACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATC
CTGCCGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGA
ACTGGATATTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCC
TGAAAACAGGACGGACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCA

FIG. 15 (Cont'd)

AGCAACATGGTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGC
TCCCTCAAGGAGATAAGTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGC
AAATACAATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCA
ACAGAGGTGAAAACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCG
AGGGCTGCTGGGGCCCGGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGC
AGGGAATGCGTGGACAAGTGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAAC
TCTGAGTGCATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACA
GGACGGGGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTC
AAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGAC
GCCGGCCATGTGTGCCACCTGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGT
CTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGG
GCCCTCCTCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATGTGAGCGGCCGCT
CTAGACCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAACCTCTGGATTACAAA
ATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCT
GCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTAT
AAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGG
TGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCT
CCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGC
CTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCG
GGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGA
CGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCT
GCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCT
TTGGGCCGCCTCCCCGCATCGATACCGTCGACTAGCCGTACCTTTAAGACCAATGACTTAC
AAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTC
ACTCCCAAAGAAGACAAGATCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATC
TGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGC
CTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCT
CAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGAATTCGATATCAAGCTTATCGATACC
GTCGACCTCGAGGGGGGGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCA
CTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCC
TTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCC
CTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAAATTGTAAGCGTTAATATTTTGTTAA
AATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAA
TCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAG
AGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCG
ATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGC
ACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAA
CGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTG
TAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCG
CGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATA
CATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAA
AGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTG
CCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGG
GTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCG
CCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTAT

FIG. 15 (Cont'd)

CCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTT
GGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTAT
GCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGG
AGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGAT
CGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCT
GTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG
GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC
CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTA
TCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGG
GAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTA
AGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTT
TTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACG
TGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTT
TGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCA
GATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAG
CACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAA
GTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGG
CTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAG
ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAG
GTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAA
ACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTG
TGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG
TTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTG
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGC
GCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCG
CGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAG
TGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTA
TGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGC
TATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCT
CCACCGCGGTGGCGGCCTCGAGGTCGAGATCCGGTCGACCAGCAACCATAGTCCCGCCCC
TAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTG
ACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGT
AGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTCGACGGTATCGATTGGCT
CATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTAC
GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCC
CGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCAT
AGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCC
ACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGT
AAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTA
CATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGC
GTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAG
TTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGA
CGCAAATGGGCGGTAGGCGTGTACGGAATTCGGAGTGGCGAGCCCTCAGATCCTGCATATA
AGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTG

FIG. 15 (Cont'd)

Leader _R11- Hinge-CH2-CH3- CD28tm/41BB-Z-T2A-tEGFR (SEQ ID NO:54)

Leader

MLLLVTSLLLCELPHPAFLLIP

R11 scFv

QSVKESEGDLVTPAGNLTLTCTASGSDINDYPISWVRQAPGKGLEWIGFINSGGSTWYASW
VKGRFTISRTSTTVDLKMTSLTTDDTATYFCARGYSTYYGDFNIWGPGTLVTISSGGGGSGG
GGSGGGGSELVMTQTPSSTSGAVGGTVTINCQASQSIDSNLAWFQQKPGQPPTLLIYRASN
LASGVPSRFSGSRSGTEYTLTISGVQREDAATYYCLGGVGNVSYRTSFGGGTEVVVK

Hinge/Spacer

ESKYGPPCPPCP

CH2

APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK

CH3

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

CD28tm

MFWVLVVVGGVLACYSLLVTVAFIIFWV

4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD3zeta

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY
NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

T2A

LEGGGEGRGSLLTCGDVEENPGPR

tEGFR

MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFR
GDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVV
SLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQV
CHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP
QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNC
TYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

FIG. 16

R11 intermediate spacer CAR: PJ_R11-CH3-41BB-Z-T2A-tEGFR (SEQ ID NO:74)

GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAG
CCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACT
CTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGG
CGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGG
ACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTAC
GCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAG
TATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAA
GAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCA
GTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTAC
AACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACC
CTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGAT
AGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGG
ACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAATG
GTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAA
GGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCA
CAAGATTTAAACACCATGCTAAACACAGTGGGGGGGACATCAAGCAGCCATGCAAATGT
TAAAAGAGACCATCAATGAGGAAGCTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAA
AAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC
ACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTA
TAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCA
ACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA
CCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACC
ACTGCTGTGCCTTGGATCTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGG
GGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATA
CAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGG
GACAGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTTAGGCG
TTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGC
GCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGT
GCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGC
CTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTC
TTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTC
TCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGT
TCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTA
AAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTC
AGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTT
TTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACG GCTAGC

R11 scFV

GAATTCGCCACC ATG CTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCCCAC
CCCGCCTTTCTGCTGATCCCCCAGAGCGTGAAAGAGTCCGAGGGCGACCTGGTCACA
CCAGCCGGCAACCTGACCCTGACCTGTACCGCCAGCGGCAGCGACATCAACGACTAC
CCCATCTCTTGGGTCCGCCAGGCTCCTGGCAAGGGACTGGAATGGATCGGCTTCATC
AACAGCGGCGGCAGCACTTGGTACGCCAGCTGGGTCAAAGGCCGGTTCACCATCAGC

FIG. 17

CGGACCAGCACCACCGTGGACCTGAAGATGACAAGCCTGACCACCGACGACACCGCCACCT
ACTTTTGCGCCAGAGGCTACAGCACCTACTACGGCGACTTCAACATCTGGGGCCCTGGCACC
CTGGTCACAATCTCTAGCGGCGGAGGCGGCAGCGGAGGTGGAGGAAGTGGCGGCGGAGGA
TCCGAGCTGGTCATGACCCAGACCCCCAGCAGCACATCTGGCGCCGTGGGCGGCACCGTGA
CCATCAATTGCCAGGCCAGCCAGAGCATCGACAGCAACCTGGCCTGGTTCCAGCAGAAGCCC
GGCCAGCCCCCCACCCTGCTGATCTACAGAGCCTCCAACCTGGCCAGCGGCGTGCCAAGCA
GATTCAGCGGCAGCAGATCTGGCACCGAGTACACCCTGACCATCTCCGGCGTGCAGAGAGA
GGACGCCGCTACCTATTACTGCCTGGGCGGCGTGGGCAACGTGTCCTACAGAACCAGCTTCG
GCGGAGGTACTGAGGTGGTCGTCAAA

Hinge/spacer

TAGGACCGCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCCAGCGTGTTCCT
GTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTGACCTGCGTG
GTGGTGGACGTGAGCCAGGAAGATCCCGAGGTCCAGTTCAATTGGTACGTGGACGGCGTGG
AAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAACAGTTCAACAGCACCTACCGGGTGGTG
TCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTC
CAACAAGGGCCTGCCCAGCAGCATCGAAAAGACCATCAGCAAGGCCAAG

CH3

GGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGATGACCAAGA
ACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTG
GGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCGAC
GGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACG
TCTTTAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGC
CTGTCCCTGGGCAAG

4-1BB

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGACAG
TGGCCTTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC
CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAG
AAGAAGAAGGAGGATGTGAACTG

CD3zeta

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTGT
ACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCG
GGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATAACGAA
CTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGAGGC
GGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGGATACCTACGA
CGCCCTGCACATGCAGGCCCTGCCCCCAAGG

T2A

CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATC
CCGGCCCTAGG

tEGFR

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATC
CCACGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTA
CGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGG
CATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTCTGA
AAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACC
TCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTC

FIG. 17 (Cont'd)

TTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGTGAT
GGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGAAAAAA
CTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAAAACAGCTGCAA
GGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCC
CAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCAAC
CTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCACCCAG
AGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTGTATCCA
GTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAGTCATGGGA
GAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATC
CAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTAA
GATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTGGTGGCCCTG
GGGATCGGCCTCTTCATGTGAGCGGCCGCTCTAGACCCGGGCTGCAGGAATTCGATATCAA
GCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTAT
GTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCC
GTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTG
GCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGT
TGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGC
CACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGG
CACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTG
TTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGC
GGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGC
CCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCGTCGACTAGCCG
TACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGG
GGGGACTGGAAGGGCTAATTCACTCCCAAAGAAGACAAGATCTGCTTTTTGCCTGTACTGGG
TCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCT
TAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTC
TGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGAATTCGATA
TCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCGGTACCCAATTCGCCCTATAGTG
AGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTT
ACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGC
CCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAAATTGTAAGCG
TTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCC
GAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCA
GTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGT
CTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGT
GCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAA
GCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGC
TGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCT
ACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTT
TCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATA
TTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGC
ATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCA
GTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT
TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTAT
TATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATG

FIG. 17 (Cont'd)

ACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAG
AATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAAC
GATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCG
CCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCA
CGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCT
AGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCT
GCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTG
GGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTA
TCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAG
GTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATT
GATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATG
ACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCA
AAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACC
ACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGT
AACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGG
CCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCA
GTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTA
CCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGG
AGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACG
CTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAG
AGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTT
CGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATG
GAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA
CATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGA
GCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAG
CGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGC
AGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGT
GAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTG
TGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCC
AAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGG
CCTCGAGGTCGAGATCCGGTCGACCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATC
CCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA
TTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGC
TTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTCGACGGTATCGATTGGCTCATGTCCAAC
ATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT
TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTG
GCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTA
AATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGT
ACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGG
GCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGG
GAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCA
TTGACGCAAATGGGCGGTAGGCGTGTACGGAATTCGGAGTGGCGAGCCCTCAGATCCTG
CATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTG

FIG. 17 (Cont'd)

Leader _R11- Hinge-CH3- CD28tm/41BB-Z-T2A-tEGFR (SEQ ID NO:55)

