FIG. 1A

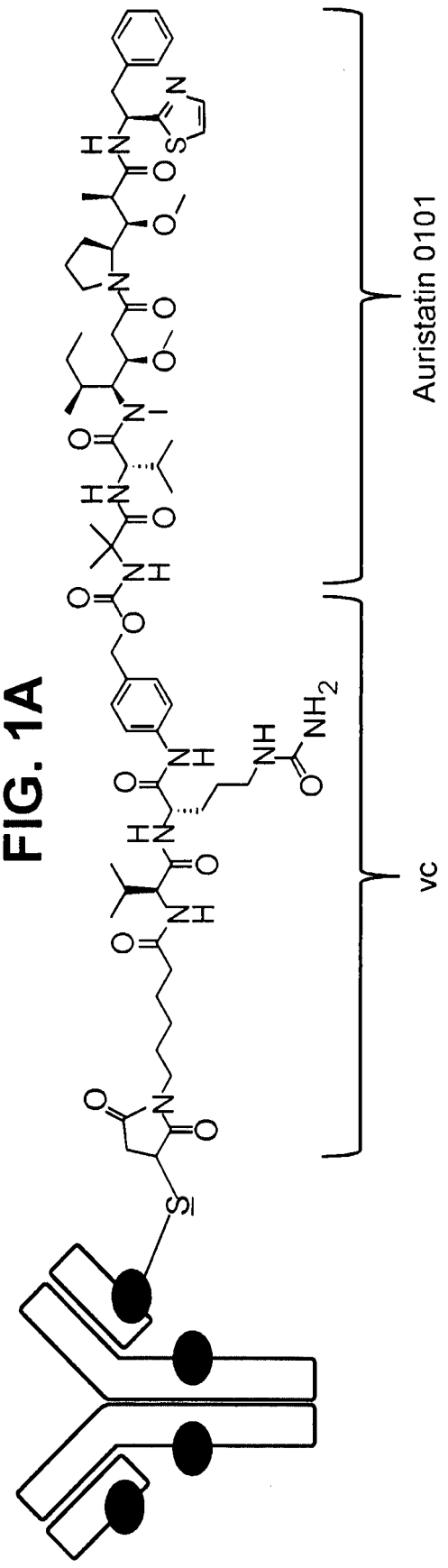


FIG. 1B

FIG. 2A

T(kK183+290)-vc0101

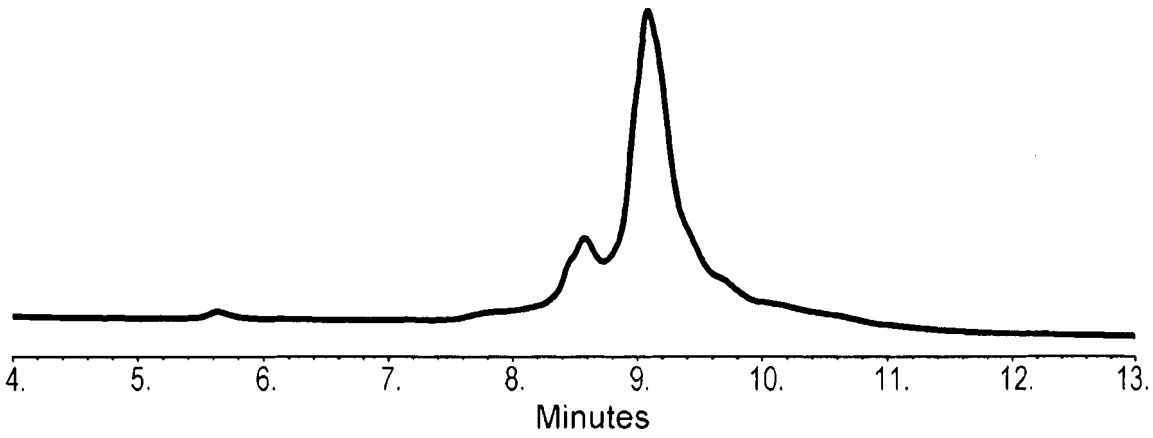


FIG. 2B

T(K334C+K392C)-vc0101

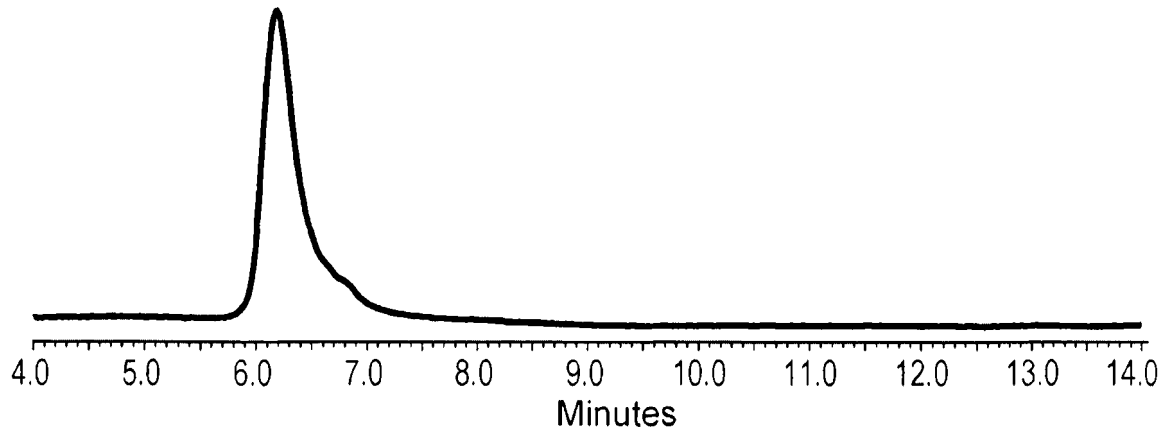


FIG. 2C

T(LCQ05+K222R)-AcLysvc0101

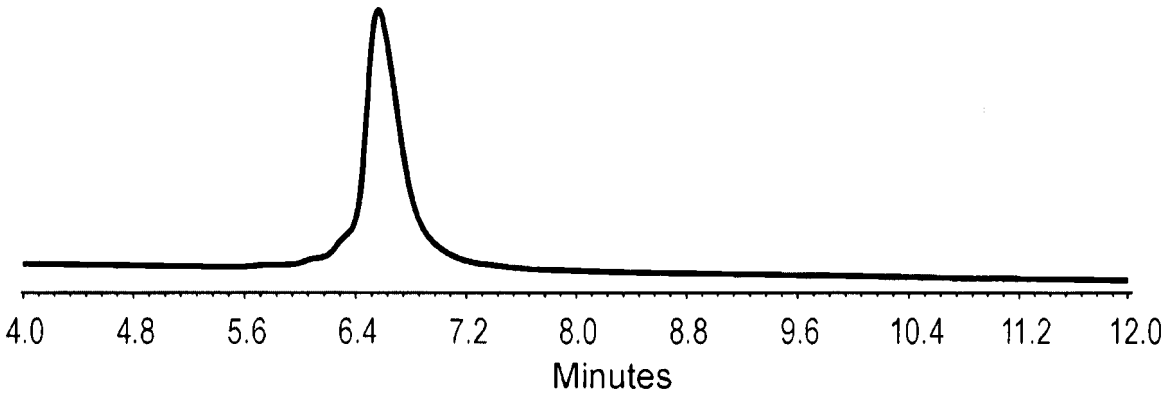


FIG. 2D

T-vc0101

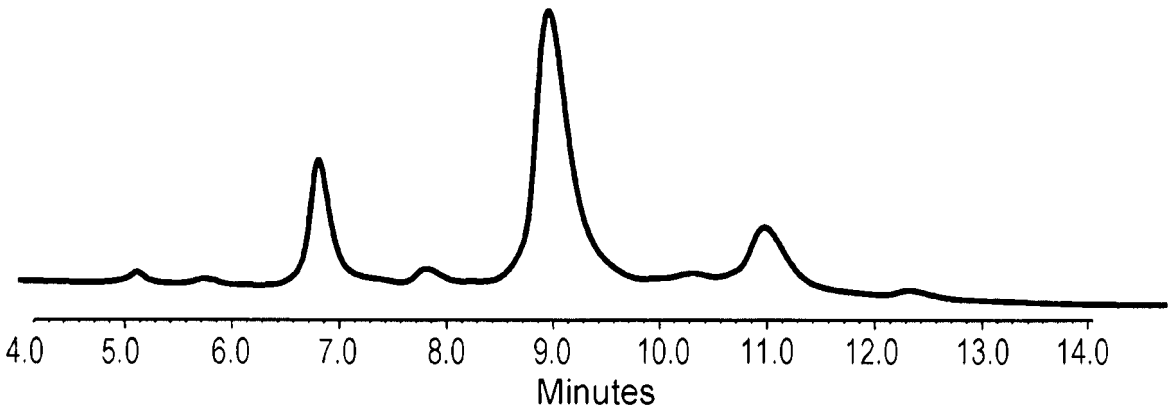


FIG. 2E

T-DM1

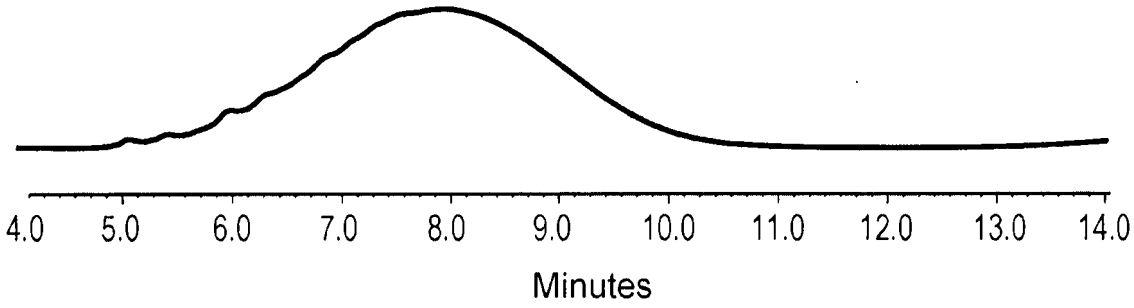


FIG. 3A

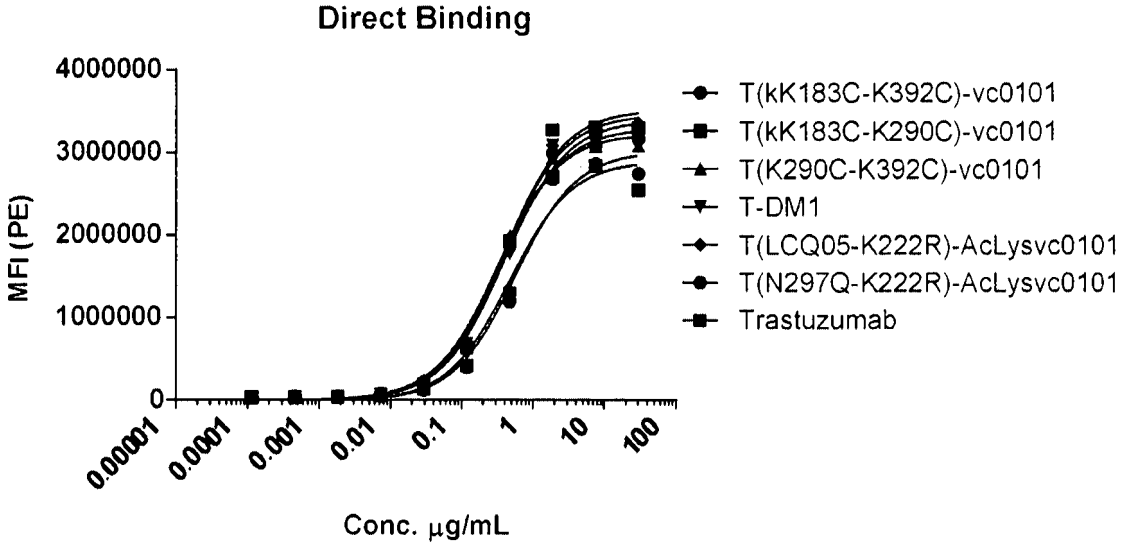

Direct Binding
MFI (PE)
4000000
3000000
2000000
1000000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Conc. µg/mL
T(kK183C-K392C)-vc0101
T(kK183C-K290C)-vc0101
T(K290C-K392C)-vc0101
T-DM1
T(LCQ05-K222R)-AcLysvc0101
T(N297Q-K222R)-AcLysvc0101
Trastuzumab

FIG. 3B

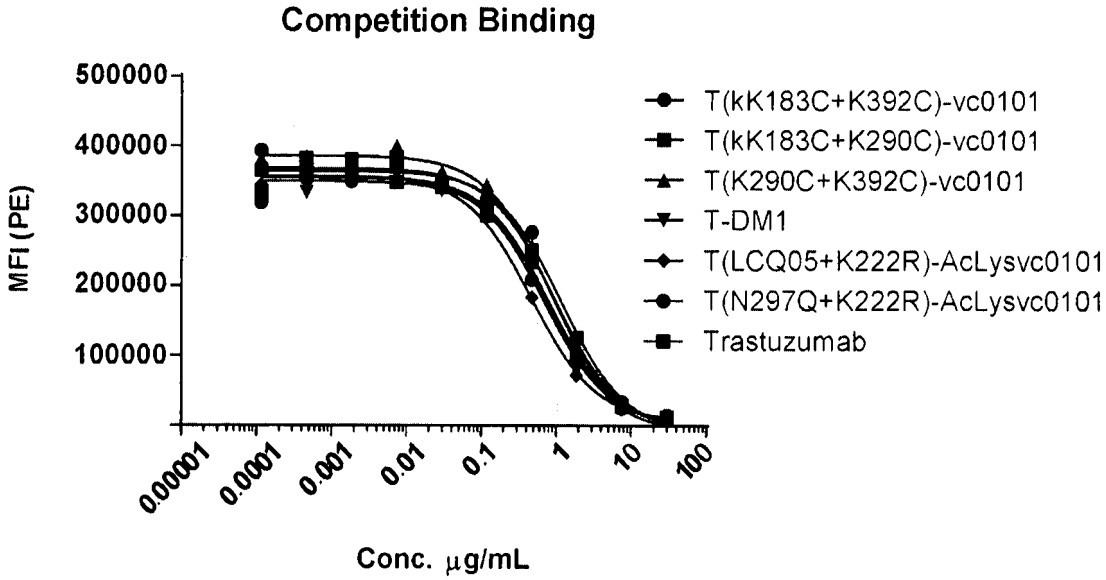

Competition Binding
MFI (PE)
500000
400000
300000
200000
100000
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Conc. µg/mL
T(kK183C+K392C)-vc0101
T(kK183C+K290C)-vc0101
T(K290C+K392C)-vc0101
T-DM1
T(LCQ05+K222R)-AcLysvc0101
T(N297Q+K222R)-AcLysvc0101
Trastuzumab

FIG. 4

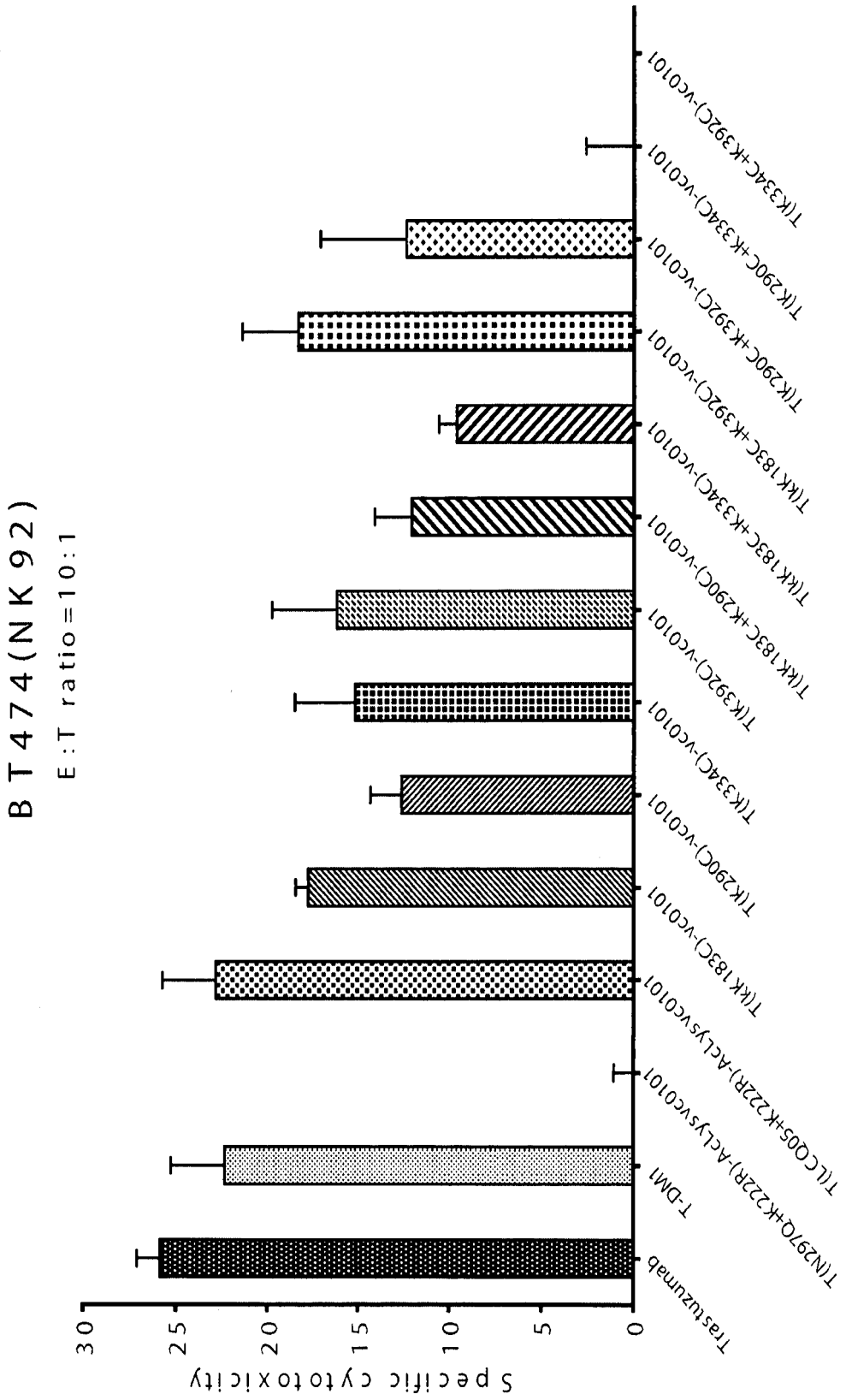

BT474(NK92)
E:T ratio=10:1
Specific cytotoxicity
0
5
10
15
20
25
30
Trastuzumab
T-DM1
T(N297Q+K222R)-AcLysvc0101
T(LCQ05+K222R)-AcLysvc0101
T(K183C)-vc0101
T(K290C)-vc0101
T(K334C)-vc0101
T(K392C)-vc0101
T(K183C+K290C)-vc0101
T(K183C+K334C)-vc0101
T(K183C+K392C)-vc0101
T(K290C+K334C)-vc0101
T(K290C+K392C)-vc0101
T(K334C+K392C)-vc0101

FIG. 5

ADC	Cell Line (with HER2 expression level)									
	3+	3+	3+	3+	2+	2+	2+	1+	-	-
	N87	BT474	HCC 1954	SKBR3	MDA-MB-361-DYT2	JIMT1	MDA-MB-453	MDA-MB-175-VII	MDA-MB-468	HT29
T-DM1	1.66 +/- 1.53 (37)	4.82 +/- 3.74 (26)	0.4 +/- 0.28 (11)	0.16 +/- 0.06 (8)	0.99 +/- 1.33 (37)	29 +/- 12 (16)	1.02 +/- 0.47 (6)	87 +/- 67 (10)	80 +/- 36 (25)	152 +/- 70 (17)
T-vc0101	0.44 +/- 0.15 (79)	0.31 +/- 0.12 (47)	0.27 +/- 0.08 (28)	0.13 +/- 0.04 (4)	0.28 +/- 0.09 (75)	8 +/- 9 (40)	0.66 +/- 0.13 (7)	19 +/- 8 (10)	460 +/- 141 (47)	325 +/- 160 (37)
T(LCQ05+K222R)-AcLysvc0101	0.67 +/- 0.29 (14)	0.25 +/- 0.07 (8)	0.24 +/- 0.1 (2)	0.13 +/- 0.01 (2)	0.97 +/- 1.3 (17)	72 +/- 79 (7)	3.1 +/- 3 (5)	75 +/- 23 (5)	434 +/- 226 (8)	318 +/- 159 (14)
T(N297Q+K222R)-AcLysvc0101	0.49 +/- 0.08 (8)	0.21 +/- 0.06 (4)	0.23 +/- 0.04 (3)	0.13 +/- 0.02 (2)	0.29 +/- 0.08 (6)	19 +/- 14 (3)	0.38 +/- 0.04 (4)	4.9 +/- 1.7 (3)	526 +/- 157 (4)	637 +/- 240 (3)
T(N297A+K222R+LCQ05)-AcLysvc0101	0.51 +/- 0.03 (2)	0.29 +/- 0.08 (3)	0.24 +/- 0.07 (2)	0.15 +/- 0 (2)	0.31 +/- 0.09 (2)	61 +/- 76 (2)	0.42 +/- 0.03 (3)	7.8 +/- 0.2 (2)	493 +/- 94 (3)	827 (1)
T(N297Q)-AcLysvc0101	0.46 +/- 0.07 (4)	0.27 +/- 0.11 (6)	0.25 +/- 0.07 (3)	0.14 +/- 0 (2)	0.23 +/- 0.09 (5)	21 +/- 37 (6)	0.39 +/- 0.04 (4)	9.1 +/- 7.3 (5)	542 +/- 93 (5)	716 +/- 137 (5)
T(kK183C+K290C)-vc0101	0.43 +/- 0.01 (2)	0.37 +/- 0.11 (3)	0.21 +/- 0.03 (2)	0.14 +/- 0.01 (2)	0.33 +/- 0.09 (2)	16 +/- 9 (2)	0.54 +/- 0.1 (3)	9.6 +/- 2.8 (2)	385 +/- 22 (3)	382 +/- 24 (2)
T(kK183C+K334C)-vc0101	0.71 +/- 0.29 (3)	0.29 +/- 0.19 (2)	0.22 (1)	0.15 +/- 0.01 (2)	0.45 +/- 0.21 (3)	18 (1)	0.44 +/- 0.03 (2)	16 (1)	455 +/- 39 (2)	564 +/- 357 (2)

continued

FIG. 5 continued

T(kK183C+K392C)-vc0101	0.57 +/- 0.14 (2)	0.27 +/- 0.09 (2)	0.22 +/- 0.01 (2)	0.15 +/- 0.01 (2)	0.35 +/- 0.03 (2)	8.1 +/- 3.9 (2)	0.41 +/- 0.04 (2)	9.5 +/- 3.4 (2)	483 +/- 168 (2)	528 +/- 245 (2)
T(kK183C+L443C)-vc0101	0.42 +/- 0.22 (4)	0.28 +/- 0.05 (4)	0.37 +/- 0.07 (4)	(0)	0.33 +/- 0.23 (8)	5.2 +/- 2.7 (8)	0.62 (1)	10.7 +/- 1.9 (2)	461 +/- 94 (7)	240 +/- 35 (3)
T(K290C+K334C)-vc0101	0.61 +/- 0.13 (6)	0.34 +/- 0.11 (3)	0.21 +/- 0.01 (2)	0.16 +/- 0.01 (2)	0.32 +/- 0.08 (6)	8.8 +/- 6.8 (2)	0.46 +/- 0.02 (3)	18 (1)	662 +/- 383 (3)	658 +/- 169 (3)
T(K290C+K392C)-vc0101	0.53 +/- 0.01 (2)	0.35 +/- 0.14 (3)	0.22 +/- 0.03 (2)	0.14 +/- 0.03 (2)	0.36 +/- 0.08 (2)	9.7 +/- 4.9 (2)	0.47 +/- 0.08 (3)	9.5 +/- 2.2 (2)	774 +/- 85 (3)	(0)
T(K334C+K392C)-vc0101	0.59 +/- 0.15 (5)	0.36 +/- 0.25 (2)	0.21 +/- 0.02 (2)	0.16 +/- 0.02 (2)	0.25 +/- 0.06 (5)	6.4 +/- 1.4 (2)	0.45 +/- 0.06 (2)	11 +/- 8 (2)	884 (1)	885 +/- 111 (3)
T(kK183C)-vc0101	0.34 +/- 0.12 (4)	0.31 +/- 0.07 (3)	0.29 +/- 0.08 (2)	(0)	0.64 +/- 0.47 (4)	133 +/- 174 (3)	1.26 (1)	115 (1)	414 +/- 226 (3)	459 +/- 114 (3)
T(K290C)-vc0101	0.57 +/- 0.28 (5)	0.28 +/- 0.16 (2)	0.23 (1)	0.14 +/- 0.05 (2)	0.68 +/- 0.58 (6)	235 (1)	1.45 +/- 0.05 (2)	147 (1)	499 +/- 151 (2)	575 +/- 199 (4)
T(K334C)-vc0101	0.47 +/- 0.13 (5)	0.28 +/- 0.16 (2)	0.17 (1)	0.11 +/- 0.02 (2)	3.57 +/- 7.91 (6)	289 (1)	1.14 +/- 0.24 (2)	123 (1)	588 +/- 73 (2)	775 +/- 49 (2)
T(K392C)-vc0101	0.41 +/- 0.09 (5)	0.29 +/- 0.11 (3)	0.19 (1)	0.13 +/- 0.03 (2)	1 +/- 1.43 (5)	172 +/- 180 (2)	1.24 +/- 0.13 (2)	164 (1)	645 +/- 229 (3)	423 +/- 9 (2)
T(L443C)-vc0101	0.4 +/- 0.27 (5)	0.23 +/- 0.08 (3)	0.32 +/- 0.06 (3)	(0)	0.48 +/- 0.28 (5)	41 +/- 34 (3)	4.4 (1)	118 (1)	457 +/- 207 (4)	88 (1)

