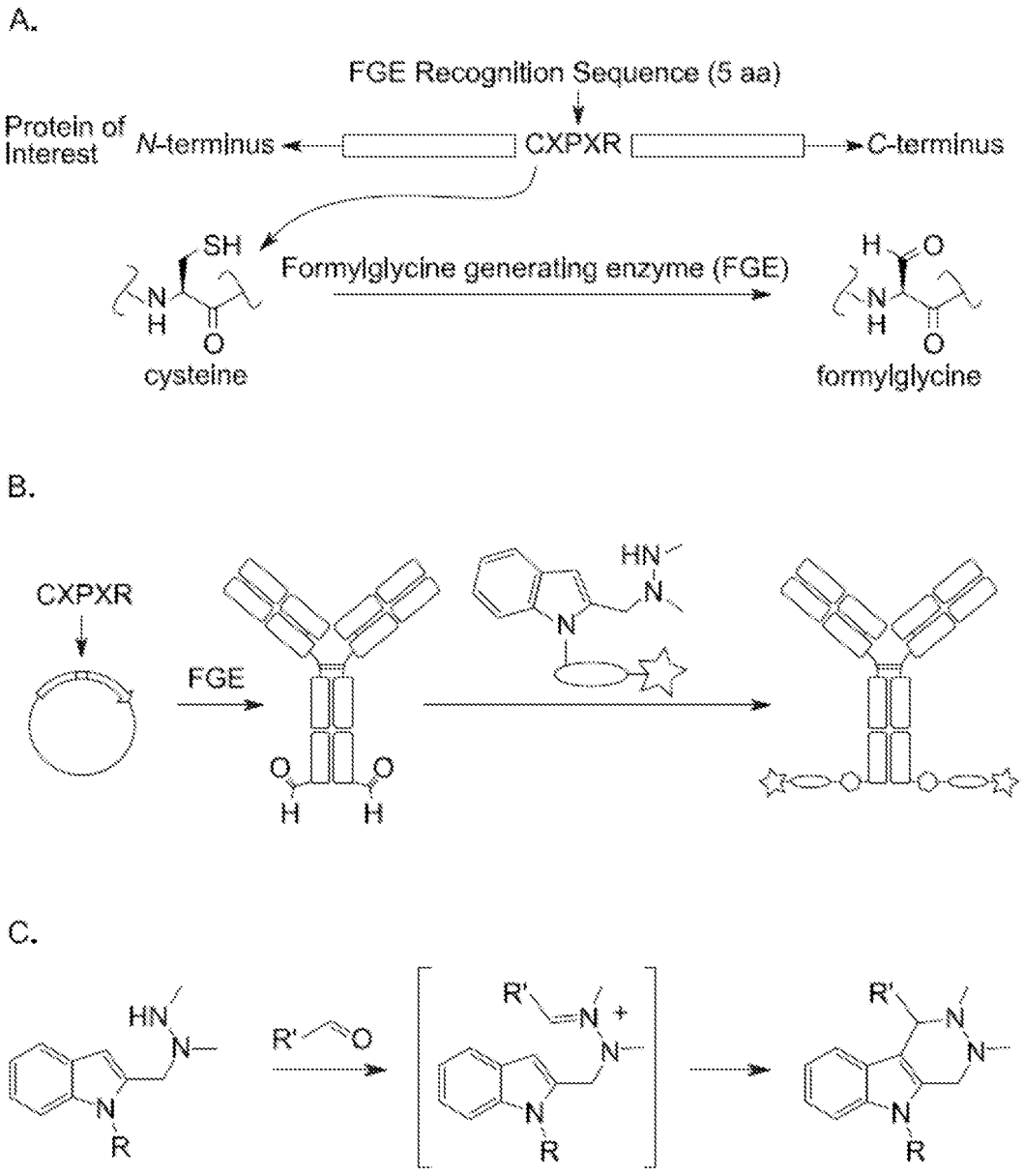

A.
FGE Recognition Sequence (5 aa)
Protein of Interest
N-terminus
CXPXR
C-terminus
SH
Formylglycine generating enzyme (FGE)
cysteine
formylglycine
B.
CXPXR
FGE
C.