Leader

MLLLVTSLLLCELPHPAFLLIP

scFV R11

QSVKESEGDLVTPAGNLTLTCTASGSDINDYPISWVRQAPGKGLEWIGFINSGGSTWYAS
WVKGRFTISRTSTTVDLKMTSLTTDDTATYFCARGYSTYYGDFNIWGPGTLVTISSGGGG
SGGGGSGGGGSELVMTQTPSSTSGAVGGTVTINCQASQSIDSNLAWFQQKPGQPPTLLI
YRASNLASGVPSRFSGSRSGTEYTLTISGVQREDAATYYCLGGVGNVSYRTSFGGGTEV
VVK

Hinge/spacer

ESKYGPPCPPCP

CH3

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

CD28tm

MFWVLVVVGGVLACYSLLVTVAFIIFWV

4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD3zeta

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

T2A

LEGGGEGRGSLLTCGDVEENPGPRM

tEGFR

LLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFR
GDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLA
VVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKAT
GQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHP
ECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHL
CHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

FIG. 18

R11 short spacer CAR: PJ_R11- 41BB-Z-T2A-tEGFR (SEQ ID NO:78)

GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC
AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACT
AGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGG
ACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCG
CGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAG
GCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATG
GGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGC
AAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAG
ACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATAT
AATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCT
TTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGA
CACAGGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAAT
GGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGC
TTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAAGATTT
AAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCAT
CAATGAGGAAGCTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATA
GGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGAC
GCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAG
GGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGG
CAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCT
CTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCAGTATTCATCCACAAT
TTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGC
AACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATT
ACAGGGACAGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTTAGGC
GTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCA
CATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAG
AAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGG
GTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTG
CCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCC
TACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCT
CCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCG
GCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGC
TCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGC
GCCTACGGCTAGC

scFV R11

GAATTCGCCACCATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGC
CTTTCTGCTGATCCCCCAGAGCGTGAAAGAGTCCGAGGGCGACCTGGTCACACCAGCCGGCA
ACCTGACCCTGACCTGTACCGCCAGCGGCAGCGACATCAACGACTACCCCATCTCTTGGGTC
CGCCAGGCTCCTGGCAAGGGACTGGAATGGATCGGCTTCATCAACAGCGGCGGCAGCACTT
GGTACGCCAGCTGGGTCAAAGGCCGGTTCACCATCAGCCGGACCAGCACCACCGTGGACCT
GAAGATGACAAGCCTGACCACCGACGACACCGCCACCTACTTTTGCGCCAGAGGCTACAGCA
CCTACTACGGCGACTTCAACATCTGGGGCCCTGGCACCCTGGTCACAATCTCTAGCGGCGGA
GGCGGCAGCGGAGGTGGAGGAAGTGGCGGCGGAGGATCCGAGCTGGTCATGACCCAGACC

FIG. 19

CCCAGCAGCACATCTGGCGCCGTGGGCGGCACCGTGACCATCAATTGCCAGGCCAGCCA
GAGCATCGACAGCAACCTGGCCTGGTTCCAGCAGAAGCCCGGCCAGCCCCCCACCCTGC
TGATCTACAGAGCCTCCAACCTGGCCAGCGGCGTGCCAAGCAGATTCAGCGGCAGCAGAT
CTGGCACCGAGTACACCCTGACCATCTCCGGCGTGCAGAGAGAGGACGCCGCTACCTATT
ACTGCCTGGGCGGCGTGGGCAACGTGTCCTACAGAACCAGCTTCGGCGGAGGTACTGAG
GTGGTCGTCAAA

Hinge/spacer

TACGGACCGCCCTGCCCCCCTTGCCCTGGCCAGCCTCGCGAGCCCCAGGTGTACACCCT
GCCTCCCTCCCAGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTA
CAAGACCACCCCTCCCGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGAC
CGTGGACAAGAGCCGGTGGCAGGAAGGCAACGTCTTTAGCTGCAGCGTGATGCACGAGG
CCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAG

4-1BB

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGACA
GTGGCCTTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACA
ACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCA
GAAGAAGAAGAAGGAGGATGTGAACTG

CD3zeta

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCT
GTACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAG
GCCGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTAT
AACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGA
GCGGAGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGG
ATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCAAGG

T2A

CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAA
TCCCGGCCCTAGG

tEGFR

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGA
TCCCACGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAAT
GCTACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGC
CGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGA
TATTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACA
GGACGGACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATG
GTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAG
GAGATAAGTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAAT
AAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGT
GAAAACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGC
TGGGGCCCGGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATG
CGTGGACAAGTGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTG
CATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGG
ACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTG
CCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCC
ATGTGTGCCACCTGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAG
GCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCC
TCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATGTGAGCGGCCGCTCTAGACC

FIG. 19 (Cont'd)

CGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTG
AAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAA
TGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCT
GGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCAC
TGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCC
GGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCC
CGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAA
TCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCT
TCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGG
CTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGC
CGCCTCCCCGCATCGATACCGTCGACTAGCCGTACCTTTAAGACCAATGACTTACAAGGCA
GCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCC
AAAGAAGACAAGATCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCC
TGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAG
TGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACC
CTTTTAGTCAGTGTGGAAAATCTCTAGCAGAATTCGATATCAAGCTTATCGATACCGTCGAC
CTCGAGGGGGGGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCC
GTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAG
CACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCC
AACAGTTGCGCAGCCTGAATGGCGAATGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCG
CGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTT
ATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCA
CTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCC
CACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAAT
CGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGC
GAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGG
TCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAG
GTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCA
AATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAA
GAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCC
TGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCA
CGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCG
AAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGT
ATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTG
AGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAG
TGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTT
GGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAG
CAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCA
ACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTT
CCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATC
ATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGG
AGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTA
AGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTT
TTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACG

FIG. 19 (Cont'd)

TGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTA
CCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAC
TGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAG
GCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTG
TTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAA
GACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCA
CACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGT
AAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACG
CCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTT
TTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCC
TTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTA
TCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCG
CCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGC
GCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTG
GCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTG
AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTAT
GTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATG
ATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCA
CCGCGGTGGCGGCCTCGAGGTCGAGATCCGGTCGACCAGCAACCATAGTCCCGC
CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCC
CCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTG
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAG
CTTCGACGGTATCGATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTA
TTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATG
GAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG
ACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG
ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT
GGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACA
TCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCAT
TGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTC
GTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGAATTCGGA
GTGGCGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTC
TCTCTG

FIG. 19 (Cont'd)

Leader _R11- Hinge- CD28tm/41BB-Z-T2A-tEGFR (SEQ ID NO:53)

Leader

MLLLVTSLLLCELPHPAFLLIP

ScFv R11

QSVKESEGDLVTPAGNLTLTCTASGSDINDYPISWVRQAPGKGLEWIGFINSGGSTWYA
SWVKGRFTISRTSTTVDLKMTSLTTDDTATYFCARGYSTYYGDFNIWGPGTLVTISSGG
GGSGGGGSGGGGSELVMTQTPSSTSGAVGGTVTINCQASQSIDSNLAWFQQKPGQPP
TLLIYRASNLASGVPSRFSGSRSGTEYTLTISGVQREDAATYYCLGGVGNVSYRTSFGG
GTEVVVK

Spacer/Hinge

ESKYGPPCPPCP

CD28tm

MFWVLVVVGGVLACYSLLVTVAFIIFWV

4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD3zeta

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

T2A

LEGGGEGRGSLLTCGDVEENPGPR

tEGFR

MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPV
AFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQ
FSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGEN
SCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENS
ECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYA
DAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

FIG. 20

Intermediate Spacer (SEQ ID NO:52)

Hinge/Spacer

ESKYGPPCPPCP

CH3

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Long Spacer (SEQ ID NO:61)

Hinge

ESKYGPPCPPCP

CH2

APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK

CH3

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLGK

FIG. 21

Her2 construct-short spacer

(SEQ ID NO:36)

GMCSFss-Her2scFv-IgG4hinge-CD28tm-41BB-Zeta-T2A-EGFRt

Leader

Atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccca

Her2scFV

gatatccagatgacccagtccccgagctccctgtccgcctctgtgggcgatagggtcaccatcacctgccgtgccagtcaggatgt
gaatactgctgtagcctggtatcaacagaaaccaggaaaagctccgaaactactgatttactcggcatccttcctctactctggagtc
ccttctcgcttctctggttccagatctgggacggatttcactctgaccatcagcagtctgcagccggaagacttcgcaacttattactgtc
agcaacattatactactcctcccacgttcggacagggtaccaaggtggagatcaaaggcagtactagcggcggtggctccgggg
gcggatccggtgggggcggcagcagcgaggttcagctggtggagtctggcggtggcctggtgcagccaggggctcactccgtt
tgtcctgtgcagcttctggcttcaacattaaagacacctatatacactgggtgcgtcaggccccgggtaagggcctggaatgggttgc
aaggatttatcctacgaatggttatactagatatgccgatagcgtcaagggccgtttcactataagcgcagacacatccaaaaaca
cagcctacctgcagatgaacagcctgcgtgctgaggacactgccgtctattattgttctagatggggaggggacggcttctatgctat
ggactactggggtcaaggaaccctggtcaccgtctcgagt

Hinge spacer

Gagagcaagtacggaccgccctgccccccttgccct

CD28tm

atgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagcctgctggtcaccgtggccttcatcatcttttgggtg

4-1BB

Aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgt
agctgccgatttccagaagaagaagaaggaggatgtgaactg

CD3ζ

Cgggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgggca
gaagggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgggcggcaagcctcggcggaagaacccc
caggaaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggcatgaagggcgagcggag
gcggggcaagggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggcc
ctgccccaagg