FIG. 6

ADC	Cell Line (with HER2 expression level)									
	3+	3+	3+	3+	2+	2+	2+	1+	-	-
	N87	BT474	HCC 1954	SKBR 3	MDA-MB-361-DYT2	JIMT1	MDA-MB-453	MDA-MB-175-VII	MDA-MB-468	HT29
T-DM1	62 +/- 54 (37)	190 +/- 143 (26)	16 +/- 12 (11)	6 +/- 2 (8)	40 +/- 59 (37)	1073 +/- 484 (16)	38 +/- 15 (6)	3626 +/- 2750 (10)	3140 +/- 1211 (25)	5488 +/- 2415 (17)
T-vc0101	17 +/- 6 (79)	12 +/- 4 (47)	10 +/- 3 (28)	5 +/- 2 (4)	10 +/- 4 (75)	294 +/- 308 (40)	24 +/- 5 (7)	718 +/- 295 (10)	17141 +/- 5658 (47)	11908 +/- 6136 (37)
T(LCQ05+K222R)-AcLysvc0101	50 +/- 21 (14)	19 +/- 6 (8)	18 +/- 7 (2)	10 +/- 1 (2)	73 +/- 97 (17)	5379 +/- 5938 (7)	233 +/- 225 (5)	5644 +/- 1743 (5)	32560 +/- 16978 (8)	23766 +/- 11898 (14)
T(N297Q+K222R)-AcLysvc0101	18 +/- 3 (8)	8 +/- 2 (4)	9 +/- 2 (3)	5 +/- 1 (2)	11 +/- 3 (6)	700 +/- 515 (3)	14 +/- 2 (4)	185 +/- 63 (3)	19737 +/- 5885 (4)	23886 +/- 8990 (3)
T(N297A+K222R +LCQ05)-AcLysvc0101	19 +/- 1 (2)	11 +/- 3 (3)	9 +/- 3 (2)	6 +/- 0 (2)	12 +/- 4 (2)	2280 +/- 2851 (2)	16 +/- 1 (3)	291 +/- 9 (2)	18489 +/- 3511 (3)	31002 (1)
T(N297Q)-AcLysvc0101	17 +/- 3 (4)	10 +/- 4 (6)	9 +/- 2 (3)	5 +/- 0 (2)	9 +/- 3 (5)	770 +/- 1362 (6)	15 +/- 1 (4)	333 +/- 262 (5)	20532 +/- 3846 (5)	26609 +/- 5265 (5)
T(kK183C+K290C) -vc0101	16 +/- 0 (2)	14 +/- 4 (3)	8 +/- 1 (2)	5 +/- 0 (2)	12 +/- 3 (2)	603 +/- 327 (2)	20 +/- 4 (3)	351 +/- 94 (2)	14134 +/- 1027 (3)	14305 +/- 912 (2)
T(kK183C+K334C) -vc0101	29 +/- 11 (3)	12 +/- 8 (2)	9 (1)	6 +/- 0 (2)	18 +/- 8 (3)	717 (1)	18 +/- 1 (2)	660 (1)	18375 +/- 1564 (2)	23284 +/- 15156 (2)
T(kK183C+K392C) -vc0101	20 +/- 5 (2)	9 +/- 3 (2)	8 +/- 0 (2)	5 +/- 0 (2)	12 +/- 1 (2)	280 +/- 133 (2)	14 +/- 1 (2)	328 +/- 118 (2)	16685 +/- 5787 (2)	18645 +/- 9043 (2)
T(kK183C+L443C) -vc0101	16 +/- 8 (4)	11 +/- 2 (4)	15 +/- 3 (4)	(0)	13 +/- 9 (8)	204 +/- 109 (8)	23 (1)	255 +/- 128 (2)	18005 +/- 3005 (7)	8900 +/- 1269 (3)

continued

FIG. 6 continued

T(K290C+K334C)-vc0101	22 +/- 5 (6)	13 +/- 4 (3)	8 +/- 1 (2)	6 +/- 0 (2)	12 +/- 3 (6)	317 +/- 246 (2)	17 +/- 1 (3)	640 (1)	24104 +/- 13727 (3)	23859 +/- 6116 (3)
T(K290C+K392C)-vc0101	20 +/- 0 (2)	13 +/- 5 (3)	8 +/- 1 (2)	5 +/- 1 (2)	13 +/- 3 (2)	354 +/- 178 (2)	17 +/- 3 (3)	345 +/- 78 (2)	28372 +/- 2664 (3)	(0)
T(K334C+K392C)-vc0101	22 +/- 5 (5)	13 +/- 9 (2)	8 +/- 1 (2)	6 +/- 1 (2)	9 +/- 2 (5)	231 +/- 51 (2)	16 +/- 2 (2)	417 +/- 274 (2)	32063 (1)	32519 +/- 4670 (3)
T(kK183C)-vc0101	26 +/- 8 (4)	23 +/- 5 (3)	22 +/- 7 (2)	(0)	49 +/- 37 (4)	9743 +/- 12536 (3)	91 (1)	8364 (1)	31205 +/- 16416 (3)	34516 +/- 7772 (3)
T(K290C)-vc0101	47 +/- 18 (5)	20 +/- 12 (2)	17 (1)	10 +/- 4 (2)	71 +/- 94 (6)	17052 (1)	105 +/- 4 (2)	10682 (1)	36261 +/- 10973 (2)	42511 +/- 15090 (4)
T(K334C)-vc0101	41 +/- 5 (5)	20 +/- 12 (2)	13 (1)	8 +/- 1 (2)	552 +/- 1290 (6)	21025 (1)	83 +/- 17 (2)	8956 (1)	42743 +/- 5313 (2)	57272 +/- 4844 (2)
T(K392C)-vc0101	36 +/- 9 (5)	21 +/- 9 (3)	14 (1)	10 +/- 2 (2)	129 +/- 226 (5)	12847 +/- 13493 (2)	93 +/- 9 (2)	12330 (1)	48108 +/- 16750 (3)	31531 +/- 931 (2)
T(L443C)-vc0101	30 +/- 20 (5)	17 +/- 6 (3)	24 +/- 5 (3)	(0)	36 +/- 21 (5)	3015 +/- 2485 (3)	326 (1)	8744 (1)	34157 +/- 15675 (4)	6520 (1)

FIG. 7A

T(kK183C+K290C)-vc0101

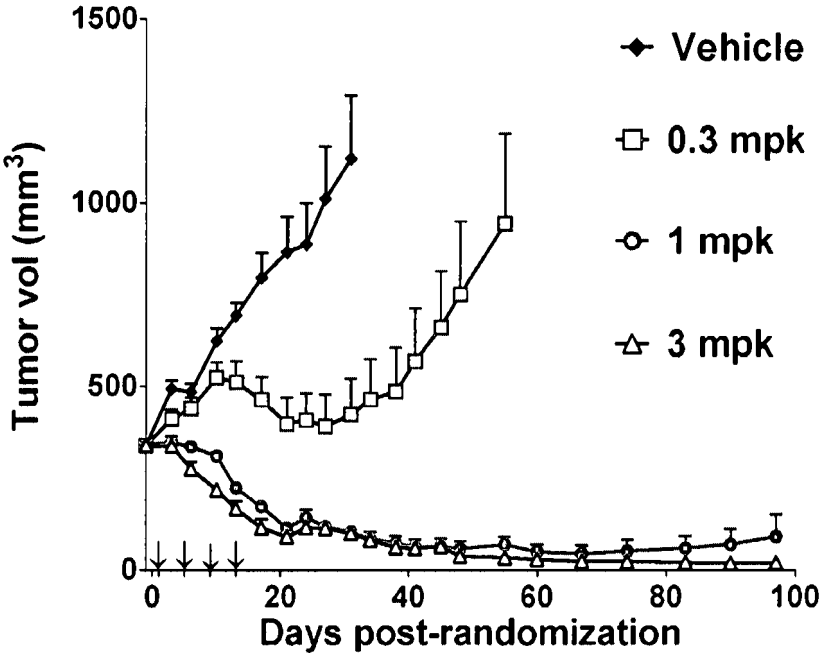


FIG. 7B

T(kK183C)-vc0101

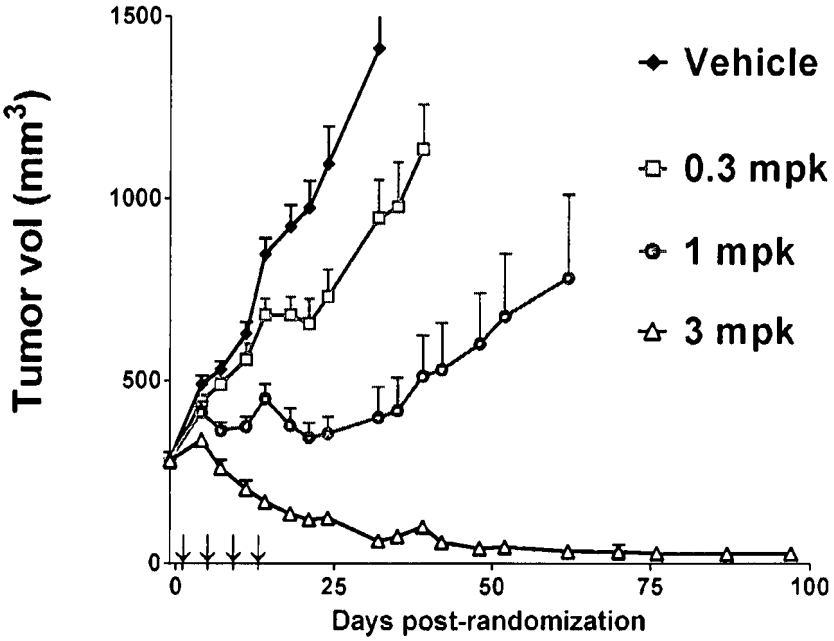


FIG. 7C

T(K290C)-vc0101

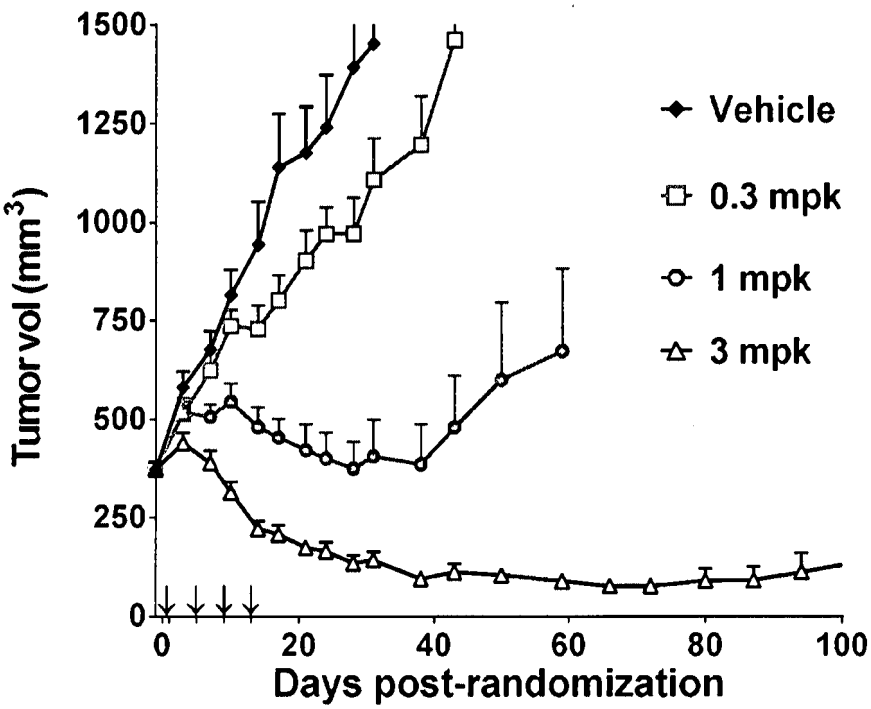


FIG. 7D

T(LCQ05+K222R)-AcLysvc0101

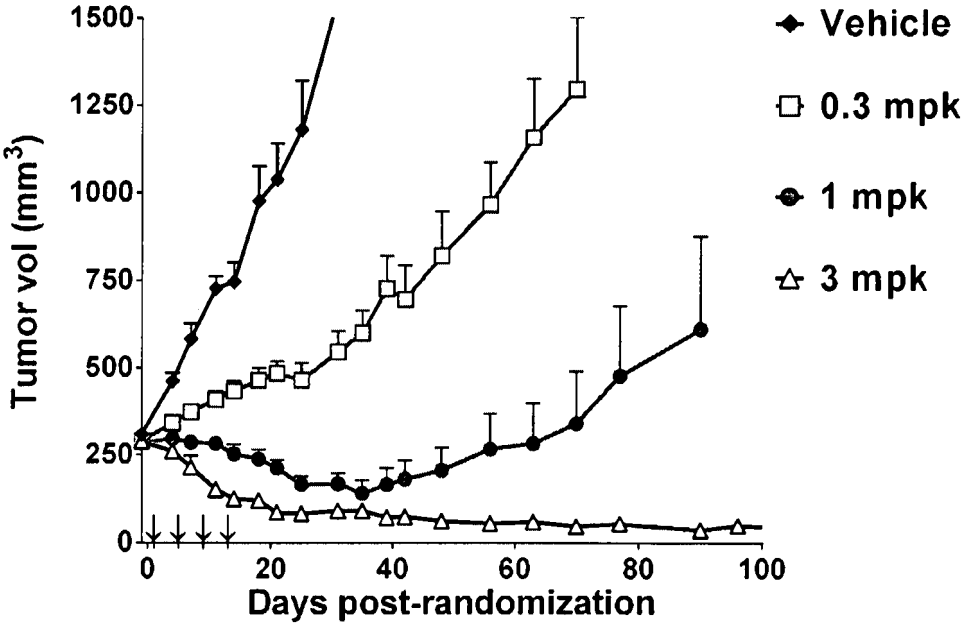


FIG. 7E

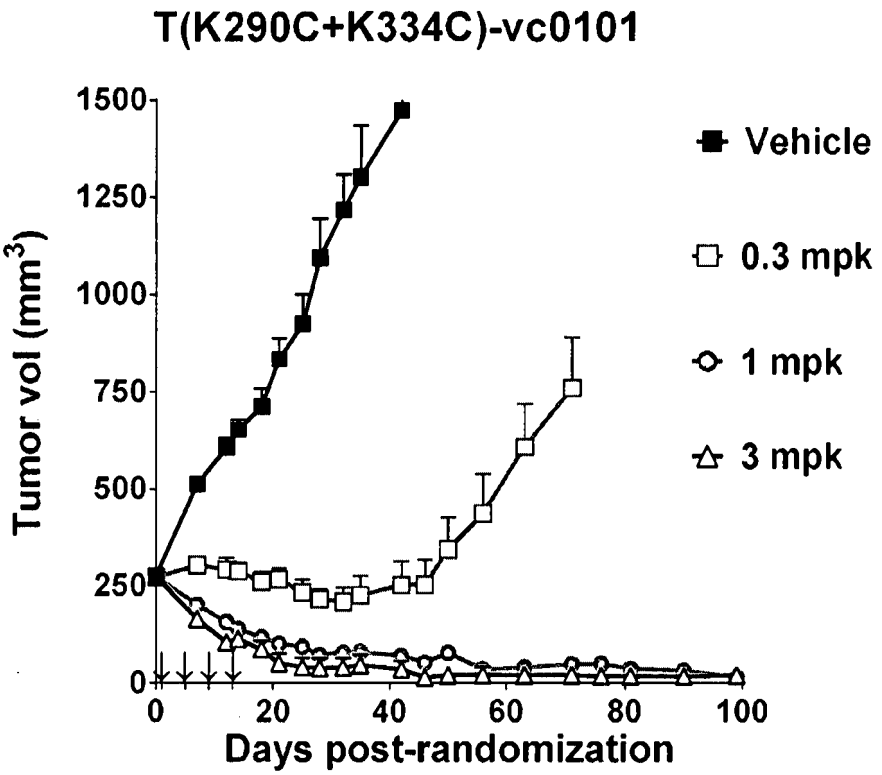

T(K290C+K334C)-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 7F

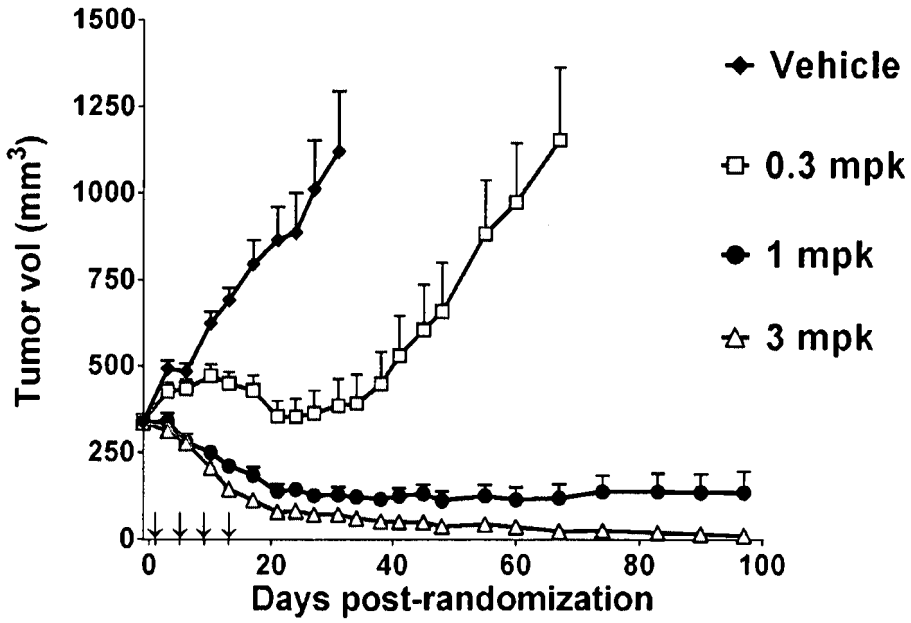

T(K334C+K392C)-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 7G

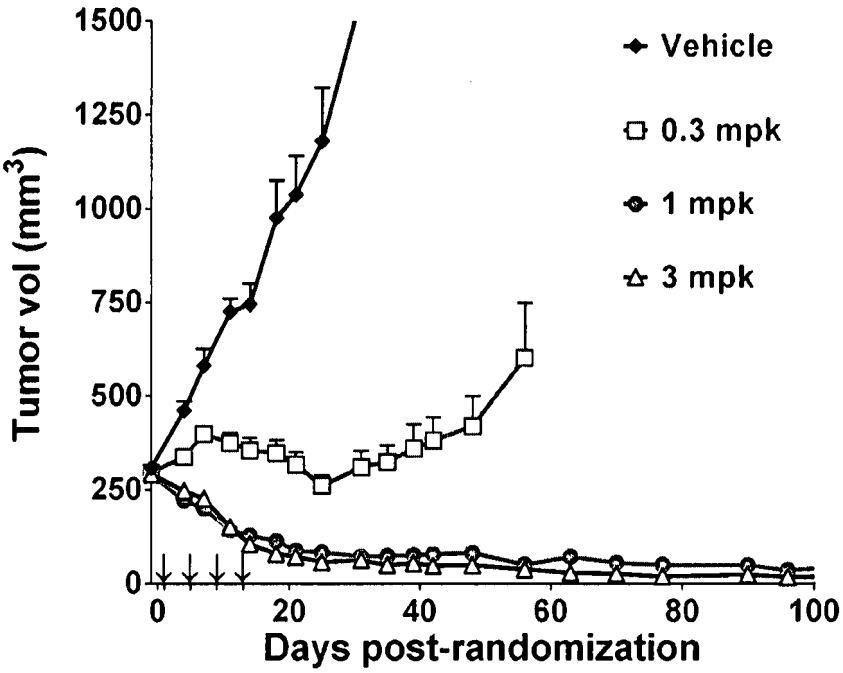

T(N297Q+K222R)-AcLysvc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 7H

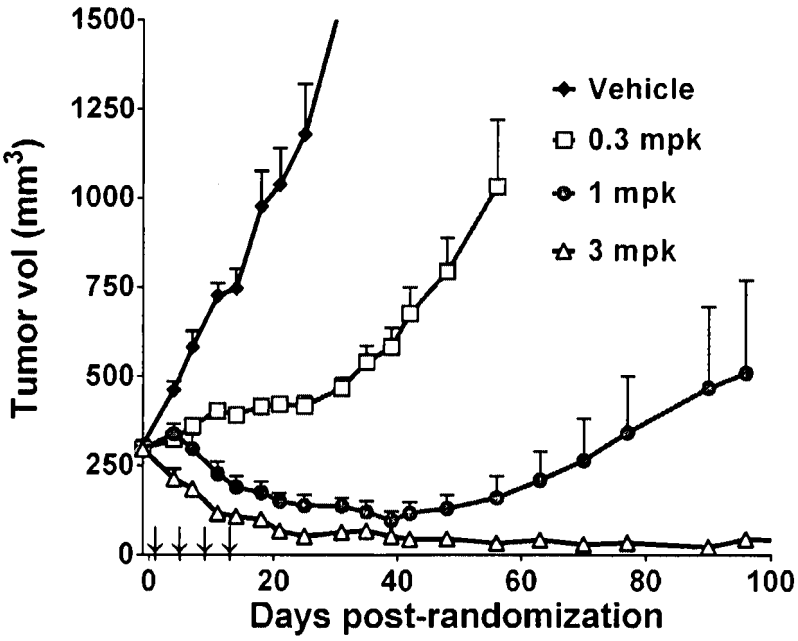

T-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 7I

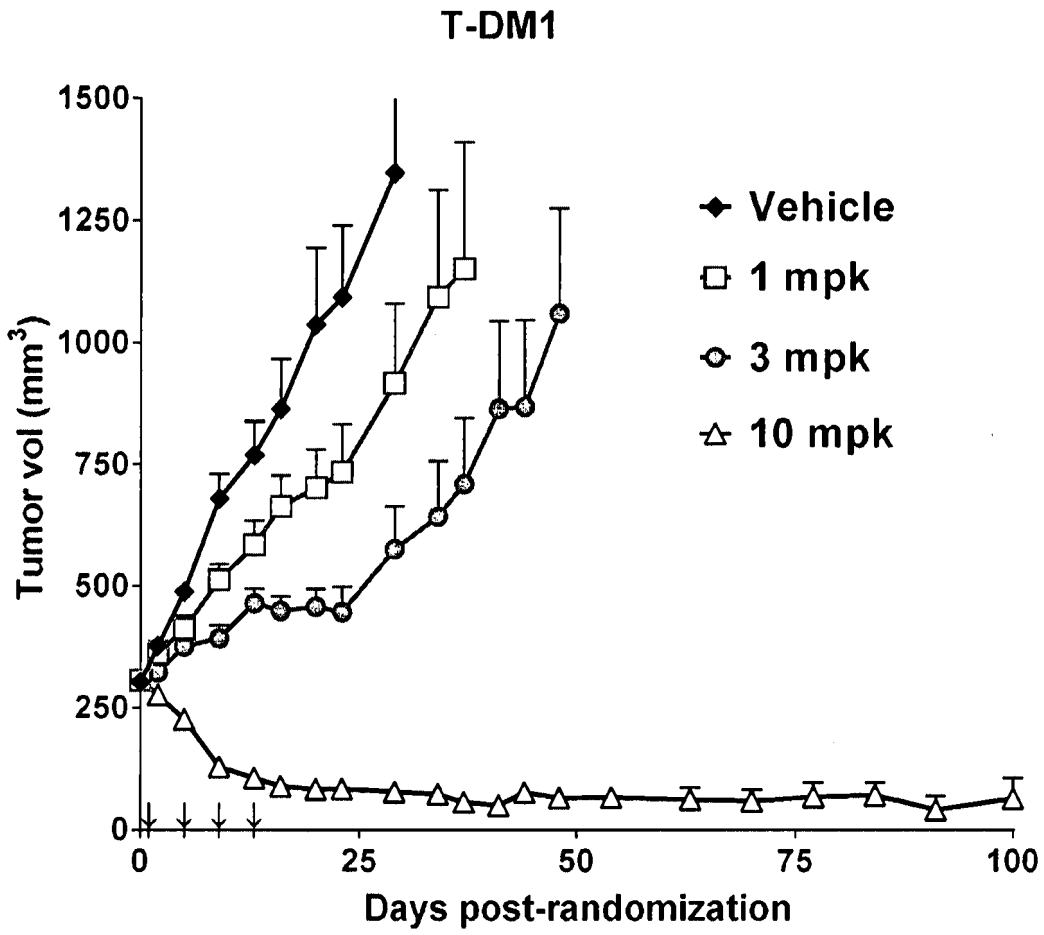

T-DM1
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
1 mpk
3 mpk
10 mpk

FIG. 8A

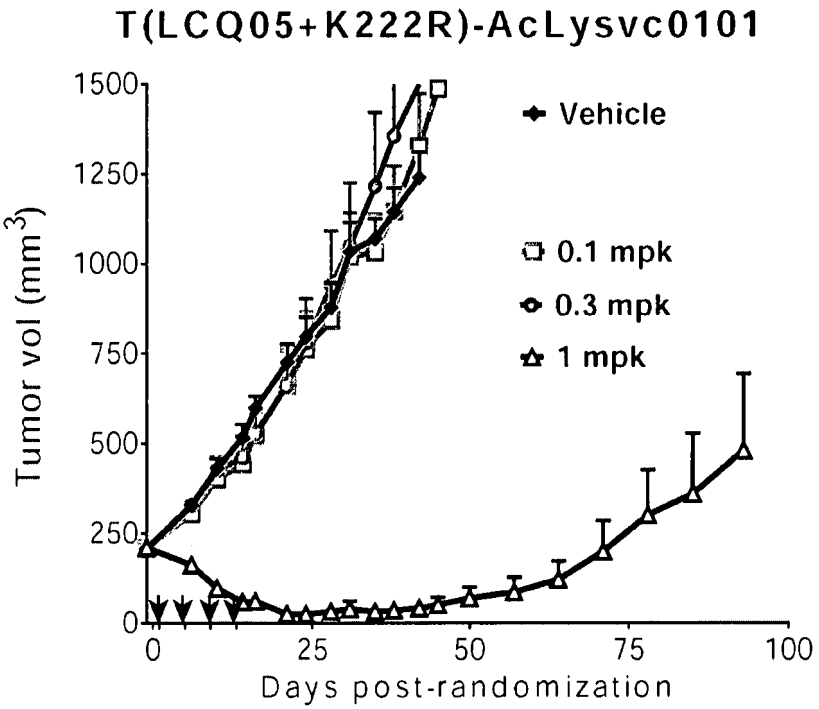

T(LCQ05+K222R)-AcLysvc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
0.1 mpk
0.3 mpk
1 mpk

