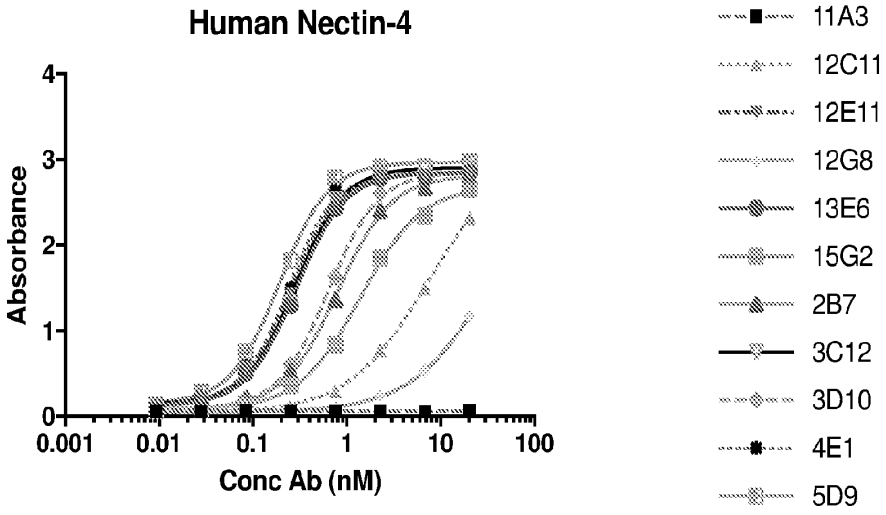

Human Nectin-4
Absorbance
Conc Ab (nM)
0.001
0.01
0.1
1
10
100
0
1
2
3
4
11A3
12C11
12E11
12G8
13E6
15G2
2B7
3C12
3D10
4E1
5D9

FIG. 1

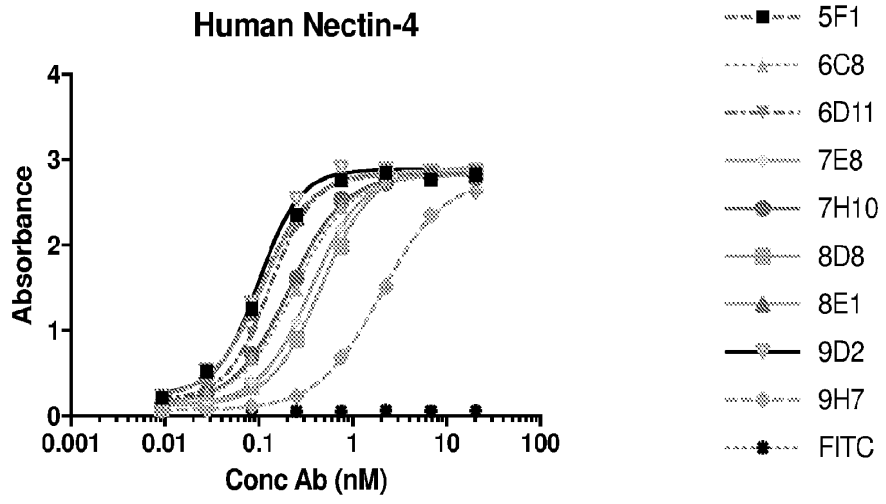

Human Nectin-4
Absorbance
Conc Ab (nM)
0.001
0.01
0.1
1
10
100
0
1
2
3
4
5F1
6C8
6D11
7E8
7H10
8D8
8E1
9D2
9H7
FITC

FIG. 2

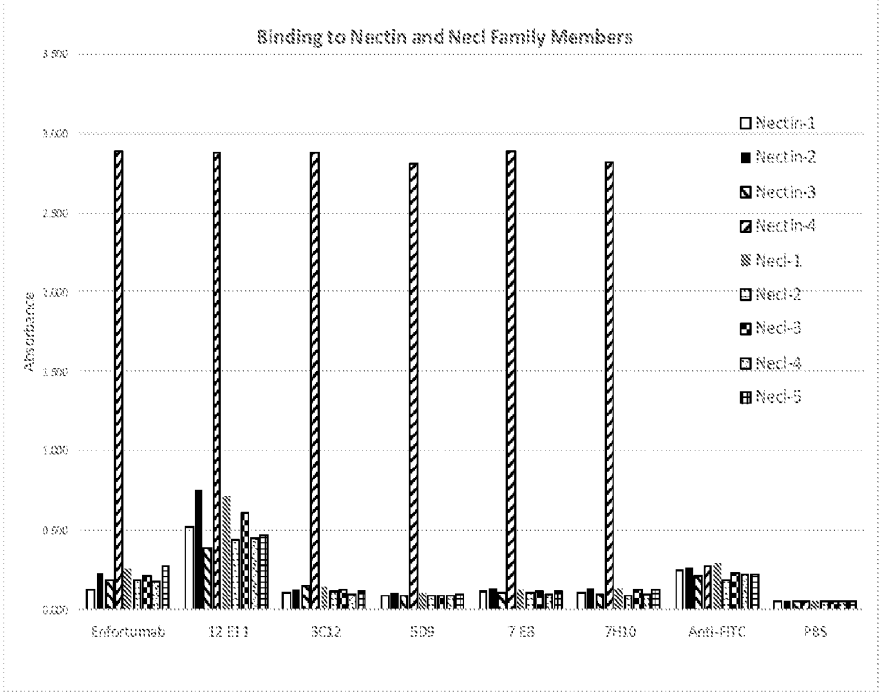

Binding to Nectin and Necl Family Members
Absorbance
Nectin-1
Nectin-2
Nectin-3
Nectin-4
Necl-1
Necl-2
Necl-3
Necl-4
Necl-5
Enfortumab
12 E1 1
3C12
5D9
7 E8
7H10
Anti-FITC
PBS

FIG. 3

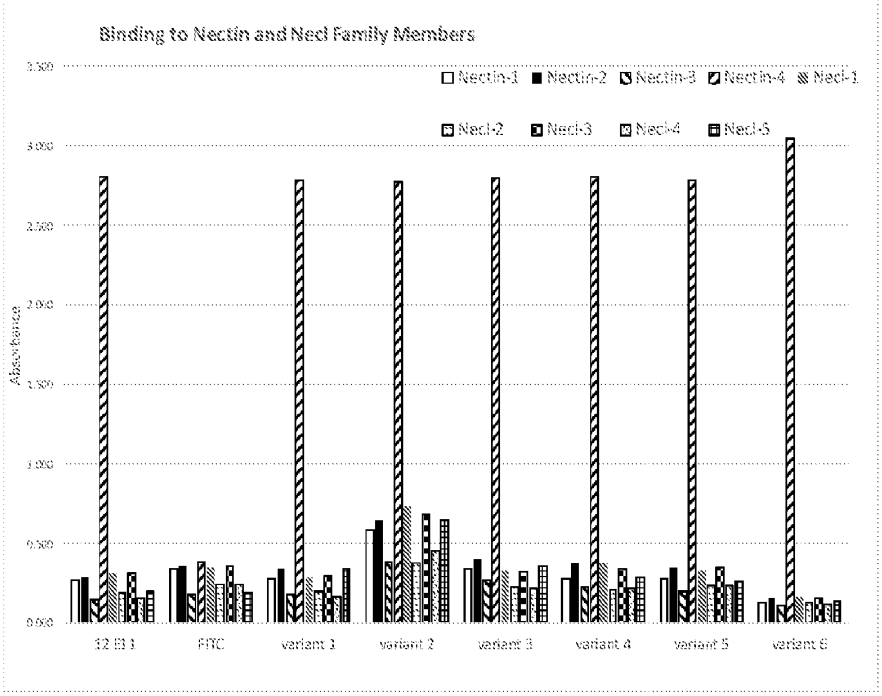

Binding to Nectin and Necl Family Members
Nectin-1
Nectin-2
Nectin-3
Nectin-4
Necl-1
Necl-2
Necl-3
Necl-4
Necl-5
Absorbance
12 B1
FITC
variant 1
variant 2
variant 3
variant 4
variant 5
variant 6

FIG. 4

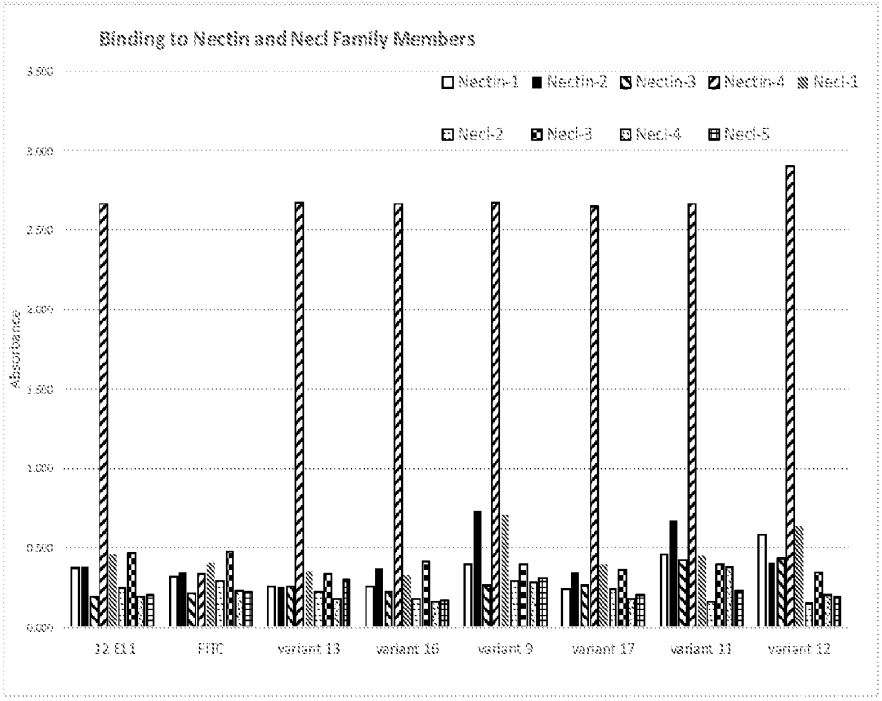

Binding to Nectin and Necl Family Members
Nectin-1
Nectin-2
Nectin-3
Nectin-4
Necl-1
Necl-2
Necl-3
Necl-4
Necl-5
Absorbance
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
32 E11
FHC
variant 13
variant 16
variant 9
variant 17
variant 11
variant 12

FIG. 5

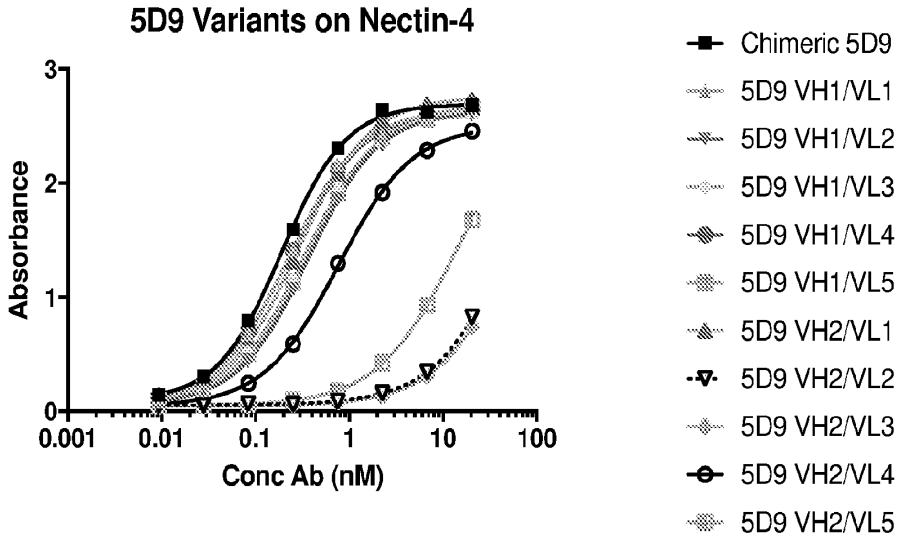

5D9 Variants on Nectin-4
Absorbance
Conc Ab (nM)
0.001
0.01
0.1
1
10
100
0
1
2
3
Chimeric 5D9
5D9 VH1/VL1
5D9 VH1/VL2
5D9 VH1/VL3
5D9 VH1/VL4
5D9 VH1/VL5
5D9 VH2/VL1
5D9 VH2/VL2
5D9 VH2/VL3
5D9 VH2/VL4
5D9 VH2/VL5