T2A

Ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctagg

tEGFR

atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgcaaagtgtgtaacggaatagg
tattggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcgatctccaca
tcctgccggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaaccgtaaagga
aatcacagggtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgagaacctagaaatcatacgcggcag
gaccaagcaacatggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgat
ggagatgtgataatttcaggaaacaaaaatttgtgctatgcaaatacaataaactggaaaaaactgtttgggacctccggtcagaa
aaccaaaattataagcaacagaggtgaaaacagctgcaaggccacaggccaggtctgccatgccttgtgctcccccgagggct
gctggggcccggagcccagggactgcgtctcttgccggaatgtcagccgaggcagggaatgcgtggacaagtgcaaccttctgg
agggtgagccaagggagtttgtggagaactctgagtgcatacagtgccacccagagtgcctgcctcaggccatgaacatcacctg
cacaggacggggaccagacaactgtatccagtgtgcccactacattgacggcccccactgcgtcaagacctgcccggcaggag
tcatgggagaaaacaacaccctggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacg
gatgcactgggccaggtcttgaaggctgtccaacgaatgggcctaagatcccgtccatcgccactgggatggtgggggccctcctc
ttgctgctggtggtggccctggggatcggcctcttcatgtga

FIG. 22

Her2 construct-intermediate spacer (SEQ ID NO:37)

Leader

Atgcttctcctggtgacaagccttctgctctgtgagttaccacaccca

Her2scFv

Gcattcctcctgatcccagatatccagatgacccagtccccgagctccctgtccgcctctgtgggcgatagggtcaccatcacctgccgtg
ccagtcaggatgtgaatactgctgtagcctggtatcaacagaaaccaggaaaagctccgaaactactgatttactcggcatccttcctcta
ctctggagtcccttctcgcttctctggttccagatctgggacggatttcactctgaccatcagcagtctgcagccggaagacttcgcaacttatt
actgtcagcaacattatactactcctcccacgttcggacagggtaccaaggtggagatcaaaggcagtactagcggcggtggctccgg
gggcggatccggtgggggcggcagcagcgaggttcagctggtggagtctggcggtggcctggtgcagccaggggctcactccgtttg
tcctgtgcagcttctggcttcaacattaaagacacctatatacactgggtgcgtcaggccccgggtaagggcctggaatgggttgcaagg
atttatcctacgaatggttatactagatatgccgatagcgtcaagggccgtttcactataagcgcagacacatccaaaaacacagcctacc
tgcagatgaacagcctgcgtgctgaggacactgccgtctattattgttctagatggggaggggacggcttctatgctatggactactggggt
caaggaaccctggtcaccgtctcgagt

Hinge spacer

GagagcaagtacggaccgccctgccccccttgccctGgccagcctagagaaccccaggtgtacaccctgcctcccagccaggaag
agatgaccaagaaccaggtgtccctgacctgcctggtcaaaggcttctaccccagcgatatcgccgtggaatgggagagcaacggcc
agcccgagaacaactacaagaccacccccccctgtgctggacagcgacggcagcttcttcctgtactcccggctgaccgtggacaaga
gccggtggcaggaaggcaacgtcttcagctgcagcgtgatgcacgaggccctgcacaaccactacacccagaagtccctgagcctg
agcctgggcaag

CD28tm

Atgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagcctgctggtcaccgtggccttcatcatcttttgggtg

4-1BB

Aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtagct
gccgatttccagaagaagaagaaggaggatgtgaactg

CD3 zeta

Cgggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgggcagaa
gggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgggcggcaagcctcggcggaagaacccccaggaa
ggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggcatgaagggcgagcggaggcggggcaa
gggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggccctgcccccaagg

T2A

Ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctagg

tEGFR

atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgcaaagtgtgtaacggaataggtatt
ggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcgatctccacatcctgcc
ggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaaccgtaaaggaaatcacagg
gtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgagaacctagaaatcatacgcggcaggaccaagcaac
atggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgatggagatgtgataatttc
aggaaacaaaaatttgtgctatgcaaatacaataaactggaaaaaactgtttgggacctccggtcagaaaaccaaaattataagcaac
agaggtgaaaacagctgcaaggccacaggccaggtctgccatgccttgtgctcccccgagggctgctggggcccggagcccaggga
ctgcgtctcttgccggaatgtcagccgaggcagggaatgcgtggacaagtgcaaccttctggagggtgagccaagggagtttgtggag
aactctgagtgcatacagtgccacccagagtgcctgcctcaggccatgaacatcacctgcacaggacggggaccagacaactgtatc
cagtgtgcccactacattgacggcccccactgcgtcaagacctgcccggcaggagtcatgggagaaaacaacaccctggtctggaag
tacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggccaggtcttgaaggctgtccaacga
atgggcctaagatcccgtccatcgccactgggatggtgggggccctcctcttgctgctggtggtggccctggggatcggcctcttcatgtga

FIG. 23

Her2 construct-long spacer (SEQ ID NO:38)

Leader

Atgcttctcctggtgacaagccttctgctctgtgagttaccacaccca

Her2scFV

gcattcctcctgatcccagatatccagatgacccagtccccgagctccctgtccgcctctgtgggcgatagggtcaccatcacct
gccgtgccagtcaggatgtgaatactgctgtagcctggtatcaacagaaaccaggaaaagctccgaaactactgatttactcg
gcatccttcctctactctggagtcccttctcgcttctctggttccagatctgggacggatttcactctgaccatcagcagtctgcagcc
ggaagacttcgcaacttattactgtcagcaacattatactactcctcccacgttcggacagggtaccaaggtggagatcaaagg
cagtactagcggcggtggctccgggggcggatccggtgggggcggcagcagcgaggttcagctggtggagtctggcggtg
gcctggtgcagccaggggctcactccgtttgtcctgtgcagcttctggcttcaacattaaagacacctatatacactgggtgcgt
caggccccgggtaagggcctggaatgggttgcaaggatttatcctacgaatggttatactagatatgccgatagcgtcaaggg
ccgtttcactataagcgcagacacatccaaaaacacagcctacctgcagatgaacagcctgcgtgctgaggacactgccgtc
tattattgttctagatggggaggggacggcttctatgctatggactactggggtcaaggaaccctggtcaccgtctcgagt

long spacer

gagagcaagtacggaccgccctgccccccttgccctgccccgagttcctgggcggacccagcgtgttcctgttcccccccaa
gcccaaggacaccctgatgatcagccggacccccgaggtgacctgcgtggtggtggacgtgagccaggaagatcccgag
gtccagttcaattggtacgtggacggcgtggaagtgcacaacgccaagaccaagcccagagaggaacagttcaacagca
cctaccgggtggtgtctgtgctgaccgtgctgcaccaggactggctgaacggcaaagaatacaagtgcaaggtgtccaaca
agggcctgcccagcagcatcgaaaagaccatcagcaaggccaagggccagcctcgcgagccccaggtgtacaccctgc
ctccctcccaggaagagatgaccaagaaccaggtgtccctgacctgcctggtgaagggcttctaccccagcgacatcgccgt
ggagtgggagagcaacggccagcctgagaacaactacaagaccacccctcccgtgctggacagcgacggcagcttcttcc
tgtacagccggctgaccgtggacaagagccggtggcaggaaggcaacgtctttagctgcagcgtgatgcacgaggccctgc
acaaccactacacccagaagagcctgagcctgtccctgggcaag

CD28tm

atgttctgggtgctggtggtggtgggcggggtgctggcctgctacagcctgctggtgacagtggccttcatcatcttttgggtg

4-1BB

aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaagaggaagatggc
tgtagctgccgatttccagaagaagaagaaggaggatgtgaactg

CD3zeta

Cgggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgg
gcagaagggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgggcggcaagcctcggcggaag
aacccccaggaaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggcatgaagggc
gagcggaggcggggcaagggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgccctgc
acatgcaggccctgcccccaagg

T2A

Ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctagg

tEGFR

atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgcaaagtgtgtaacggaat
aggtattggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcgat
ctccacatcctgccggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaac
cgtaaaggaaatcacagggtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgagaacctagaaat
catacgcggcaggaccaagcaacatggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggattacgctccctc
aaggagataagtgatggagatgtgataatttcaggaaacaaaaatttgtgctatgcaaatacaataaactggaaaaaactgttt

FIG. 24

gggacctccggtcagaaaaccaaaattataagcaacagaggtgaaaacagctgcaaggccacaggccaggtc
tgccatgccttgtgctcccccgagggctgctggggcccggagcccagggactgcgtctcttgccggaatgtcagcc
gaggcagggaatgcgtggacaagtgcaaccttctggagggtgagccaagggagtttgtggagaactctgagtgc
atacagtgccacccagagtgcctgcctcaggccatgaacatcacctgcacaggacggggaccagacaactgtat
ccagtgtgcccactacattgacggcccccactgcgtcaagacctgcccggcaggagtcatgggagaaaacaaca
ccctggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgg
gccaggtcttgaaggctgtccaacgaatgggcctaagatcccgtccatcgccactgggatggtgggggccctcctc
ttgctgctggtggtggccctggggatcggcctcttcatgtga

FIG. 24 (Cont'd)