FIG. 8B

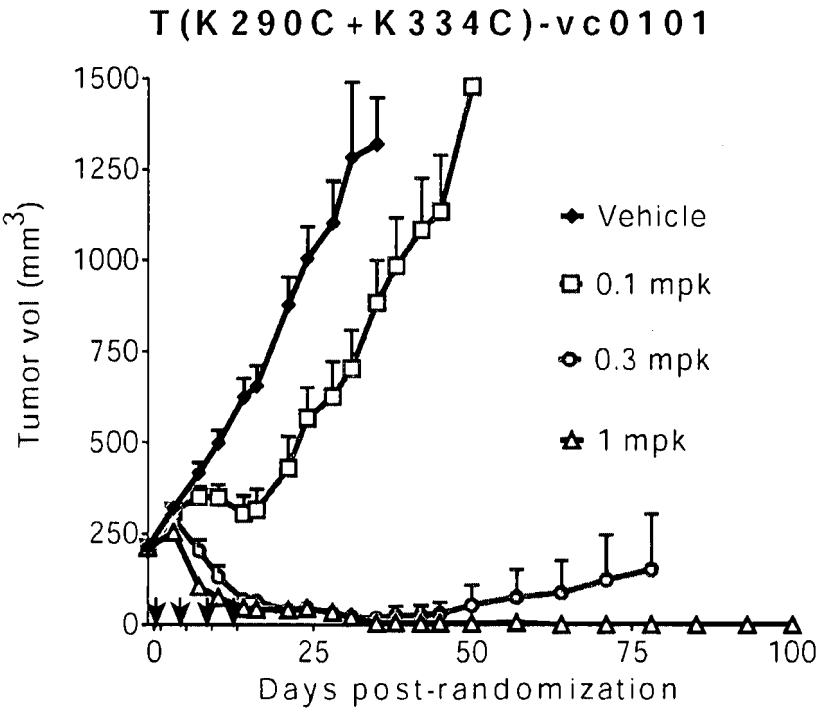

T(K290C+K334C)-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
0.1 mpk
0.3 mpk
1 mpk

FIG. 8C

T(K334C+K392C)-vc0101

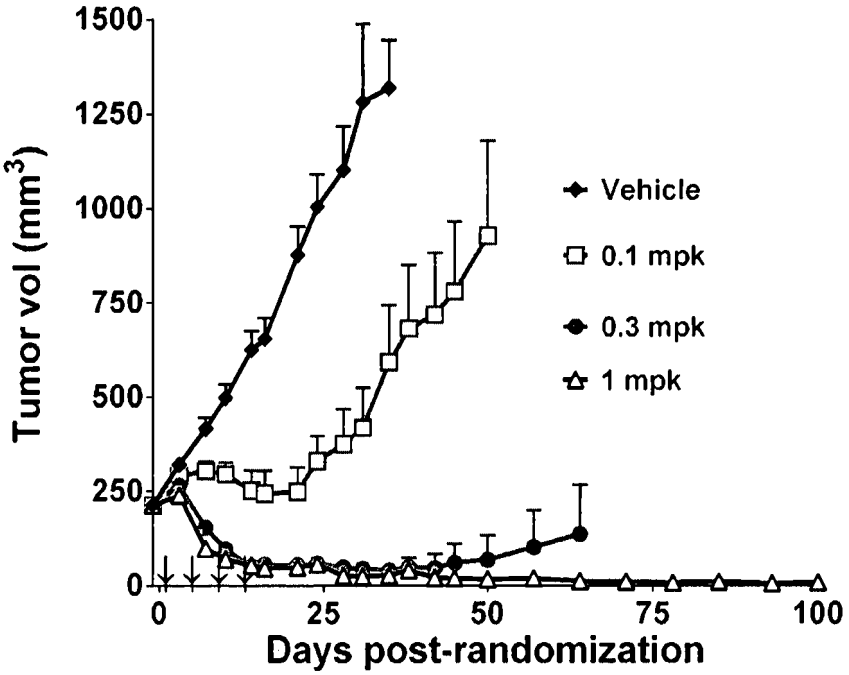


FIG. 8D

T(N297Q+K222R)-AcLysvc0101

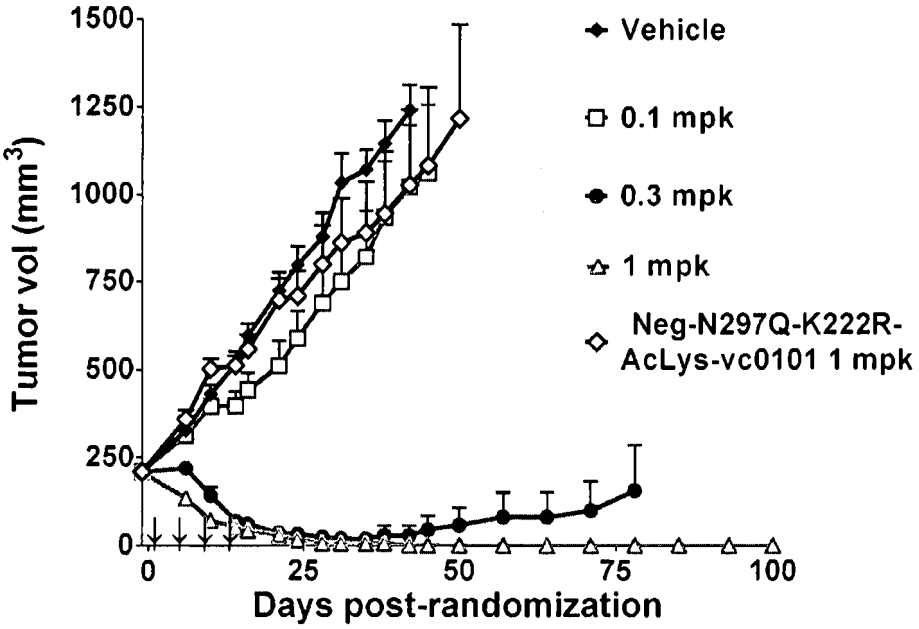


FIG. 8E

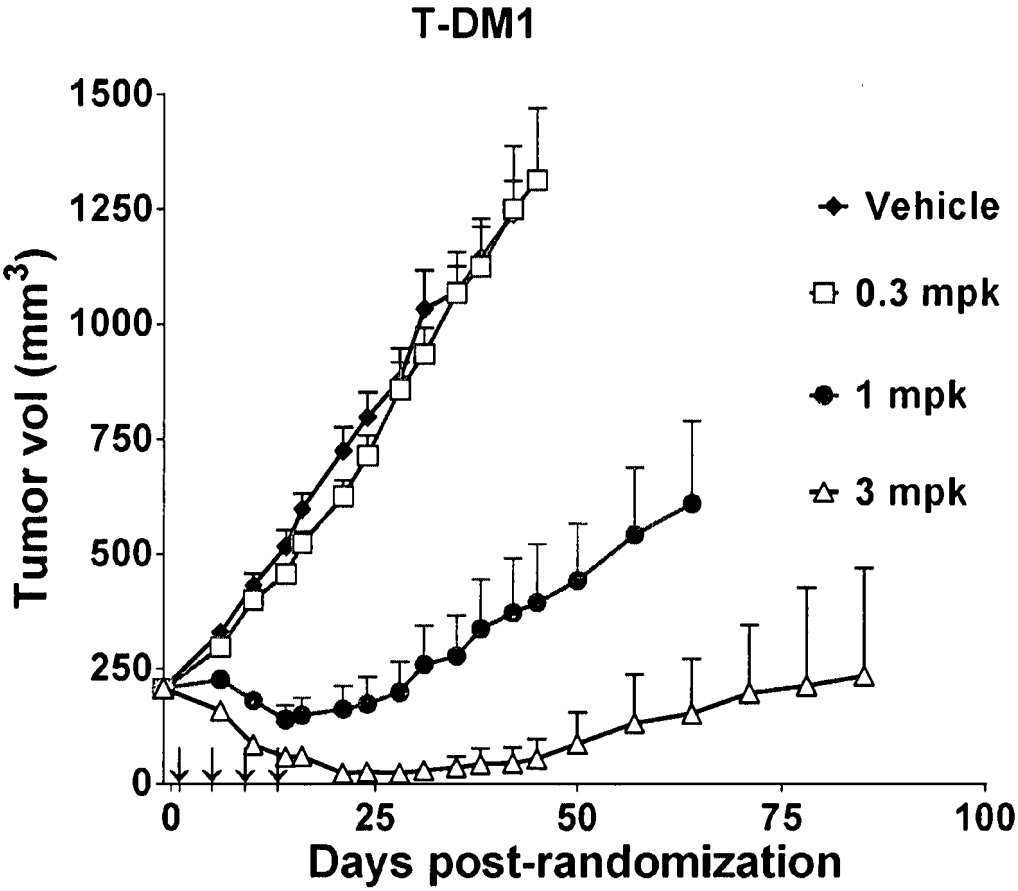

T-DM1
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 9A

T(kK183C+K290C)-vc0101

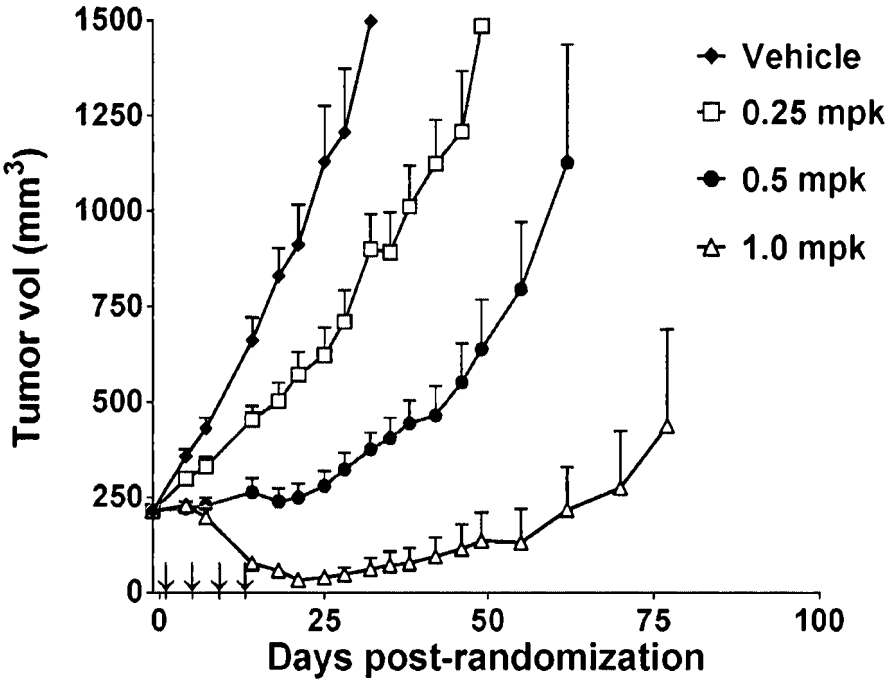


FIG. 9B

T(LCQ05+K222R)-AcLysvc0101

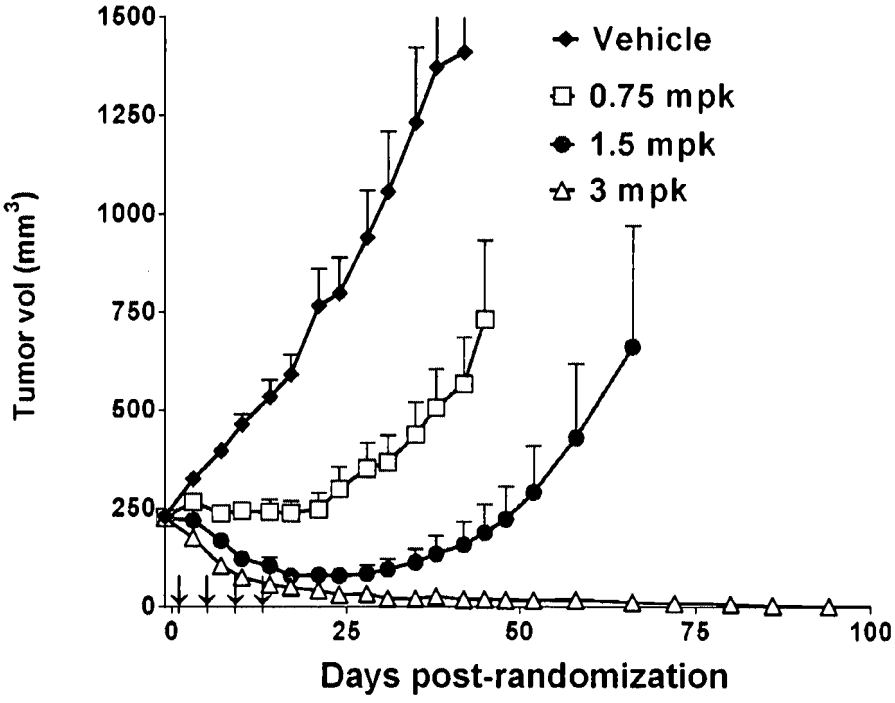


FIG. 9C

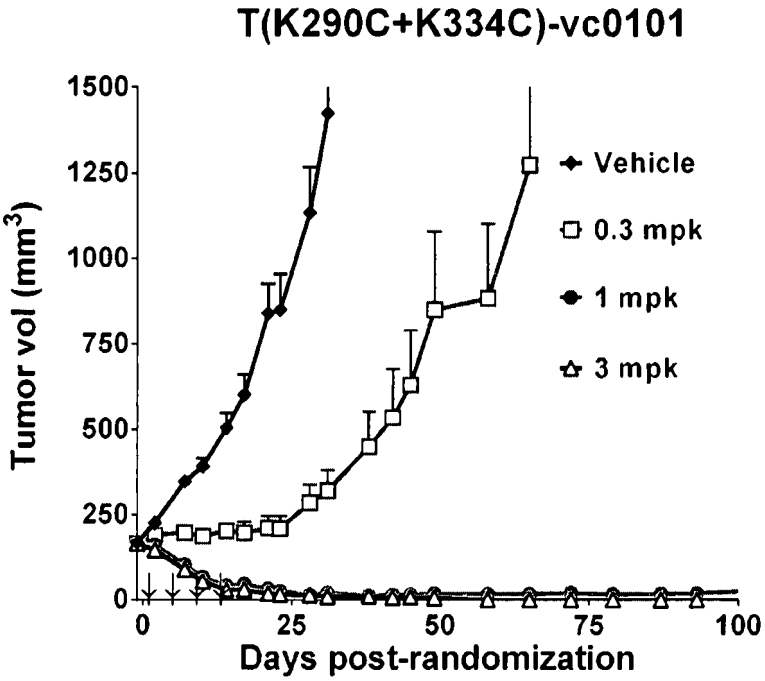

T(K290C+K334C)-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 9D

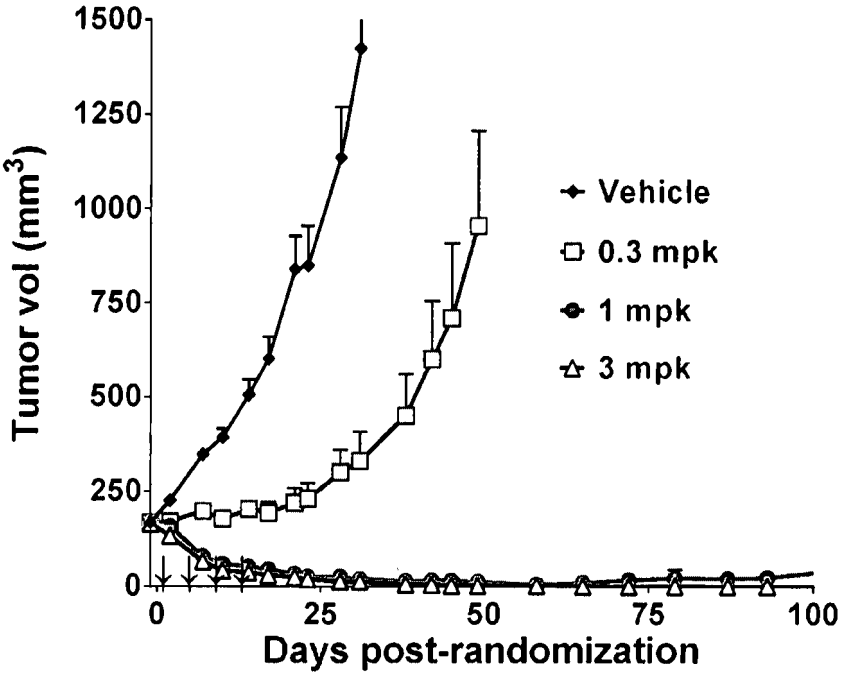

T(K334C+K392C)-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 9E

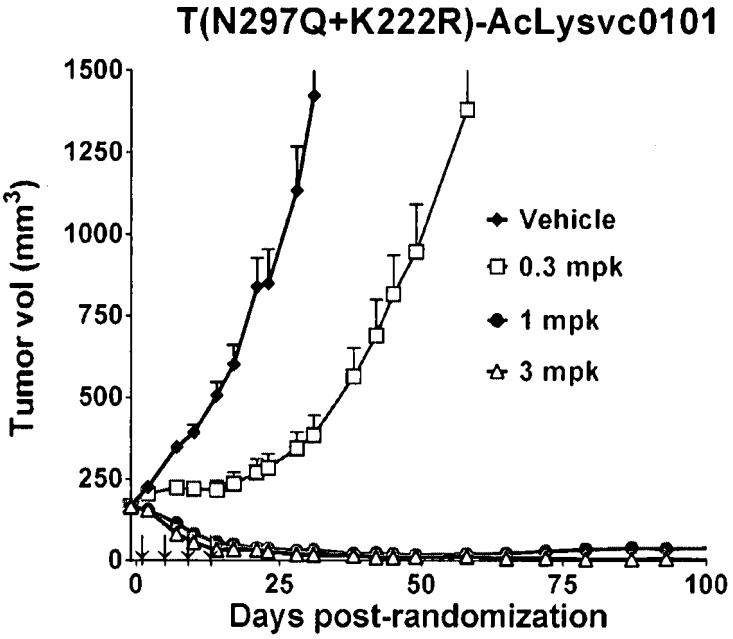

T(N297Q+K222R)-AcLysvc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
Vehicle
0.3 mpk
1 mpk
3 mpk

FIG. 9F

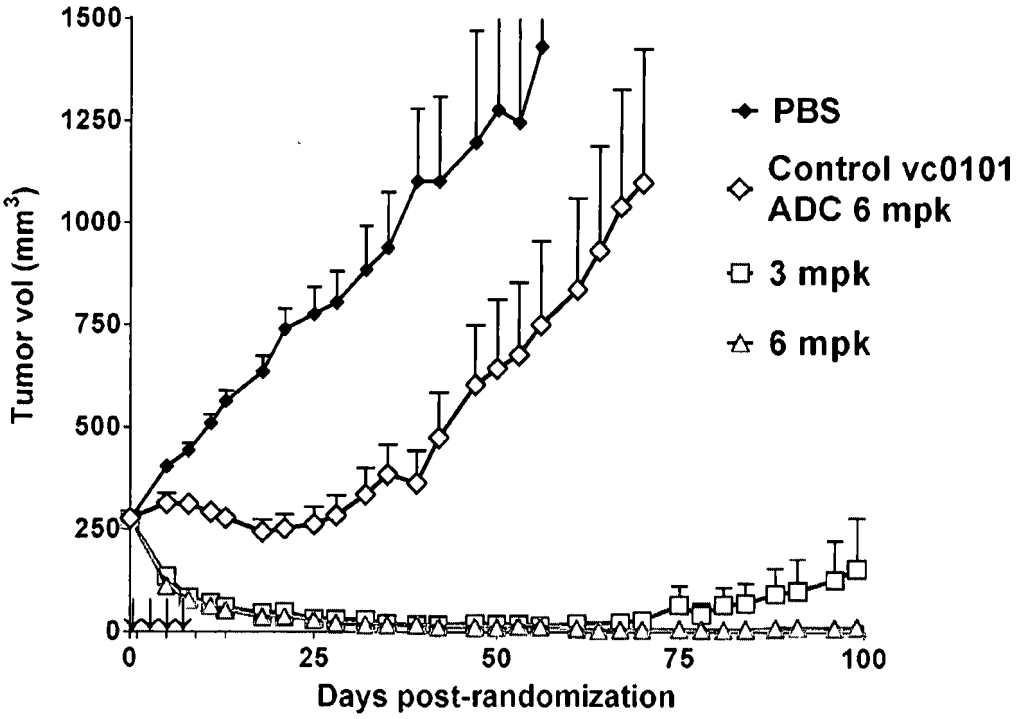

T-vc0101
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
25
50
75
100
Days post-randomization
PBS
Control vc0101 ADC 6 mpk
3 mpk
6 mpk

FIG. 9G

T-DM1

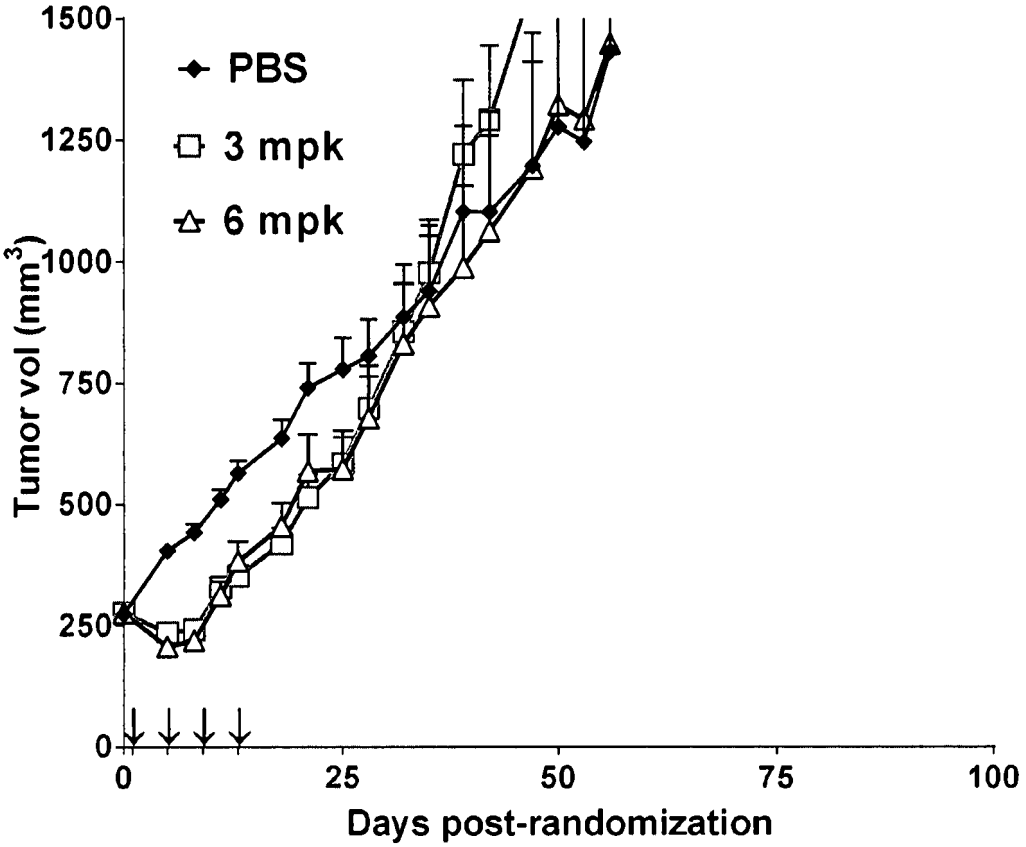

1500
1250
1000
750
500
250
0
Tumor vol (mm3)
PBS
3 mpk
6 mpk
0
25
50
75
100
Days post-randomization