FIG. 6

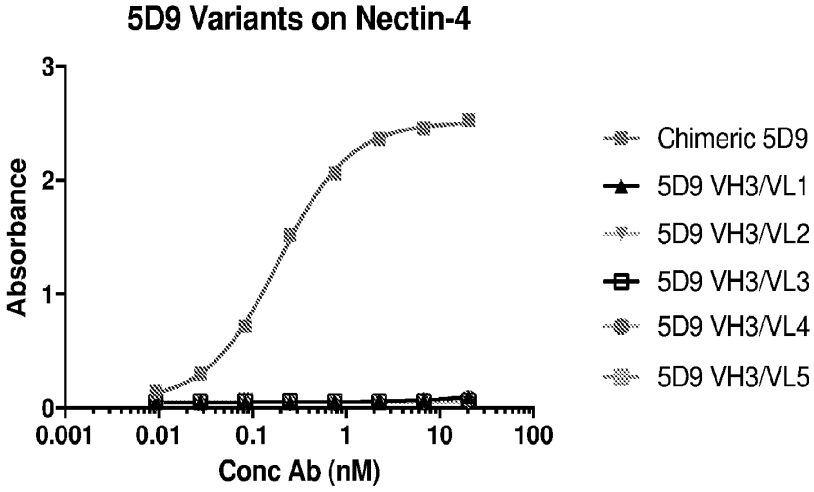

5D9 Variants on Nectin-4
Absorbance
Conc Ab (nM)
0.001
0.01
0.1
1
10
100
0
1
2
3
Chimeric 5D9
5D9 VH3/VL1
5D9 VH3/VL2
5D9 VH3/VL3
5D9 VH3/VL4
5D9 VH3/VL5

FIG. 7

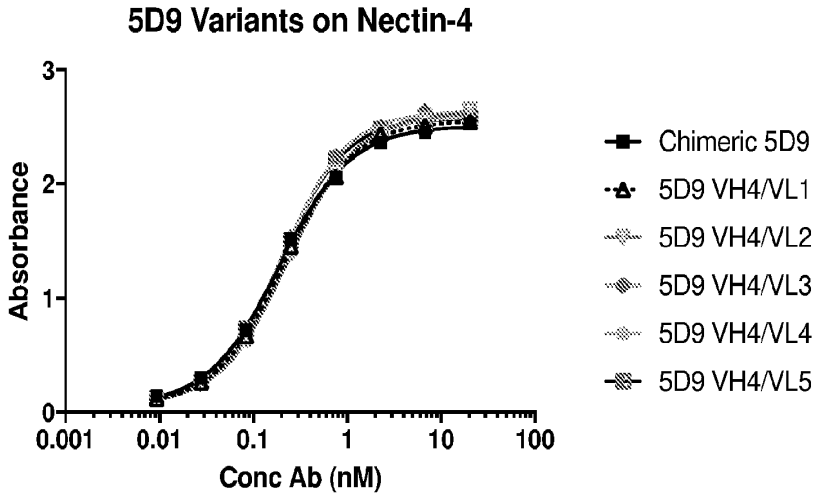

5D9 Variants on Nectin-4
Absorbance
Conc Ab (nM)
0.001
0.01
0.1
1
10
100
0
1
2
3
Chimeric 5D9
5D9 VH4/VL1
5D9 VH4/VL2
5D9 VH4/VL3
5D9 VH4/VL4
5D9 VH4/VL5

FIG. 8

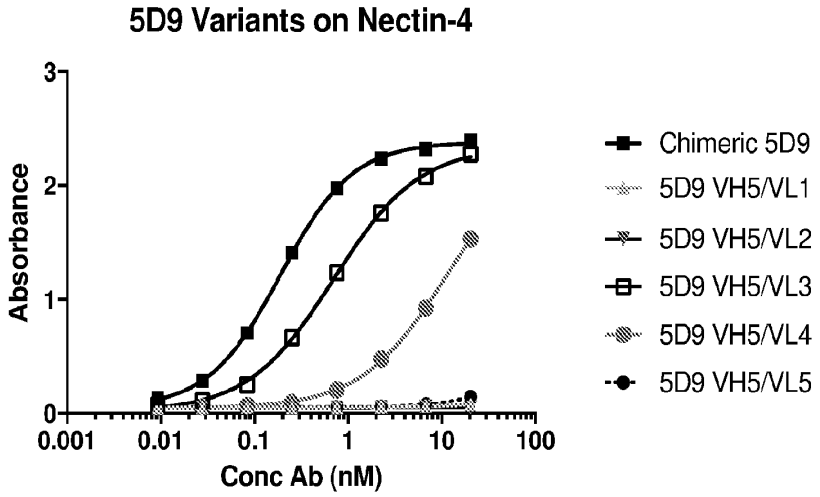

5D9 Variants on Nectin-4
Absorbance
Conc Ab (nM)
0.001
0.01
0.1
1
10
100
0
1
2
3
Chimeric 5D9
5D9 VH5/VL1
5D9 VH5/VL2
5D9 VH5/VL3
5D9 VH5/VL4
5D9 VH5/VL5

FIG. 9

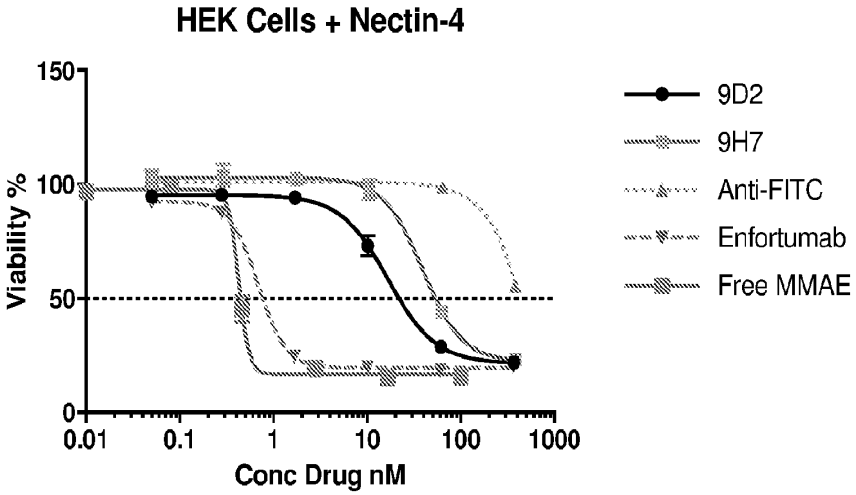

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
9D2
9H7
Anti-FITC
Enfortumab
Free MMAE

FIG. 10

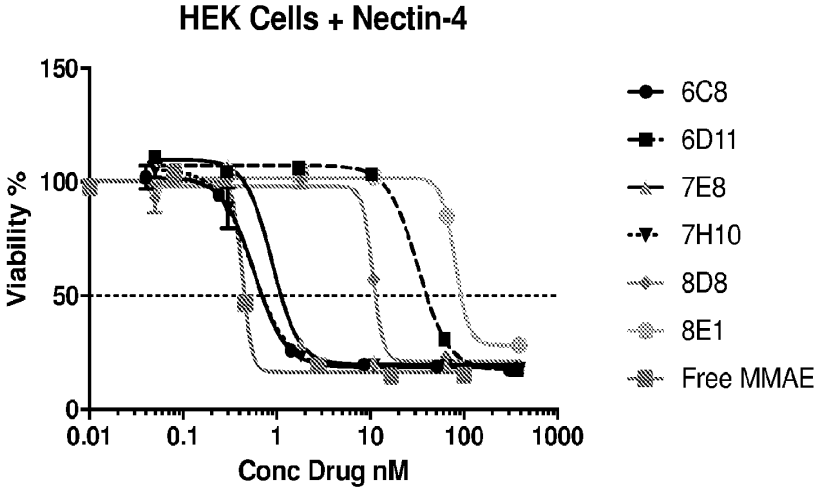

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
6C8
6D11
7E8
7H10
8D8
8E1
Free MMAE

FIG. 11

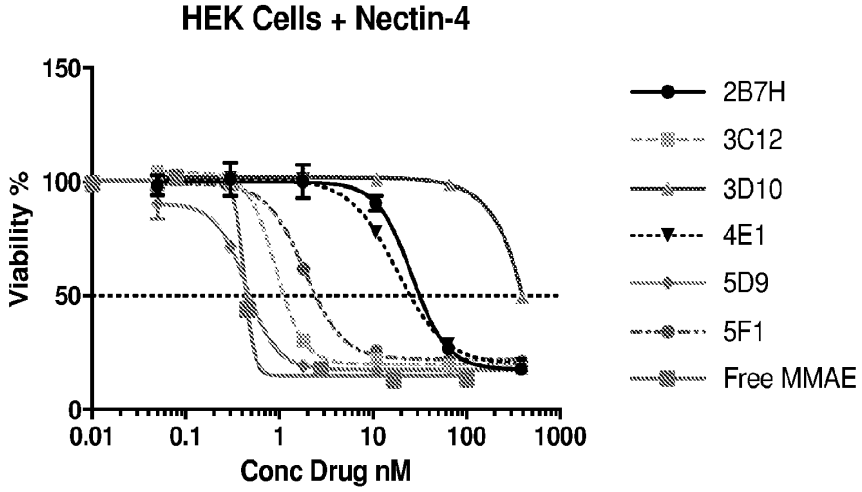

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
2B7H
3C12
3D10
4E1
5D9
5F1
Free MMAE

FIG. 12

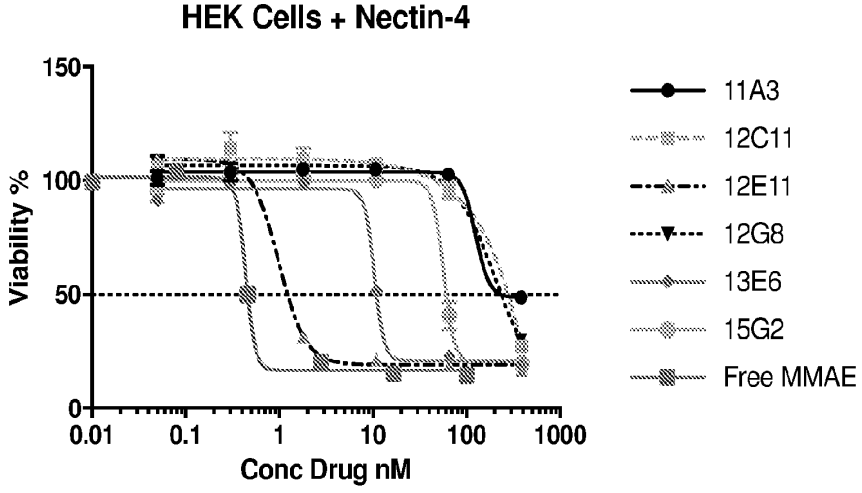

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
11A3
12C11
12E11
12G8
13E6
15G2
Free MMAE