FIG. 1

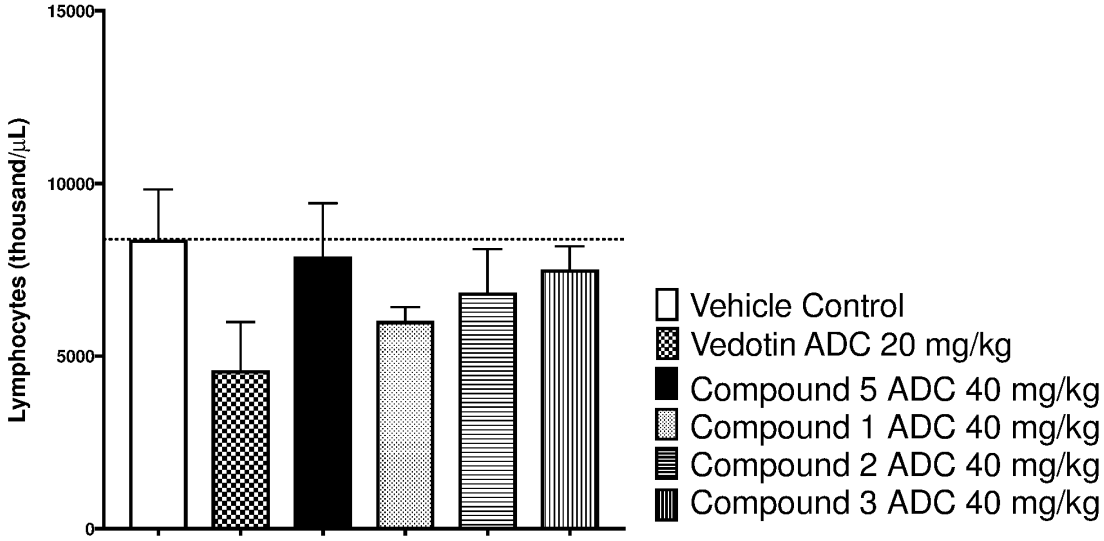

15000
10000
5000
0
Lymphocytes (thousand/μL)
Vehicle Control
Vedotin ADC 20 mg/kg
Compound 5 ADC 40 mg/kg
Compound 1 ADC 40 mg/kg
Compound 2 ADC 40 mg/kg
Compound 3 ADC 40 mg/kg

FIG. 2

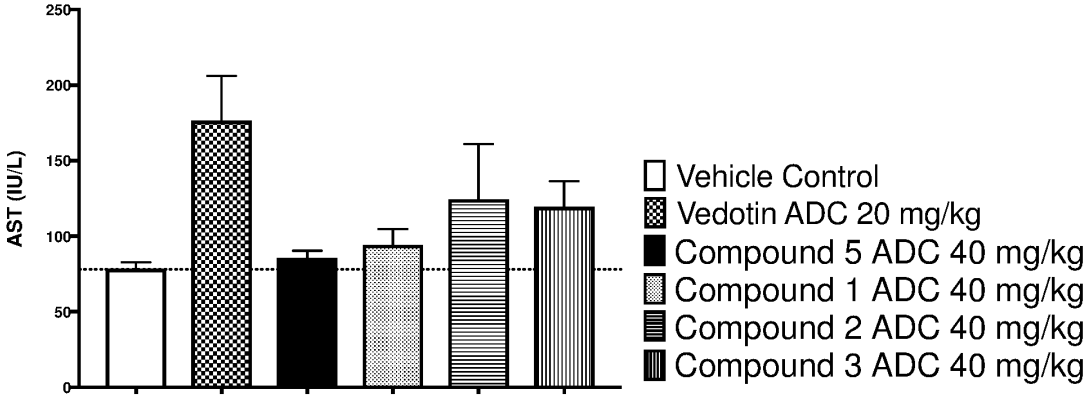

250
200
150
100
50
0
AST (IU/L)
Vehicle Control
Vedotin ADC 20 mg/kg
Compound 5 ADC 40 mg/kg
Compound 1 ADC 40 mg/kg
Compound 2 ADC 40 mg/kg
Compound 3 ADC 40 mg/kg

FIG. 3

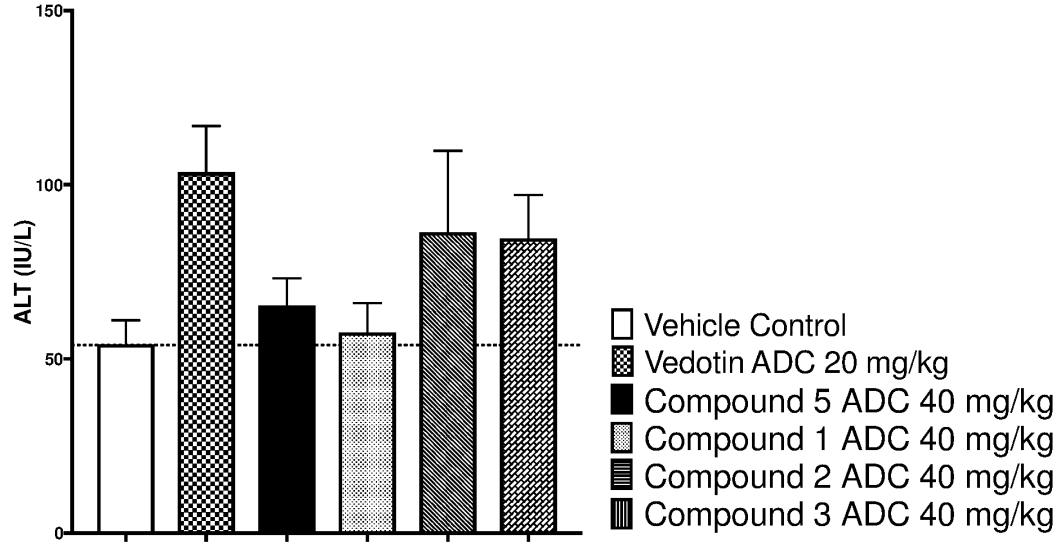

150
100
50
0
ALT (IU/L)
Vehicle Control
Vedotin ADC 20 mg/kg
Compound 5 ADC 40 mg/kg
Compound 1 ADC 40 mg/kg
Compound 2 ADC 40 mg/kg
Compound 3 ADC 40 mg/kg