FIG. 10A

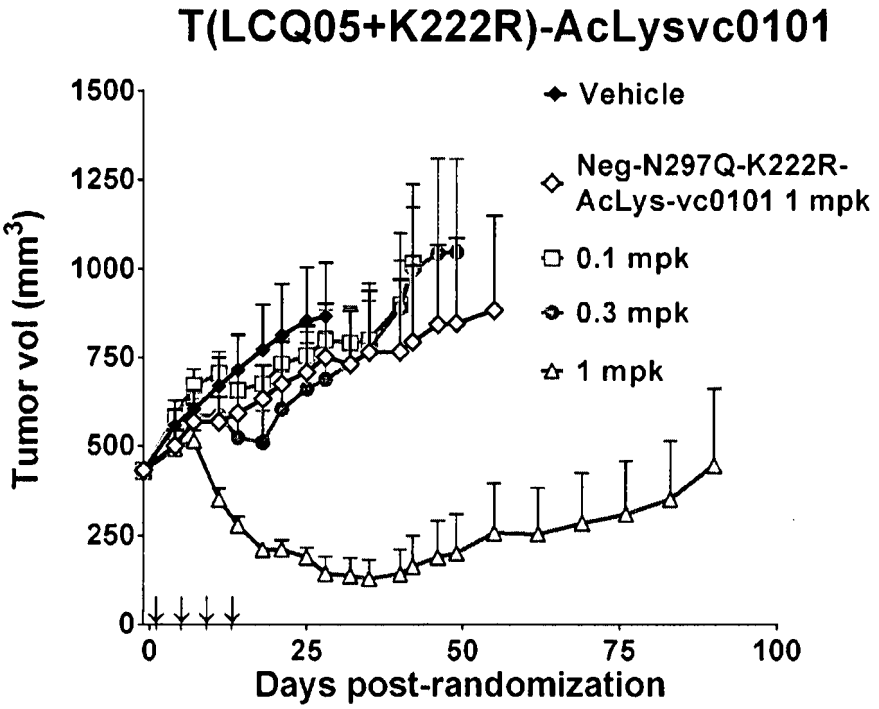


FIG. 10B

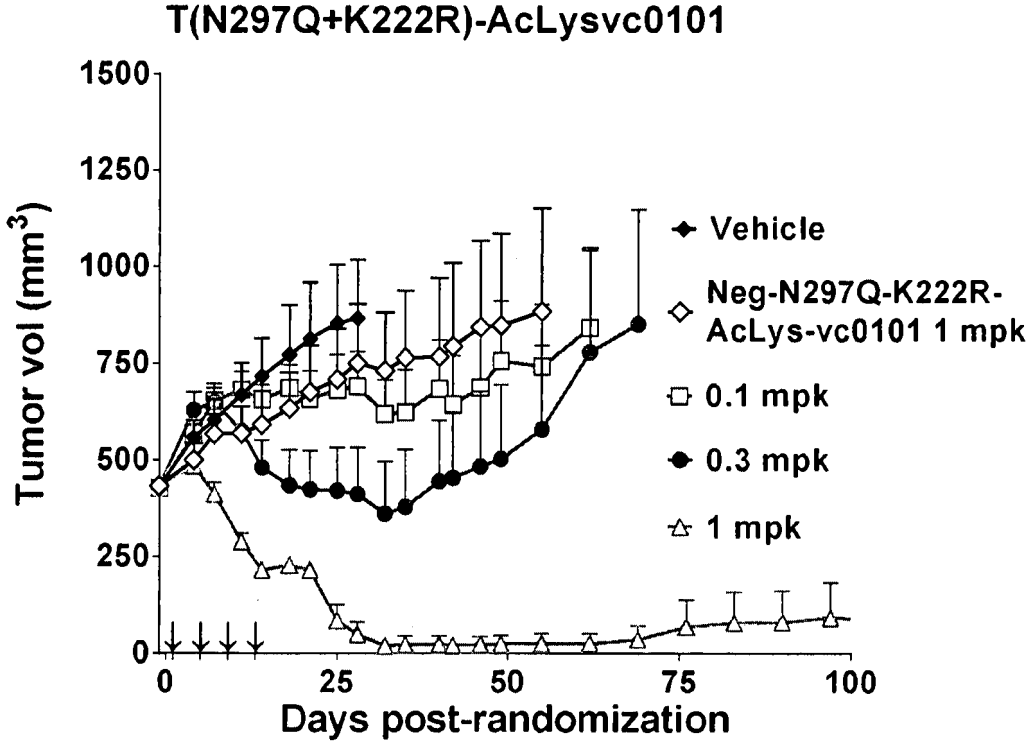


FIG. 10C

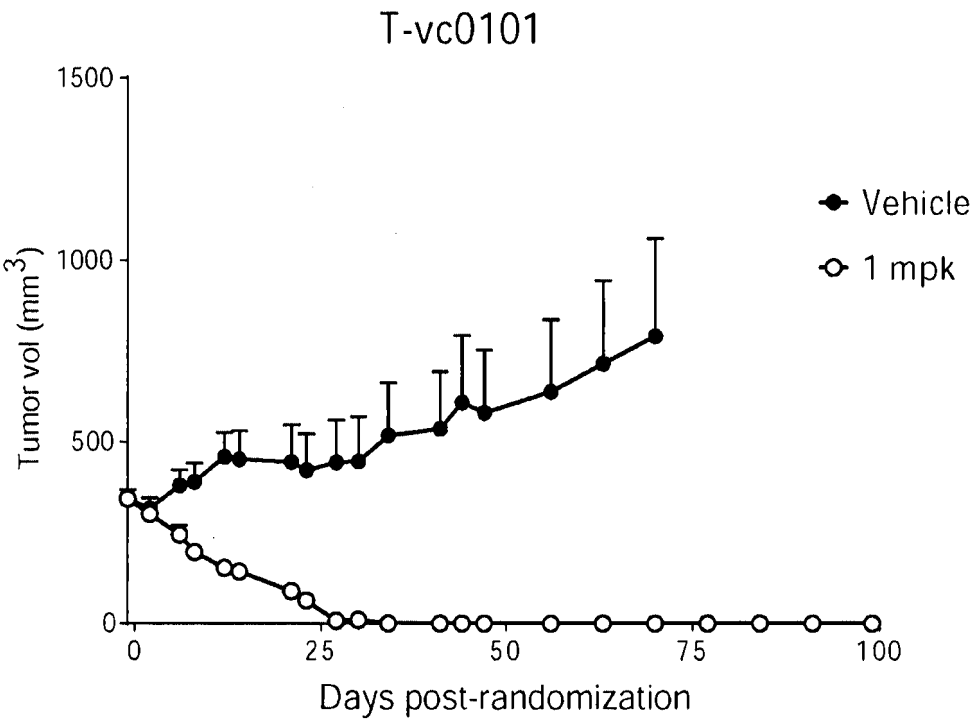


FIG. 10D

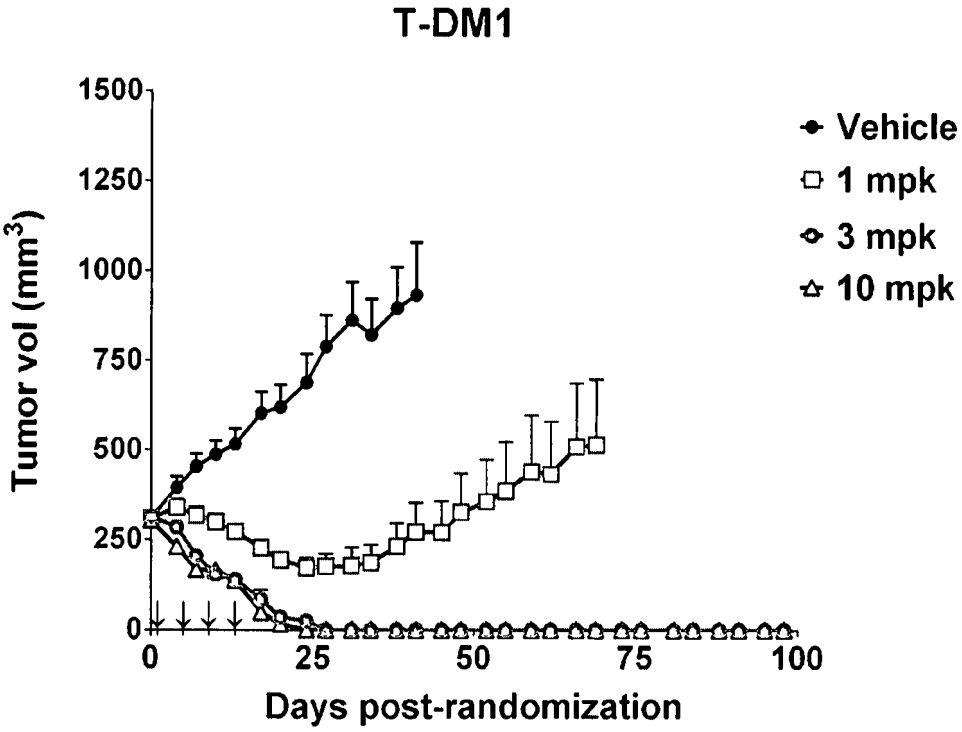


FIG. 11A

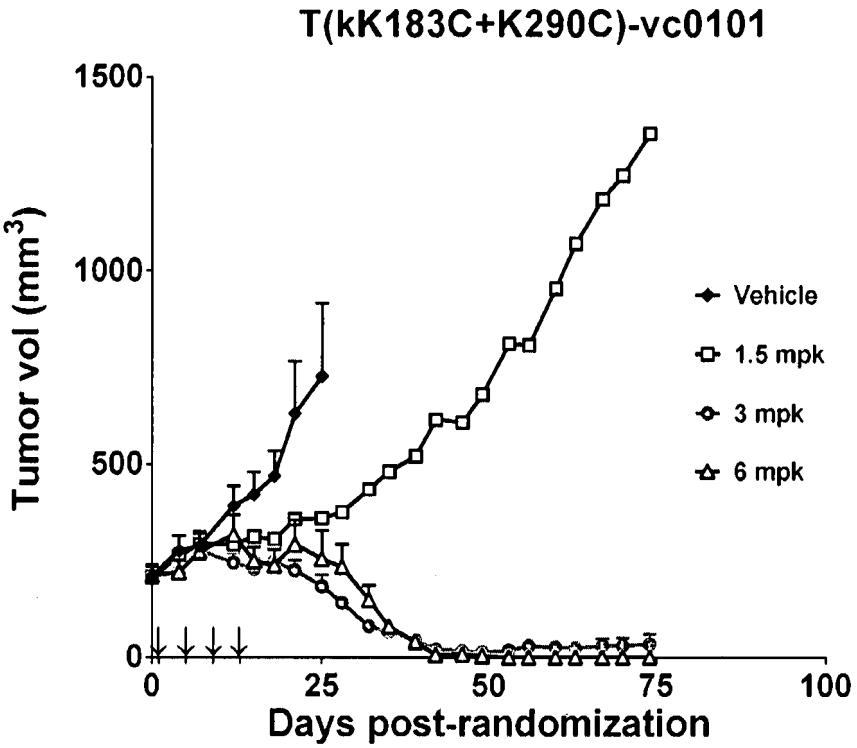


FIG. 11B

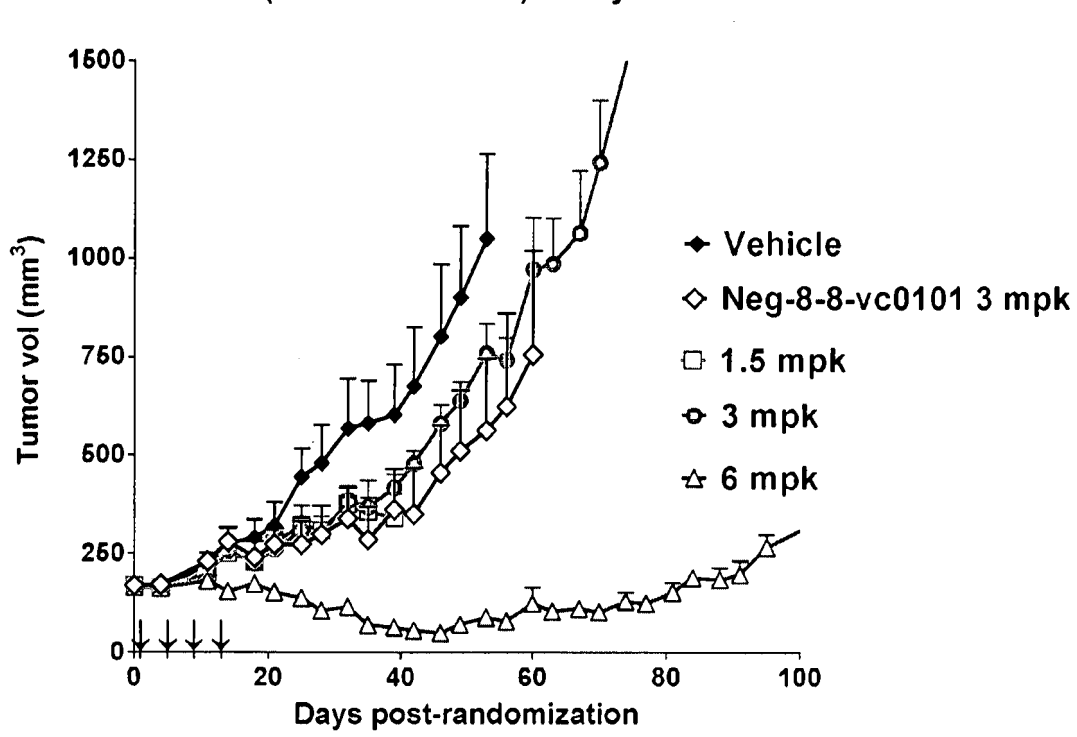


FIG. 11C

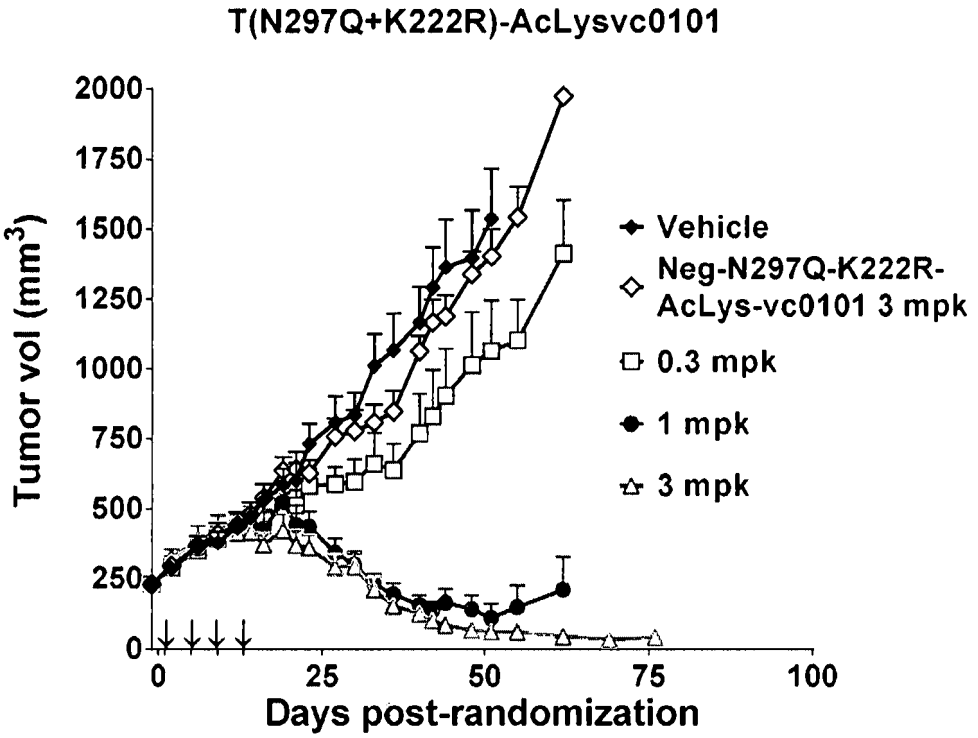


FIG. 11D

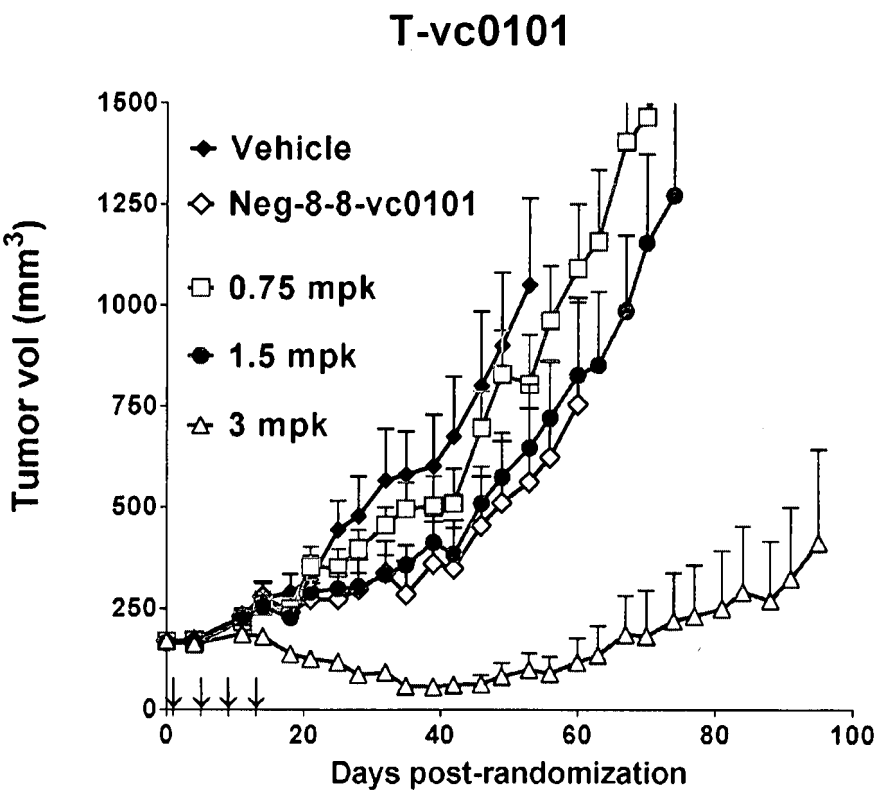


FIG. 11E

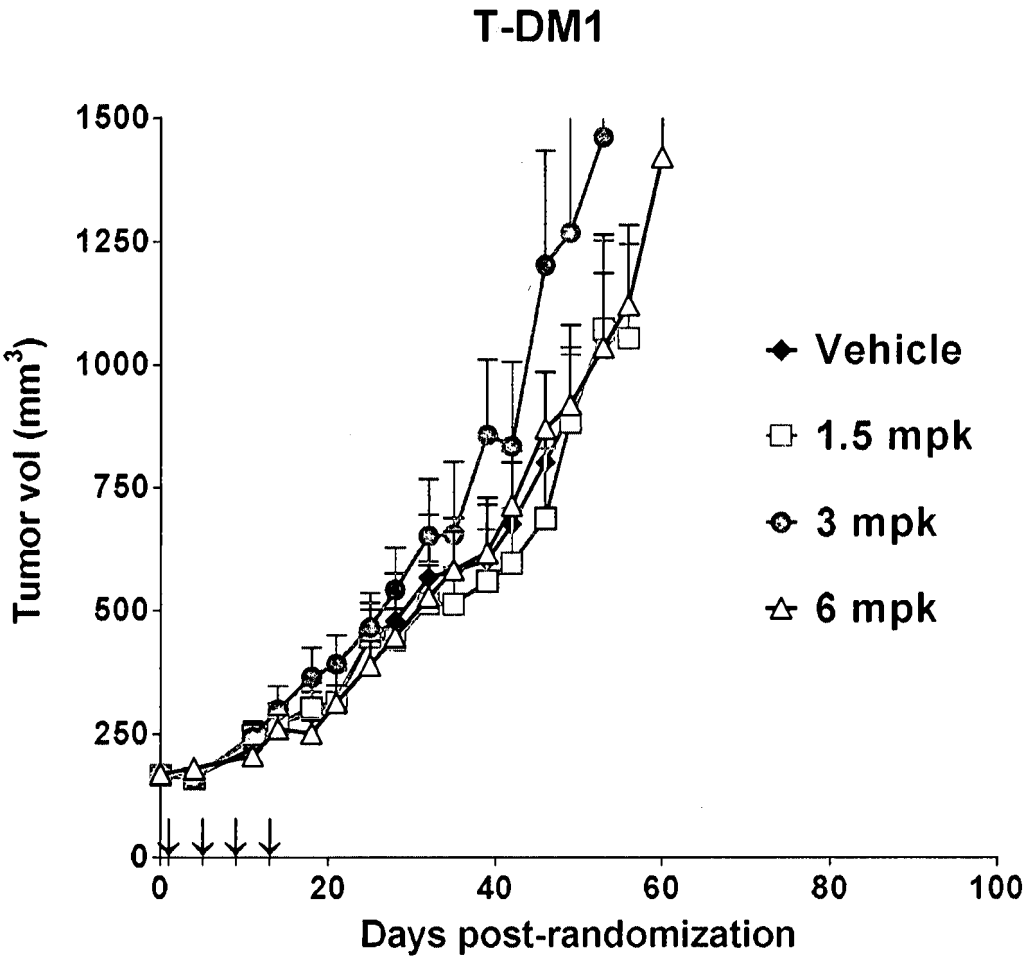

T-DM1
Tumor vol (mm3)
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
Days post-randomization
Vehicle
1.5 mpk
3 mpk
6 mpk