FIG. 13

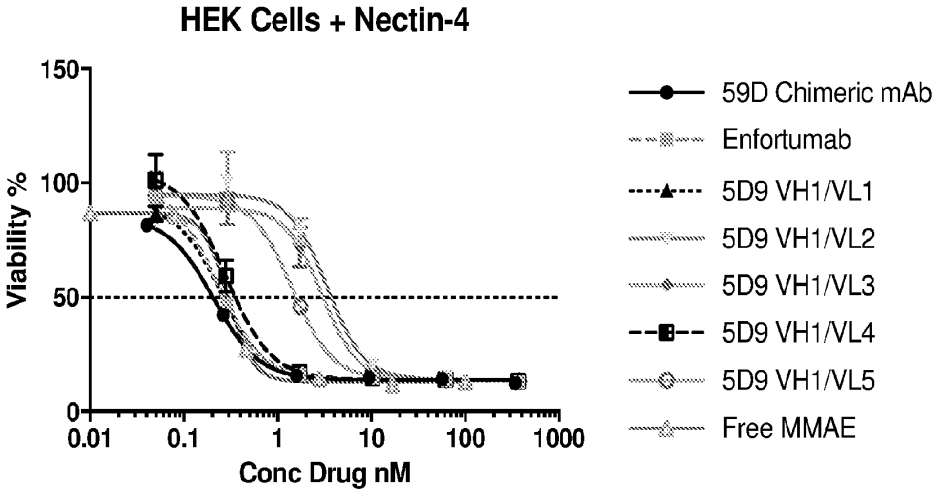

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH1/VL1
5D9 VH1/VL2
5D9 VH1/VL3
5D9 VH1/VL4
5D9 VH1/VL5
Free MMAE

FIG. 14

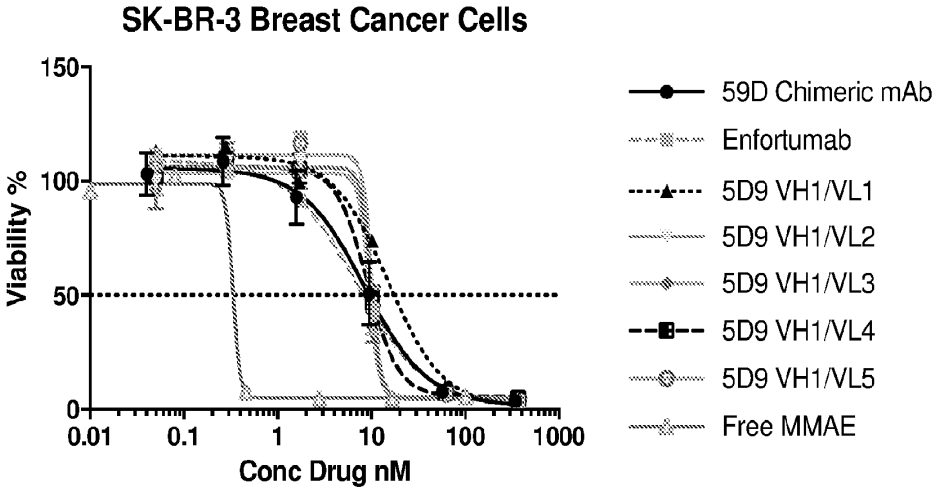

SK-BR-3 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH1/VL1
5D9 VH1/VL2
5D9 VH1/VL3
5D9 VH1/VL4
5D9 VH1/VL5
Free MMAE

FIG. 15

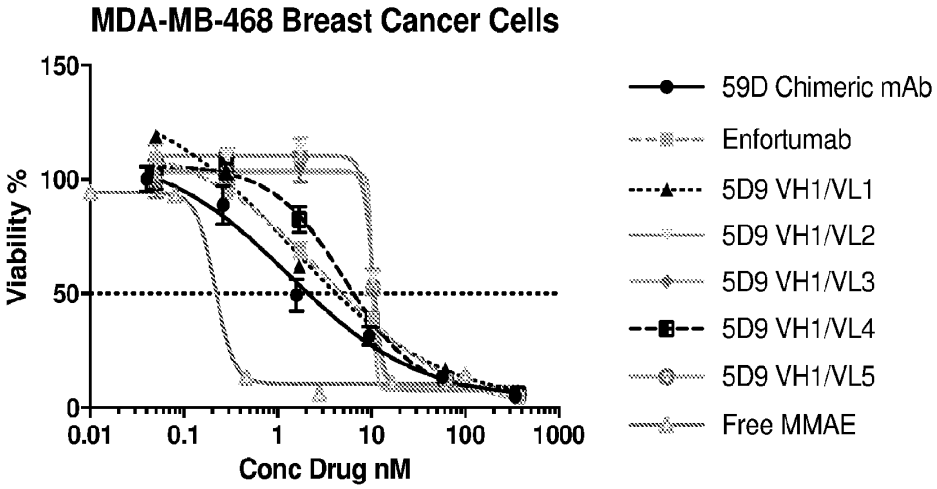

MDA-MB-468 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH1/VL1
5D9 VH1/VL2
5D9 VH1/VL3
5D9 VH1/VL4
5D9 VH1/VL5
Free MMAE

FIG. 16

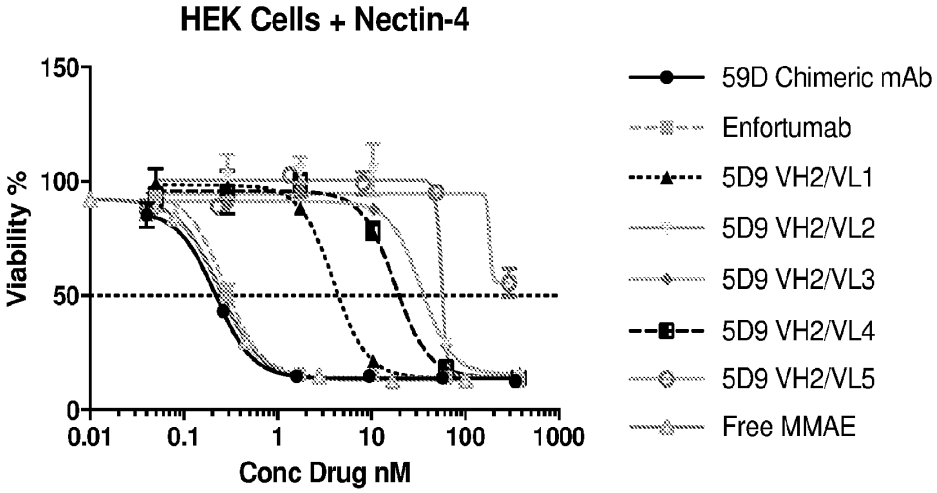

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH2/VL1
5D9 VH2/VL2
5D9 VH2/VL3
5D9 VH2/VL4
5D9 VH2/VL5
Free MMAE

FIG. 17

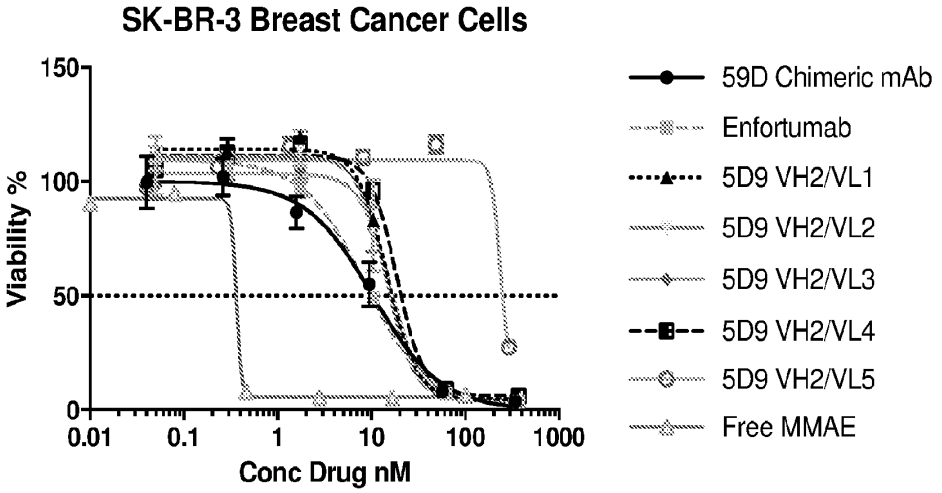

SK-BR-3 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH2/VL1
5D9 VH2/VL2
5D9 VH2/VL3
5D9 VH2/VL4
5D9 VH2/VL5
Free MMAE

FIG. 18

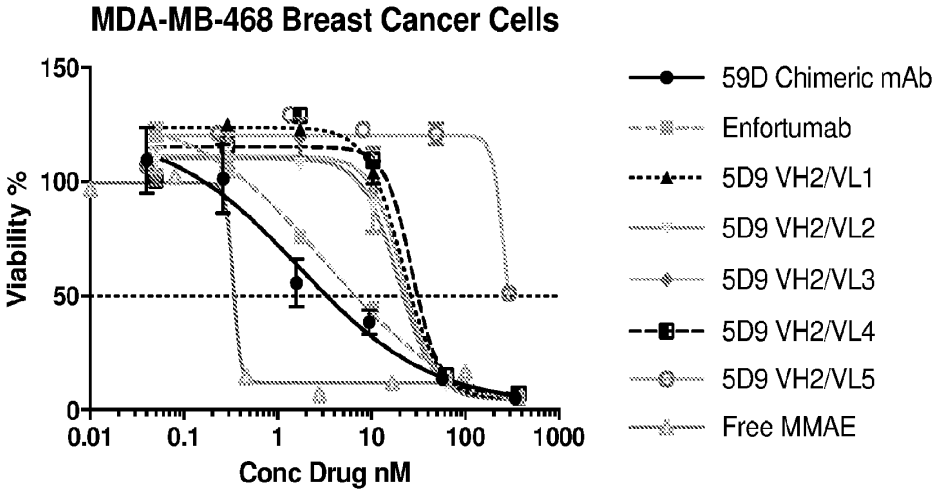

MDA-MB-468 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH2/VL1
5D9 VH2/VL2
5D9 VH2/VL3
5D9 VH2/VL4
5D9 VH2/VL5
Free MMAE

FIG. 19

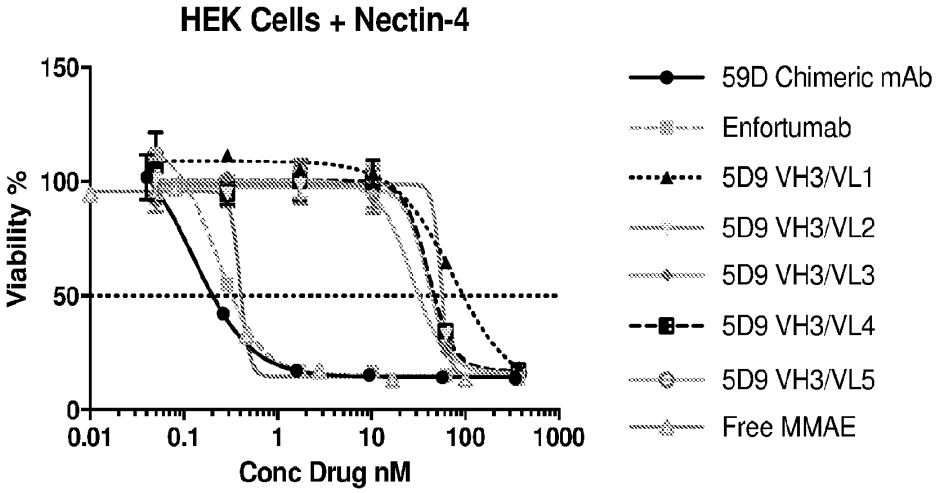

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH3/VL1
5D9 VH3/VL2
5D9 VH3/VL3
5D9 VH3/VL4
5D9 VH3/VL5
Free MMAE