FIG. 4

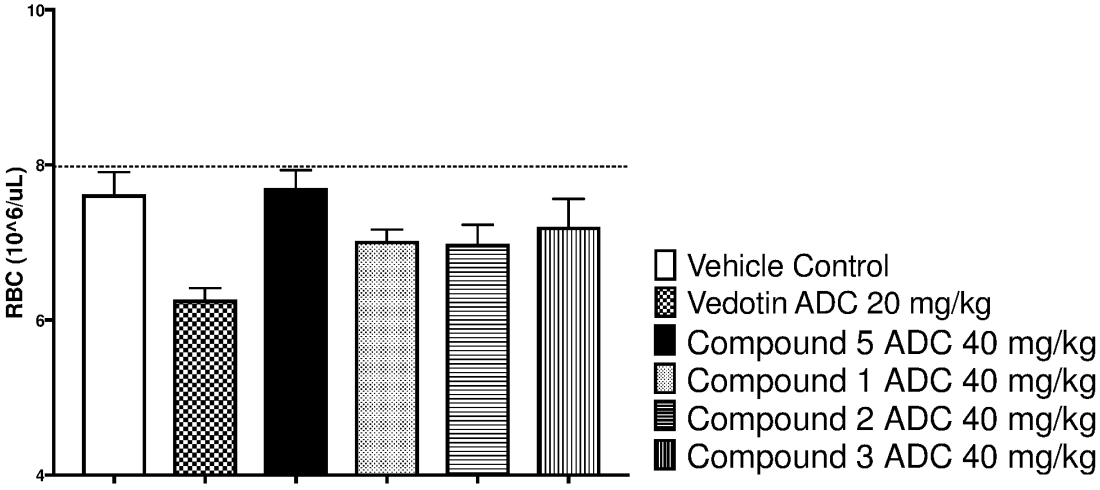

10
8
6
4
RBC (10^6/uL)
Vehicle Control
Vedotin ADC 20 mg/kg
Compound 5 ADC 40 mg/kg
Compound 1 ADC 40 mg/kg
Compound 2 ADC 40 mg/kg
Compound 3 ADC 40 mg/kg

FIG. 5

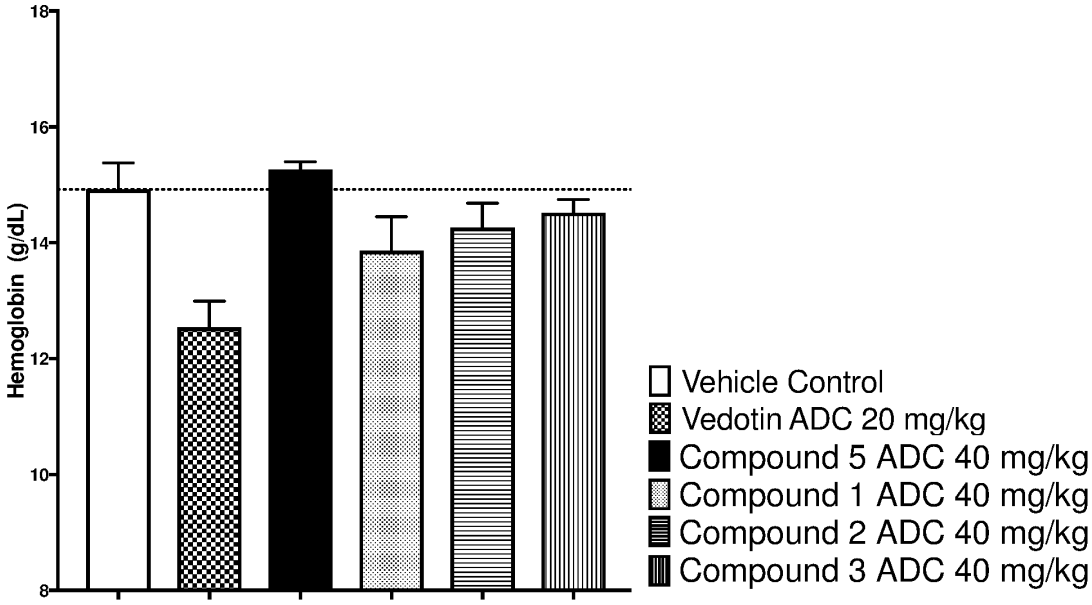

Hemoglobin (g/dL)
18
16
14
12
10
8
Vehicle Control
Vedotin ADC 20 mg/kg
Compound 5 ADC 40 mg/kg
Compound 1 ADC 40 mg/kg
Compound 2 ADC 40 mg/kg
Compound 3 ADC 40 mg/kg