FIG. 12A

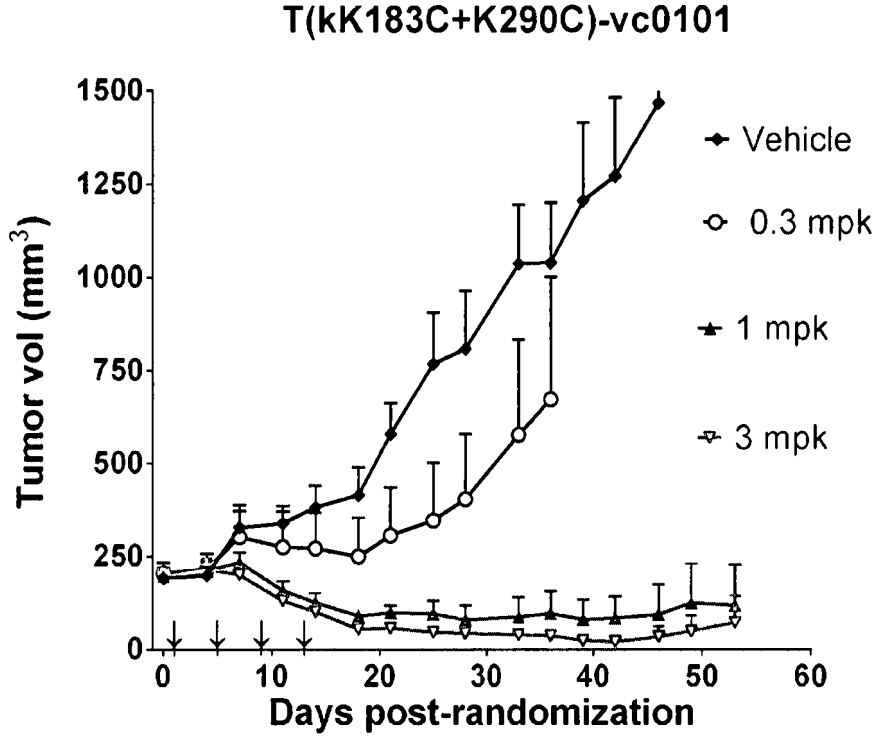


FIG. 12B

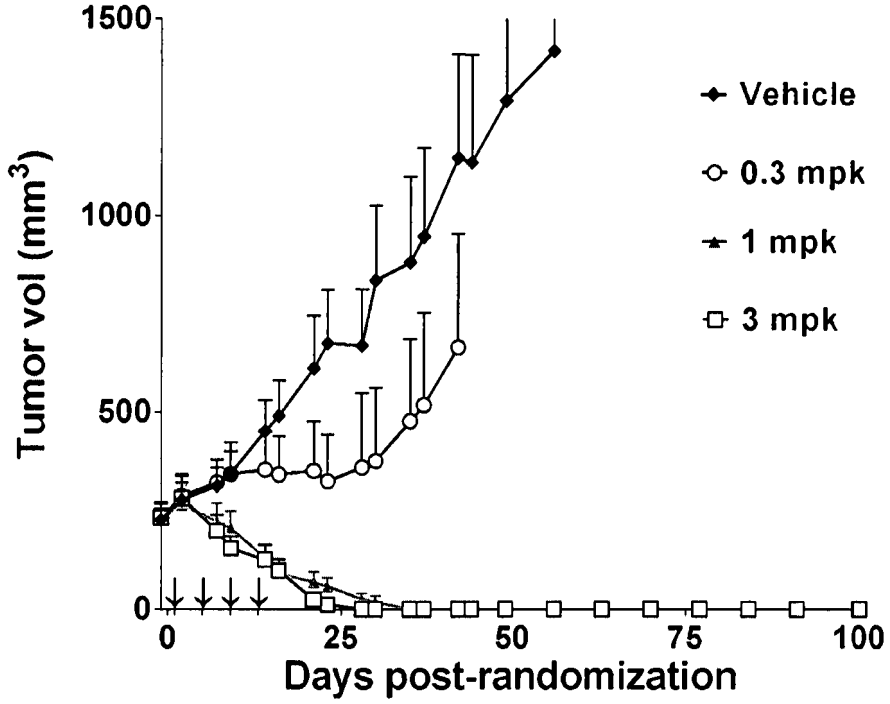


FIG. 12C

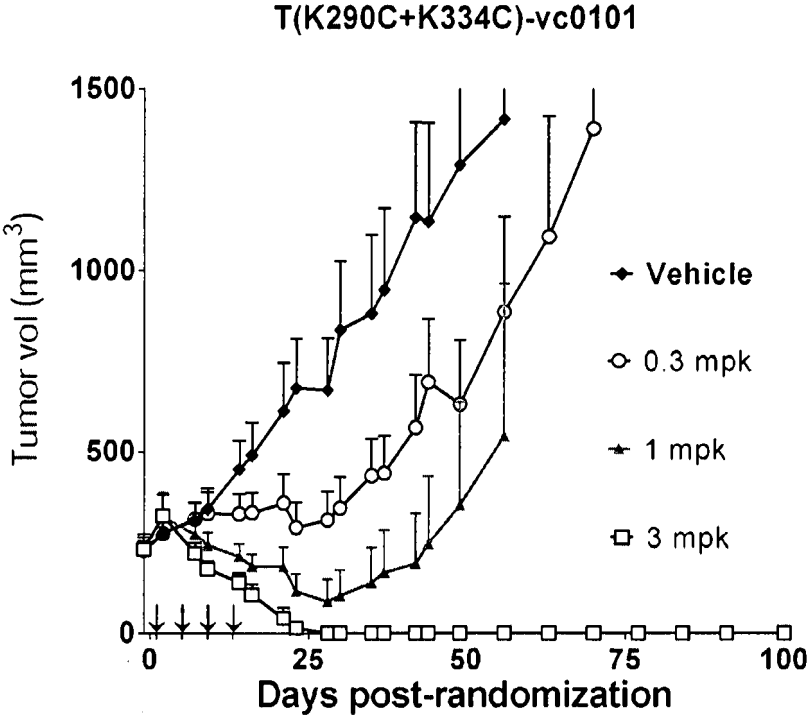


FIG. 12D

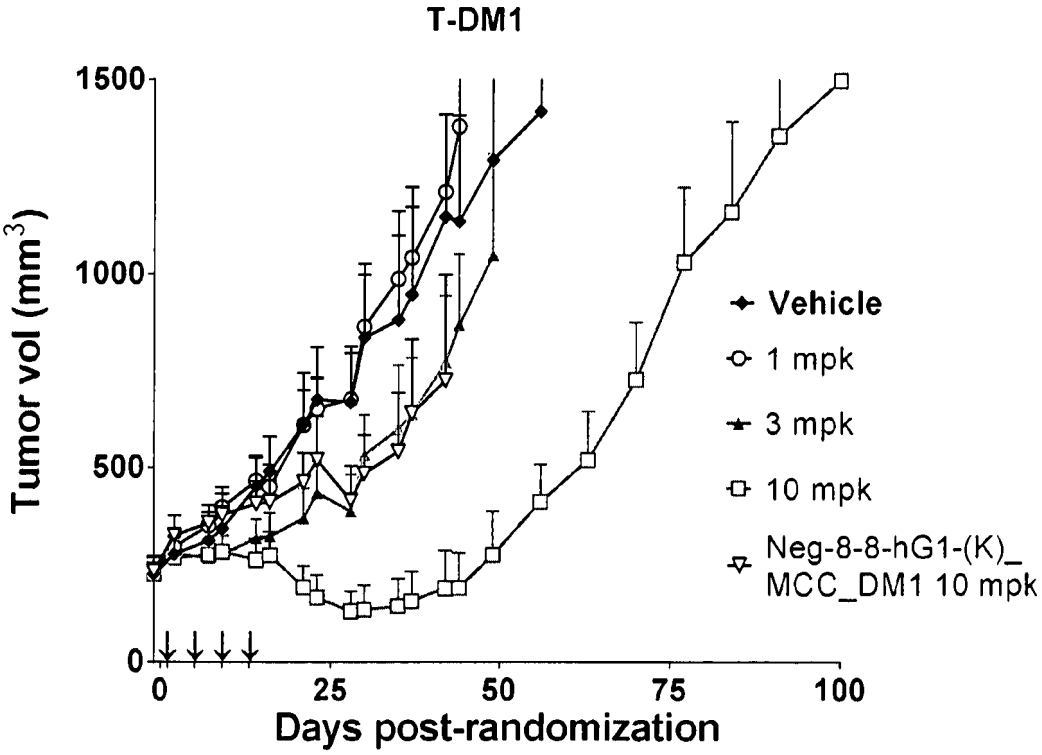


FIG. 13A

FIG. 13B

FIG. 14

In vitro resistance profile of N87 parental vs. N87-TM resistant cell lines.											Relative Resistance	
		N87			N87-TM-1			N87-TM-2			Avg IC50 (nM) Payload Fold Change compared to N87	
ADC	Linker Type	Avg ± SD IC50 (nM) Payload	Avg ± SD IC50 (ng/mL) Antibody	n	Avg ± SD IC50 (nM) Payload	Avg ± SD IC50 (ng/mL) Antibody	n	Avg ± SD IC50 (nM) Payload	Avg ± SD IC50 (ng/mL) Antibody	n	N87-TM-1	N87-TM-2
T-DM1	N	1.3 ± 0.6	51.9 ± 32.6	25	150 ± 58	5335 ± 1815	12	191 ± 109	6806 ± 3835	24	114	146
T-mc8261	N	3.0 ± 1.8	107 ± 61	9	>1000 ± ND	>35247 ± ND	9	>1000 ± ND	>35247 ± ND	9	>330	>330
T-m(H20)c8261	N	39.9 ± 75.9	1639 ± 3123	4	>1000 ± ND	>41127 ± ND	5	>1000 ± ND	20781 ± ND	4	>25.1	>25.1
T-MalPeg8261	N	3.7 ± 2.6	176 ± 123	3	>1000 ± ND	>47767 ± ND	4	>1000 ± ND	>47767 ± ND	2	>272	>272
T-mc6121	N	0.41 ± ND	17.4 ± ND	1	3.9 ± 2.2	166 ± 93	2	1.5 ± ND	64.8 ± ND	1	9.5	3.70
T-MalPeg6121	N	0.45 ± ND	18.8 ± ND	1	ND ± ND	ND ± ND	ND	>1000 ± ND	>42133 ± ND	1	ND	>2242
T-MalPegMMAD	N	4.3 ± 1.6	143 ± 53	8	>1000 ± ND	>33649 ± ND	7	>1000 ± ND	>33649 ± ND	4	>235	>235
T-mc0101	N	50.61 ± 14	1536.2 ± 370	2	ND ± ND	ND ± ND	0	>1000 ± ND	>31260 ± ND	2	ND	>19
T-vc0101	C	0.64 ± 0.28	23.4 ± 10.7	17	0.60 ± 0.20	21.9 ± 7.2	7	0.73 ± 0.27	26.7 ± 9.9	14	0.94	1.1
T-vc8261	C	0.43 ± 0.1	15 ± 3.4	8	0.5 ± 0.2	17.1 ± 7.0	6	0.5 ± 0.2	18.2 ± 6.8	8	1.1	1.2
T-vc8254	C	0.44 ± ND	16.6 ± ND	1	ND ± ND	ND ± ND	0	0.58 ± ND	21.7 ± ND	1	ND	1.31
T-vc6780	C	0.73 ± ND	25.8 ± ND	1	ND ± ND	ND ± ND	0	0.56 ± ND	19.8 ± ND	1	ND	0.77
T-vc0131	C	0.61 ± ND	20.2 ± ND	1	ND ± ND	ND ± ND	0	0.59 ± ND	19.8 ± ND	1	ND	0.98

continued

FIG. 14 continued

T-vcMMAE	C	0.54 ± 0.1	21.0 ± 3.2	2	0.8 ± ND	32.2 ± ND	1	0.9 ± ND	36.8 ± ND	1	1.5	1.8
T(LCQ05+K222R)-AcLysvc0101	C	0.63 ± 0.23	46.9 ± 17.0	8	0.58 ± 0.25	43.4 ± 18.9	7	0.60 ± 0.24	45.2 ± 18.2	8	0.93	0.96
T(N297Q+K222R)-AcLysvc0101	C	0.57 ± 0.09	21.5 ± 3.6	4	0.49 ± 0.01	18.5 ± 0.5	2	0.46 ± 0.06	17.2 ± 2.4	5	0.86	0.80
T(N297A+K222R+LCQ05)-AcLysvc0101	C	0.62 ± 0.10	23.2 ± 3.7	2	0.57 ± ND	21.4 ± ND	1	0.57 ± 0.07	21.4 ± 2.7	2	0.92	0.92
T(N297Q)-AcLysvc0101	C	0.56 ± 0.12	20.3 ± 4.3	5	0.58 ± 0.15	21.3 ± 5.5	5	0.64 ± 0.09	23.4 ± 3.6	5	1.0	1.20
T(K290C+K334C)-vc0101	C	0.62 ± 0.21	22.7 ± 7.3	3	0.51 ± 0.17	18.8 ± 5.9	2	0.47 ± 0.08	17.3 ± 2.9	3	0.82	0.76
T(K334C+K392C)-vc0101	C	0.71 ± 0.11	25.8 ± 4	3	0.63 ± ND	22.9 ± ND	1	0.59 ± 0.21	21.4 ± 7.5	3	0.89	0.83
T(kK183C+K392C)-vc0101	C	0.66 ± 0.04	22.7 ± 1.4	3	0.54 ± ND	18.8 ± ND	1	0.49 ± 0.05	17.0 ± 1.8	4	0.83	0.75
T(kK183C+K290C)-vc0101	C	0.72 ± 0.17	26.6 ± 5.6	2	0.63 ± ND	23.7 ± ND	1	0.71 ± 0.23	26.2 ± 8	2	0.88	0.99
T(kK183C+K334C)-vc0101	C	1.59 ± ND	62.4 ± ND	1	ND ± ND	ND ± ND	0	1.4 ± ND	56 ± ND	1	ND	0.90
T(K290C+K392C)-vc0101	C	0.65 ± 0.01	23.4 ± 0.2	2	0.67 ± ND	24.5 ± ND	1	0.65 ± 0.1	23.7 ± 3.6	2	1.0	1.0
T(K290C)-vc0101	C	0.59 ± ND	43.0 ± ND	1	ND ± ND	ND ± ND	0	0.42 ± ND	30.4 ± ND	1	ND	0.71
T(K334C)-vc0101	C	0.56 ± 0.02	40.5 ± 1.7	2	ND ± ND	ND ± ND	ND	0.51 ± 0.17	37.2 ± 12.3	2	ND	0.92
T(K392C)-vc0101	C	0.55 ± 0.01	41.3 ± 0.2	2	ND ± ND	ND ± ND	ND	0.45 ± 0.17	33.6 ± 12.7	2	ND	0.81
T(kK183C)-vc0101	C	0.62 ± ND	45.1 ± ND	1	ND ± ND	ND ± ND	0	0.6 ± ND	46.6 ± ND	1	ND	1.03
DM1	--	15.1 ± 8.5	--	13	38.4 ± 17.53	--	8	33.4 ± 8.9	--	11	2.5	2.2
0101	--	0.36 ± 0.09	--	7	0.50 ± 0.11	--	4	0.49 ± 0.12	--	7	1.4	1.4

continued

FIG. 14 continued

8254	--	0.59 ± 0.12	--	4	1.1 ± 0.3	--	3	1.0 ± 0.3	--	4	1.9	1.7
6121	--	0.15 ± 0.03	--	2	0.32 ± ND	--	1	0.41 ± 0.11	--	2	2.1	2.7
Vinblastine	--	3.6 ± 0.1	--	2	5.3 ± ND	--	1	5.7 ± 1.1	--	2	1.5	1.6
Paclitaxel	--	8.2 ± 4.0	--	2	12.8 ± 9.0	--	2	11.7 ± 6.5	--	2	1.6	1.4
Docetaxel	--	2.9 ± 1.4	--	2	2.8 ± ND	--	1	4.1 ± 1.2	--	2	0.95	1.4
5-fluorouracil	--	1611 ± 203	--	2	3129 ± ND	--	1	3135 ± 1014	--	2	1.9	1.9
Gemcitabine	--	7.3 ± 2.2	--	2	8.3 ± ND	--	1	8.4 ± 1.1	--	2	1.1	1.1
Doxorubicin	--	38.2 ± 13.5	--	5	60.0 ± 18.5	--	5	38.5 ± 7.5	--	5	1.6	1.0
Oxaliplatin	--	623 ± 471	--	2	681 ± ND	--	1	1511 ± 1462	--	2	1.1	2.4
Camptothecin	--	20.6 ± 2.3	--	2	11.0 ± ND	--	1	9.8 ± 3.3	--	2	0.53	0.48