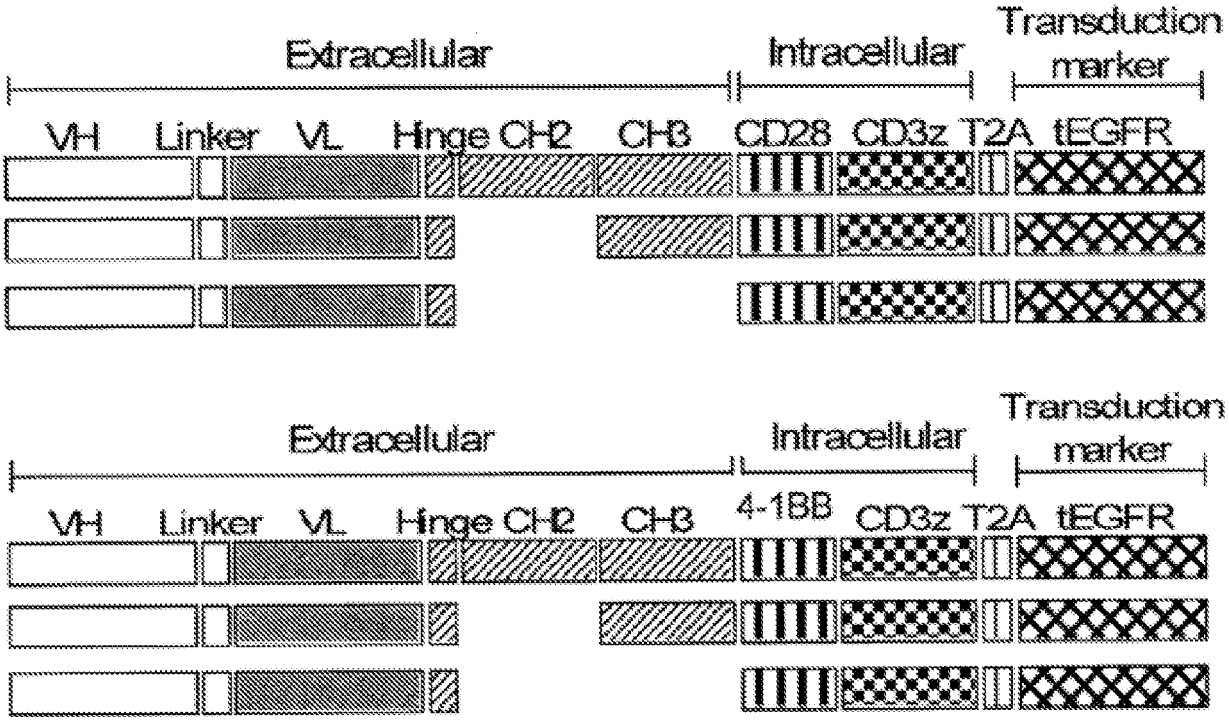

Extracellular
Intracellular
Transduction marker
VH
Linker
VL
Hinge
CH2
CH3
CD28
CD3z
T2A
tEGFR
Extracellular
Intracellular
Transduction marker
VH
Linker
VL
Hinge
CH2
CH3
4-1BB
CD3z
T2A
tEGFR

FIG. 25

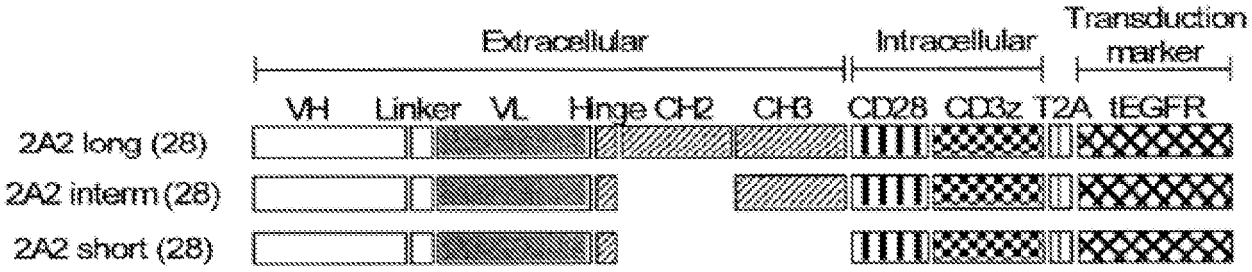


FIG. 26A

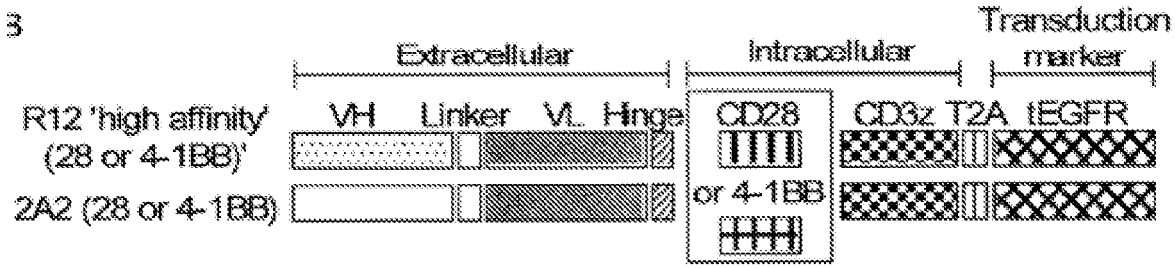


FIG. 26B

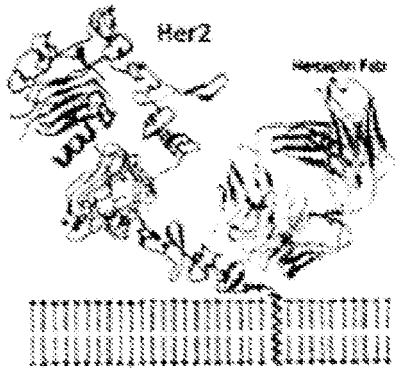


FIG. 27A

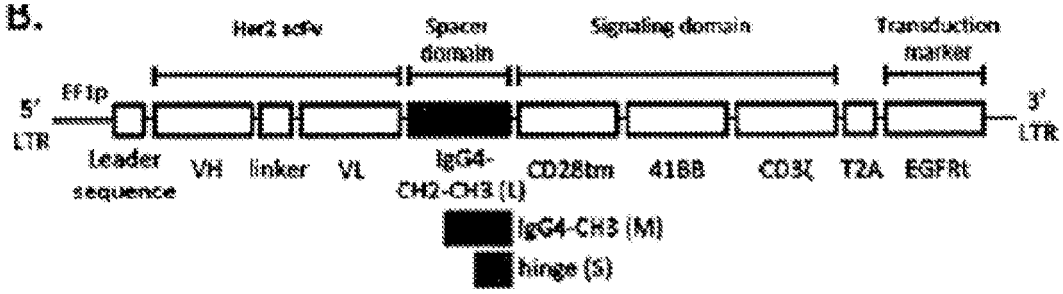


FIG. 27B

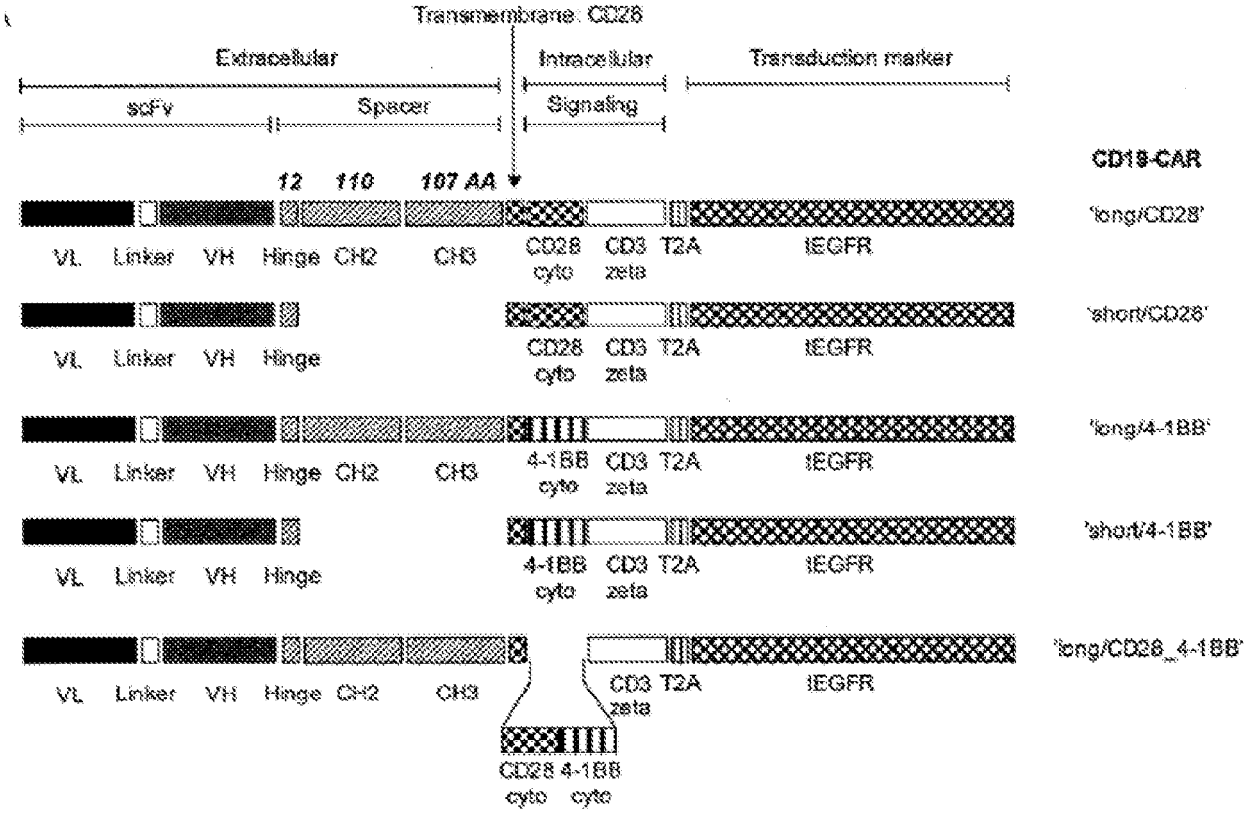

Transmembrane: CD28
Extracellular
Intracellular
Transduction marker
scFv
Spacer
Signaling
CD19-CAR
12
110
107 AA
VL
Linker
VH
Hinge
CH2
CH3
CD28 cyto
CD3 zeta
T2A
tEGFR
'long/CD28'
'short/CD28'
4-1BB cyto
'long/4-1BB'
'short/4-1BB'
'long/CD28_4-1BB'
CD28 cyto
4-1BB cyto