FIG. 6

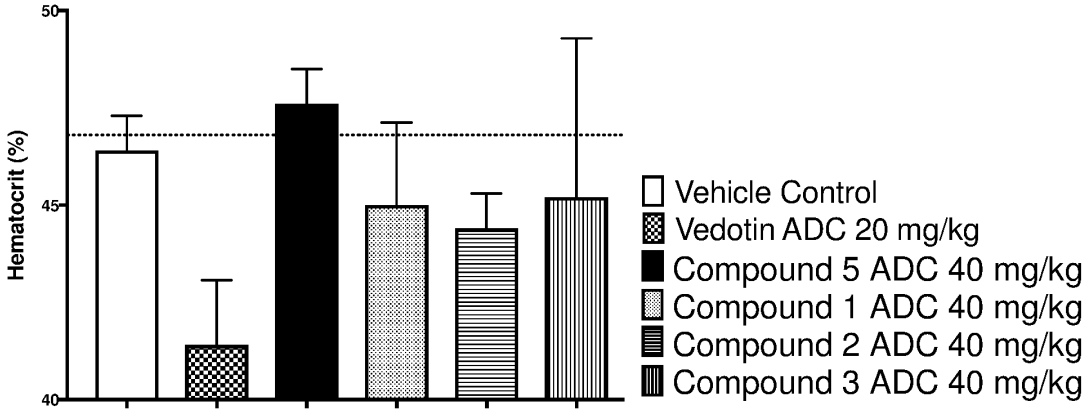

Hematocrit (%)
50
45
40
Vehicle Control
Vedotin ADC 20 mg/kg
Compound 5 ADC 40 mg/kg
Compound 1 ADC 40 mg/kg
Compound 2 ADC 40 mg/kg
Compound 3 ADC 40 mg/kg

FIG. 7

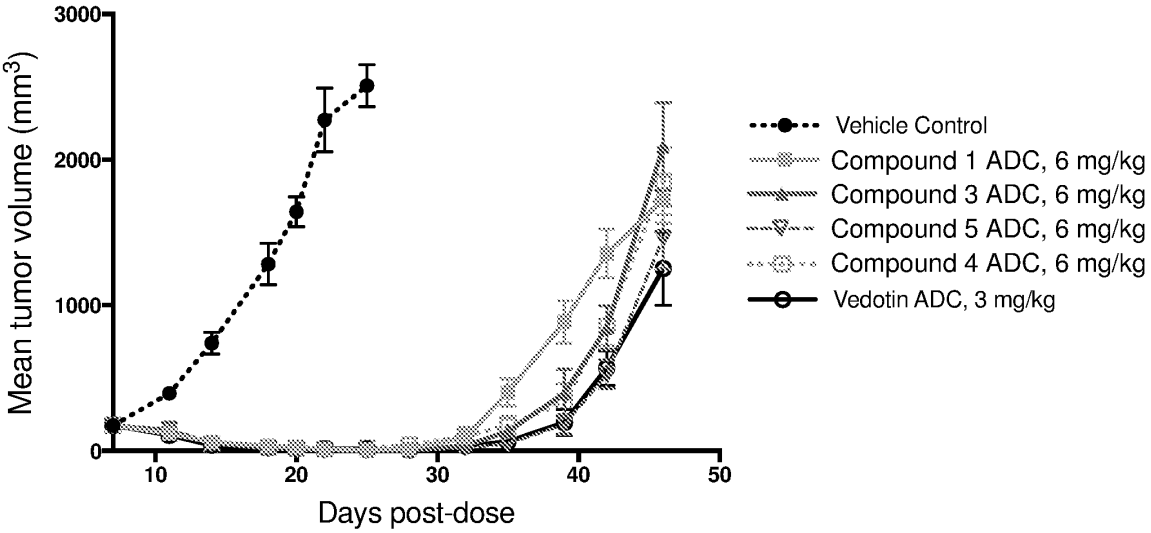

Mean tumor volume (mm3)
3000
2000
1000
0
10
20
30
40
50
Days post-dose
Vehicle Control
Compound 1 ADC, 6 mg/kg
Compound 3 ADC, 6 mg/kg
Compound 5 ADC, 6 mg/kg
Compound 4 ADC, 6 mg/kg
Vedotin ADC, 3 mg/kg

FIG. 8

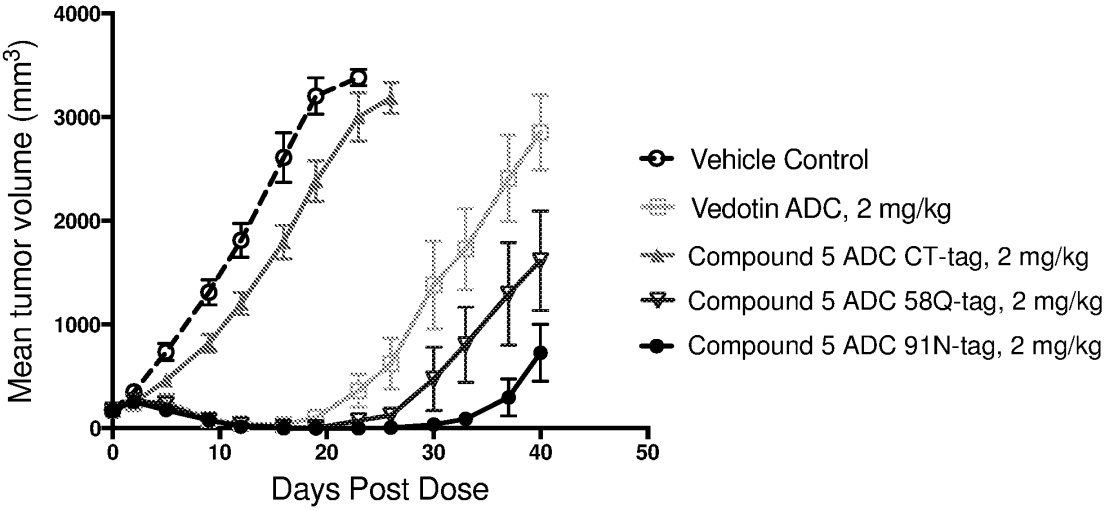

Mean tumor volume (mm3)
4000
3000
2000
1000
0
0
10
20
30
40
50
Days Post Dose
Vehicle Control
Vedotin ADC, 2 mg/kg
Compound 5 ADC CT-tag, 2 mg/kg
Compound 5 ADC 58Q-tag, 2 mg/kg
Compound 5 ADC 91N-tag, 2 mg/kg

