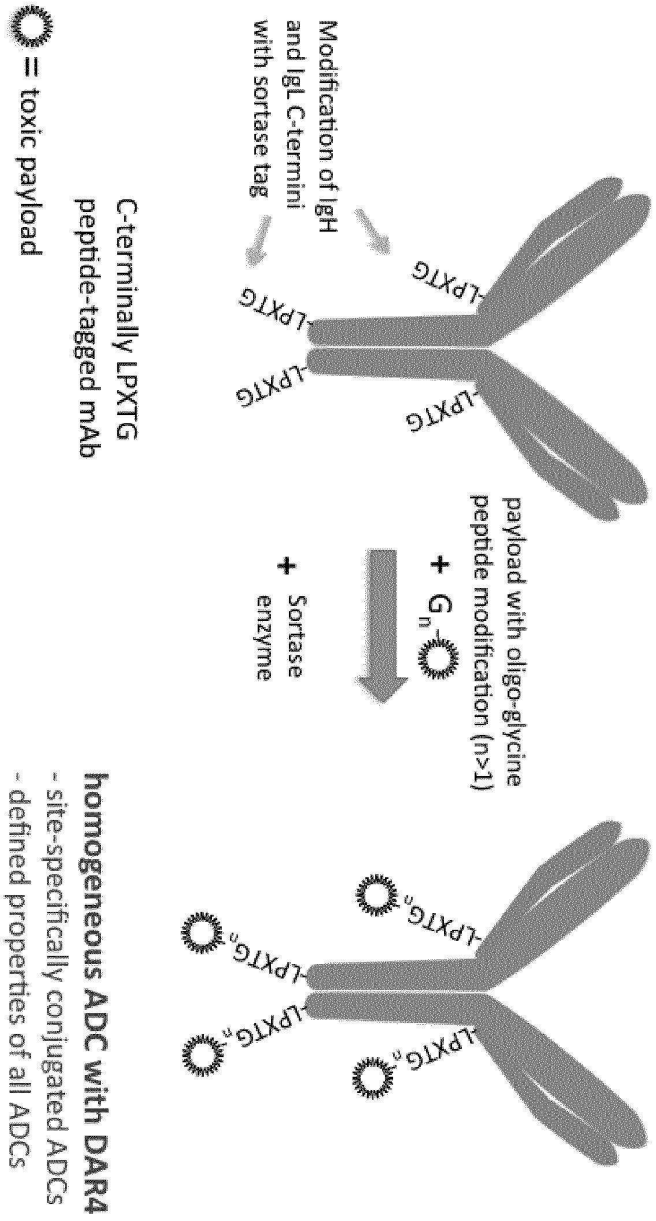
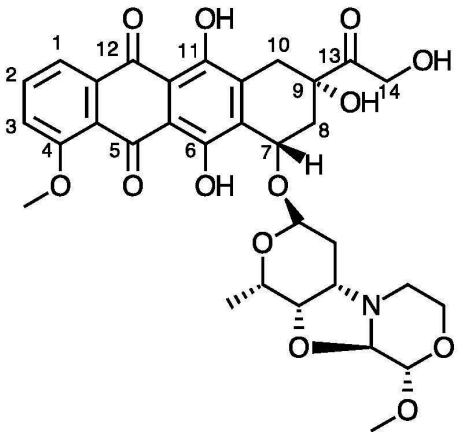
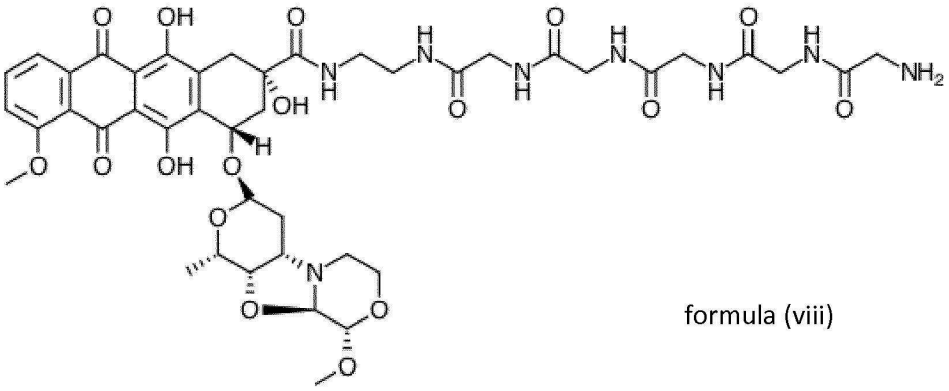