FIG. 15A

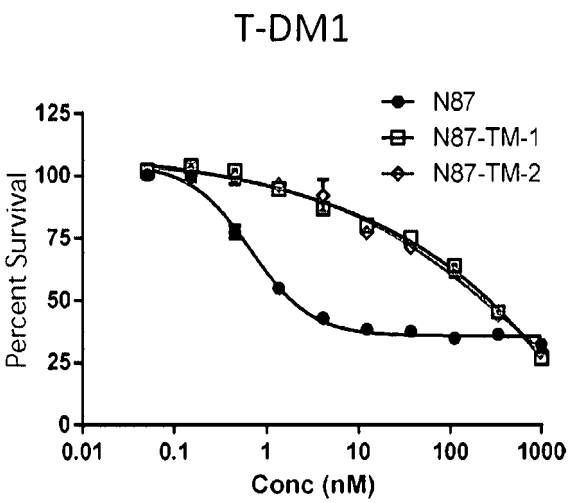


FIG. 15B

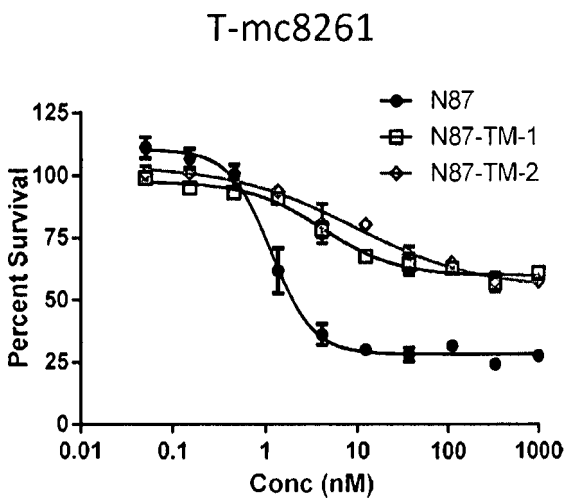


FIG. 15C

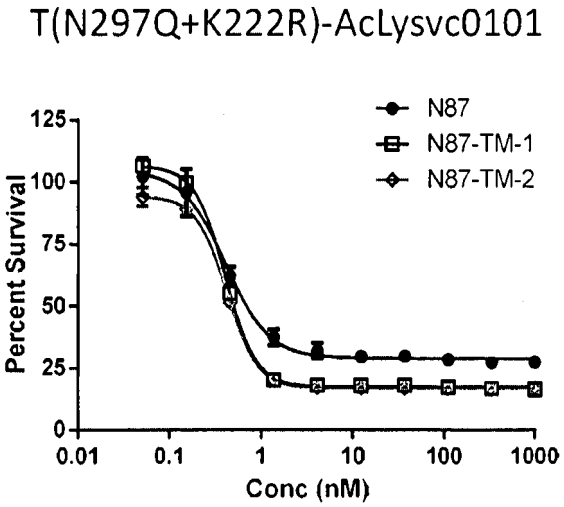


FIG. 15D

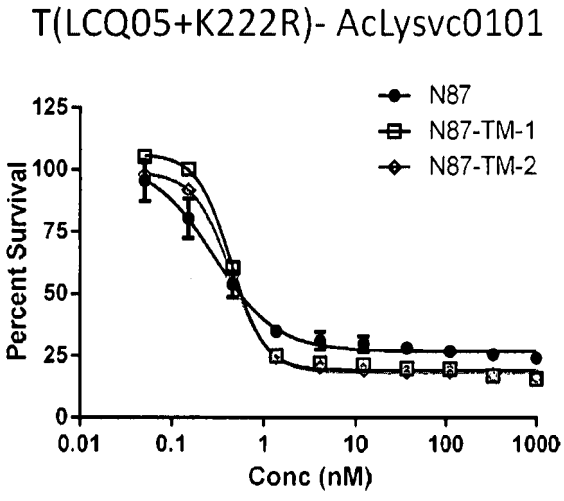


FIG. 15E

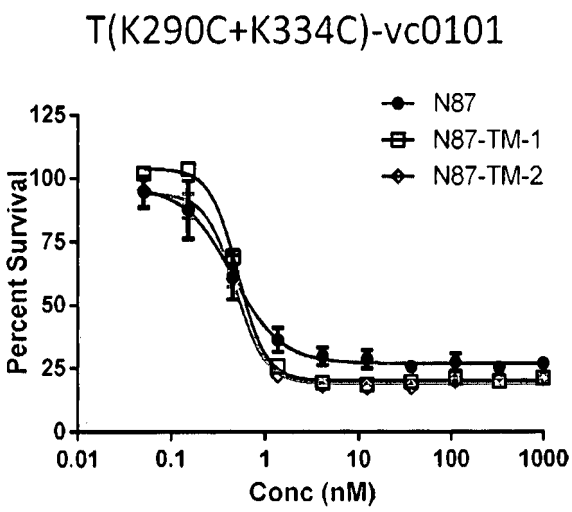


FIG. 15F

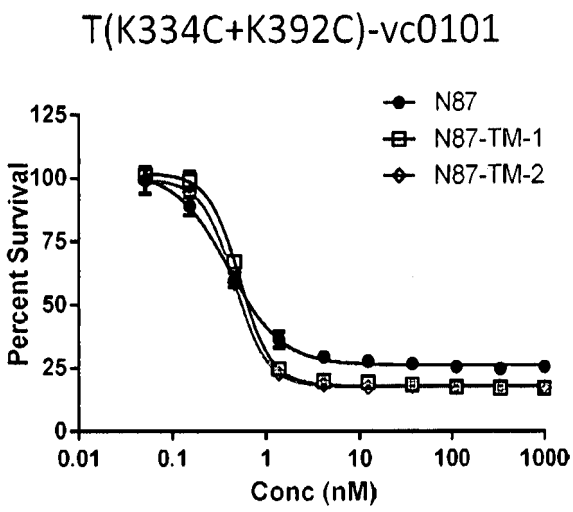


FIG. 15G

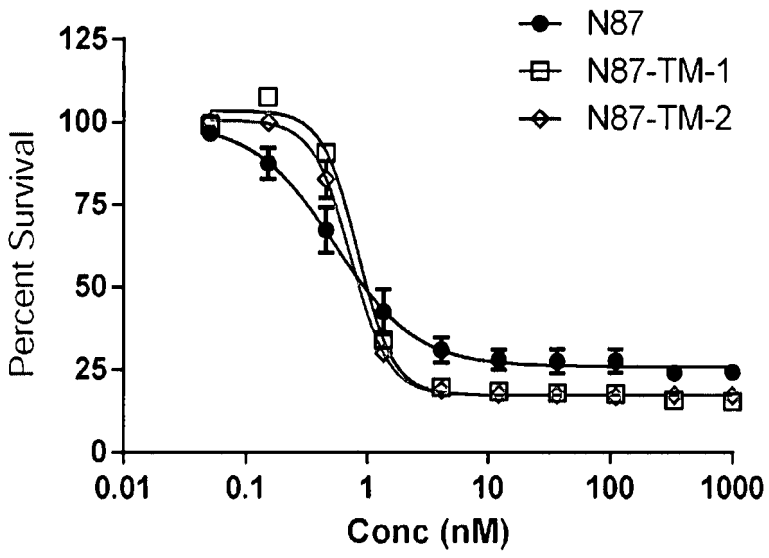


FIG. 16B

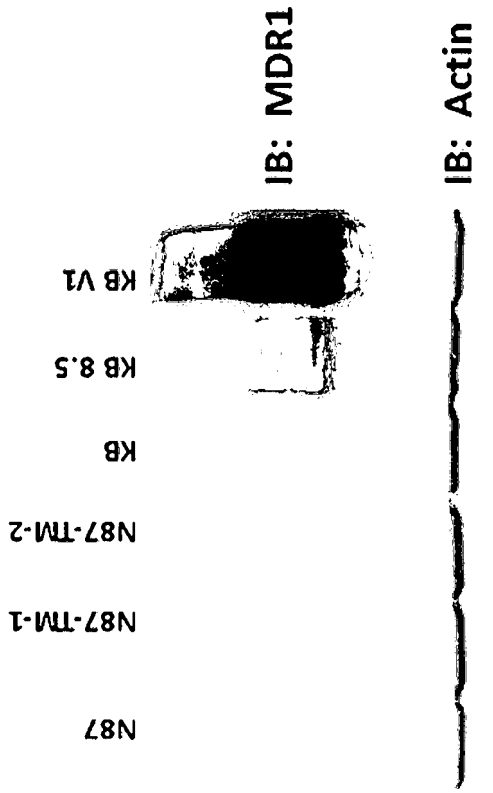


FIG. 16A

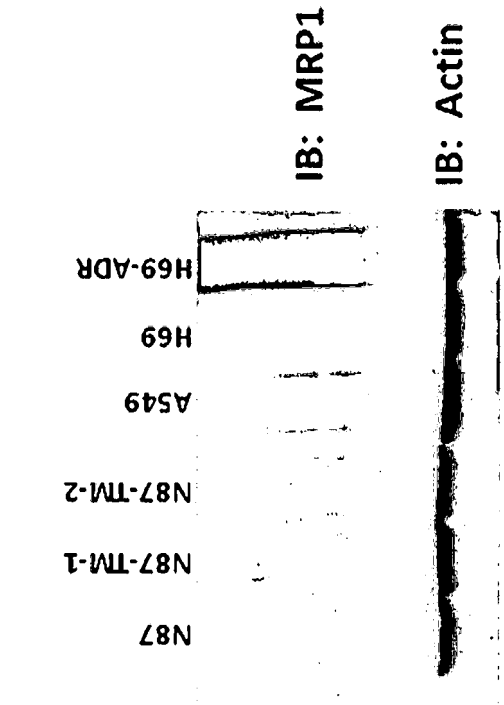


FIG. 17A

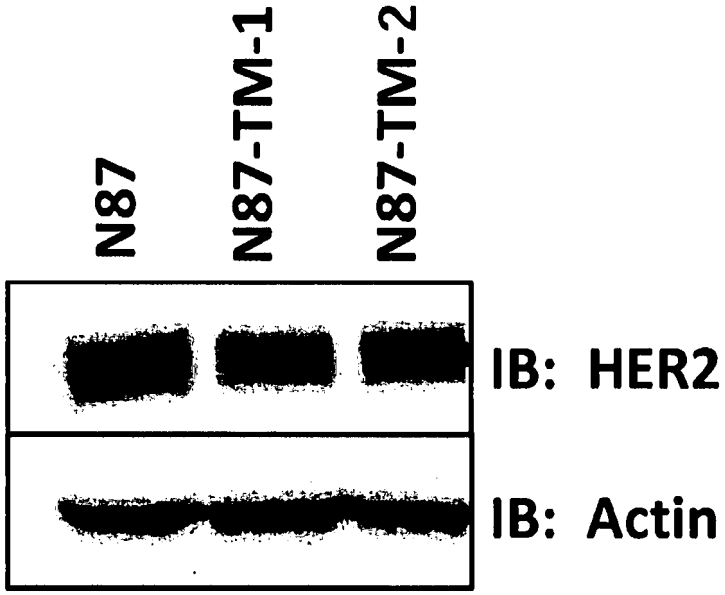


FIG. 17B

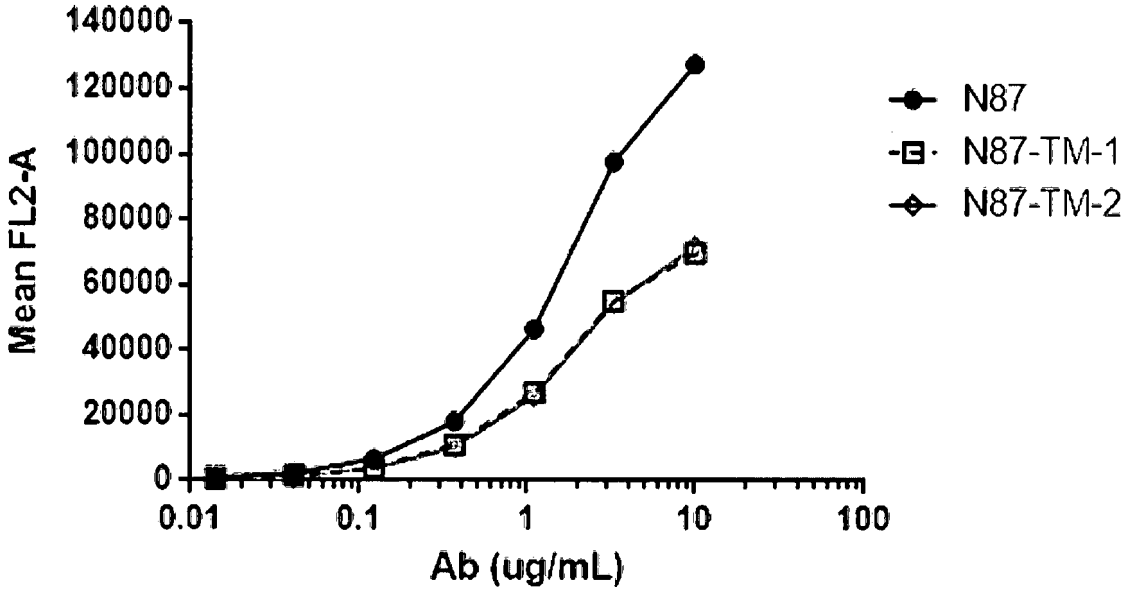


FIG. 18A

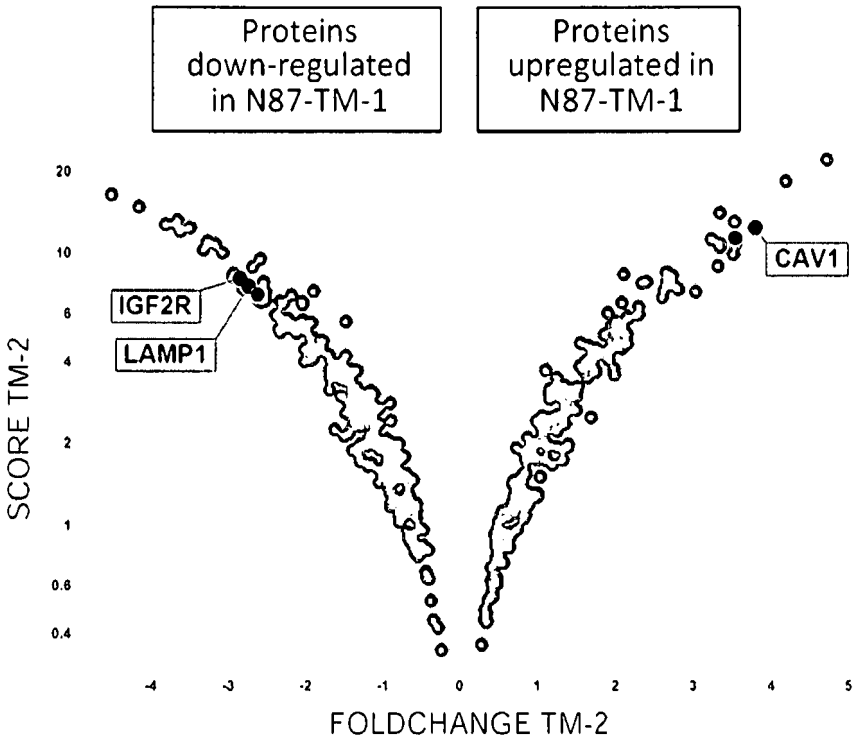


FIG. 18B

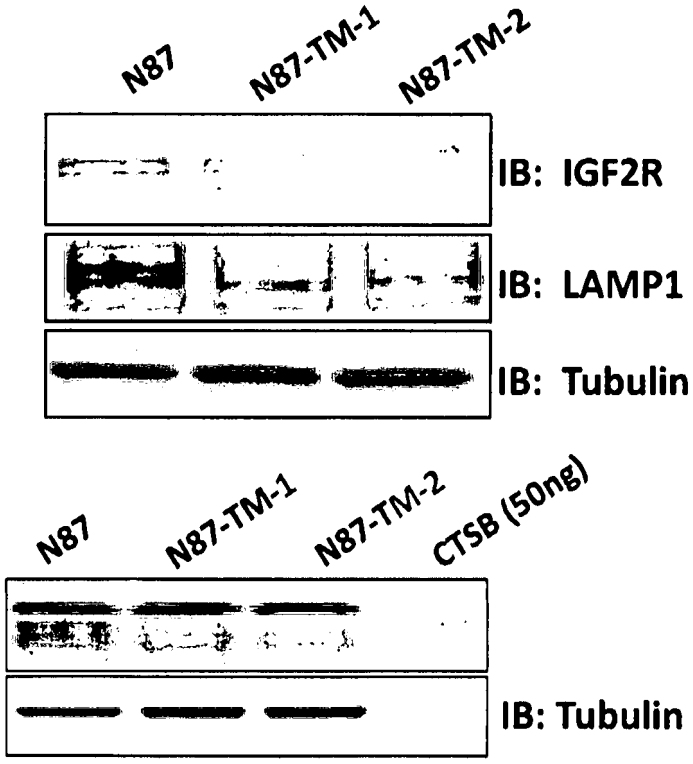


FIG. 18C

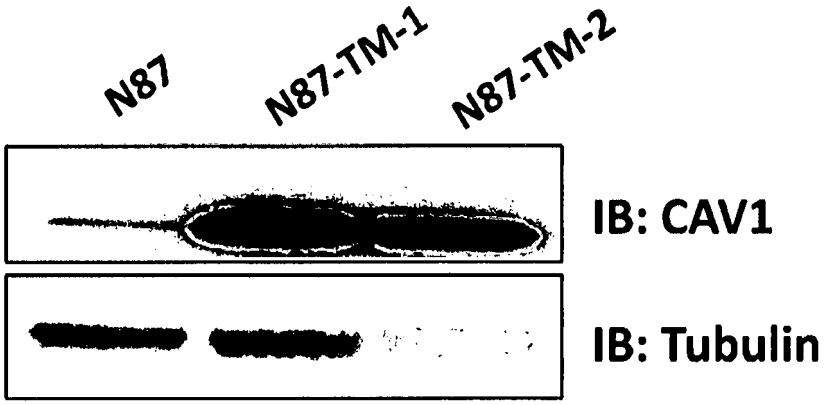


FIG. 18D

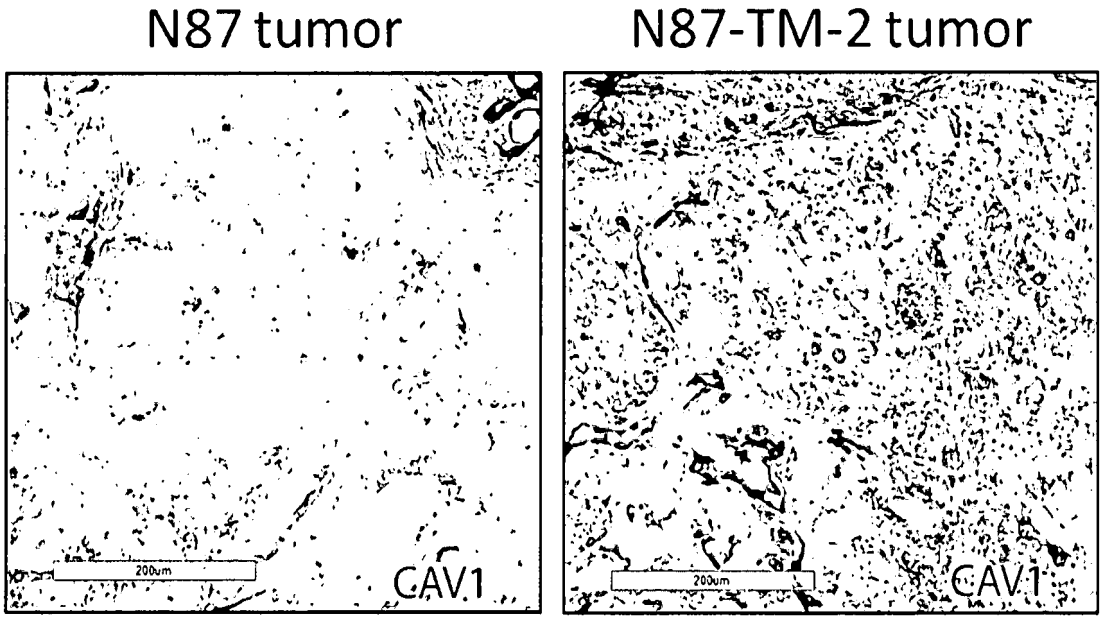


FIG. 19A

N87 model			Tumor Volume - Average ± Standard Deviation (Day)																
ADC Name	Dose (mg/kg)	n	-2	4	7	11	14	18	21	25	28	32	35	39	42	48	55	62	69
PBS	0	10	286 ± 39	507 ± 61	642 ± 121	946 ± 102	922 ± 101	1118 ± 200	1262 ± 212	1512 ± 195	1712 ± 219								
trastuzumab	13, 4.5	10	294 ± 22	435 ± 59	590 ± 73	669 ± 100	713 ± 106	732 ± 111	874 ± 118	977 ± 168	1144 ± 201	1295 ± 199	1419 ± 228	1525 ± 276	1732 ± 190				
T-vc0101	1	10	282 ± 38	383 ± 47	398 ± 61	375 ± 70	323 ± 75	254 ± 47	215 ± 56	255 ± 94	289 ± 109	351 ± 149	358 ± 146	413 ± 152	435 ± 163	528 ± 193	631 ± 191	780 ± 241	773 ± 340
T-vc0101	3	10	292 ± 42	298 ± 57	247 ± 51	205 ± 47	127 ± 29	92 ± 21	96 ± 22	92 ± 22	88 ± 26	76 ± 22	76 ± 14	82 ± 26	69 ± 28	22 ± 29	21 ± 47	32 ± 44	0 ± 0
Neg-8-8-DM1	3	10	291 ± 35	456 ± 77	492 ± 75	598 ± 93	551 ± 104	497 ± 121	515 ± 126	533 ± 111	605 ± 129	794 ± 204	826 ± 220	952 ± 239	943 ± 223	925 ± 196	1137 ± 235	1262 ± 245	1266 ± 250
T(N297Q+K222R)-AcLysvc0101	3	10	483 ± 42	483 ± 42	512 ± 101	477 ± 66	359 ± 71	203 ± 34	158 ± 29	122 ± 36	113 ± 23	120 ± 29	94 ± 33	95 ± 21	79 ± 22	57 ± 60	13 ± 29	0 ± 0	8 ± 25
T-DM1	6	10	289 ± 28	436 ± 72	426 ± 101	437 ± 125	343 ± 95	253 ± 86	219 ± 60	178 ± 53	173 ± 52	204 ± 68	211 ± 87	231 ± 104	293 ± 138	331 ± 149	458 ± 176	536 ± 217	577 ± 263
T-DM1	10	10	288 ± 23	379 ± 45	338 ± 64	306 ± 78	248 ± 56	166 ± 50	153 ± 29	158 ± 21	120 ± 21	141 ± 32	120 ± 28	112 ± 49	83 ± 52	76 ± 87	67 ± 94	89 ± 120	100 ± 213

FIG. 19B

N87-TM model: Study #1			Tumor Volume - Average ± Standard Deviation (Day)																
ADC Name	Dose (mg/kg)	n	-1	3	7	10	14	17	21	24	28	31	35	38	43	49	58	65	72	78
PBS	0	10	256 ± 28	369 ± 49	503 ± 77	574 ± 70	610 ± 104	841 ± 110	1046 ± 119	1238 ± 101	1593 ± 208	1715 ± 259	1881 ± 284							
trastuzumab	13, 4.5	10	251 ± 20	278 ± 57	341 ± 92	411 ± 97	468 ± 112	640 ± 225	845 ± 201	997 ± 283	1150 ± 334	1404 ± 313	1589 ± 322							
T-vc0101	1	10	255 ± 34	230 ± 47	333 ± 54	299 ± 58	297 ± 43	371 ± 90	493 ± 125	576 ± 93	692 ± 119	835 ± 179	1034 ± 214	1121 ± 190	1399 ± 278					
T-vc0101	3	10	253 ± 21	240 ± 44	227 ± 53	152 ± 48	118 ± 38	104 ± 49	145 ± 41	125 ± 35	97 ± 35	103 ± 35	121 ± 48	84 ± 54	93 ± 58	123 ± 86	108 ± 154	81 ± 130		
Neg-8-8-DM1	3	10	252 ± 50	319 ± 62	341 ± 68	371 ± 85	406 ± 56	536 ± 89	628 ± 113	694 ± 164	822 ± 185	1055 ± 258	1125 ± 271	1168 ± 269	1455 ± 327					
T(N297Q+K222R)-AcLysvc0101	3	10	254 ± 34	273 ± 38	268 ± 30	214 ± 33	176 ± 38	164 ± 33	194 ± 33	155 ± 26	154 ± 25	143 ± 33	134 ± 30	109 ± 41	106 ± 32	127 ± 39	114 ± 64	58 ± 44	147 ± 78	99 ± 69
T-DM1	6	10	253 ± 33	294 ± 50	368 ± 69	435 ± 133	465 ± 116	570 ± 150	638 ± 149	783 ± 201	902 ± 326	1137 ± 312	1278 ± 291	1401 ± 327	1505 ± 336					
T-DM1	10	15	243 ± 32	287 ± 41	343 ± 39	326 ± 65	315 ± 64	417 ± 92	530 ± 143	577 ± 135	706 ± 156	859 ± 187	978 ± 303	957 ± 251	1157 ± 341	1571 ± 339				
T-DM1, T(N297Q+K222R)-AcLysvc0101	10, 3	8	233 ± 32	302 ± 38	339 ± 27	312 ± 55	316 ± 66	422 ± 68	559 ± 163	593 ± 119	699 ± 155	856 ± 166	1079 ± 236	992 ± 202	966 ± 177	820 ± 129	590 ± 138	388 ± 82	510 ± 88	452 ± 160

FIG. 19C

N87-TM model: Study #2			Tumor Volume - Average ± Standard Deviation (Day)																		
ADC Name	Dose (mg/kg)	n	-1	4	7	11	14	18	21	25	28	32	35	42	49	56	63	71	77	85	91	99
PBS	0	10	249 ± 24	353 ± 42	394 ± 62	522 ± 89	645 ± 112	811 ± 111	919 ± 113	1102 ± 180	1280 ± 187	1490 ± 240	1569 ± 248	1874 ± 292	2110 ± 196	2329 ± 314						
T(K290C+K334C)-vc0101	1	9	251 ± 36	301 ± 44	257 ± 55	240 ± 45	264 ± 31	249 ± 49	234 ± 50	268 ± 80	285 ± 111	322 ± 114	345 ± 132	528 ± 204	689 ± 281	892 ± 359	1340 ± 472	1437 ± 350	1696 ± 320	2337 ± 236		
T(K334C+K392C)-vc0101	1	9	248 ± 42	253 ± 32	252 ± 56	271 ± 41	300 ± 56	341 ± 97	304 ± 79	395 ± 123	464 ± 206	484 ± 197	519 ± 213	747 ± 290	1028 ± 337	1307 ± 384	1544 ± 349	1828 ± 326	2046 ± 288	2461 ± 439		
T(K290C+K334C)-vc0101	3	9	246 ± 33	246 ± 36	188 ± 24	141 ± 37	163 ± 42	170 ± 66	136 ± 17	148 ± 35	96 ± 17	123 ± 45	111 ± 24	98 ± 30	60 ± 21	76 ± 30	55 ± 18	102 ± 23	86 ± 32	84 ± 30	75 ± 24	92 ± 29
T(K334C+K392C)-vc0101	3	9	250 ± 30	265 ± 55	210 ± 45	175 ± 30	171 ± 38	211 ± 42	160 ± 24	209 ± 59	137 ± 18	130 ± 27	83 ± 16	132 ± 25	96 ± 21	94 ± 30	67 ± 21	99 ± 11	91 ± 20	86 ± 16	84 ± 26	91 ± 24
T(K392C)-vc0101	3	9	249 ± 40	233 ± 46	195 ± 27	172 ± 20	155 ± 21	166 ± 34	125 ± 37	143 ± 23	122 ± 32	133 ± 36	101 ± 48	151 ± 84	125 ± 93	95 ± 27	142 ± 163	272 ± 254	269 ± 333	441 ± 568	506 ± 693	438 ± 599
T(K290C)-vc0101	3	9	248 ± 25	259 ± 32	216 ± 28	198 ± 41	172 ± 42	174 ± 39	127 ± 24	154 ± 29	147 ± 35	129 ± 43	127 ± 51	177 ± 101	204 ± 175	272 ± 258	342 ± 350	514 ± 498	557 ± 568	928 ± 903	403 ± 479	590 ± 633
T(K334C)-vc0101	3	9	247 ± 19	238 ± 32	224 ± 59	173 ± 34	174 ± 17	177 ± 21	120 ± 20	161 ± 35	135 ± 22	123 ± 23	112 ± 33	135 ± 34	104 ± 44	122 ± 59	136 ± 120	202 ± 251	203 ± 273	274 ± 522	327 ± 703	105 ± 17

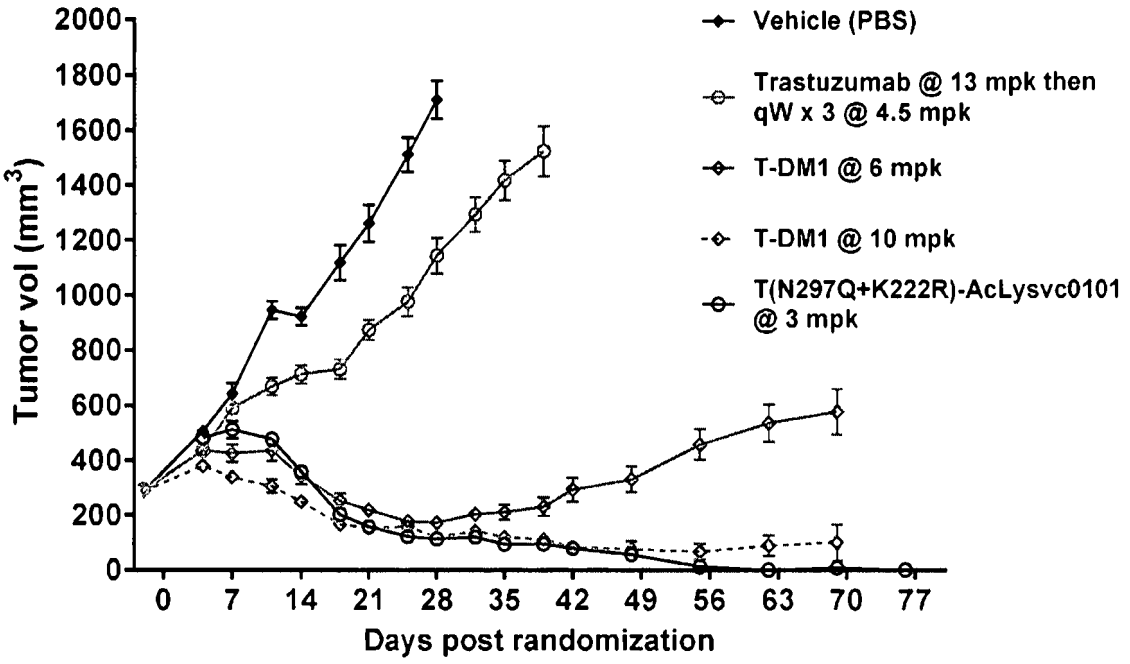
continued

FIG. 19C continued

T(LCQ05+K222R)-AcLysvc0101	3	9	251 ± 41	271 ± 42	245 ± 65	238 ± 66	224 ± 57	209 ± 49	200 ± 51	197 ± 65	192 ± 74	215 ± 99	213 ± 153	326 ± 246	368 ± 331	547 ± 468	597 ± 512	999 ± 824	968 ± 900	548 ± 410	602 ± 375	962 ± 636
T(K290C)-vc0101	6	9	252 ± 55	263 ± 39	195 ± 48	164 ± 27	156 ± 49	163 ± 48	114 ± 38	150 ± 24	119 ± 15	116 ± 30	94 ± 28	123 ± 52	89 ± 22	92 ± 29	81 ± 9	129 ± 35	98 ± 24	85 ± 25	80 ± 19	95 ± 23
T(K290C)-vc0101	6	9	245 ± 32	241 ± 30	191 ± 41	148 ± 26	147 ± 32	147 ± 36	112 ± 15	143 ± 42	123 ± 28	97 ± 22	106 ± 24	109 ± 36	85 ± 20	98 ± 22	75 ± 20	100 ± 25	91 ± 29	102 ± 26	76 ± 16	107 ± 32
T(K334C)-vc0101	6	9	247 ± 46	262 ± 49	201 ± 37	162 ± 17	172 ± 30	163 ± 26	145 ± 13	168 ± 25	122 ± 16	117 ± 26	107 ± 23	135 ± 24	85 ± 14	108 ± 19	94 ± 19	120 ± 20	91 ± 19	106 ± 21	81 ± 18	125 ± 23
T(LCQ05+K222R)-AcLysvc0101	6	9	248 ± 33	225 ± 38	197 ± 41	168 ± 22	145 ± 26	156 ± 28	134 ± 20	145 ± 21	125 ± 17	109 ± 10	101 ± 27	129 ± 30	87 ± 17	92 ± 21	76 ± 28	105 ± 21	82 ± 24	88 ± 20	91 ± 15	86 ± 15
T-DM1	6	9	247 ± 33	312 ± 32	331 ± 63	448 ± 44	520 ± 67	685 ± 127	704 ± 166	978 ± 176	1097 ± 177	1294 ± 249	1249 ± 246	1644 ± 371	1964 ± 341	1887 ± 307	2046 ± 1					
Neg-8-8-DM1	6	9	246 ± 34	328 ± 40	411 ± 43	535 ± 75	720 ± 154	869 ± 121	854 ± 114	1117 ± 138	1290 ± 125	1546 ± 272	1483 ± 241	1844 ± 382	1927 ± 287	1888 ± 337						

FIG. 20A

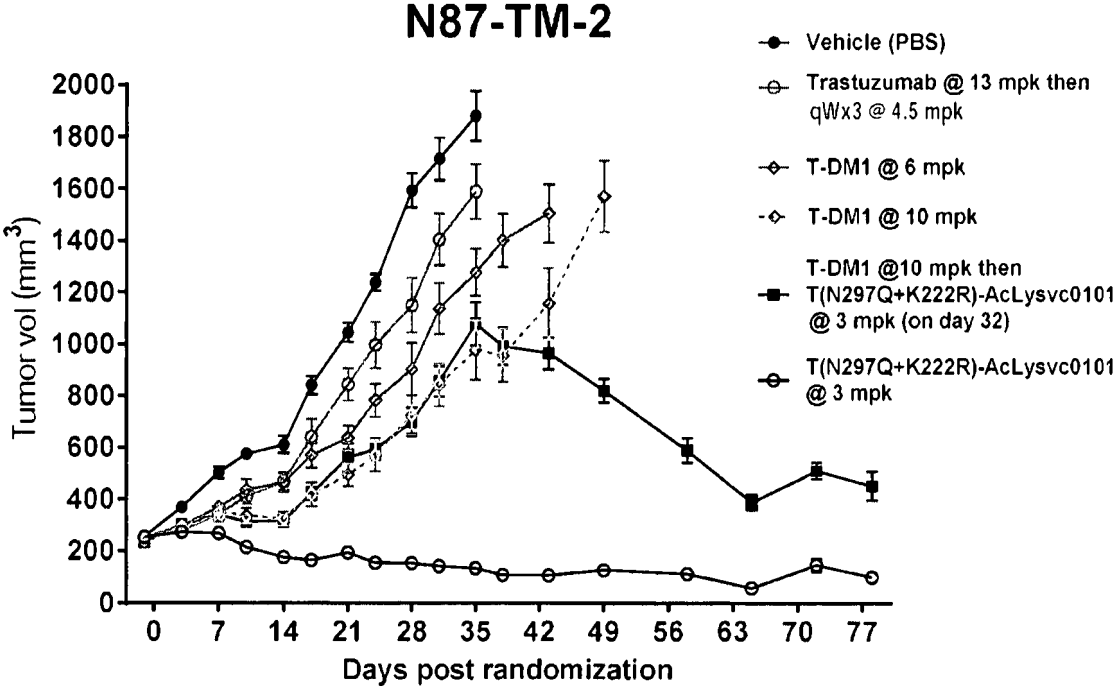
N87



Dose Schedule q4d (days 1,5,9,13)

FIG. 20B

N87-TM-2



Dose Schedule q4d (days 1,5,9,13)

FIG. 20C

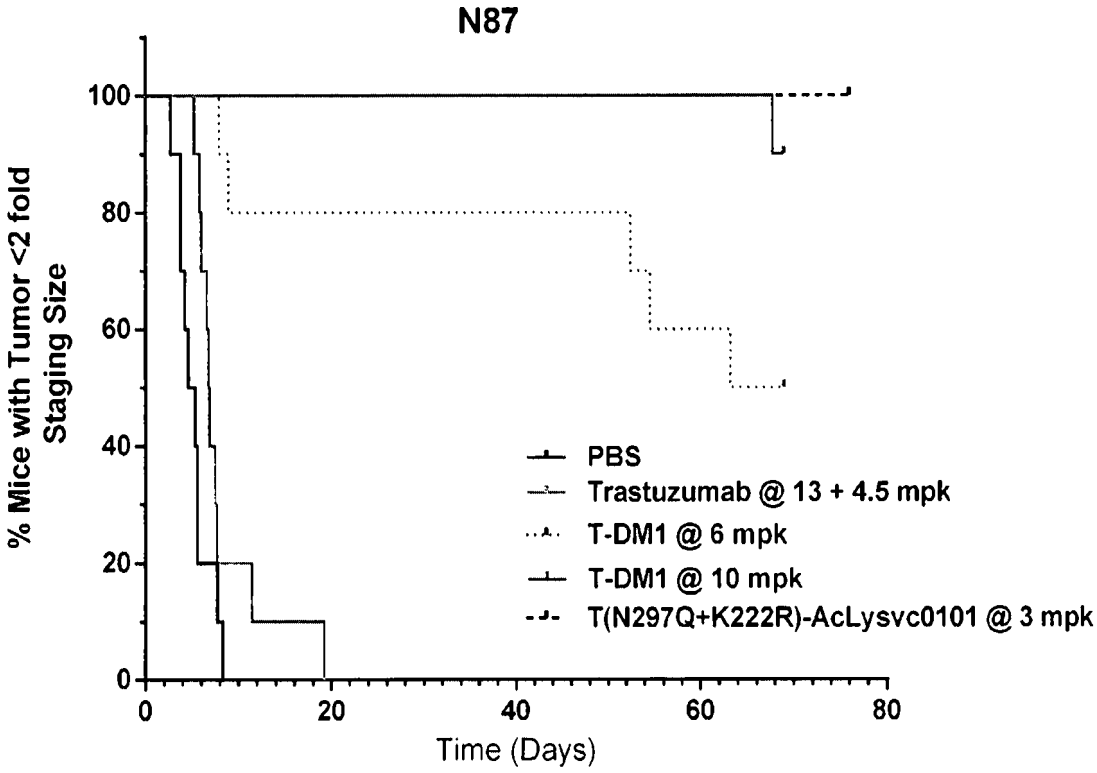


FIG. 20D

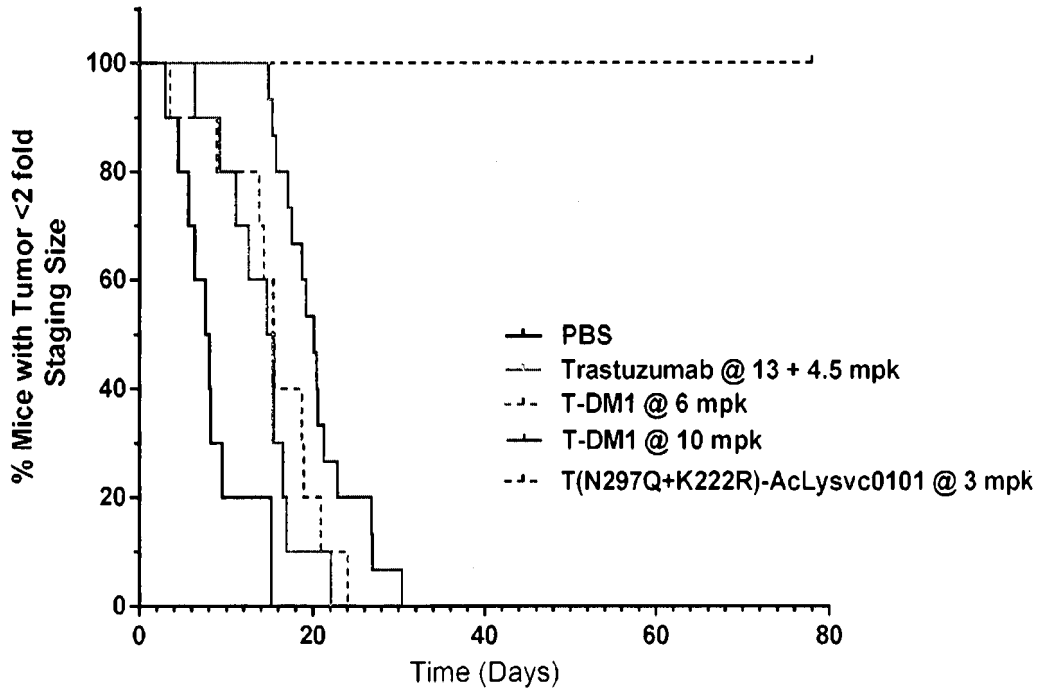
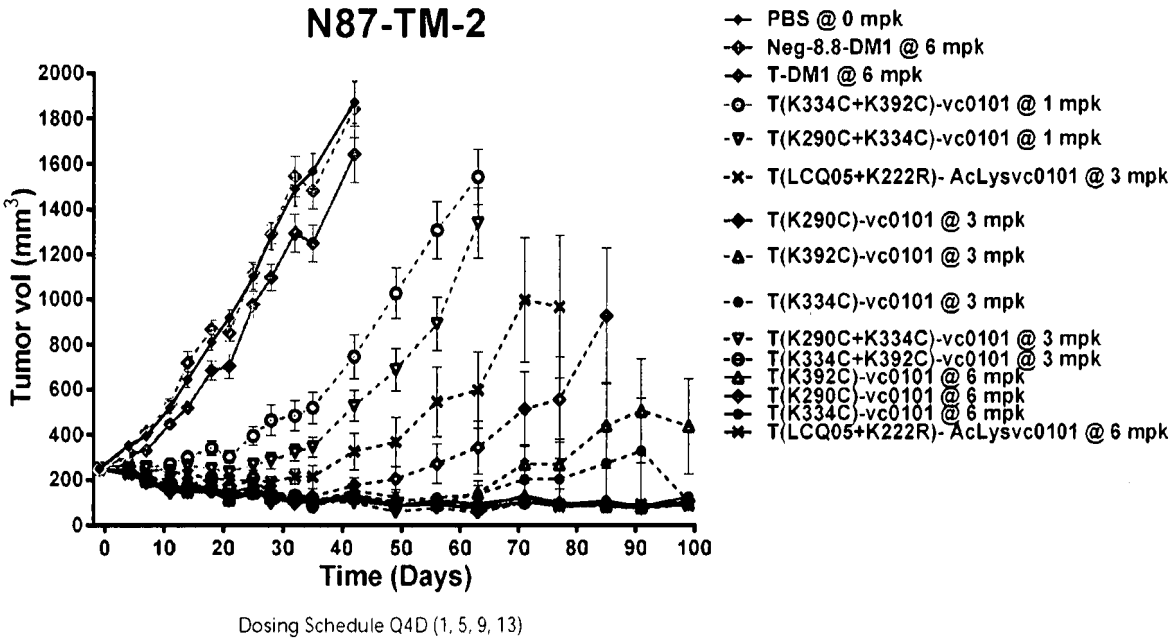