FIG. 20

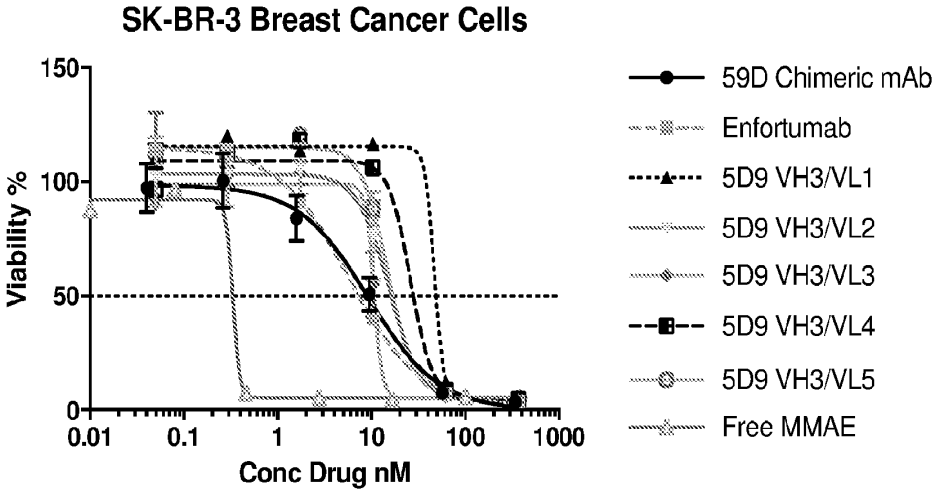

SK-BR-3 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH3/VL1
5D9 VH3/VL2
5D9 VH3/VL3
5D9 VH3/VL4
5D9 VH3/VL5
Free MMAE

FIG. 21

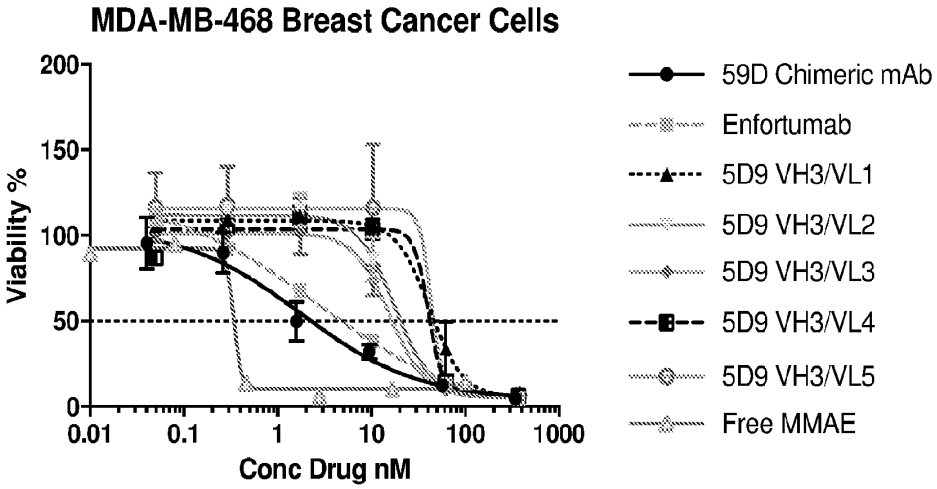

MDA-MB-468 Breast Cancer Cells
Viability %
200
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH3/VL1
5D9 VH3/VL2
5D9 VH3/VL3
5D9 VH3/VL4
5D9 VH3/VL5
Free MMAE

FIG. 22

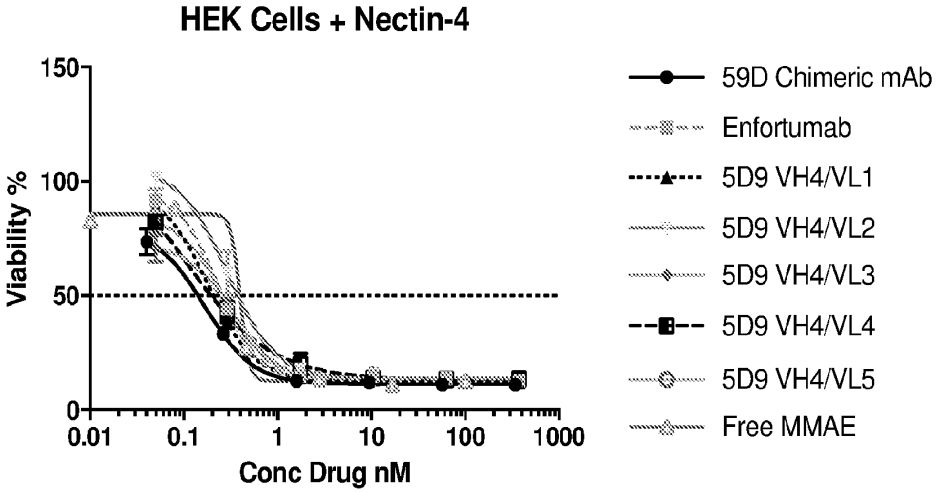

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH4/VL1
5D9 VH4/VL2
5D9 VH4/VL3
5D9 VH4/VL4
5D9 VH4/VL5
Free MMAE

FIG. 23

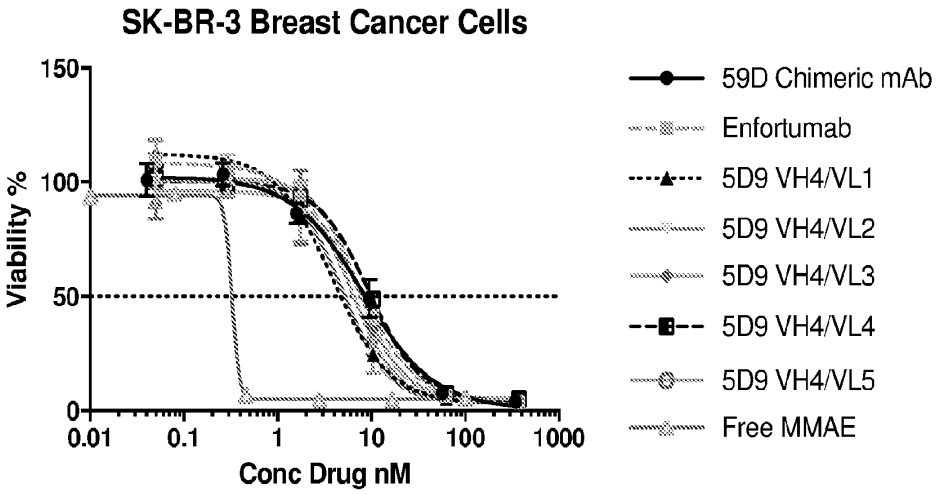

SK-BR-3 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH4/VL1
5D9 VH4/VL2
5D9 VH4/VL3
5D9 VH4/VL4
5D9 VH4/VL5
Free MMAE

FIG. 24

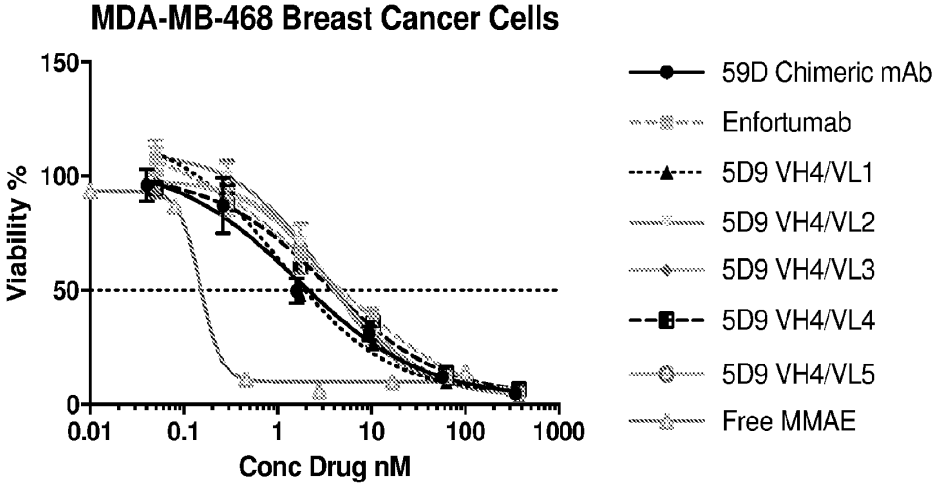

MDA-MB-468 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH4/VL1
5D9 VH4/VL2
5D9 VH4/VL3
5D9 VH4/VL4
5D9 VH4/VL5
Free MMAE

FIG. 25

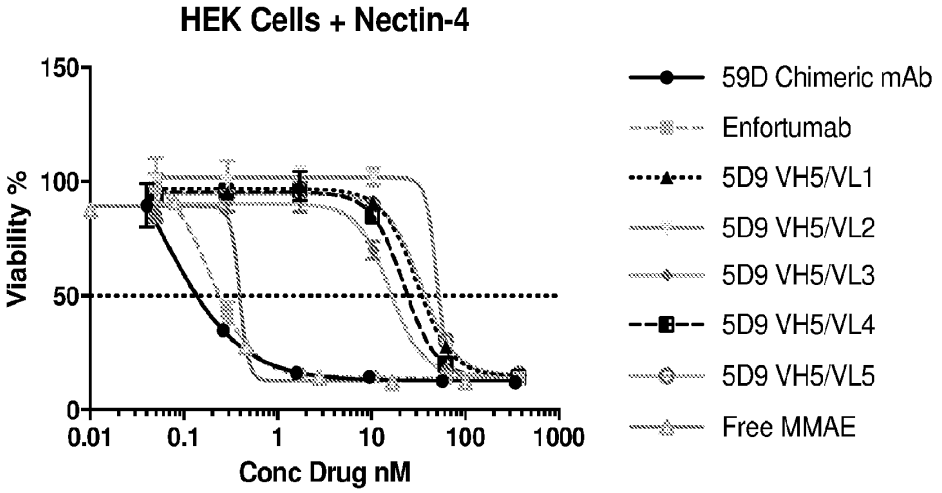

HEK Cells + Nectin-4
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH5/VL1
5D9 VH5/VL2
5D9 VH5/VL3
5D9 VH5/VL4
5D9 VH5/VL5
Free MMAE

FIG. 26

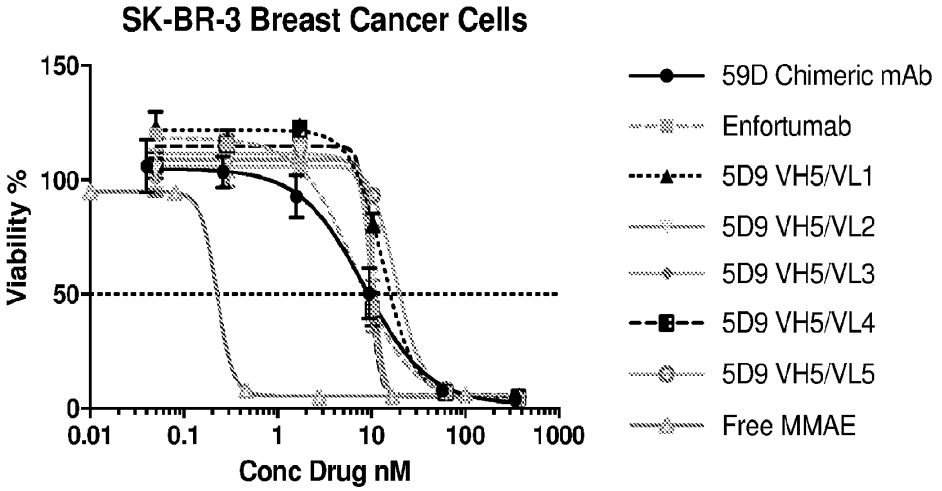

SK-BR-3 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH5/VL1
5D9 VH5/VL2
5D9 VH5/VL3
5D9 VH5/VL4
5D9 VH5/VL5
Free MMAE