Modification of IgH and IgL C-termini with sortase tag
LPXTG
LPXTG
LPXTG
LPXTG
payload with oligo-glycine peptide modification (n>1)
+ G_n
+ Sortase enzyme
$LPXTG_n$
$LPXTG_n$
$LPXTG_n$
$LPXTG_n$
C-terminally LPXTG peptide-tagged mAb
= toxic payload
homogeneous ADC with DAR4
- site-specifically conjugated ADCs
- defined properties of all ADCs

Fig. 1



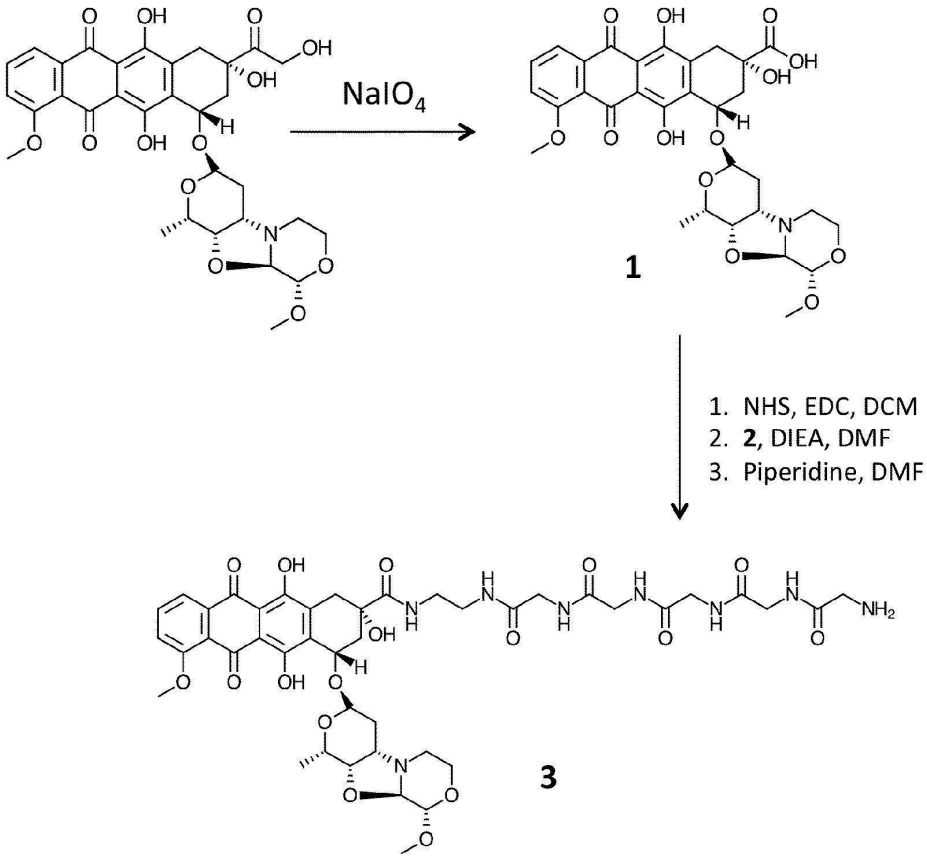
formula (vii)

Fig. 2



formula (viii)

Fig. 3 A



Where **2** corresponds to:

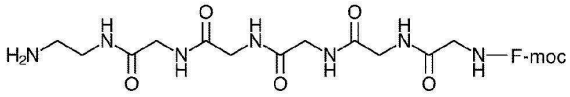


Fig. 3 B

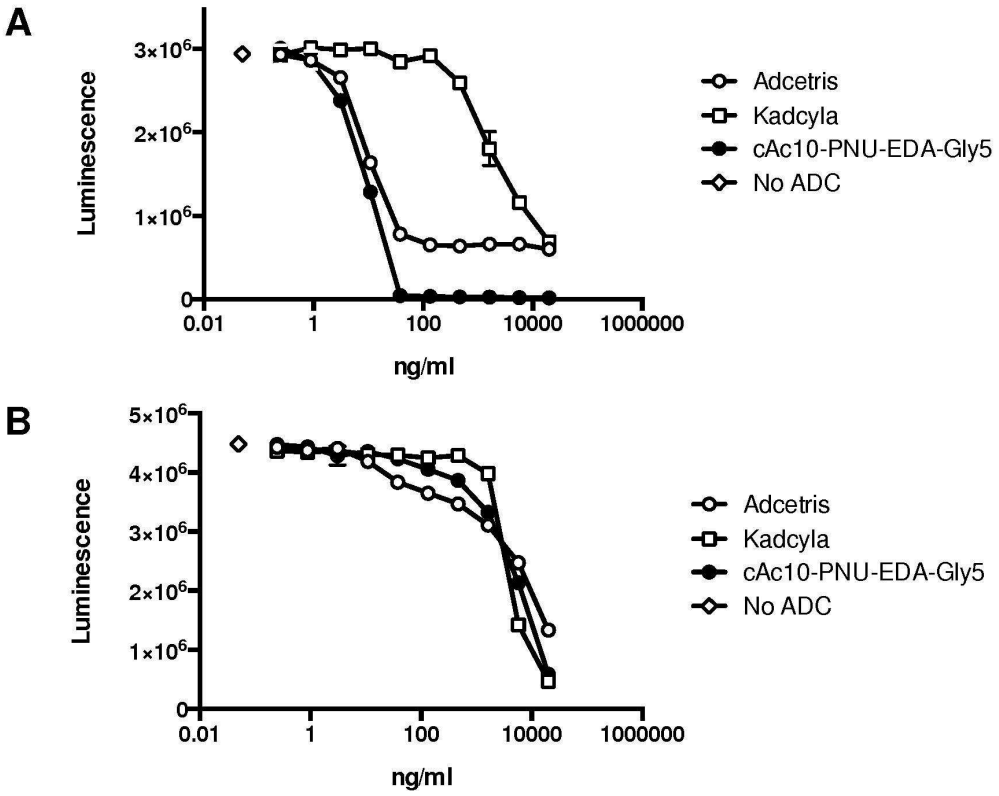

A
Luminescence
3×10^6
2×10^6
1×10^6
0
0.01
1
100
10000
1000000
ng/ml
Adcetris
Kadcyla
cAc10-PNU-EDA-Gly5
No ADC
B
Luminescence
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
0.01
1
100
10000
1000000
ng/ml
Adcetris
Kadcyla
cAc10-PNU-EDA-Gly5
No ADC