FIG. 20E



Dosing Schedule Q4D (1, 5, 9, 13)

FIG. 20F

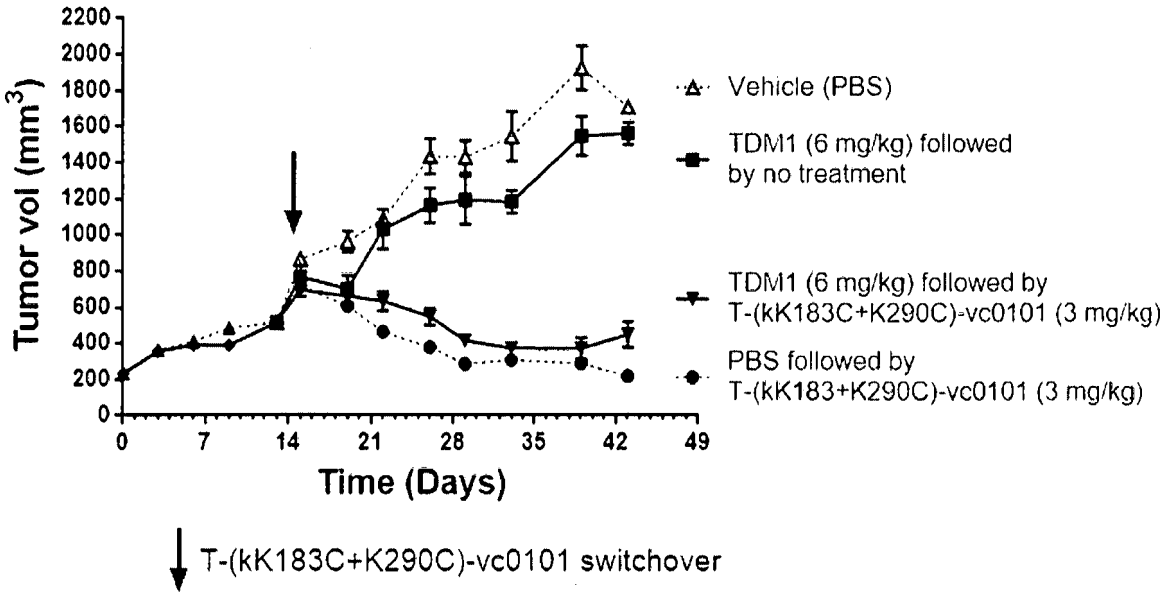


FIG. 21A

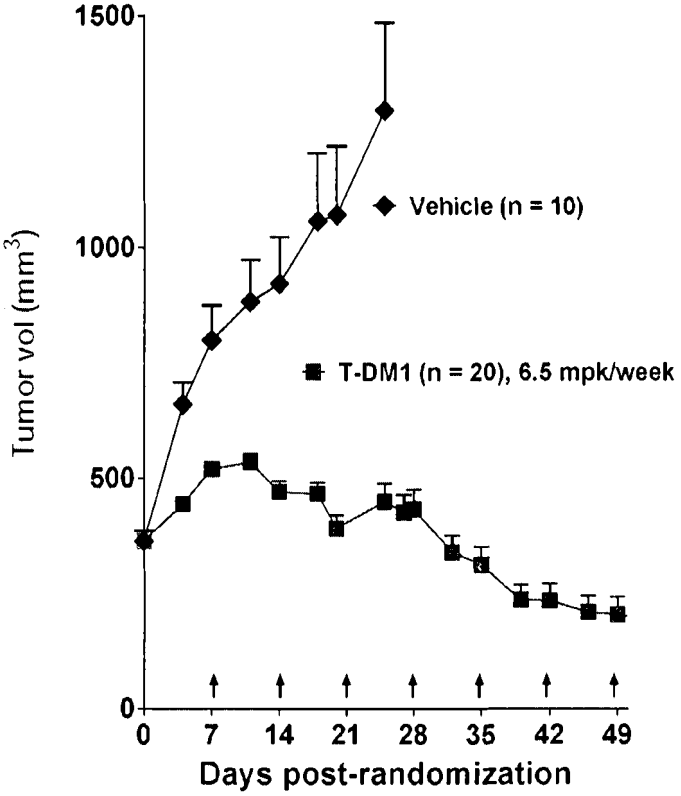


FIG. 21B

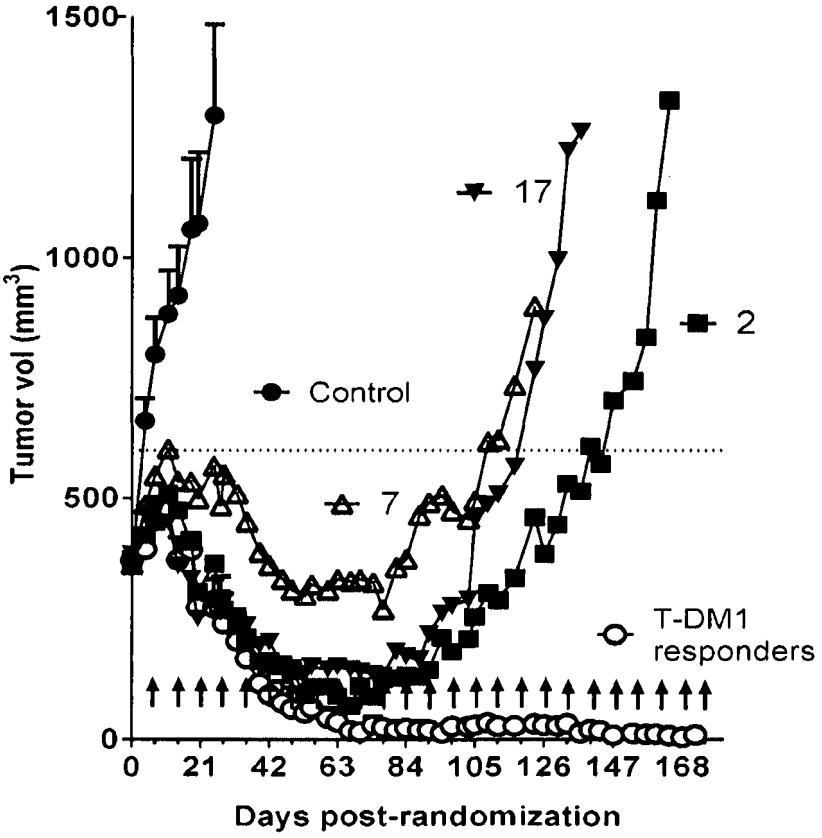


FIG. 21C

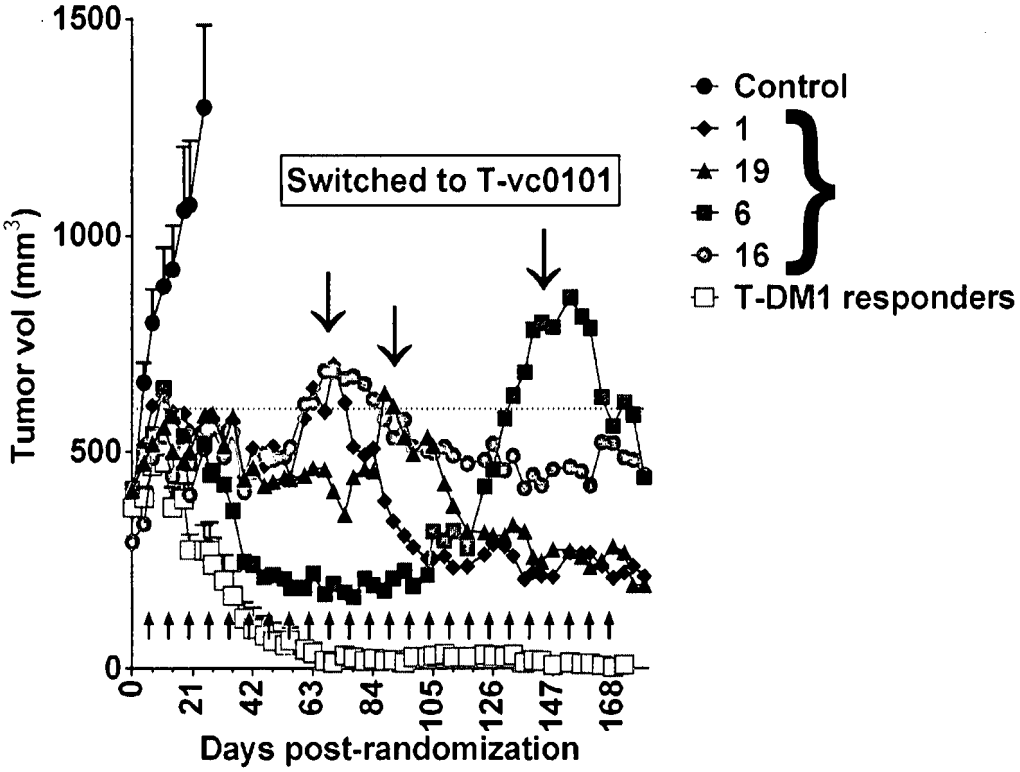


FIG. 21D

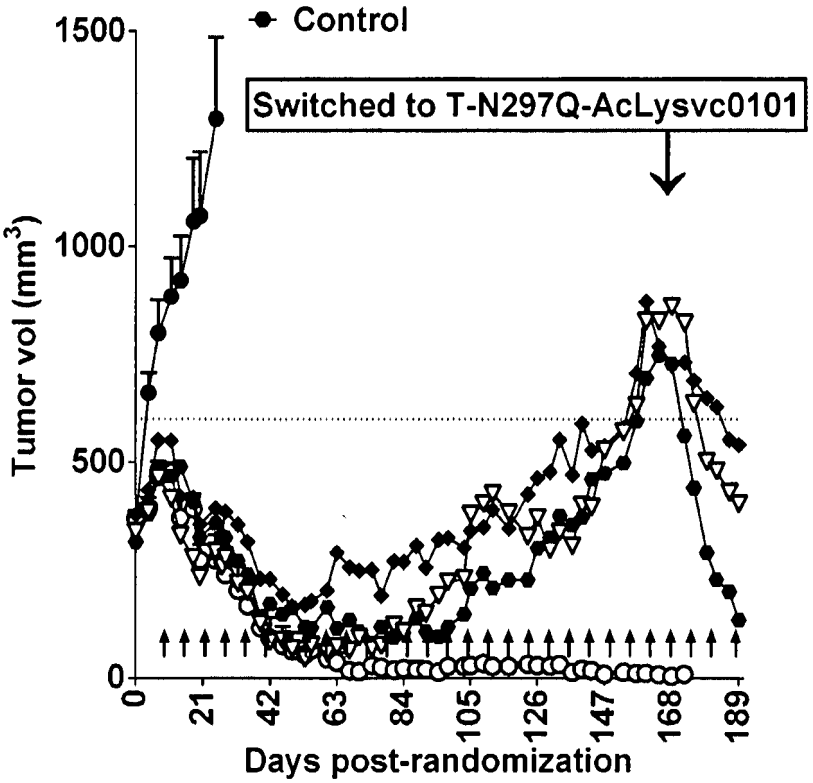


FIG. 21E

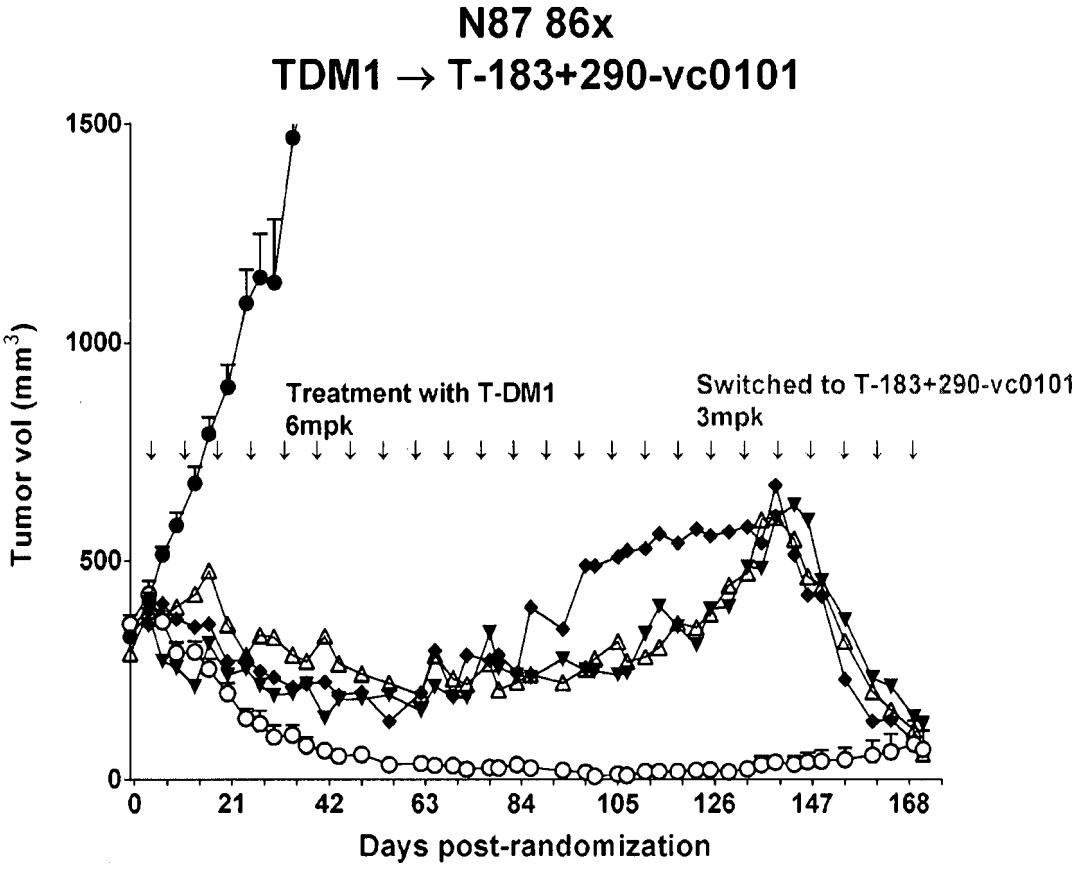

N87 86x
TDM1 → T-183+290-vc0101
Tumor vol (mm3)
1500
1000
500
0
Treatment with T-DM1
6mpk
Switched to T-183+290-vc0101
3mpk
0
21
42
63
84
105
126
147
168
Days post-randomization