FIG. 27

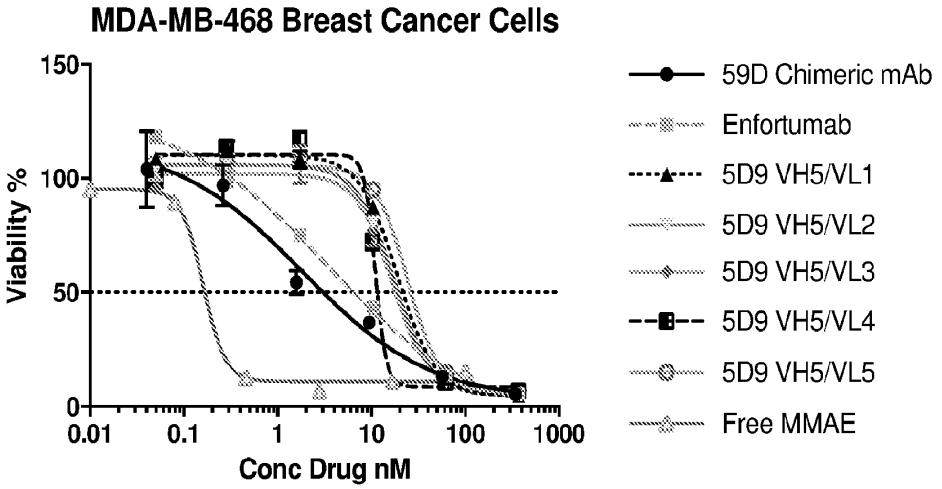

MDA-MB-468 Breast Cancer Cells
Viability %
150
100
50
0
0.01
0.1
1
10
100
1000
Conc Drug nM
59D Chimeric mAb
Enfortumab
5D9 VH5/VL1
5D9 VH5/VL2
5D9 VH5/VL3
5D9 VH5/VL4
5D9 VH5/VL5
Free MMAE

FIG. 28

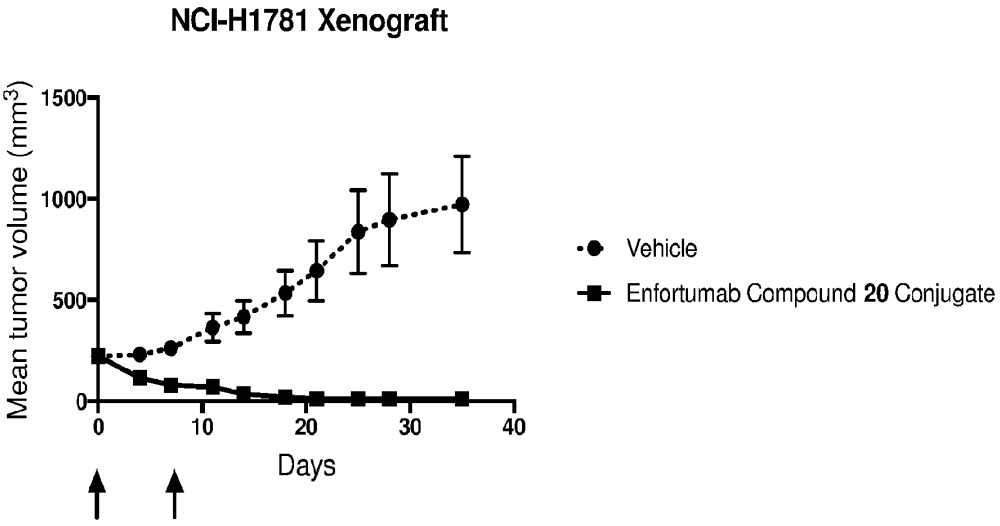

NCI-H1781 Xenograft
Mean tumor volume (mm3)
1500
1000
500
0
0
10
20
30
40
Days
Vehicle
Enfortumab Compound 20 Conjugate

FIG. 29

Light chain conserved region:

```
         140        150        160        170        180       189
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD

         200        210        220        230   236
SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
```

Heavy chain conserved region:

```
      130        140        150        160        170        180
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

      190        200        210        220        230        240
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG

      250        260        270        280        290        300
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN

      310        320        330        340        350        360
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE

      370        380        390        400        410        420
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW

      430        440        450
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
```

FIG. 30A

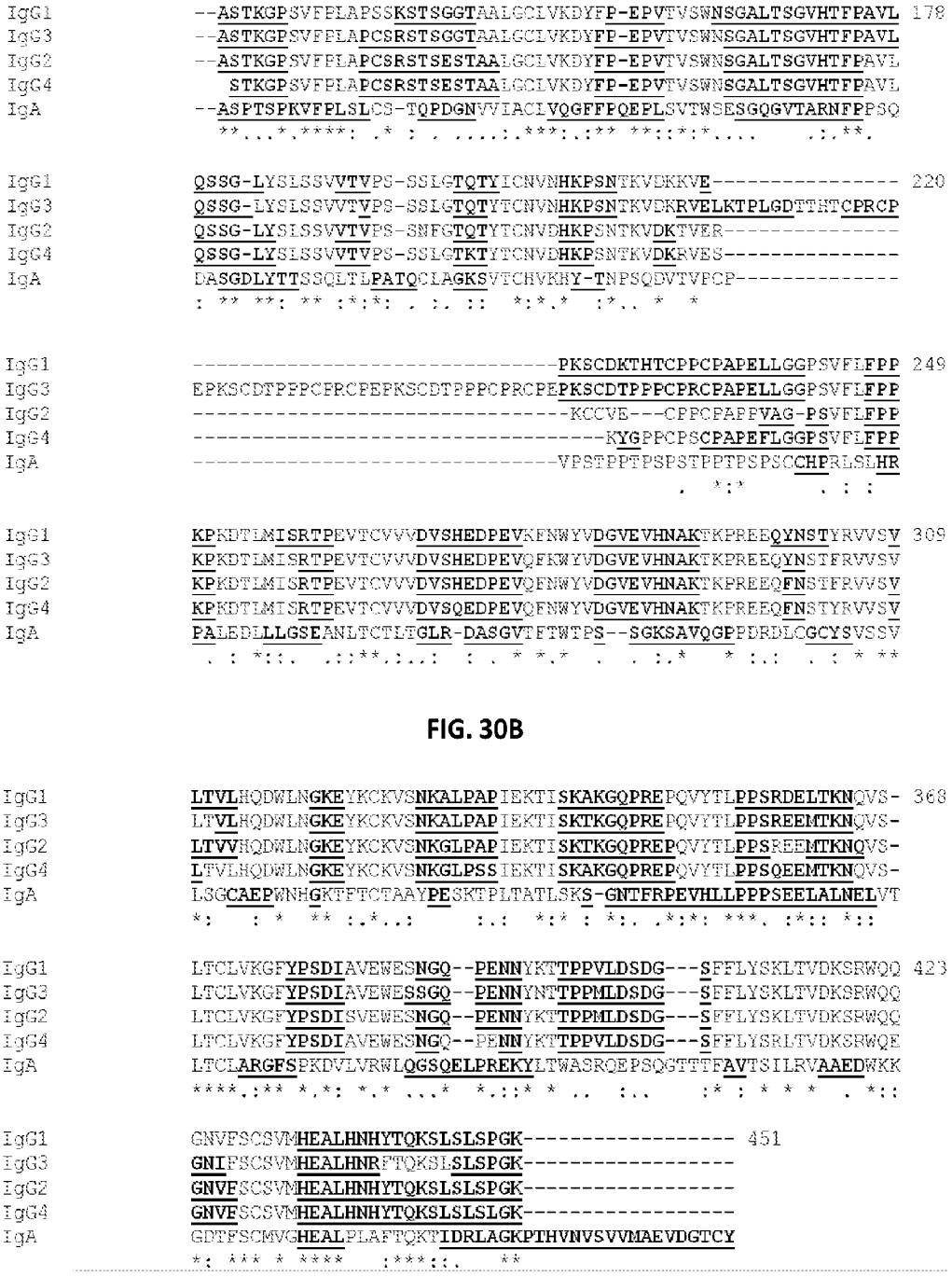


FIG. 30B

FIG. 30C

```
Seq1 = Homo sapiens kappa light chain constant region; GenBank CAA75031.1
Seq2 = Homo sapiens kappa light chain constant region; GenBank BAC01168.1
Seq3 = Homo sapiens lambda light chain constant region; GenBank CAA75033
Seq4 = Mus musculus light chain constant region; GenBank AAB09710.1
Seq5 = Rattus norvegicus light chain constant region; GenBank AAD10133

Seq1            RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD 189
Seq2            RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
Seq4            RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQD
Seq5            RADAAPTVSIFPPSMEQLTSGGATVVCFVNNFYPRDISVKWKIDGSEQRDGVLDSVTDQD
Seq3            QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQ
                :. ***:* :**** *:* :. *::**::.:***   .* ** *.:    .   : ..::

Seq1            SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
Seq2            SKDSTYSLSSTLTLSKADYEKHKLYACEVTHQGLSSPVTKSFNRGEC
Seq4            SKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRGEC
Seq5            SKDSTYSMSSTLSLTKVEYERHNLYTCEVVHKTSSSPVVKSFNRNEC |
Seq3            S-NNKYAASSYLSLTPEQWKSHKSYSCQVTHEG--STVEKTVAPTECS
                * :..*: ** *:*:  ::: *: *:*:..*:   *.: *:.   **
```

FIG. 30D

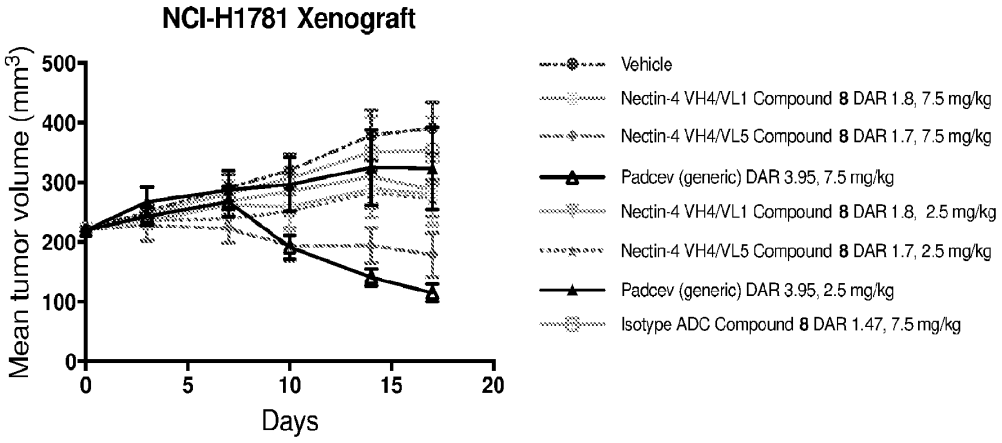

NCI-H1781 Xenograft
Mean tumor volume (mm3)
500
400
300
200
100
0
0
5
10
15
20
Days
Vehicle
Nectin-4 VH4/VL1 Compound 8 DAR 1.8, 7.5 mg/kg
Nectin-4 VH4/VL5 Compound 8 DAR 1.7, 7.5 mg/kg
Padcev (generic) DAR 3.95, 7.5 mg/kg
Nectin-4 VH4/VL1 Compound 8 DAR 1.8, 2.5 mg/kg
Nectin-4 VH4/VL5 Compound 8 DAR 1.7, 2.5 mg/kg
Padcev (generic) DAR 3.95, 2.5 mg/kg
Isotype ADC Compound 8 DAR 1.47, 7.5 mg/kg