FIG. 9

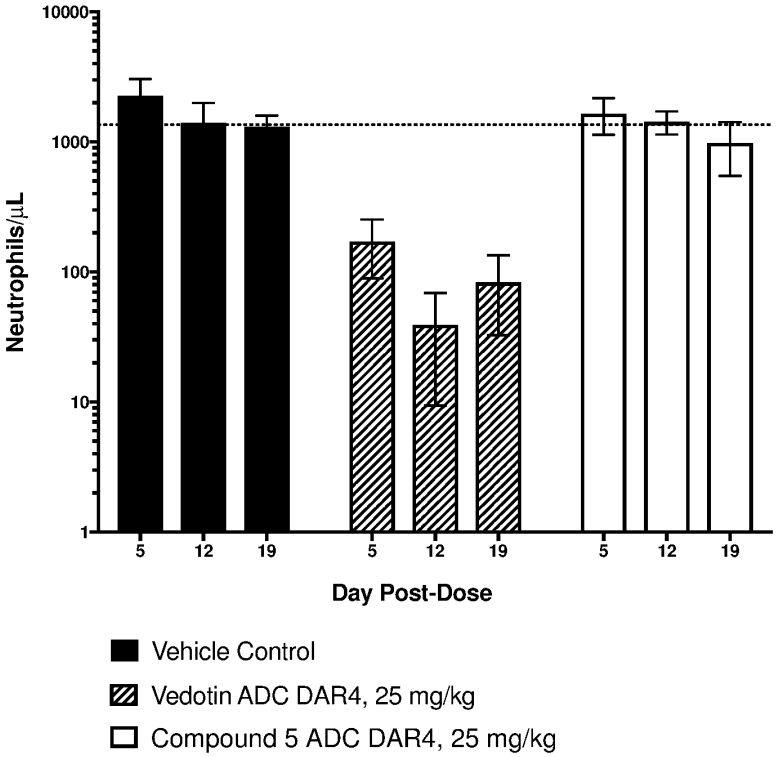

10000
1000
100
10
1
Neutrophils/μL
5
12
19
5
12
19
5
12
19
Day Post-Dose
Vehicle Control
Vedotin ADC DAR4, 25 mg/kg
Compound 5 ADC DAR4, 25 mg/kg

FIG. 10

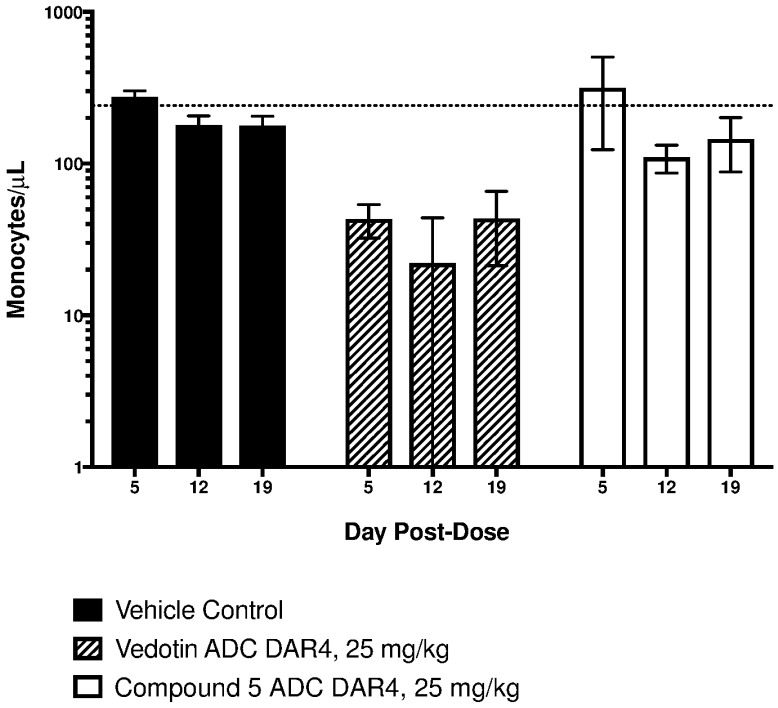

1000
100
10
1
Monocytes/μL
5
12
19
5
12
19
5
12
19
Day Post-Dose
Vehicle Control
Vedotin ADC DAR4, 25 mg/kg
Compound 5 ADC DAR4, 25 mg/kg