FIG. 28

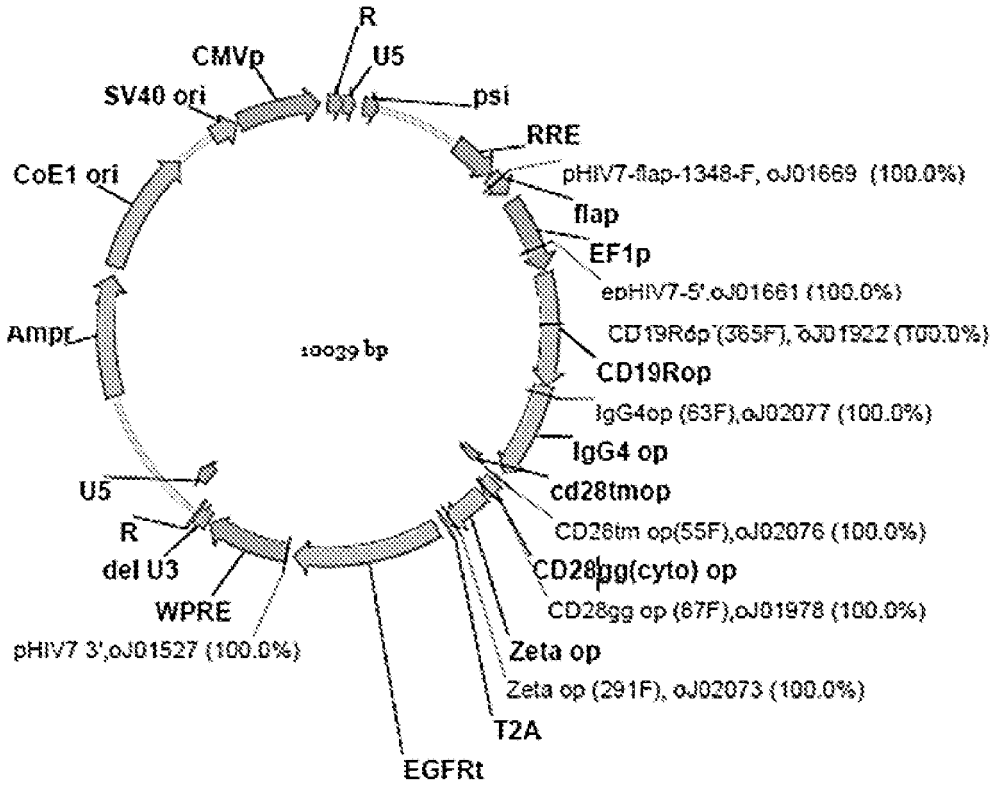


FIG. 29A

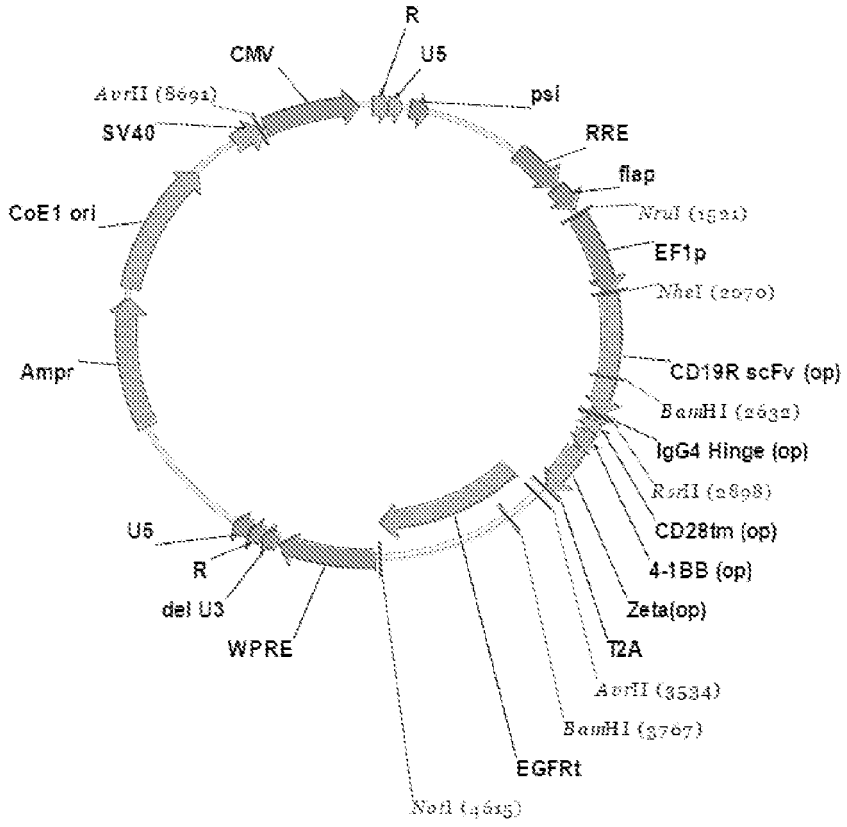


FIG. 29B

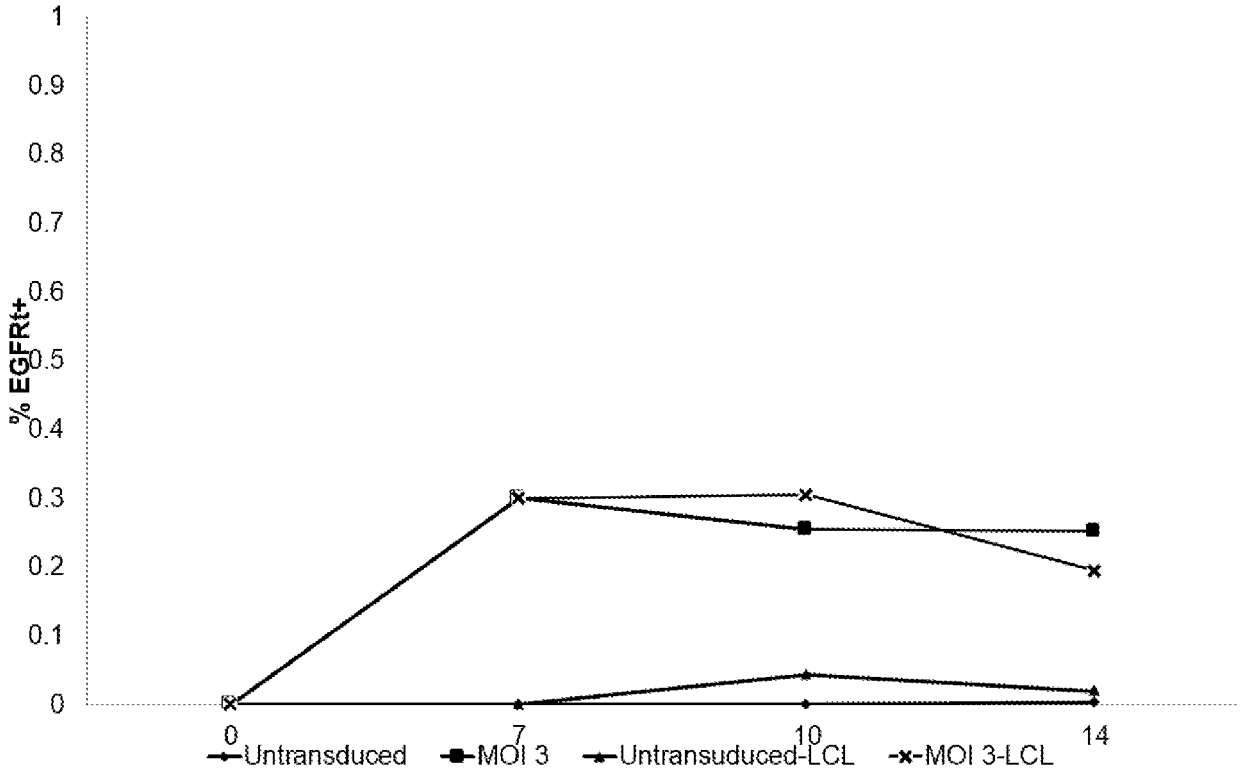

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
% EGFR+
0
7
10
14
Untransduced
MOI 3
Untransuduced-LCL
MOI 3-LCL