FIG. 31

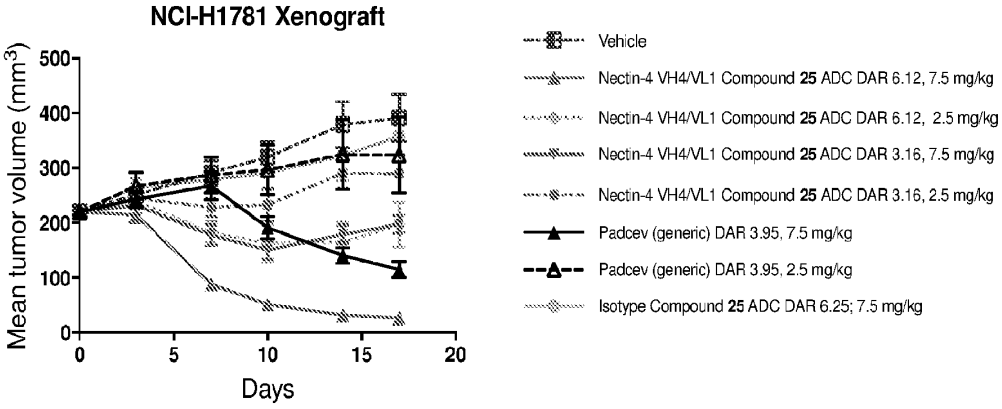

NCI-H1781 Xenograft
Mean tumor volume (mm3)
500
400
300
200
100
0
0
5
10
15
20
Days
Vehicle
Nectin-4 VH4/VL1 Compound 25 ADC DAR 6.12, 7.5 mg/kg
Nectin-4 VH4/VL1 Compound 25 ADC DAR 6.12, 2.5 mg/kg
Nectin-4 VH4/VL1 Compound 25 ADC DAR 3.16, 7.5 mg/kg
Nectin-4 VH4/VL1 Compound 25 ADC DAR 3.16, 2.5 mg/kg
Padcev (generic) DAR 3.95, 7.5 mg/kg
Padcev (generic) DAR 3.95, 2.5 mg/kg
Isotype Compound 25 ADC DAR 6.25; 7.5 mg/kg

FIG. 32

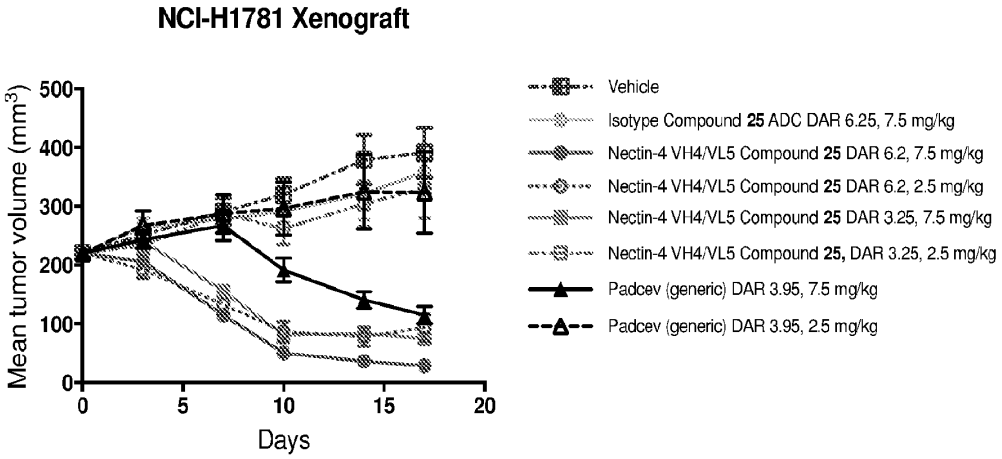

NCI-H1781 Xenograft
Mean tumor volume (mm3)
500
400
300
200
100
0
0
5
10
15
20
Days
Vehicle
Isotype Compound 25 ADC DAR 6.25, 7.5 mg/kg
Nectin-4 VH4/VL5 Compound 25 DAR 6.2, 7.5 mg/kg
Nectin-4 VH4/VL5 Compound 25 DAR 6.2, 2.5 mg/kg
Nectin-4 VH4/VL5 Compound 25 DAR 3.25, 7.5 mg/kg
Nectin-4 VH4/VL5 Compound 25, DAR 3.25, 2.5 mg/kg
Padcev (generic) DAR 3.95, 7.5 mg/kg
Padcev (generic) DAR 3.95, 2.5 mg/kg