FIG. 11

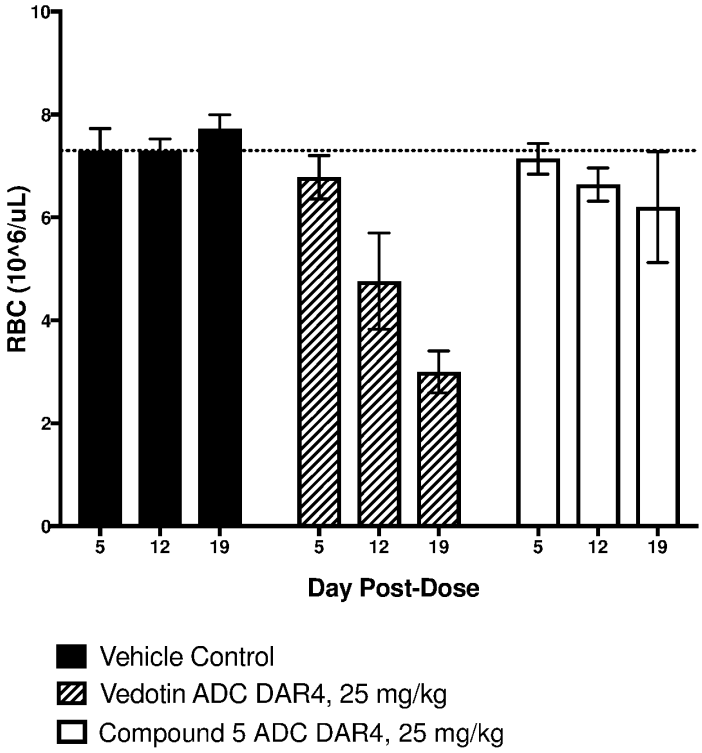

10
8
6
4
2
0
RBC (10^6/uL)
5
12
19
5
12
19
5
12
19
Day Post-Dose
Vehicle Control
Vedotin ADC DAR4, 25 mg/kg
Compound 5 ADC DAR4, 25 mg/kg

FIG. 12

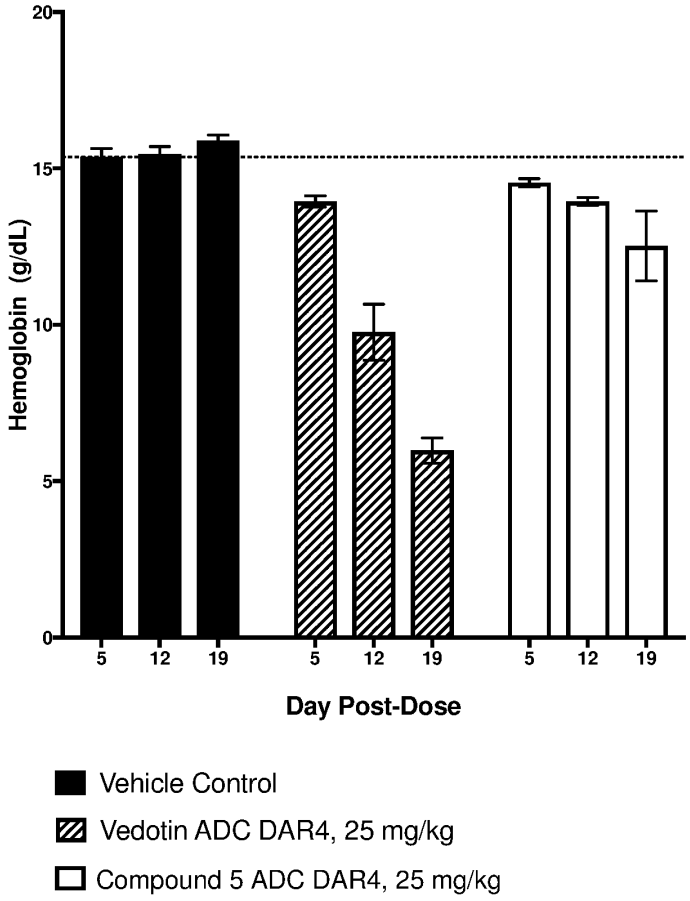

20
15
10
5
0
Hemoglobin (g/dL)
5
12
19
5
12
19
5
12
19
Day Post-Dose
Vehicle Control
Vedotin ADC DAR4, 25 mg/kg
Compound 5 ADC DAR4, 25 mg/kg

FIG. 13

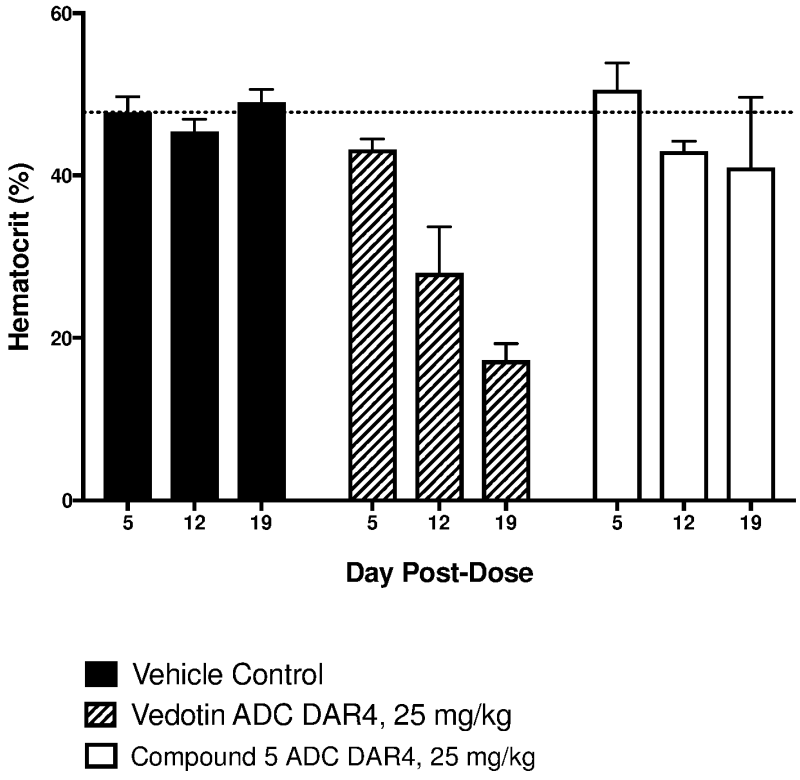

60
40
20
0
Hematocrit (%)
5
12
19
5
12
19
5
12
19
Day Post-Dose
Vehicle Control
Vedotin ADC DAR4, 25 mg/kg
Compound 5 ADC DAR4, 25 mg/kg