Fig. 4

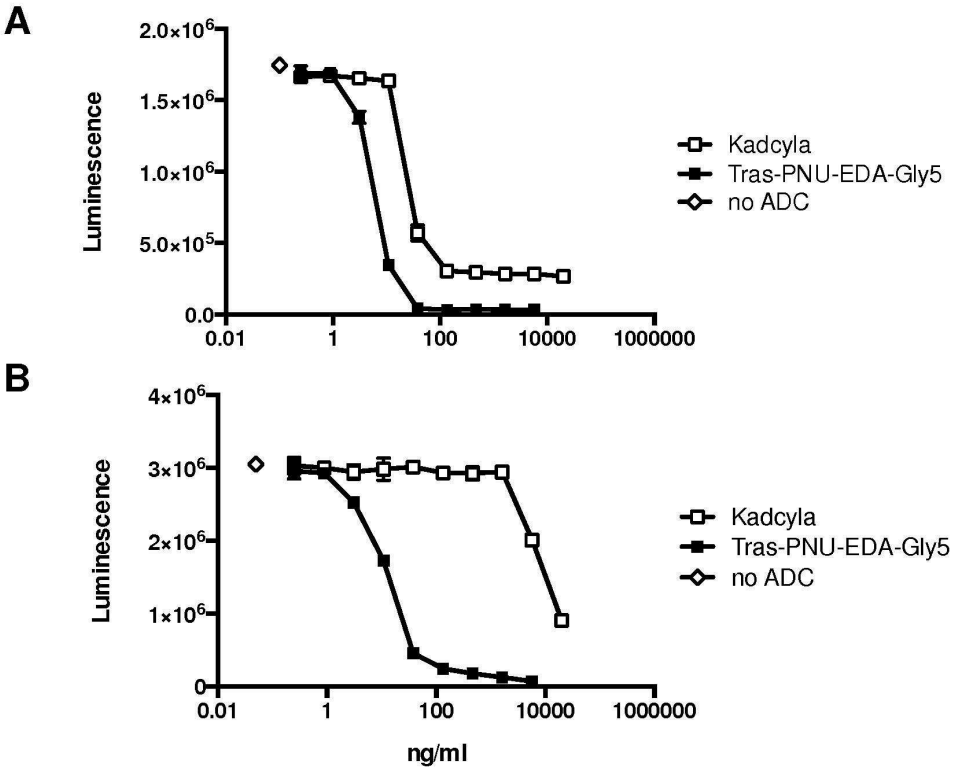

A
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
Luminescence
0.01
1
100
10000
1000000
Kadcyla
Tras-PNU-EDA-Gly5
no ADC
B
4×10^6
3×10^6
2×10^6
1×10^6
0
Luminescence
0.01
1
100
10000
1000000
ng/ml
Kadcyla
Tras-PNU-EDA-Gly5
no ADC