FIG. 30A

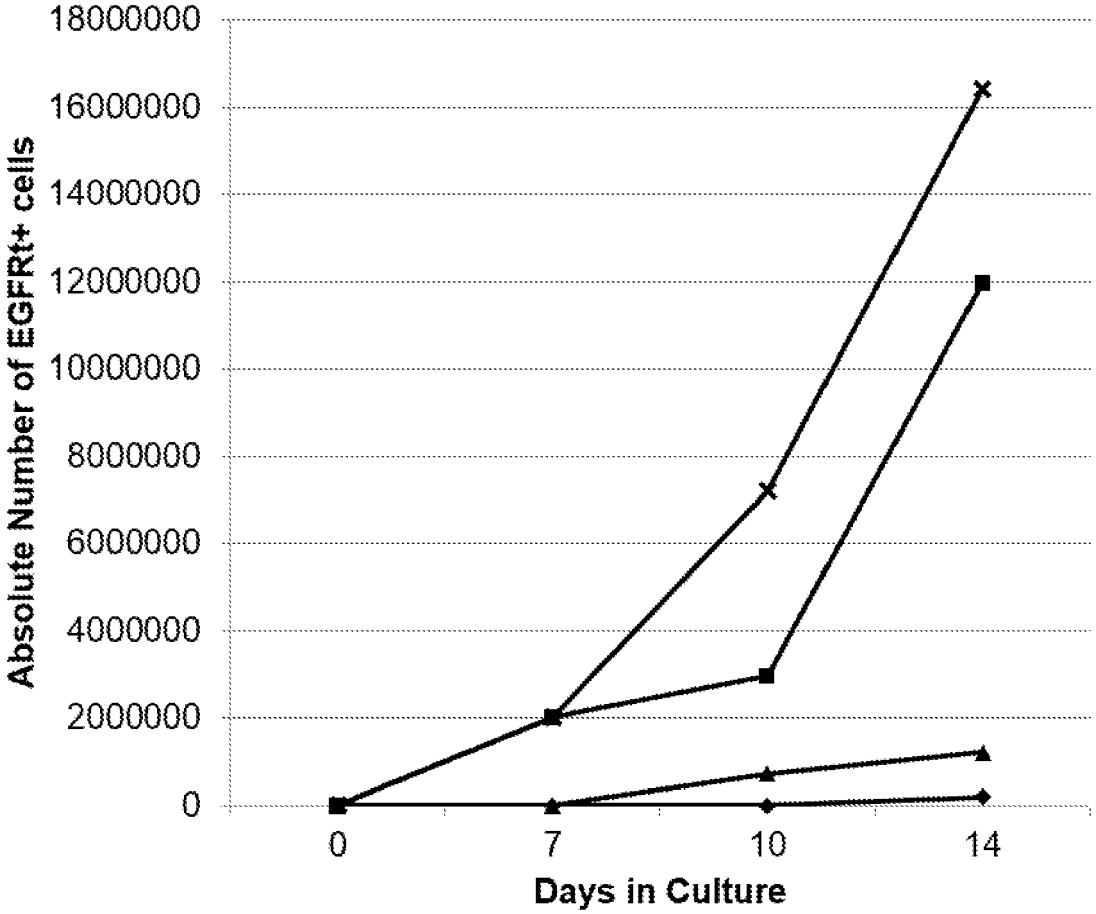
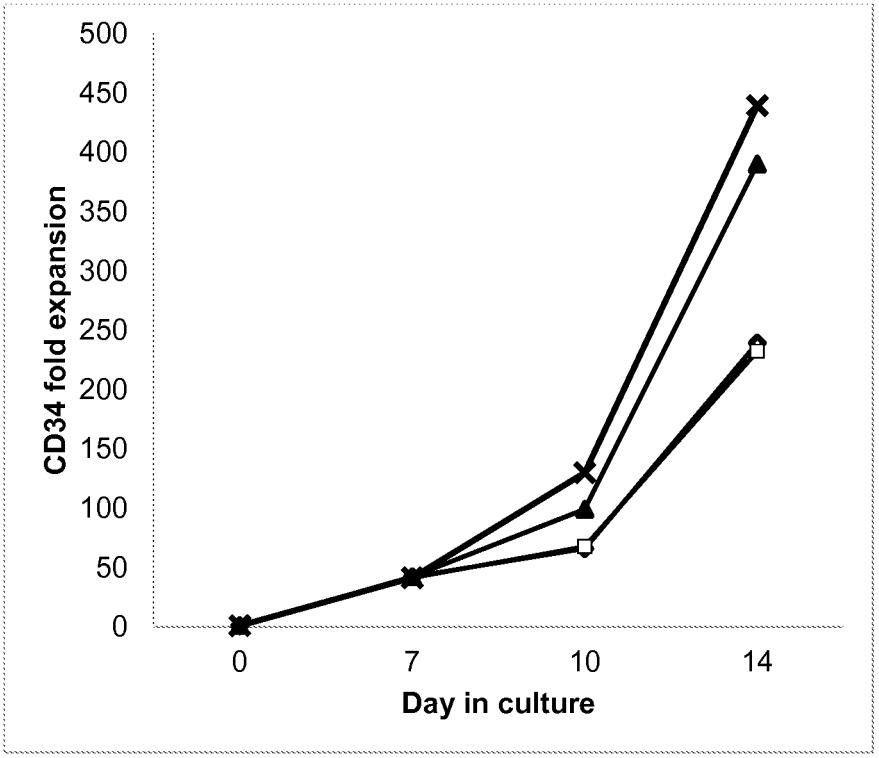

18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Absolute Number of EGFRt+ cells
0
7
10
14
Days in Culture

FIG. 30B


500
450
400
350
300
250
200
150
100
50
0
CD34 fold expansion
0
7
10
14
Day in culture

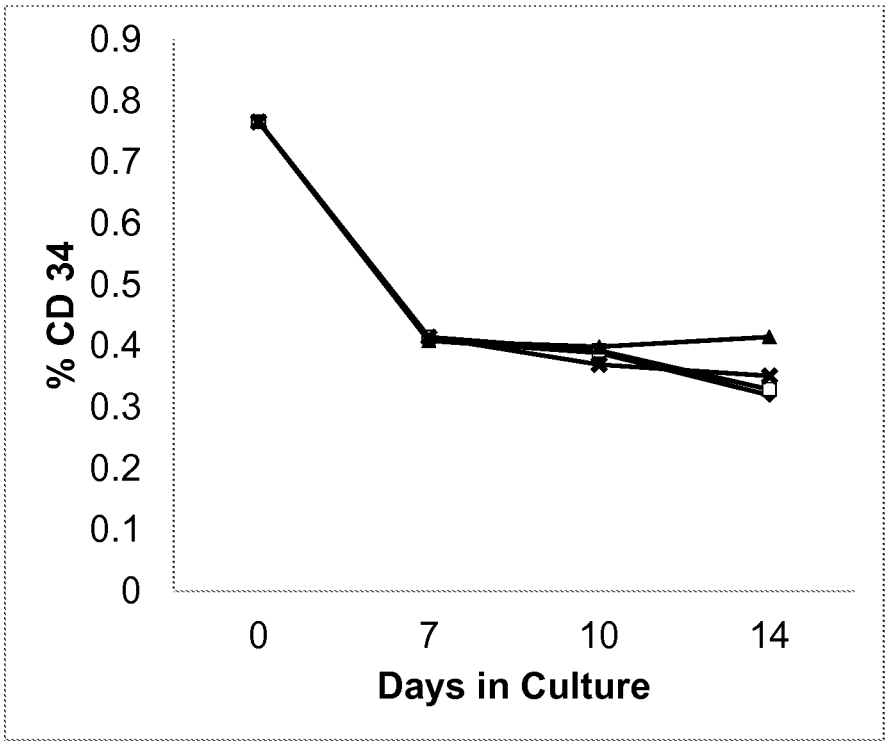

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
% CD 34
0
7
10
14
Days in Culture

FIG. 31

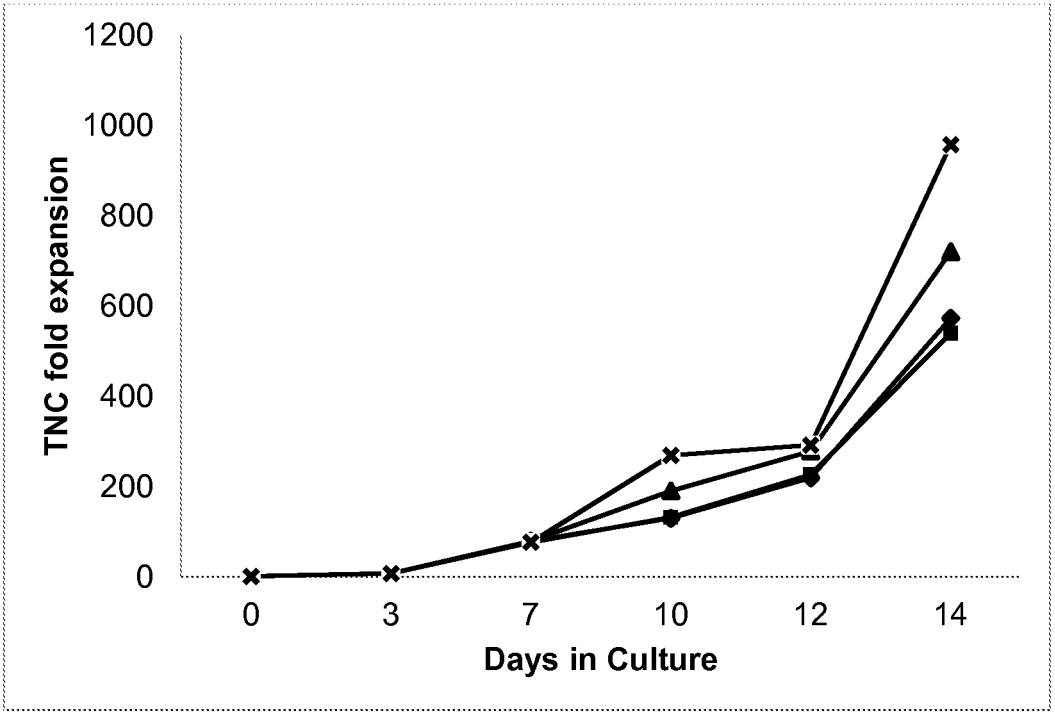

1200
1000
800
600
400
200
0
TNC fold expansion
0
3
7
10
12
14
Days in Culture

FIG. 31 (Cont'd)