FIG. 14

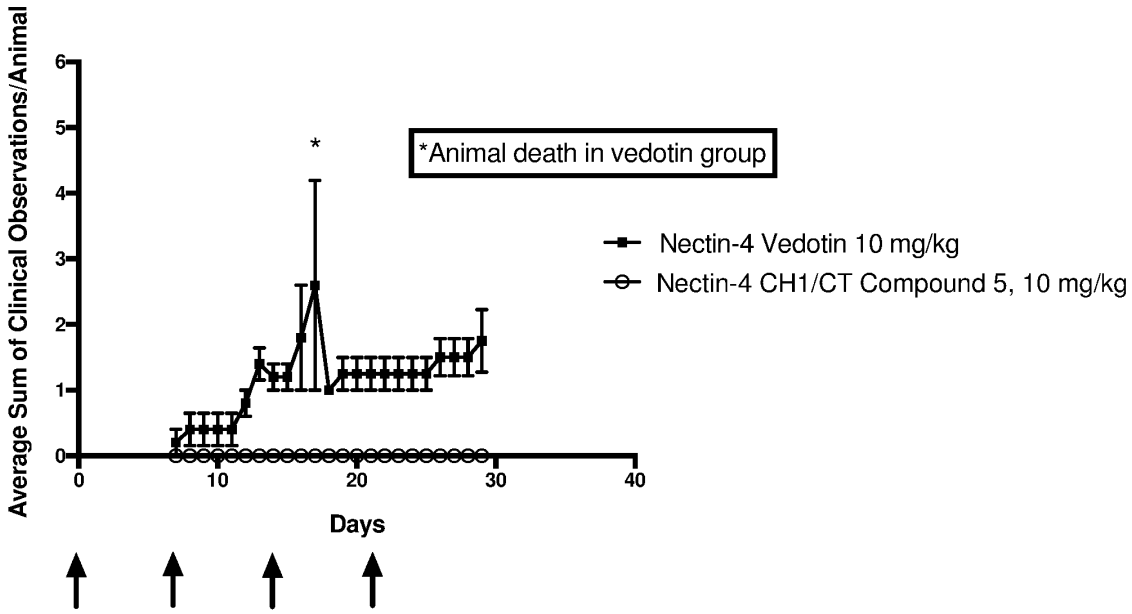

Average Sum of Clinical Observations/Animal
6
5
4
3
2
1
0
*
*Animal death in vedotin group
Nectin-4 Vedotin 10 mg/kg
Nectin-4 CH1/CT Compound 5, 10 mg/kg
0
10
20
30
40
Days

FIG. 15

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. 16A



FIG. 16B

FIG. 16C

Seq1 = *Homo sapiens* kappa light chain constant region; GenBank CAA75031.1
Seq2 = *Homo sapiens* kappa light chain constant region; GenBank BAC01681.1
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. 16D

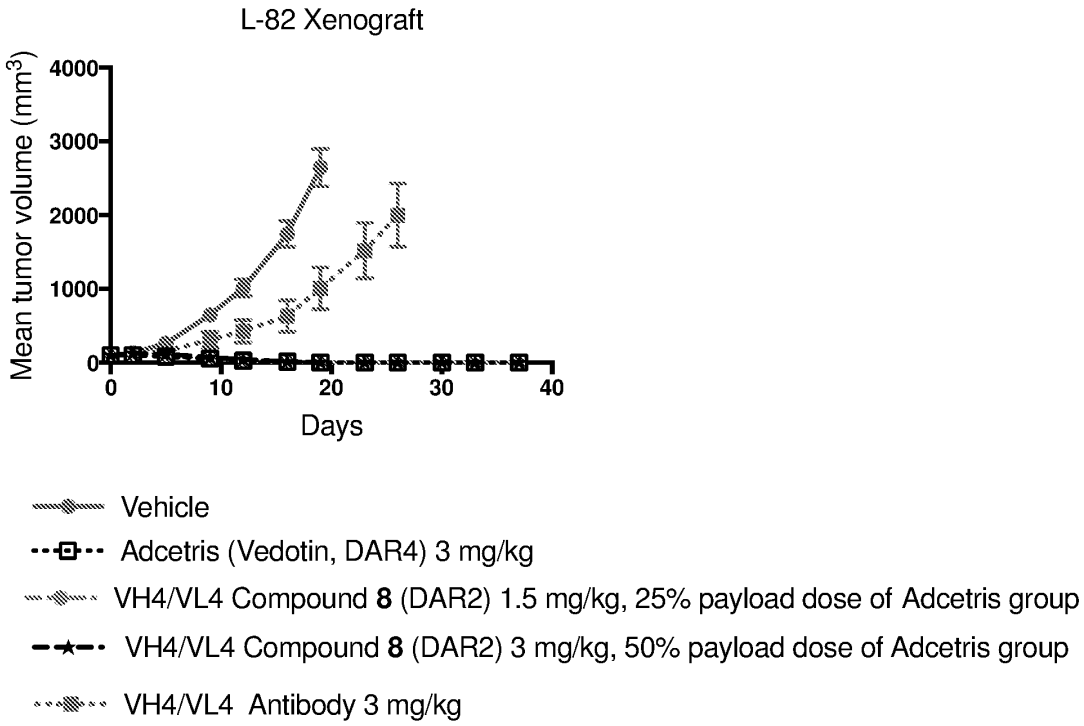
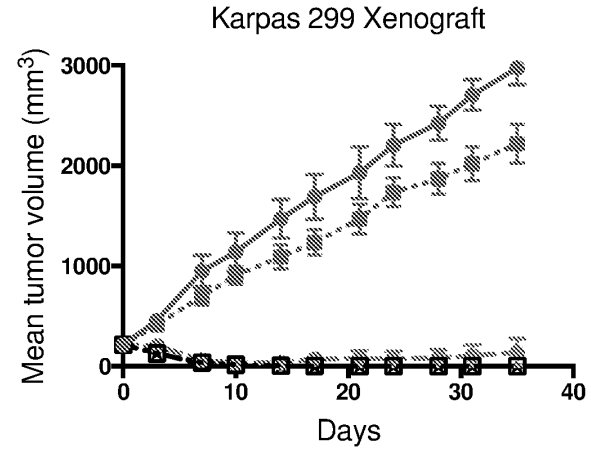