Fig. 5

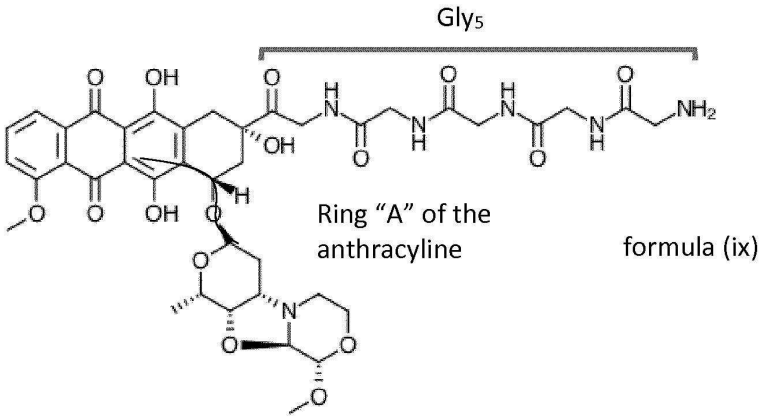


Fig. 6 A: PNU-Gly_5

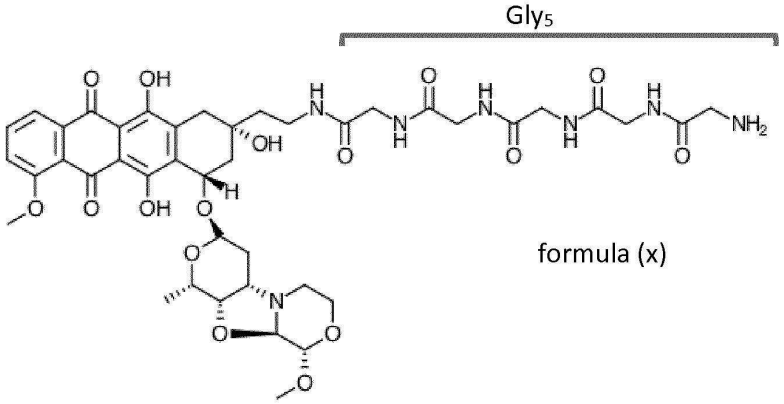


Fig. 6 B: PNU-EA-Gly_5

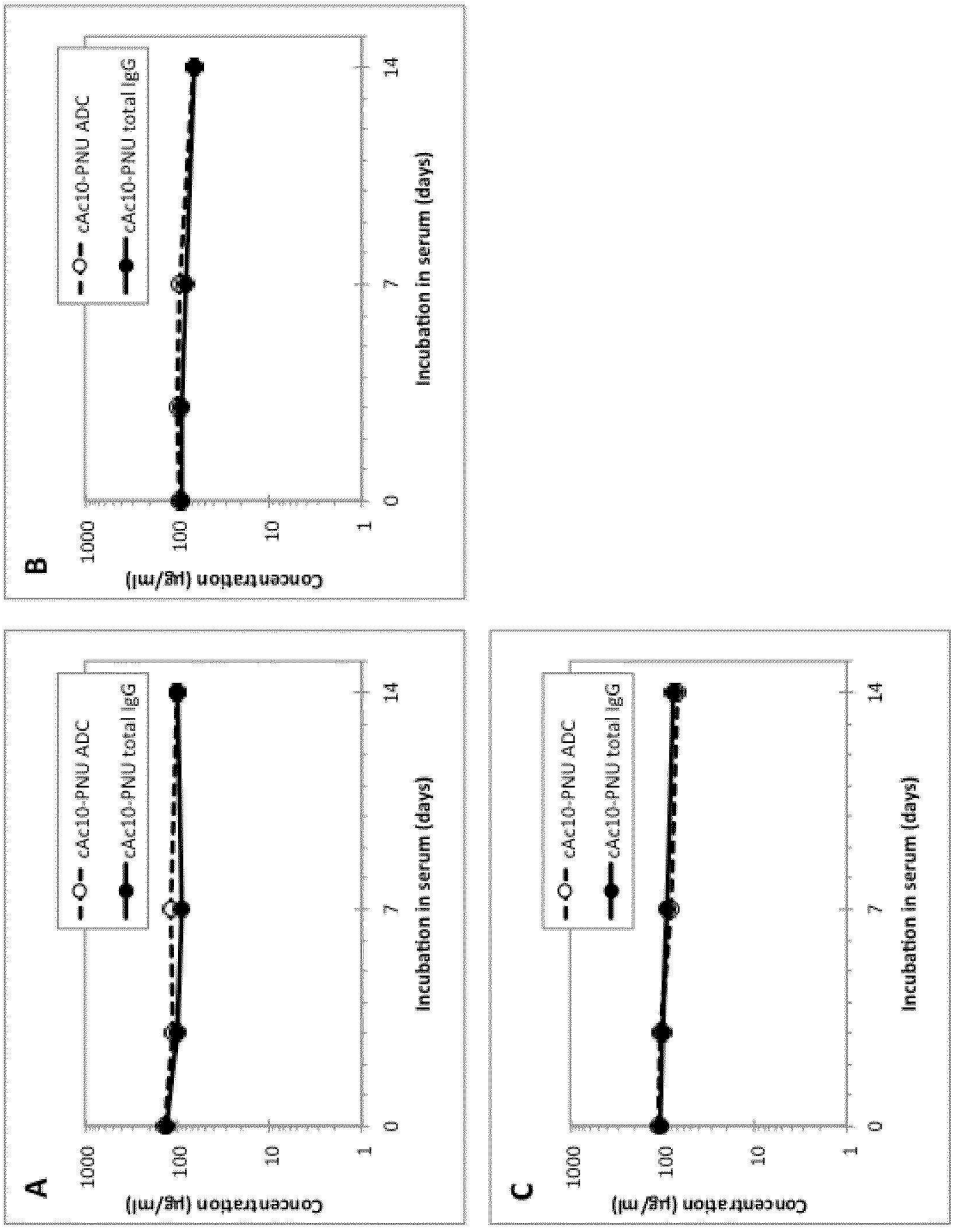

A
B
C
cAc10-PNU ADC
cAc10-PNU total IgG
Concentration (µg/ml)
Incubation in serum (days)
1000
100
10
1
0
7
14