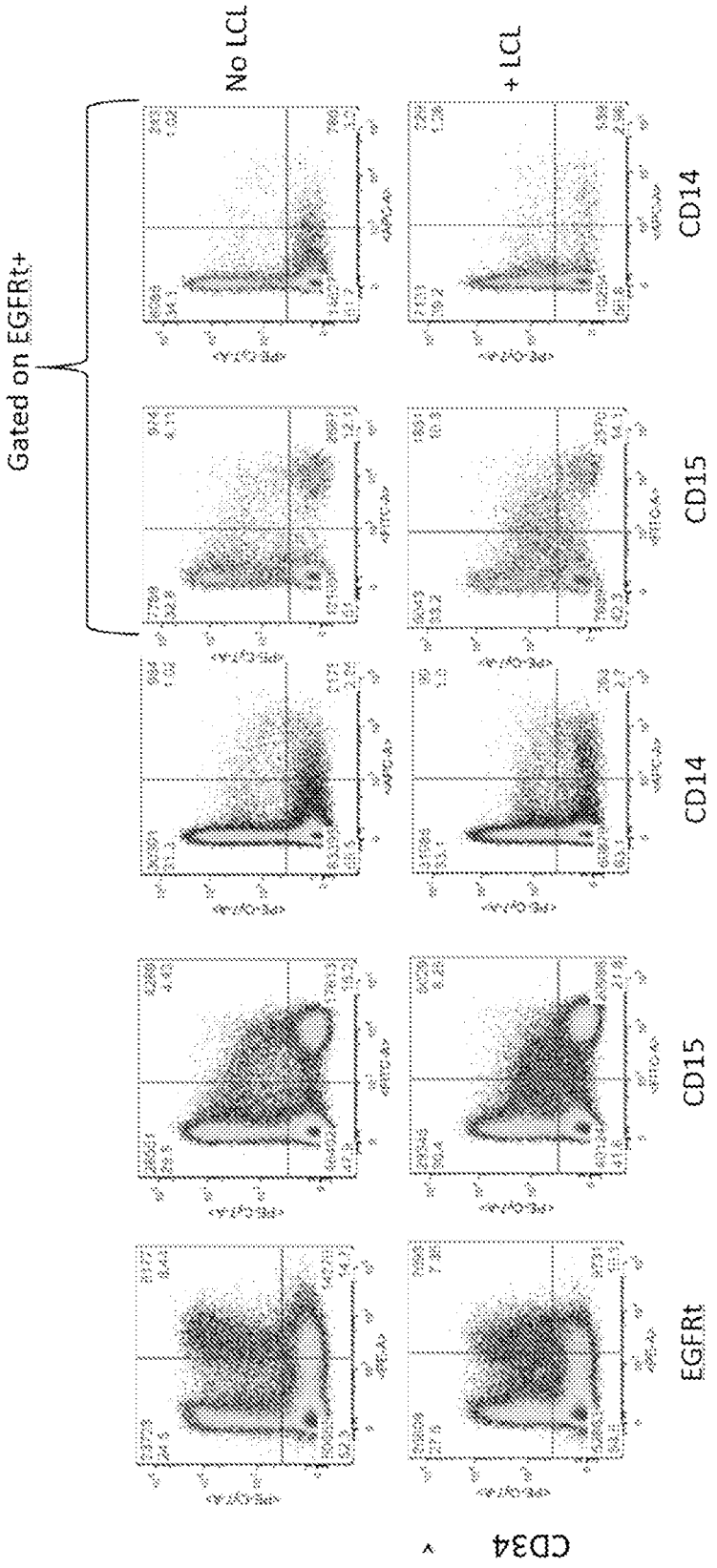


FIG. 32

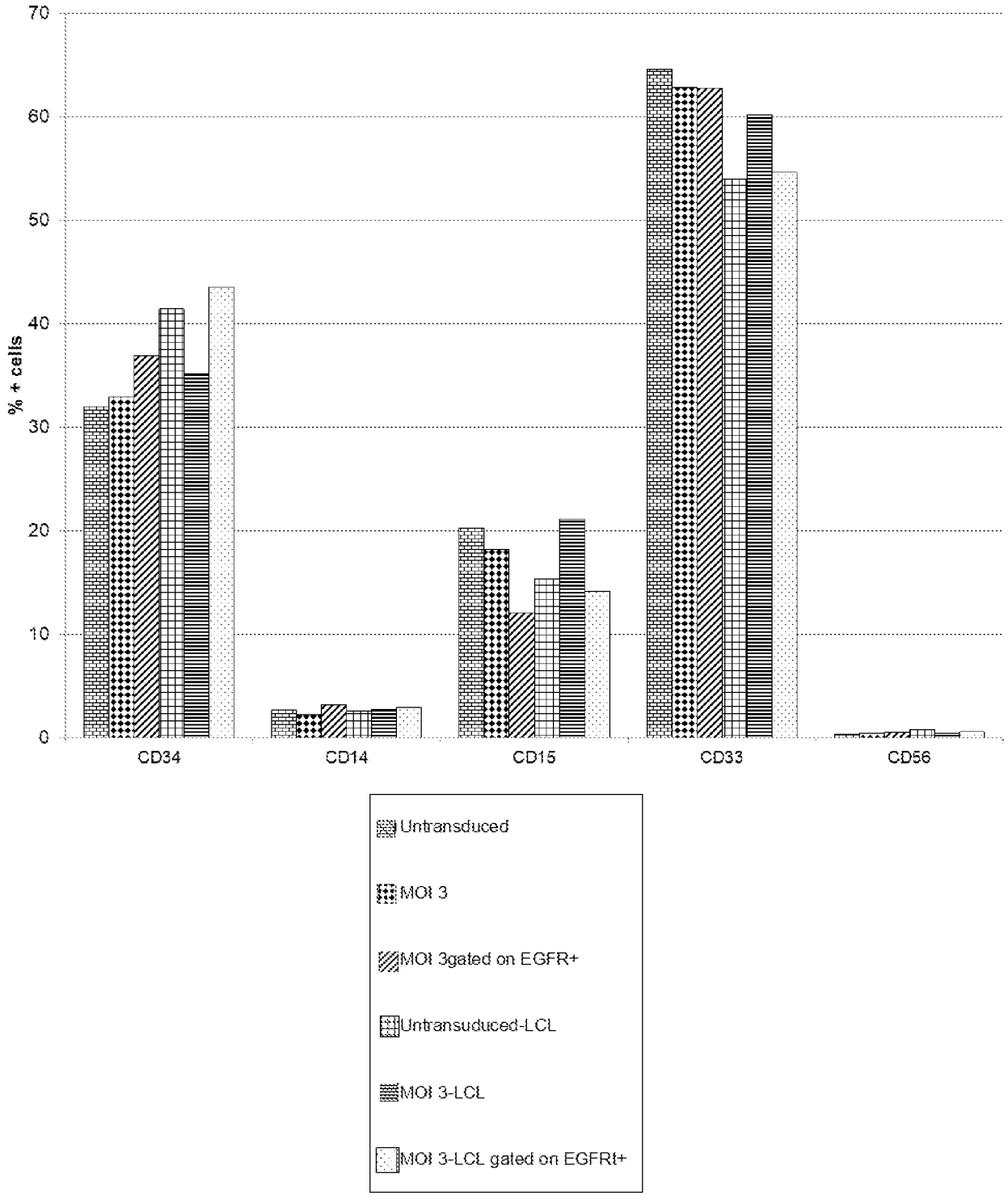

70
60
50
40
30
20
10
0
% + cells
CD34
CD14
CD15
CD33
CD56
Untransduced
MOI 3
MOI 3gated on EGFR+
Untransuduced-LCL
MOI 3-LCL
MOI 3-LCL gated on EGFRI+

FIG. 33

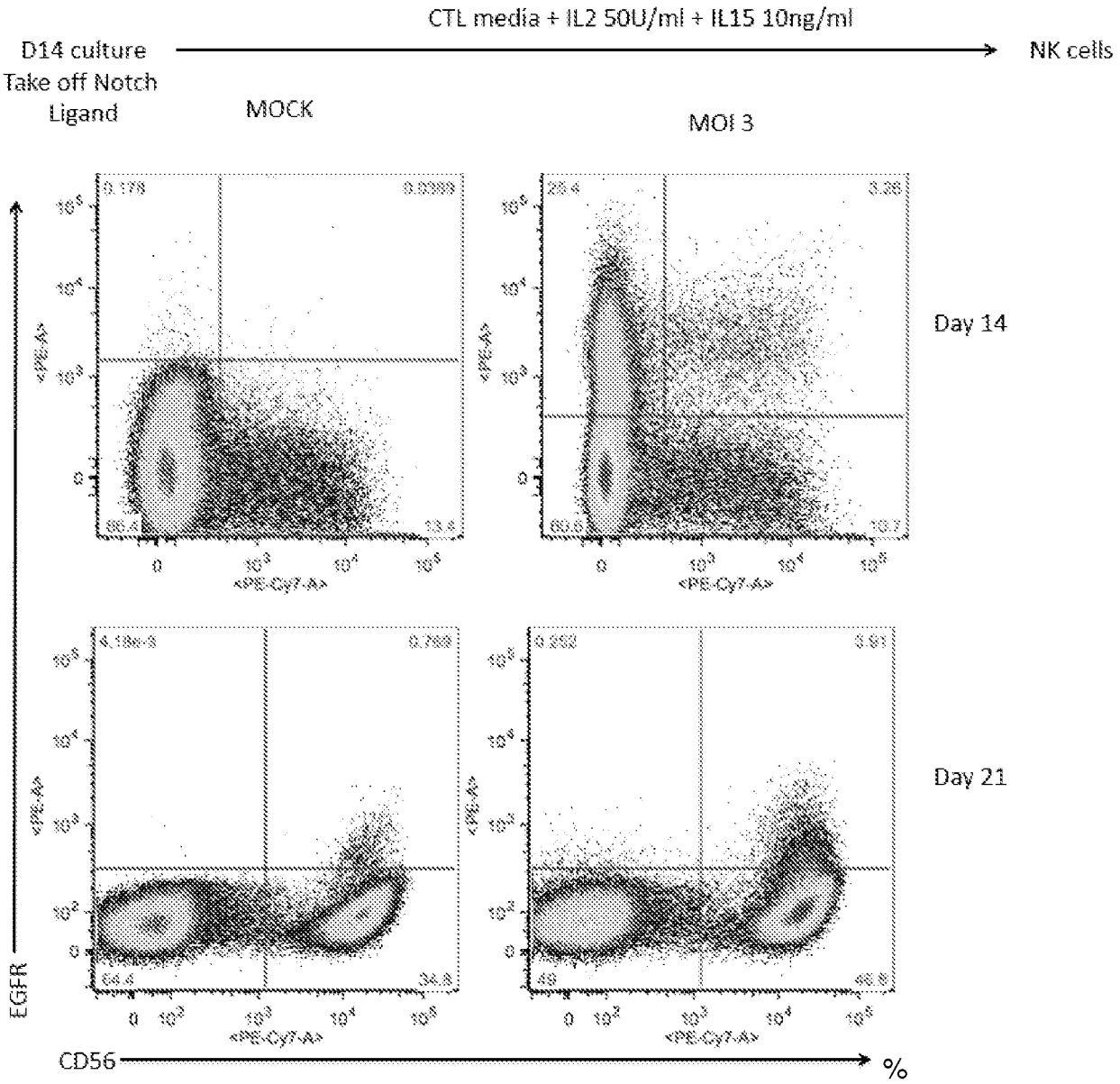

CTL media + IL2 50U/ml + IL15 10ng/ml
D14 culture
Take off Notch Ligand
NK cells
MOCK
MOI 3
Day 14
Day 21
EGFR
CD56
%
<PE-A>
<PE-Cy7-A>