L-82 Xenograft
Mean tumor volume (mm3)
4000
3000
2000
1000
0
0
10
20
30
40
Days
Vehicle
Adcetris (Vedotin, DAR4) 3 mg/kg
VH4/VL4 Compound 8 (DAR2) 1.5 mg/kg, 25% payload dose of Adcetris group
VH4/VL4 Compound 8 (DAR2) 3 mg/kg, 50% payload dose of Adcetris group
VH4/VL4 Antibody 3 mg/kg

FIG. 17



Vehicle

Adcetris (Vedotin, DAR4) 3 mg/kg

VH4/VL4 Compound **8** (DAR2) 1.5 mg/kg, 25% payload dose of Adcetris group

VH4/VL4 Compound **8** (DAR2) 3 mg/kg, 50% payload dose of Adcetris group

VH4/VL4 Antibody 3 mg/kg

FIG. 18

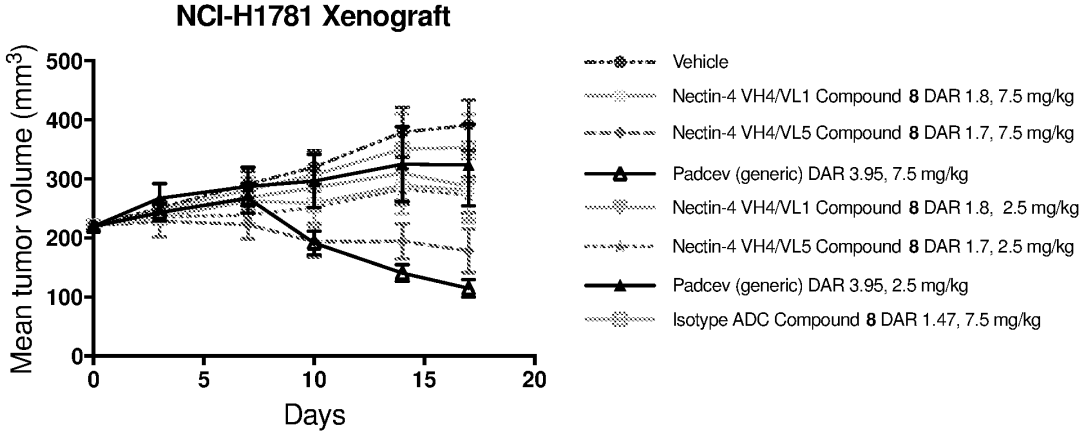
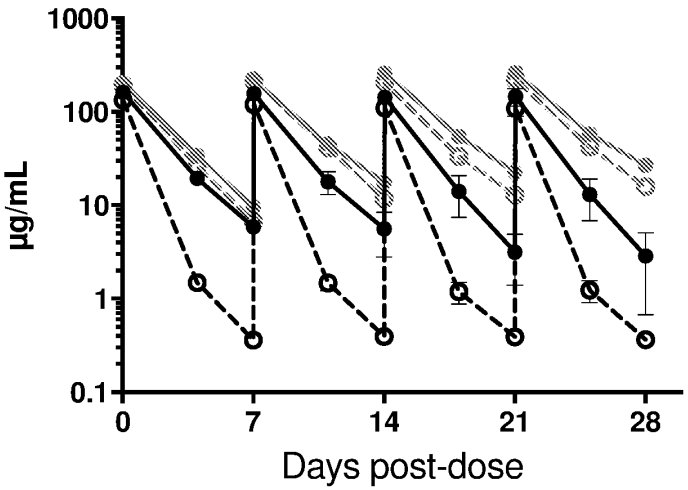


FIG. 19



Enfortumab Vedotin Total Antibody

Enfortumab Vedotin Total ADC

Enfortumab Compound **5** Total Antibody

Enfortumab Compound **5** Total ADC

FIG. 20