Fig. 7 A

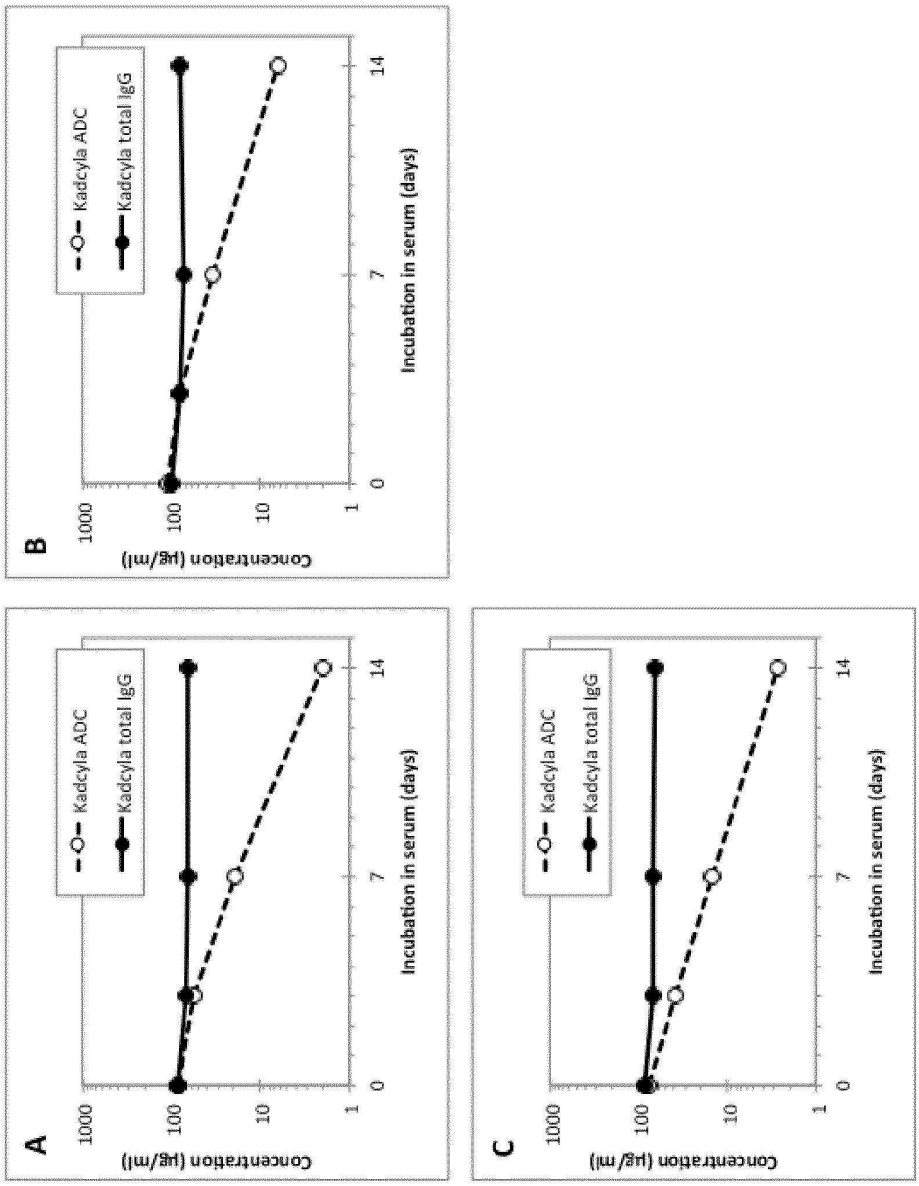

A
B
C
Kadcyla ADC
Kadcyla total IgG
Concentration (µg/ml)
1000
100
10
1
0
7
14
Incubation in serum (days)