FIG. 33

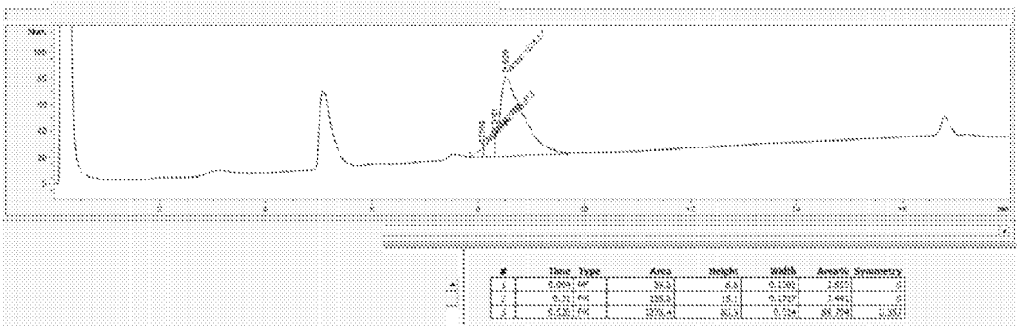


FIG. 34

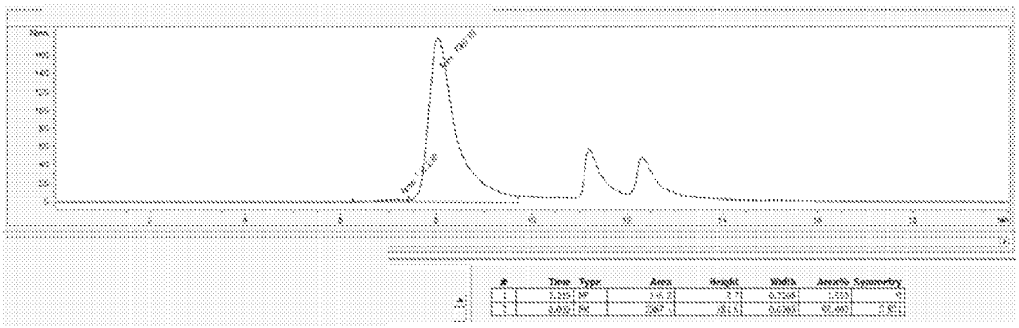


FIG. 35

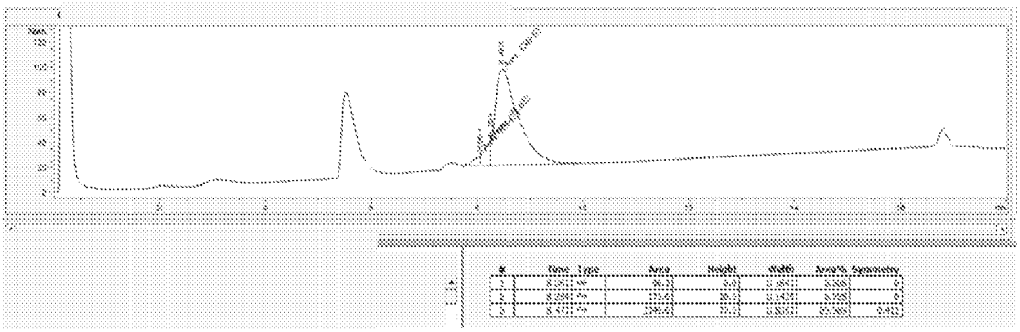


FIG. 36

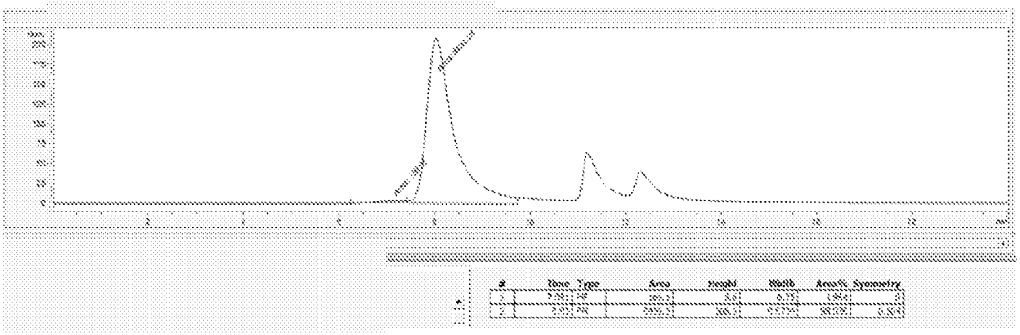


FIG. 37

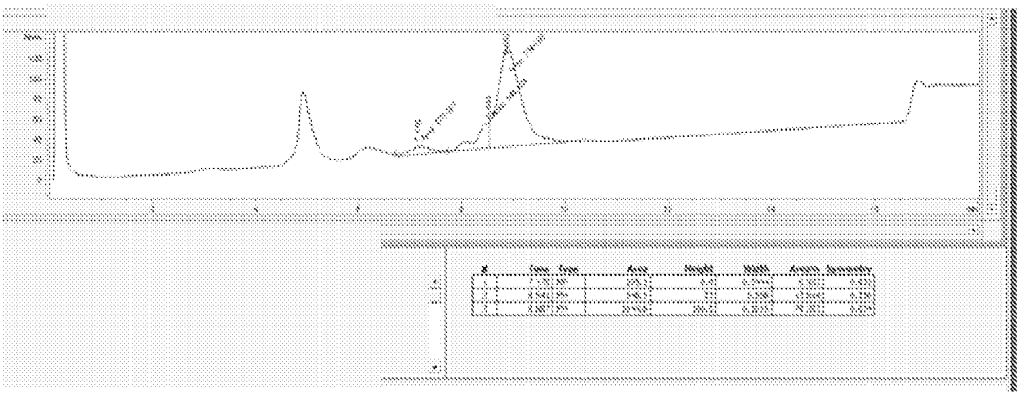


FIG. 38

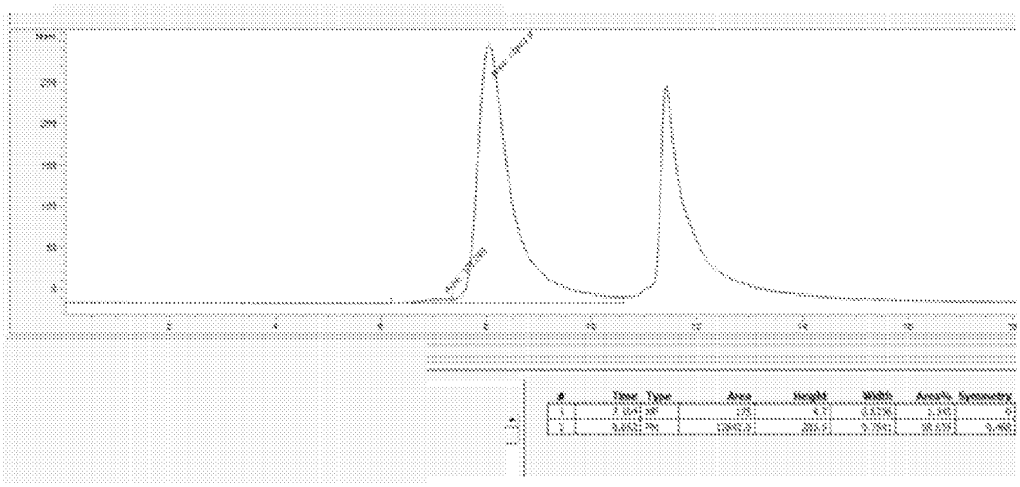


FIG. 39

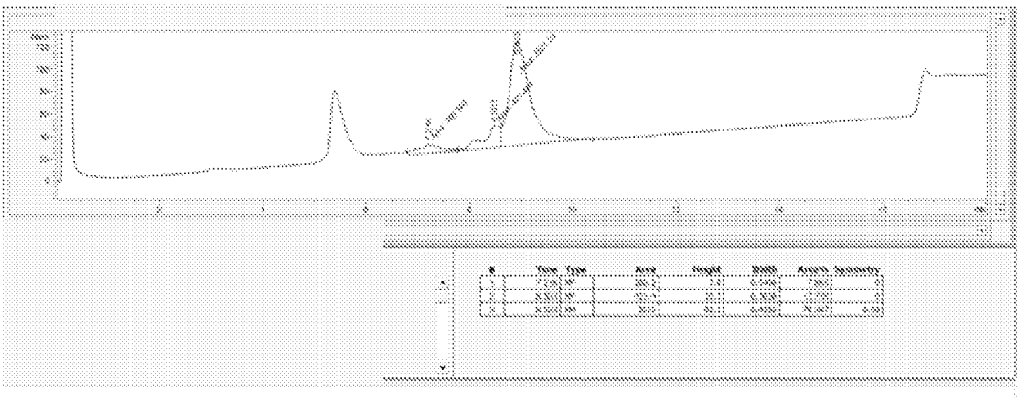


FIG. 40

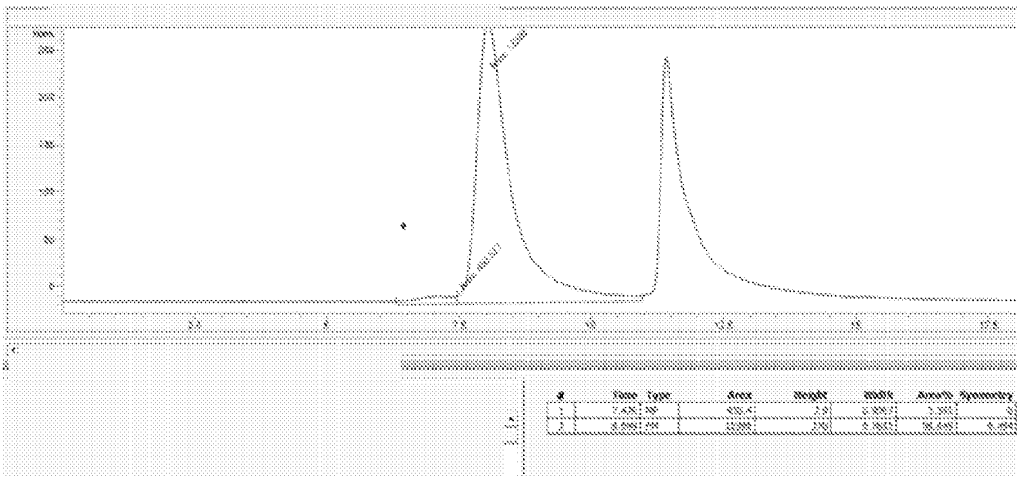


FIG. 41

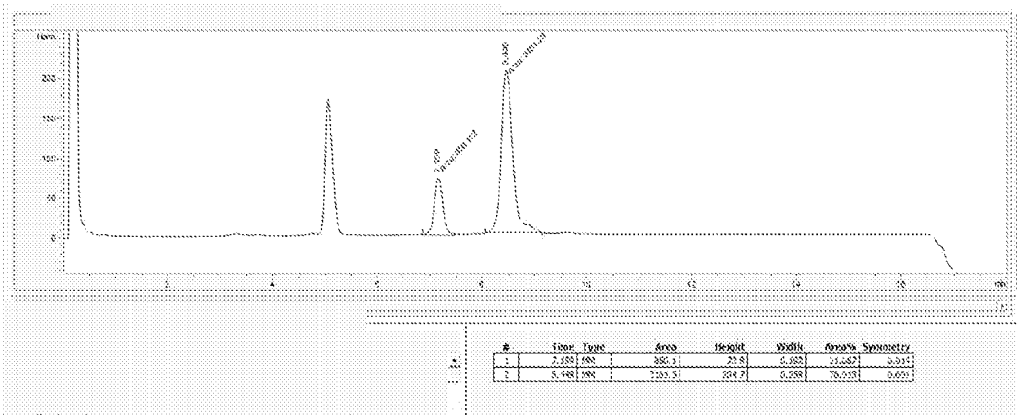


FIG. 42

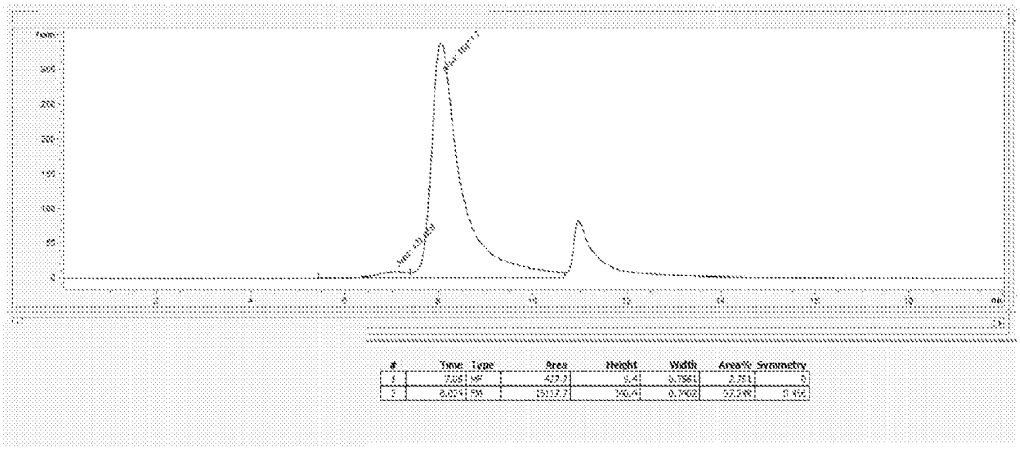


FIG. 43

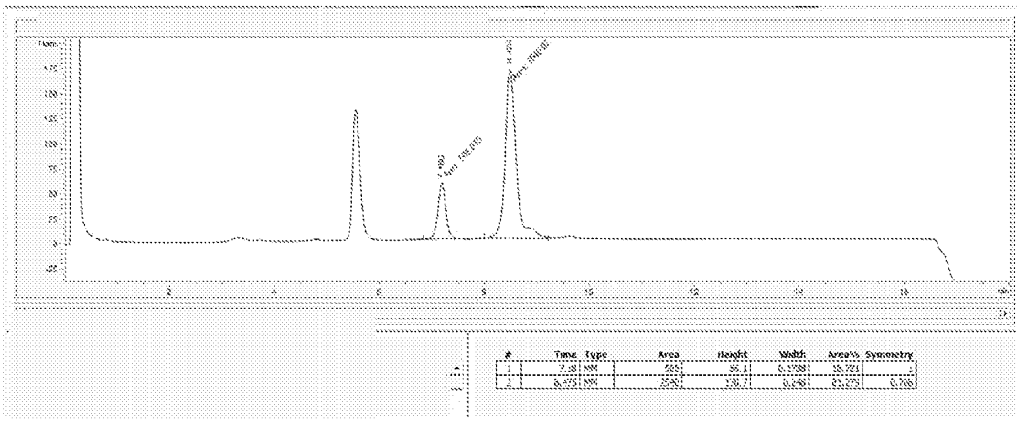
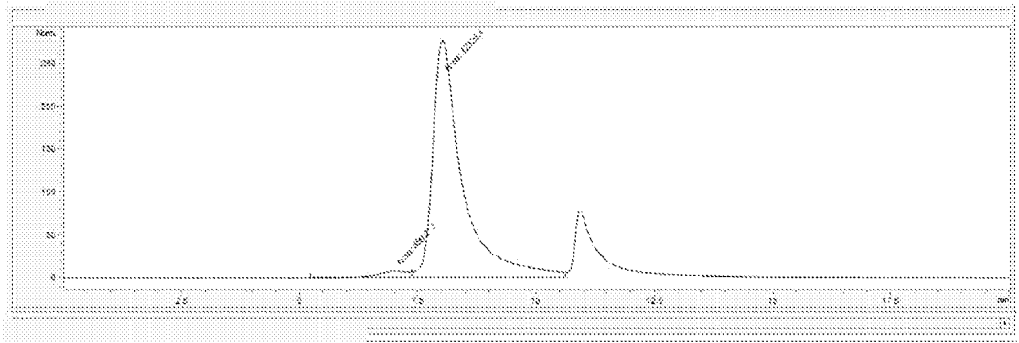


FIG. 44



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.021	NP	385.2	8.3	0.753	3.081	0
2	8.026	FM	12226.4	270.1	0.7398	96.938	0.467

FIG. 45

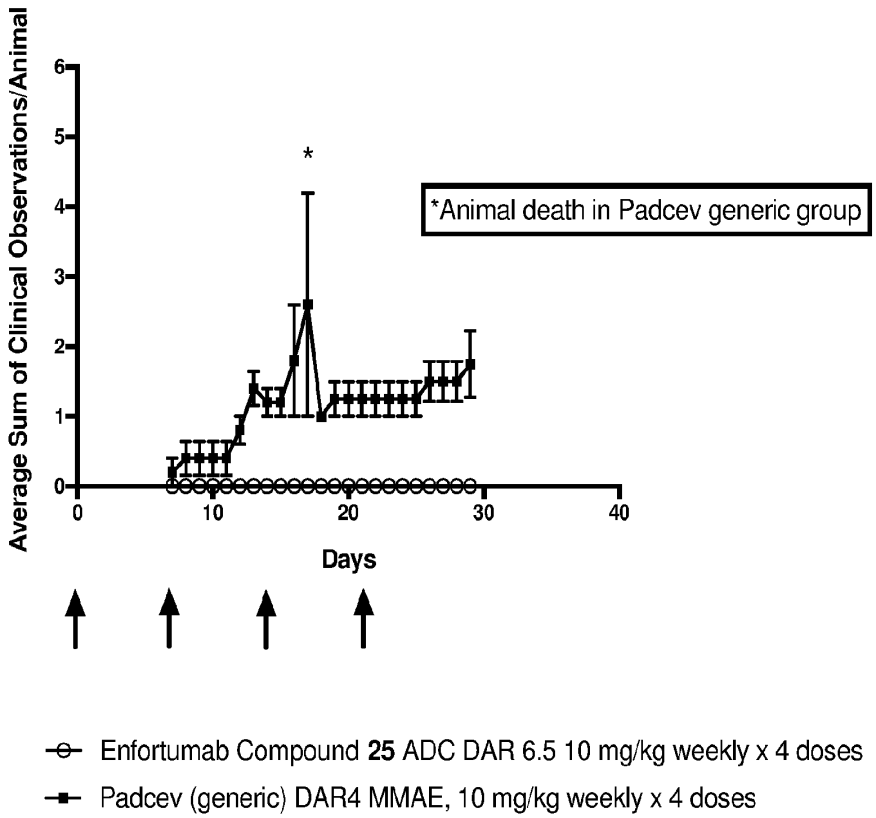

Average Sum of Clinical Observations/Animal
6
5
4
3
2
1
0
*
*Animal death in Padcev generic group
0
10
20
30
40
Days
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg weekly x 4 doses
Padcev (generic) DAR4 MMAE, 10 mg/kg weekly x 4 doses

FIG. 46

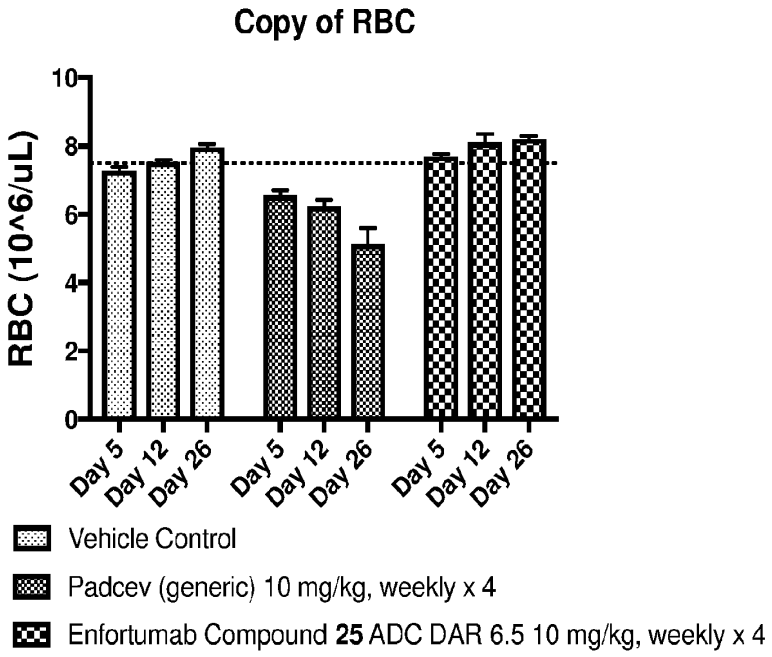

Copy of RBC
RBC (10^6/uL)
10
8
6
4
2
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 47