FIG. 34

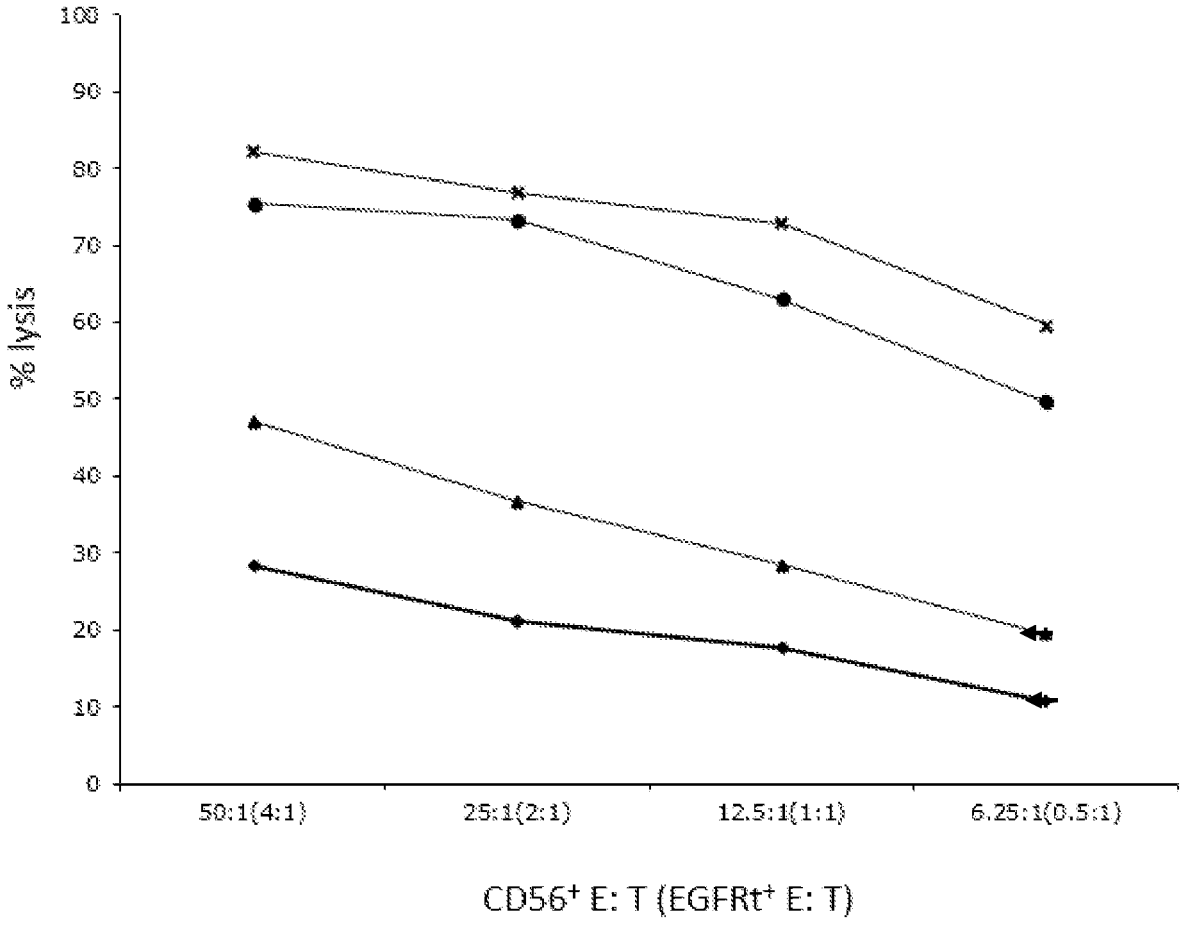

100
90
80
70
60
50
40
30
20
10
0
% lysis
50:1(4:1)
25:1(2:1)
12.5:1(1:1)
6.25:1(0.5:1)
CD56+ E: T (EGFRt+ E: T)

FIG. 35

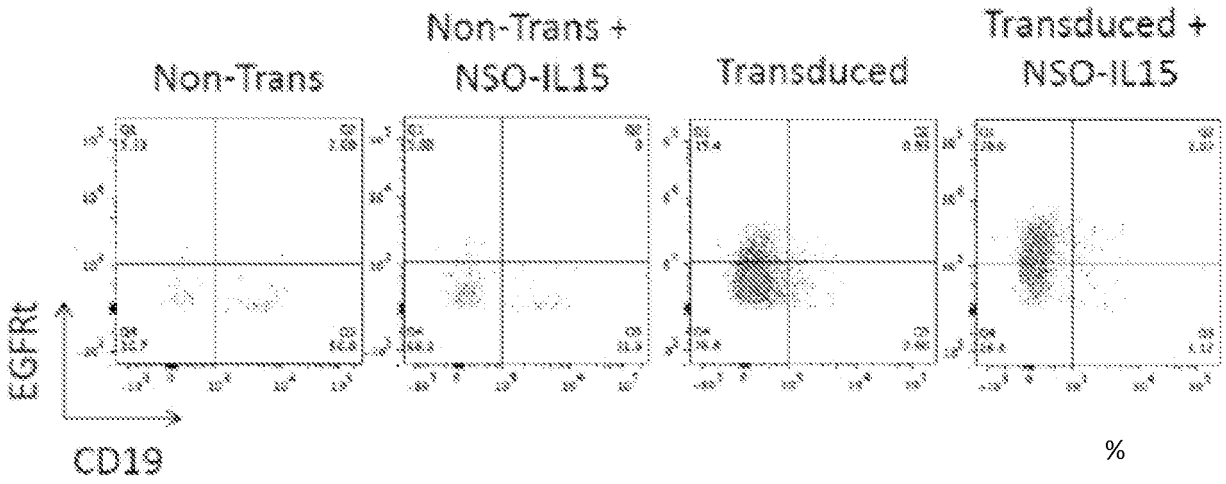

Non-Trans
Non-Trans + NSO-IL15
Transduced
Transduced + NSO-IL15
EGFRt
CD19
%

FIG. 36

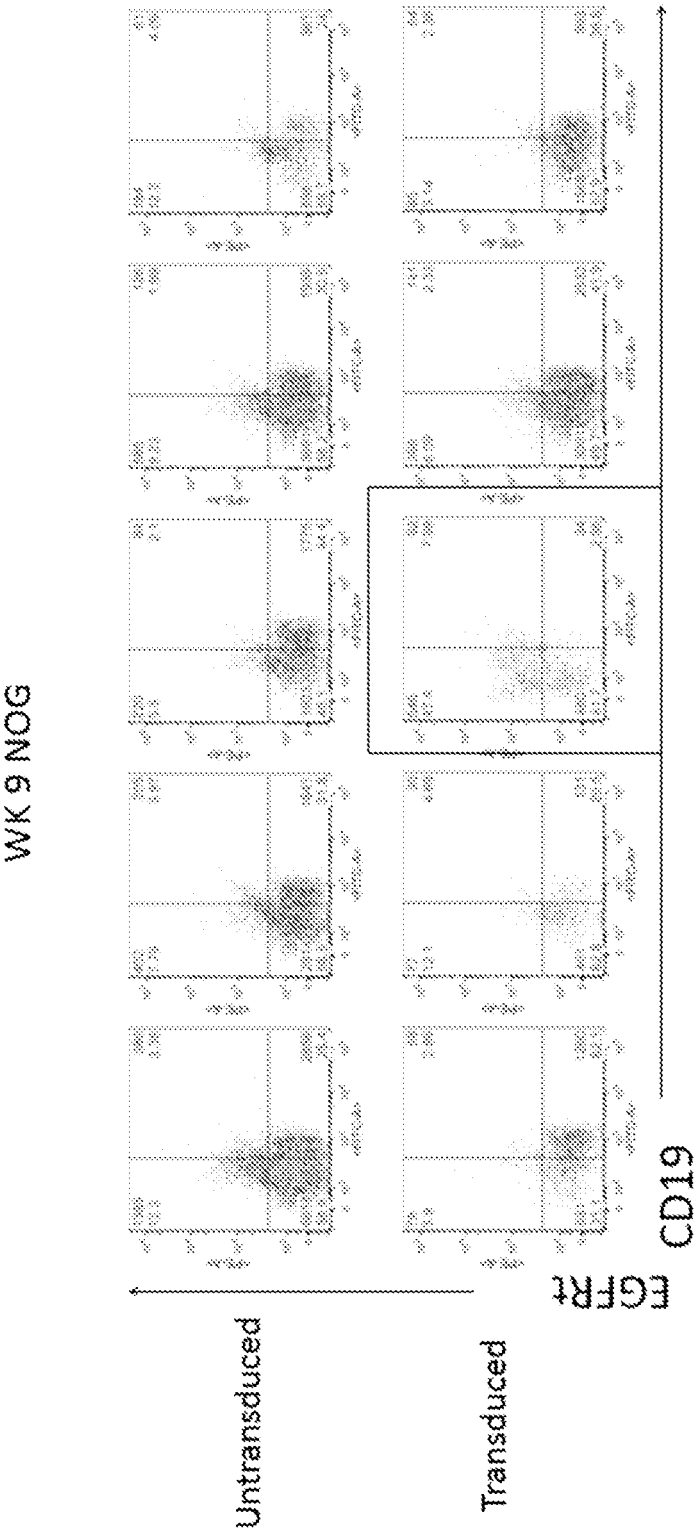


FIG. 37