Fig. 7 B

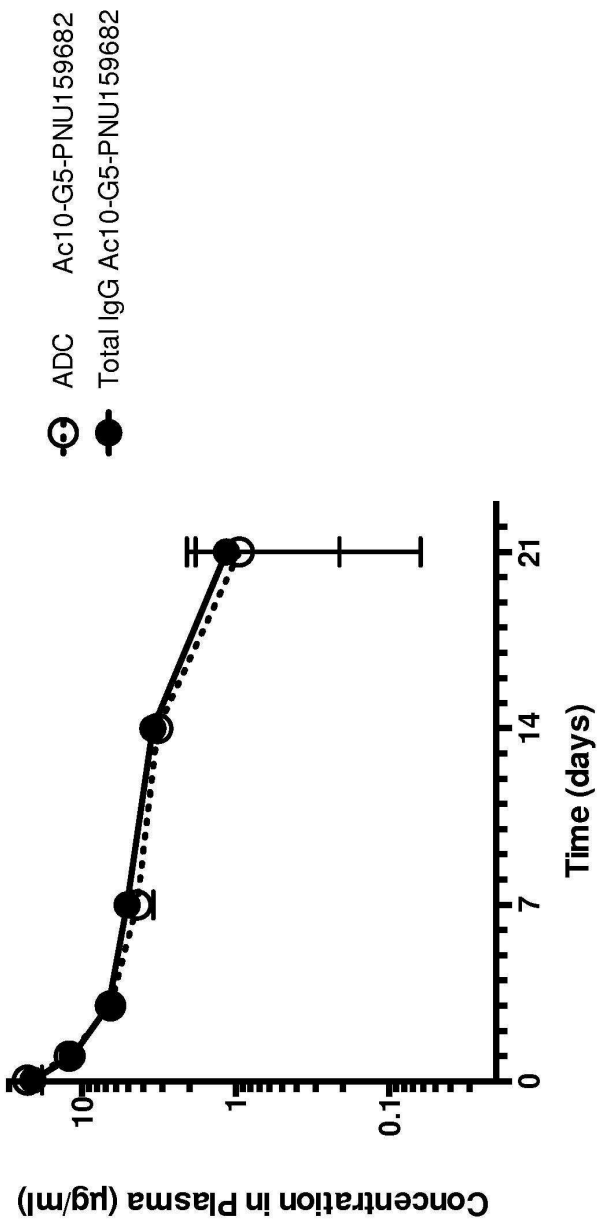

ADC Ac10-G5-PNU159682
Total IgG Ac10-G5-PNU159682
Concentration in Plasma (µg/ml)
10
1
0.1
0
7
14
21
Time (days)

Fig. 8

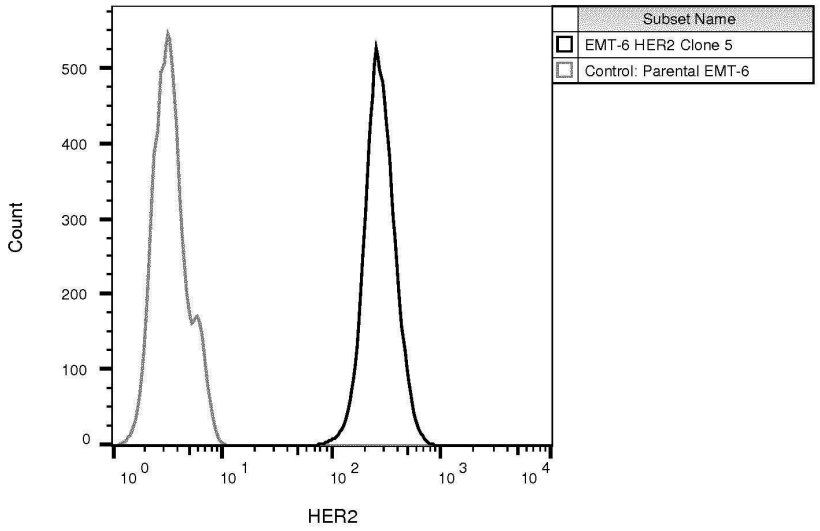

Subset Name
EMT-6 HER2 Clone 5
Control: Parental EMT-6
Count
500
400
300
200
100
0
10 0
10 1
10 2
10 3
10 4
HER2