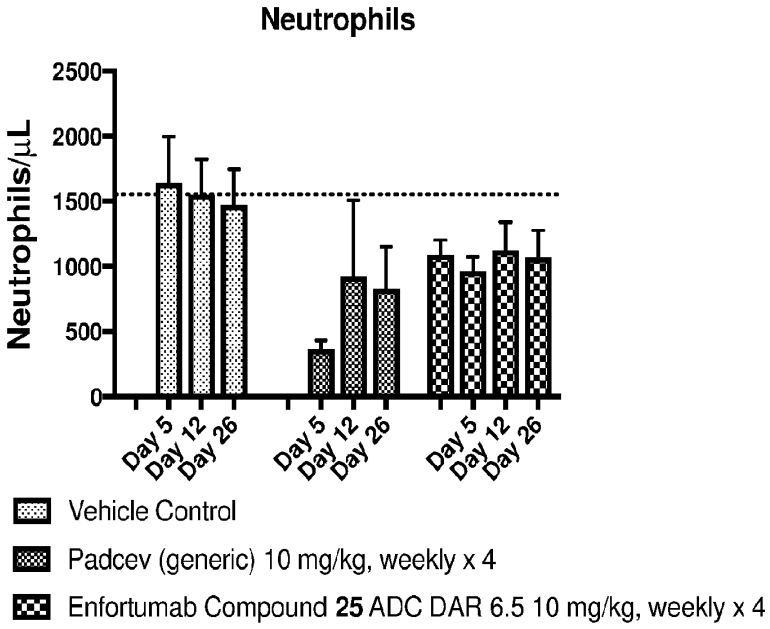

Neutrophils
Neutrophils/μL
2500
2000
1500
1000
500
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 48

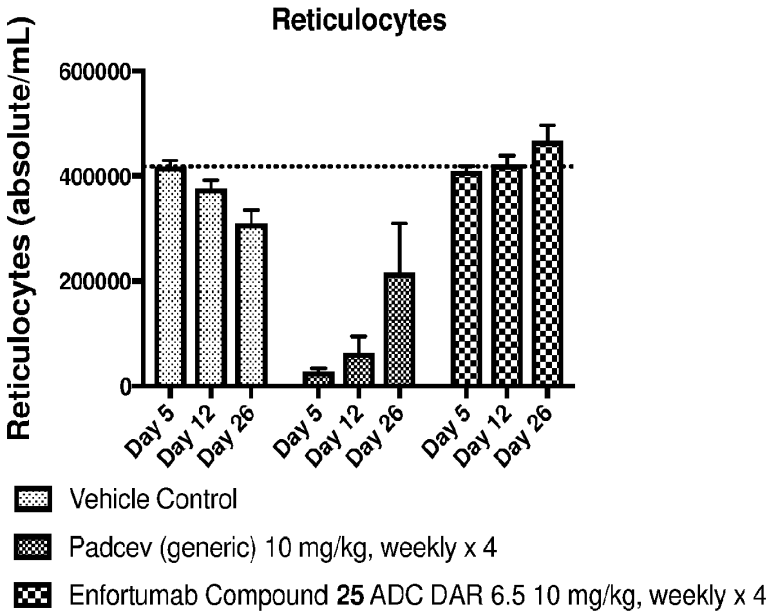

Reticulocytes
Reticulocytes (absolute/mL)
600000
400000
200000
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 49

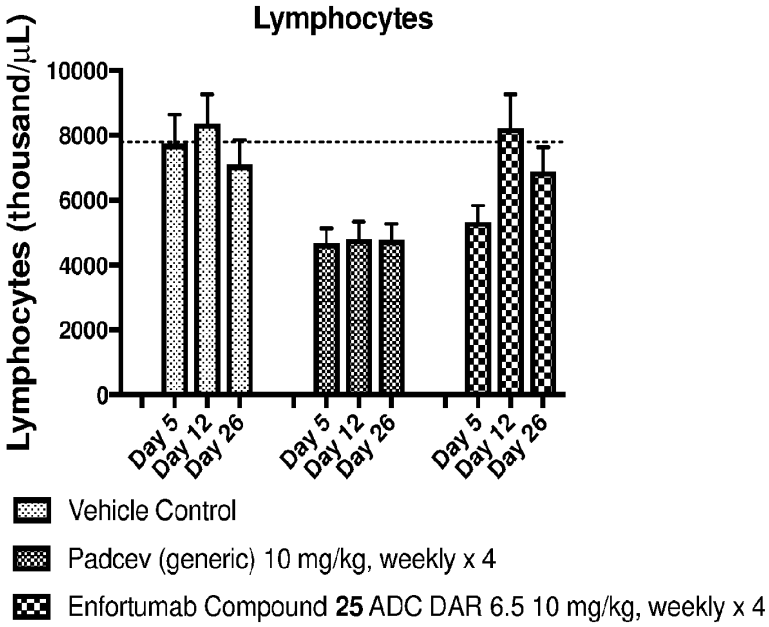

Lymphocytes
Lymphocytes (thousand/μL)
10000
8000
6000
4000
2000
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 50

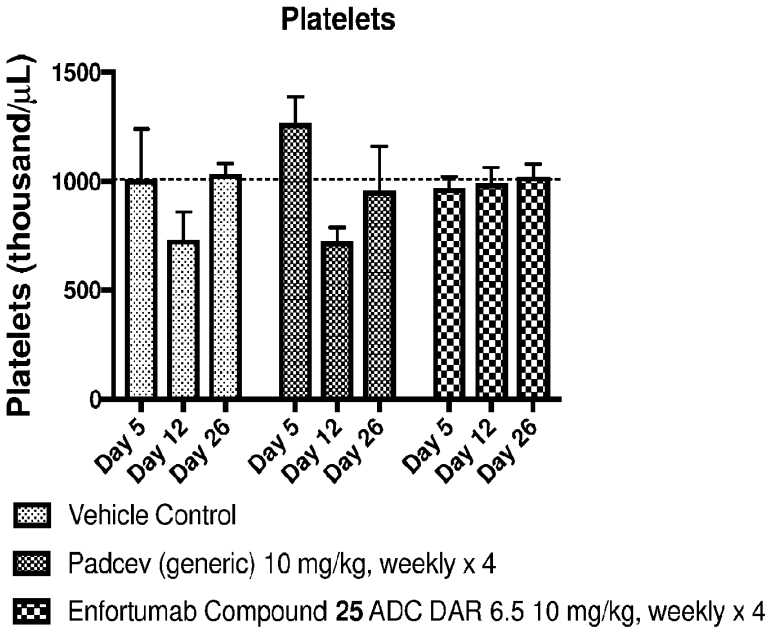

Platelets
Platelets (thousand/µL)
1500
1000
500
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 51

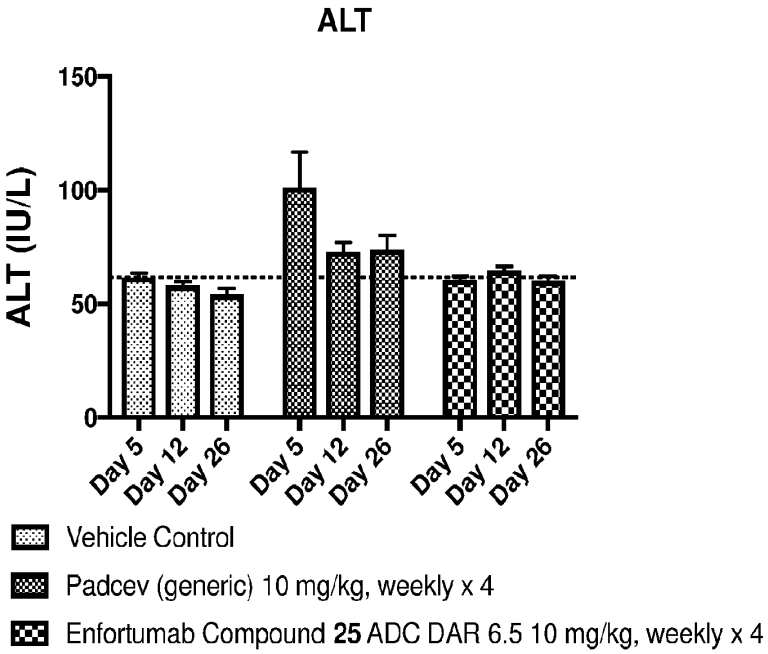

ALT
ALT (IU/L)
150
100
50
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 52

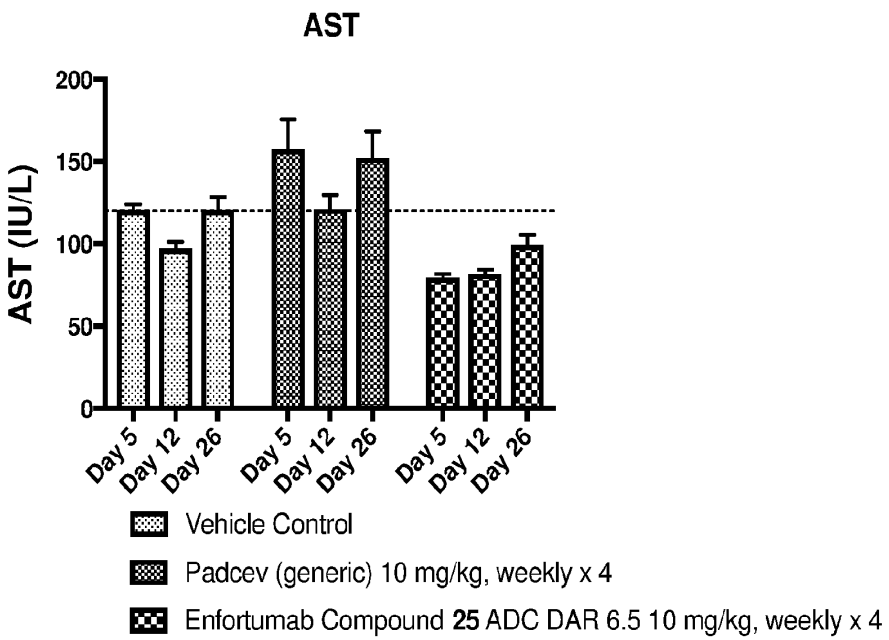
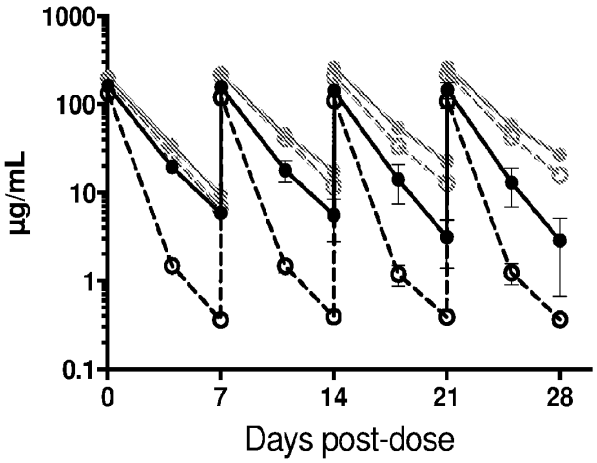

AST
AST (IU/L)
200
150
100
50
0
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Day 5
Day 12
Day 26
Vehicle Control
Padcev (generic) 10 mg/kg, weekly x 4
Enfortumab Compound 25 ADC DAR 6.5 10 mg/kg, weekly x 4

FIG. 53



Enfortumab Vedotin Total Antibody

Enfortumab Vedotin Total ADC

Enfortumab Compound 8 Total Antibody

Enfortumab Compound 8 Total ADC

FIG. 54