Fig. 9

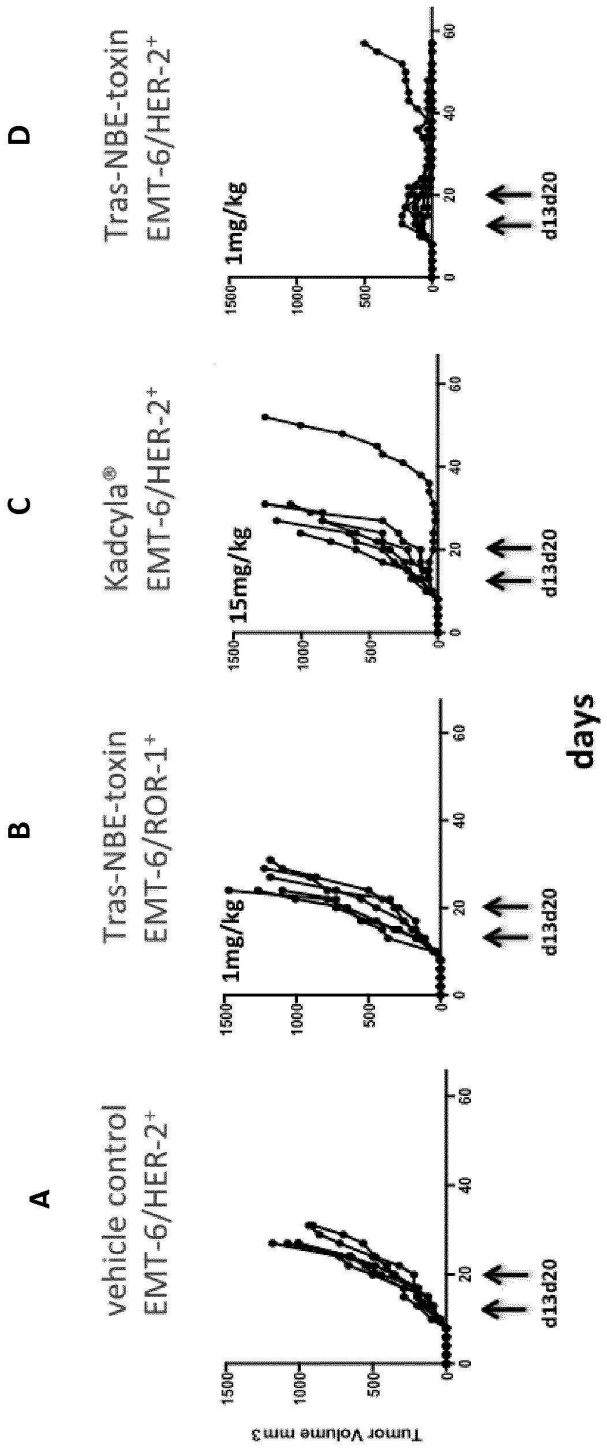

A
vehicle control
EMT-6/HER-2+
B
Tras-NBE-toxin
EMT-6/ROR-1+
1mg/kg
C
Kadcyla®
EMT-6/HER-2+
15mg/kg
D
Tras-NBE-toxin
EMT-6/HER-2+
1mg/kg
d13d20
Tumor Volume mm3
days

Fig. 10

Fig. 11 A:

Trastuzumab-HC-LPETGGGGG-PNU-toxin

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**LPET**GGGGG-PNU-
toxin

Trastuzumab-LC-GGGGS-LPETGGGGG-PNU-toxin

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP
TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC<u>GGGGS**LPET**GGGGG-PNU-toxin</u>

Fig. 11 B

Brentuximab-HC-LPETGGGGG-PNU-toxin

QIQLQQSGPEVVKPGASVKISCKASGYTFTDYYITWVKQKPGQGLEWIGWIYPGSGNTKYNEKFKGKATLTVDTSSSTAFMQLSSLTSEDT
AVYFCANYGNYWFAYWGQGTQVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**LPET**G
GGGG-PNU-toxin

Brentuximab-LC-GGGGS-LPETGGGGG-PNU-toxin

DIVLTQSPASLAVSLGQRATISCKASQSVDFDGDSYMNWYQQKPGQPPKVLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYC
QQSNEDPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**GGGGS**LPETGGGGG-PNU-toxin