FIG. 22A

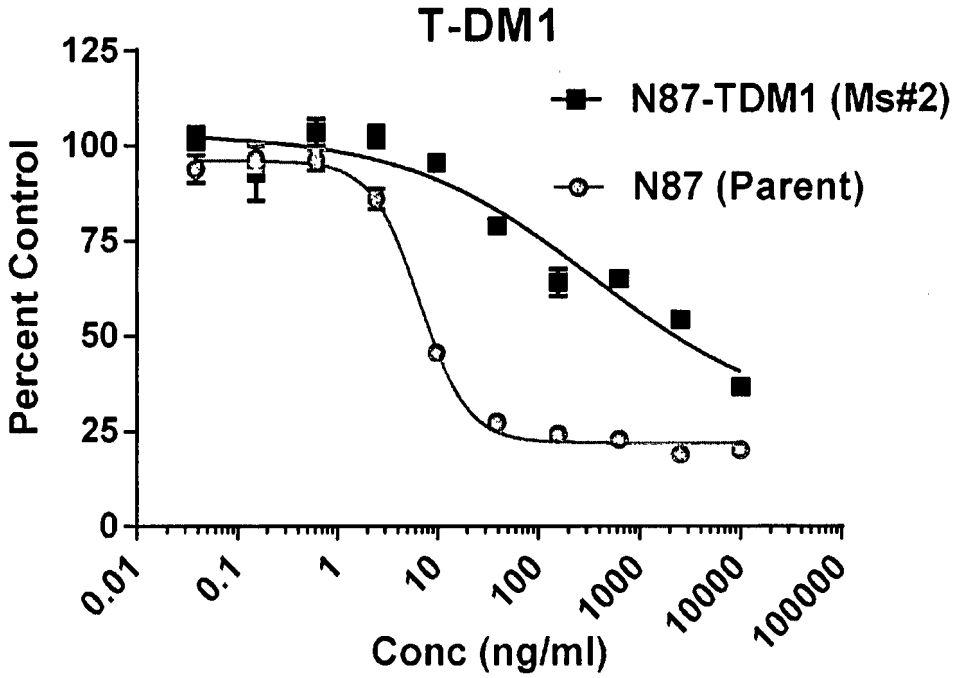


FIG. 22B

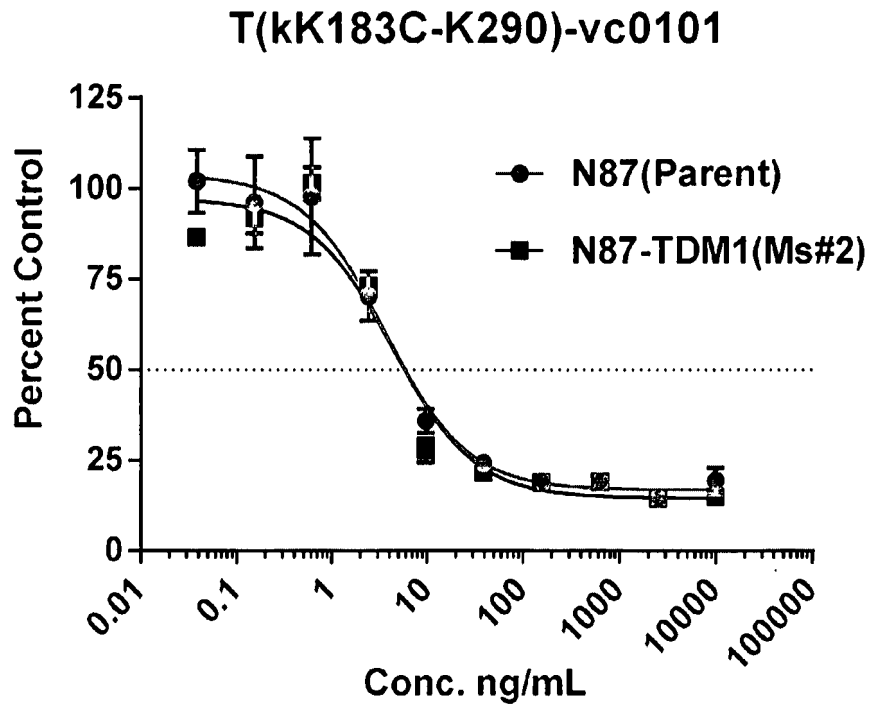


FIG. 22C

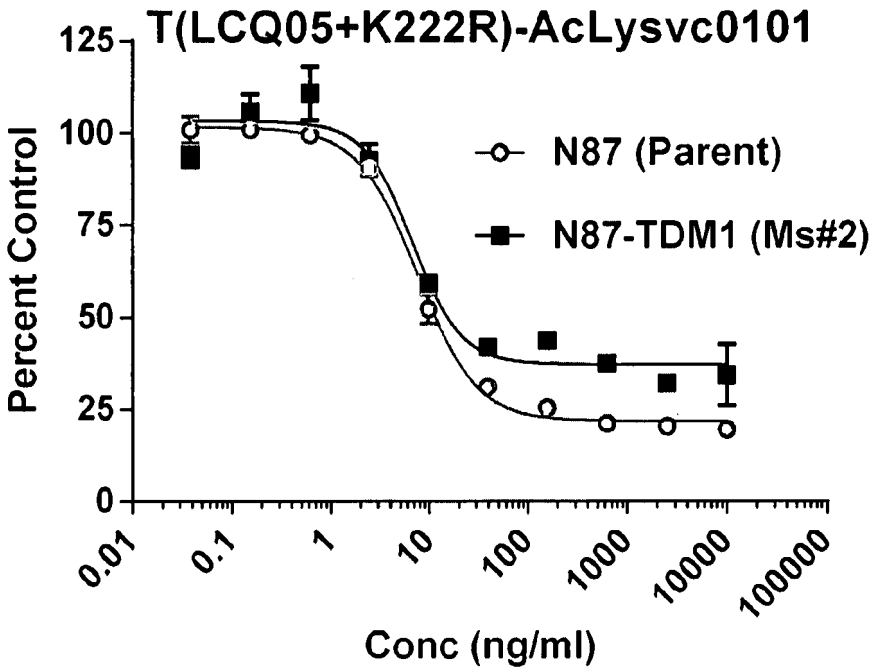


FIG. 22D

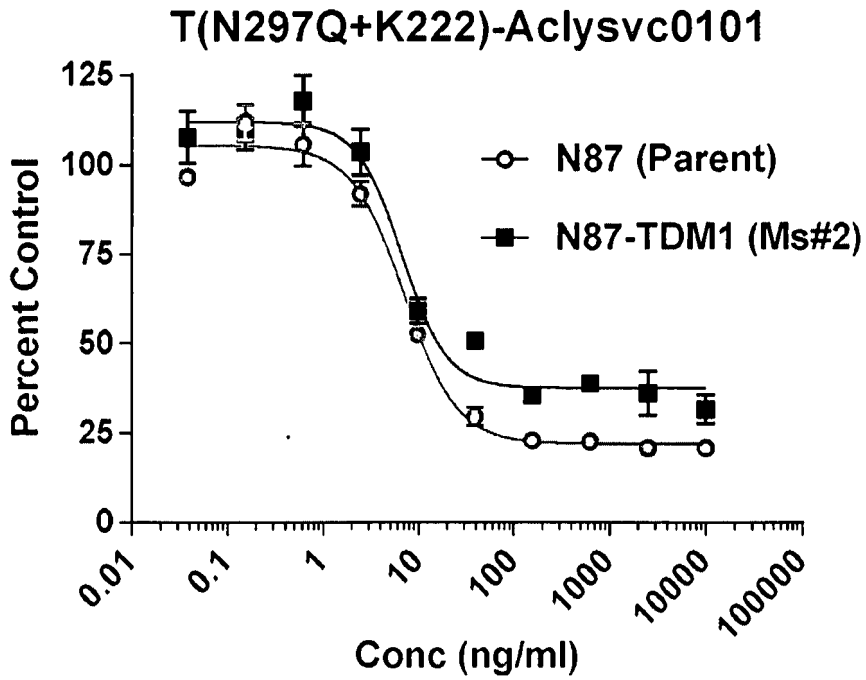


FIG. 23A

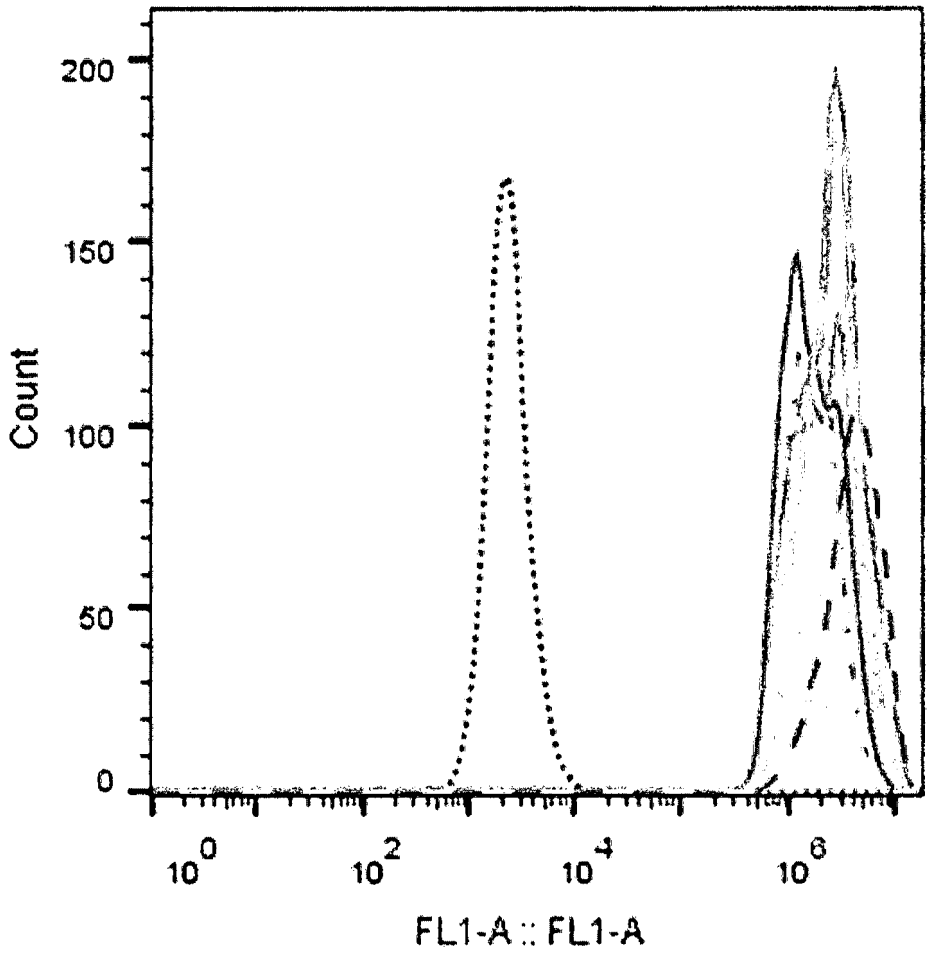


FIG. 23B

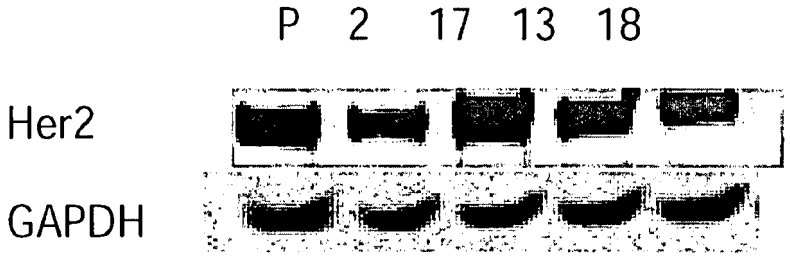


FIG. 24A

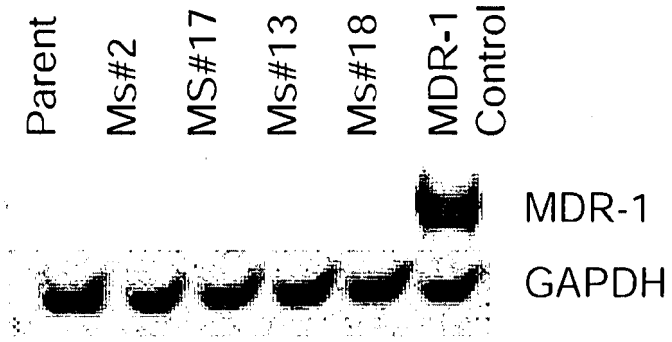


FIG. 24B

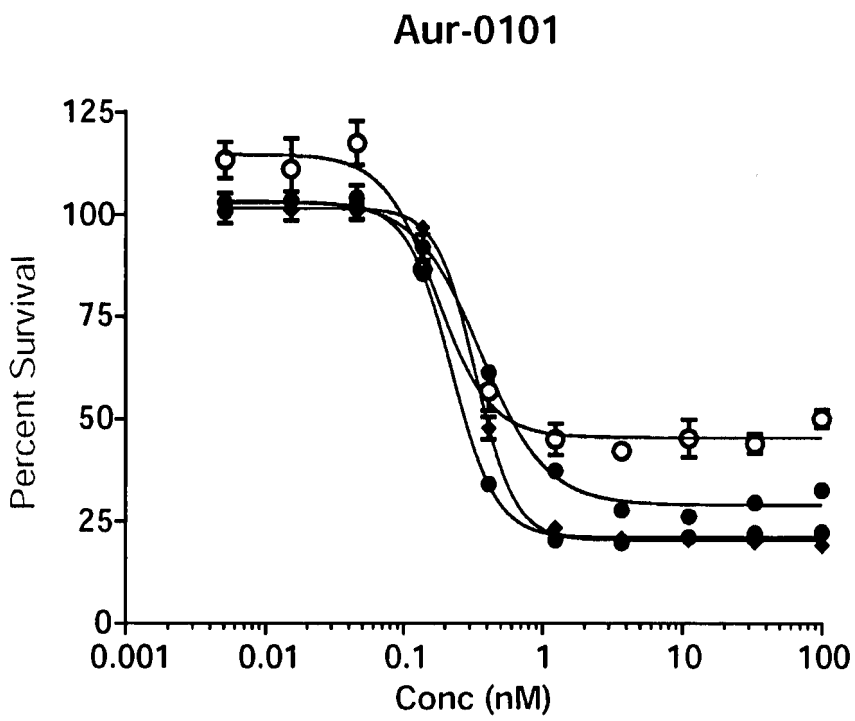


FIG. 24C

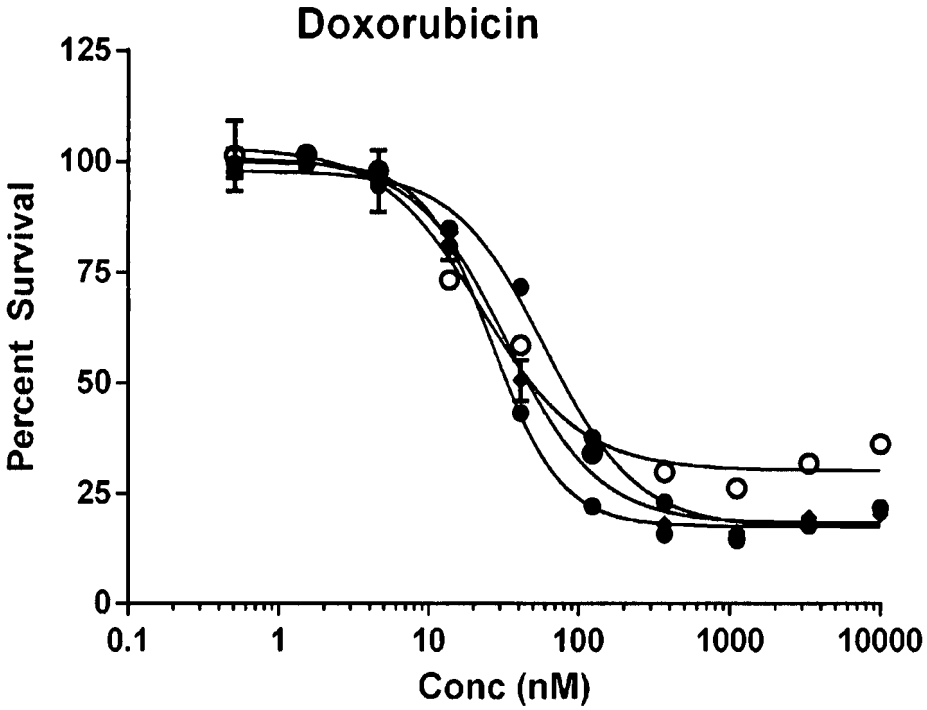


FIG. 24D

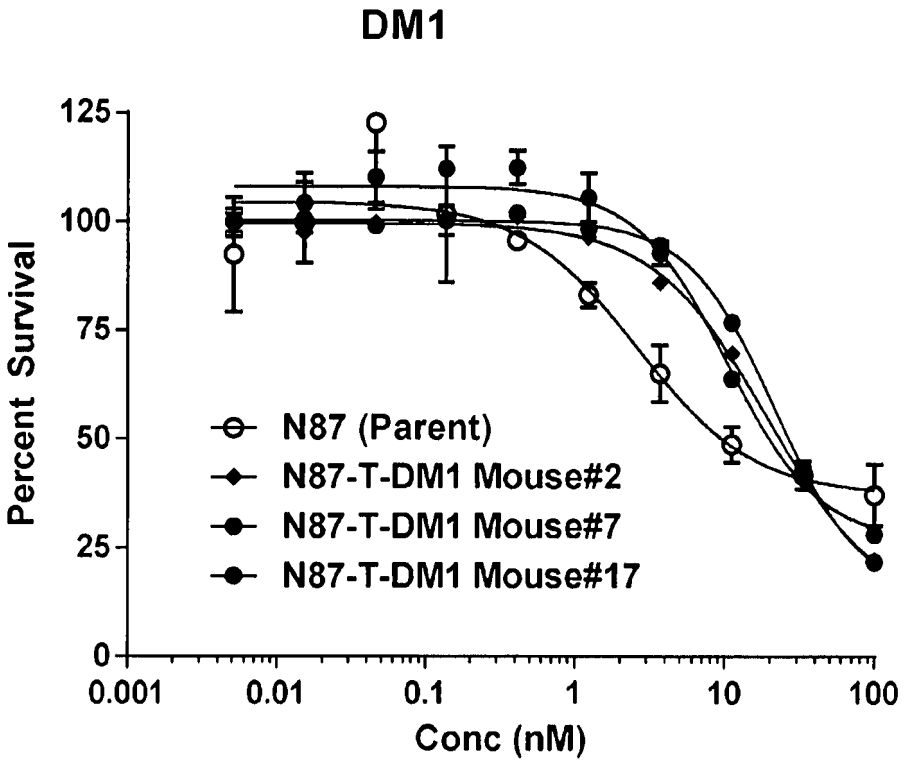


FIG. 25A

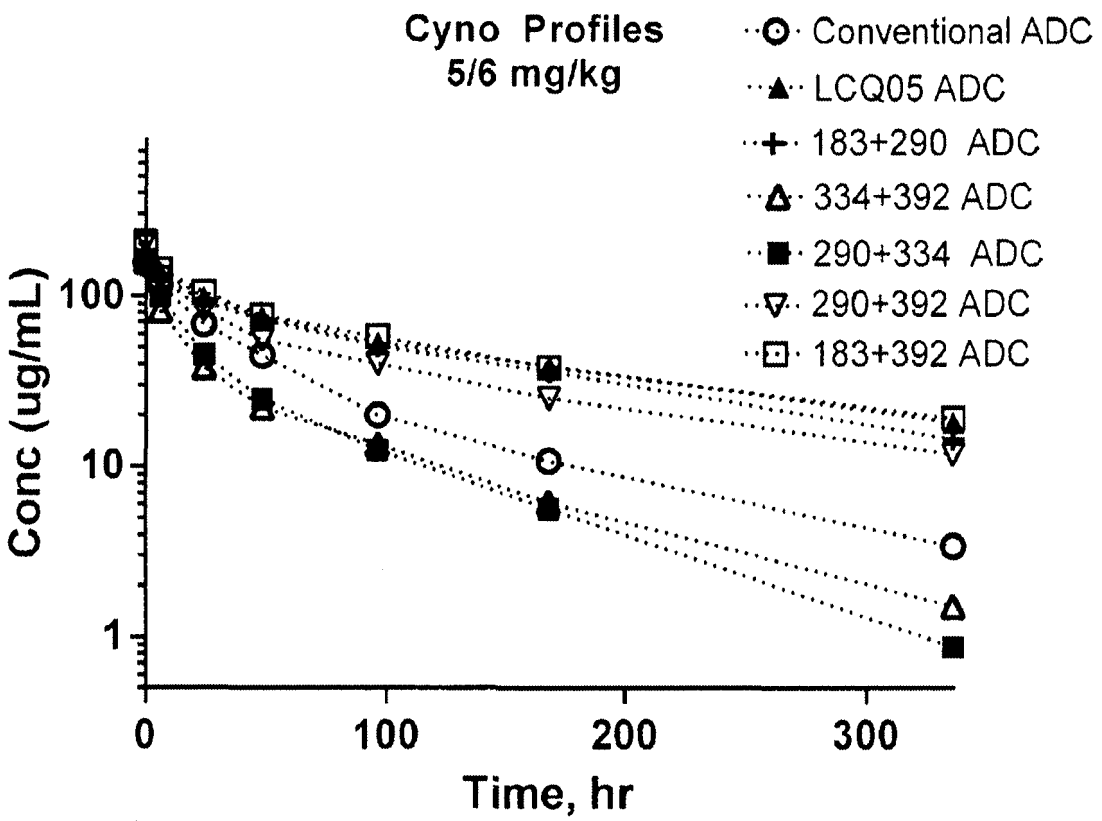
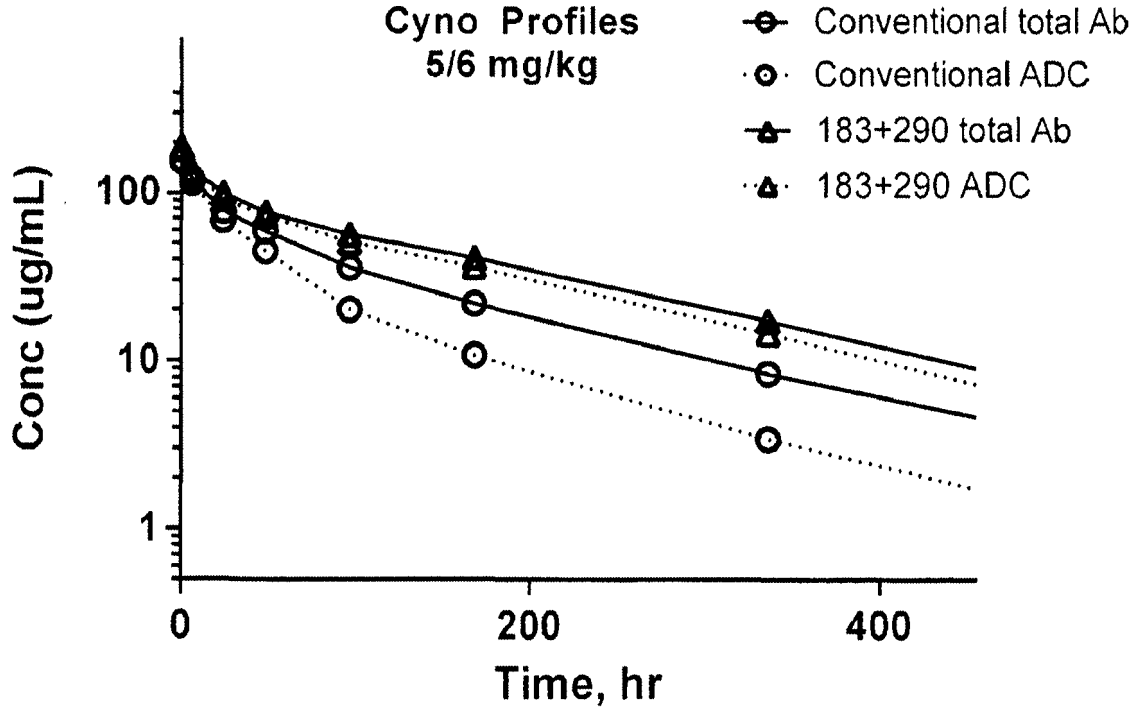


FIG. 25B



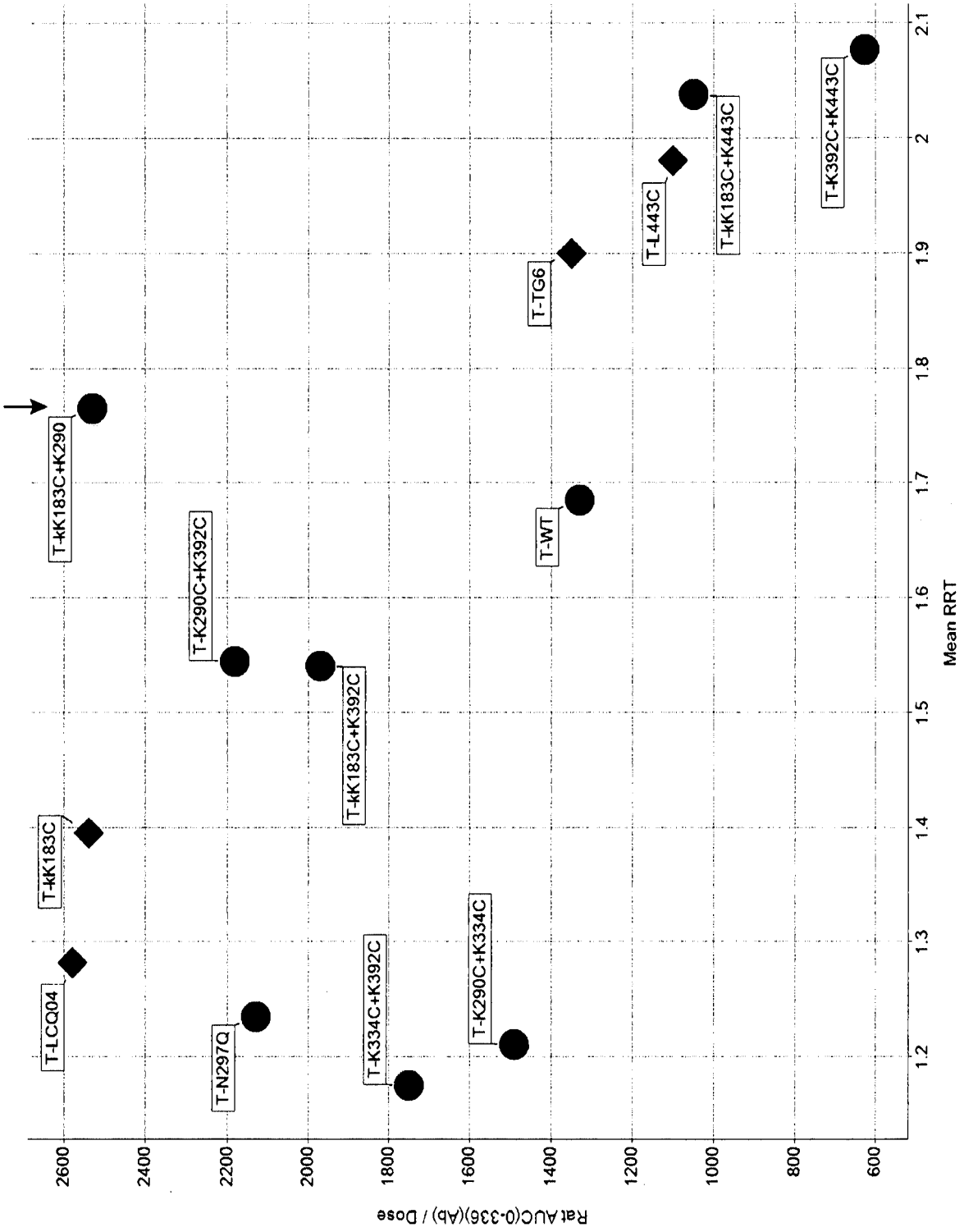

T-LCQ04
T-kK183C
T-kK183C+K290
T-N297Q
T-K334C+K392C
T-K290C+K334C
T-K290C+K392C
T-kK183C+K392C
T-WT
T-TG6
T-L443C
T-kK183C+K443C
T-K392C+K443C
Rat AUC(0-336)(Ab) / Dose
Mean RRT
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
2.1

FIG. 26

FIG. 27

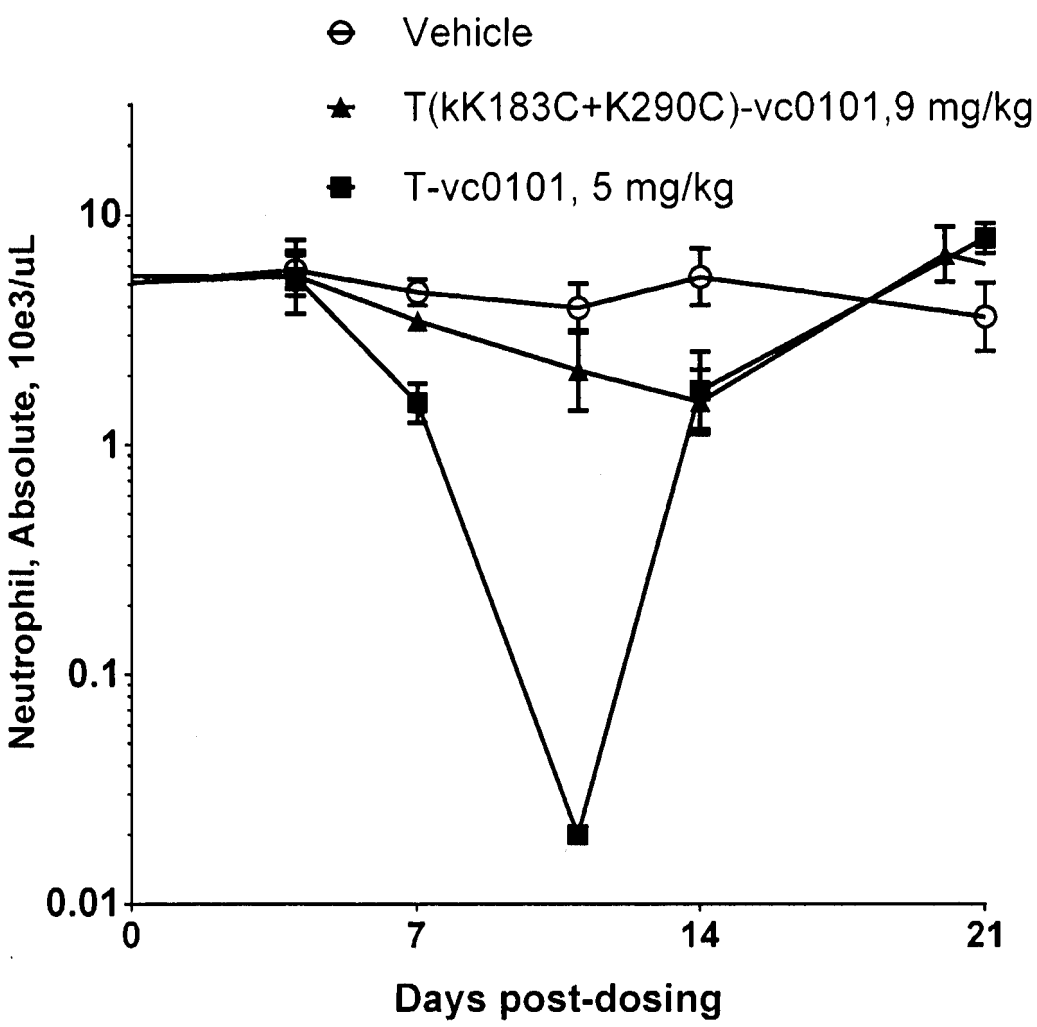

Vehicle
T(kK183C+K290C)-vc0101,9 mg/kg
T-vc0101, 5 mg/kg
Neutrophil, Absolute, 10e3/uL
10
1
0.1
0.01
0
7
14
21
Days post-dosing

FIG. 28A

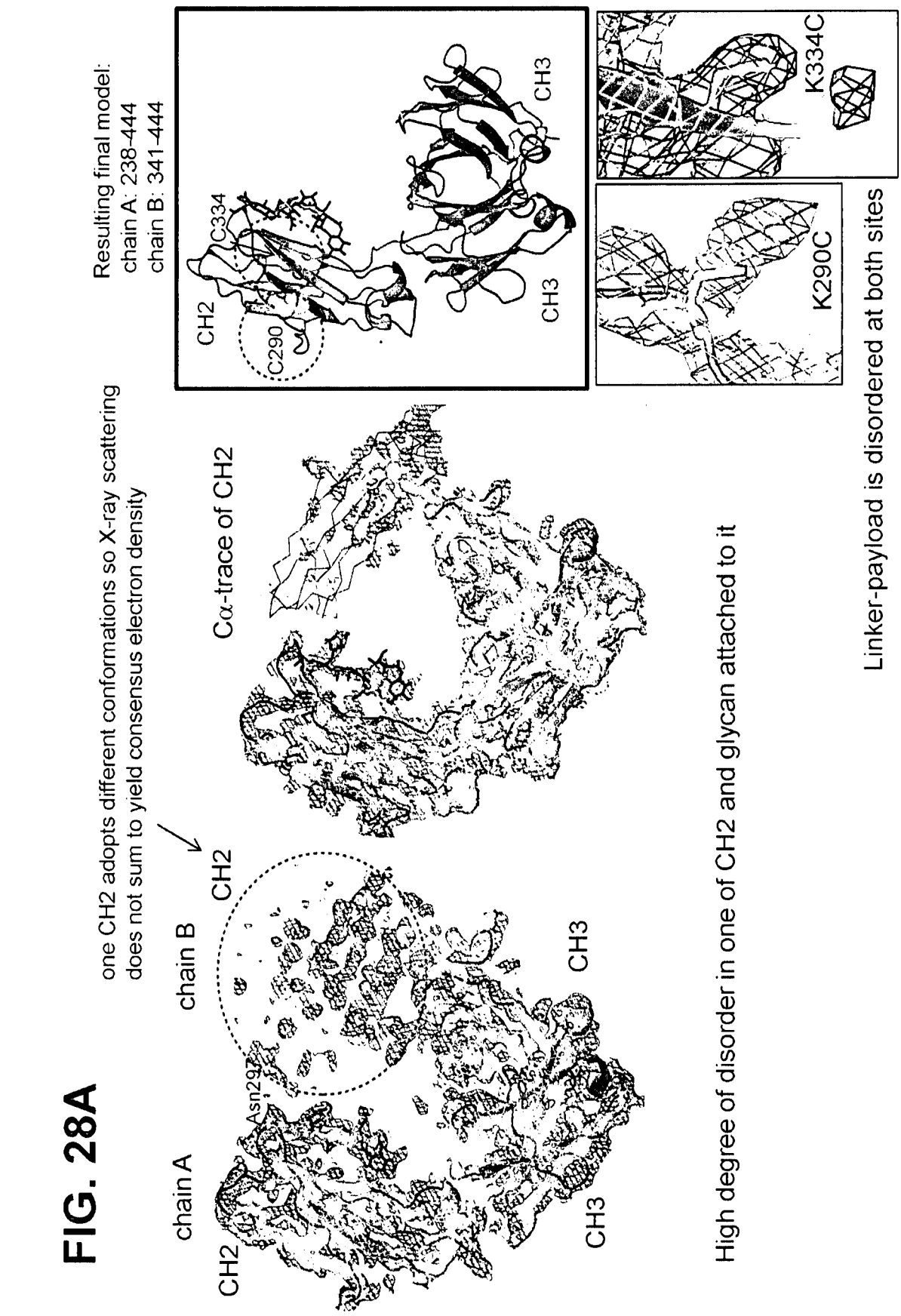

chain A
chain B
CH2
CH3
CH3
Asn297
one CH2 adopts different conformations so X-ray scattering does not sum to yield consensus electron density
Cα-trace of CH2
High degree of disorder in one of CH2 and glycan attached to it
Resulting final model:
chain A: 238-444
chain B: 341-444
CH2
C334
C290
CH3
CH3
K290C
K334C
Linker-payload is disordered at both sites

FIG. 28B

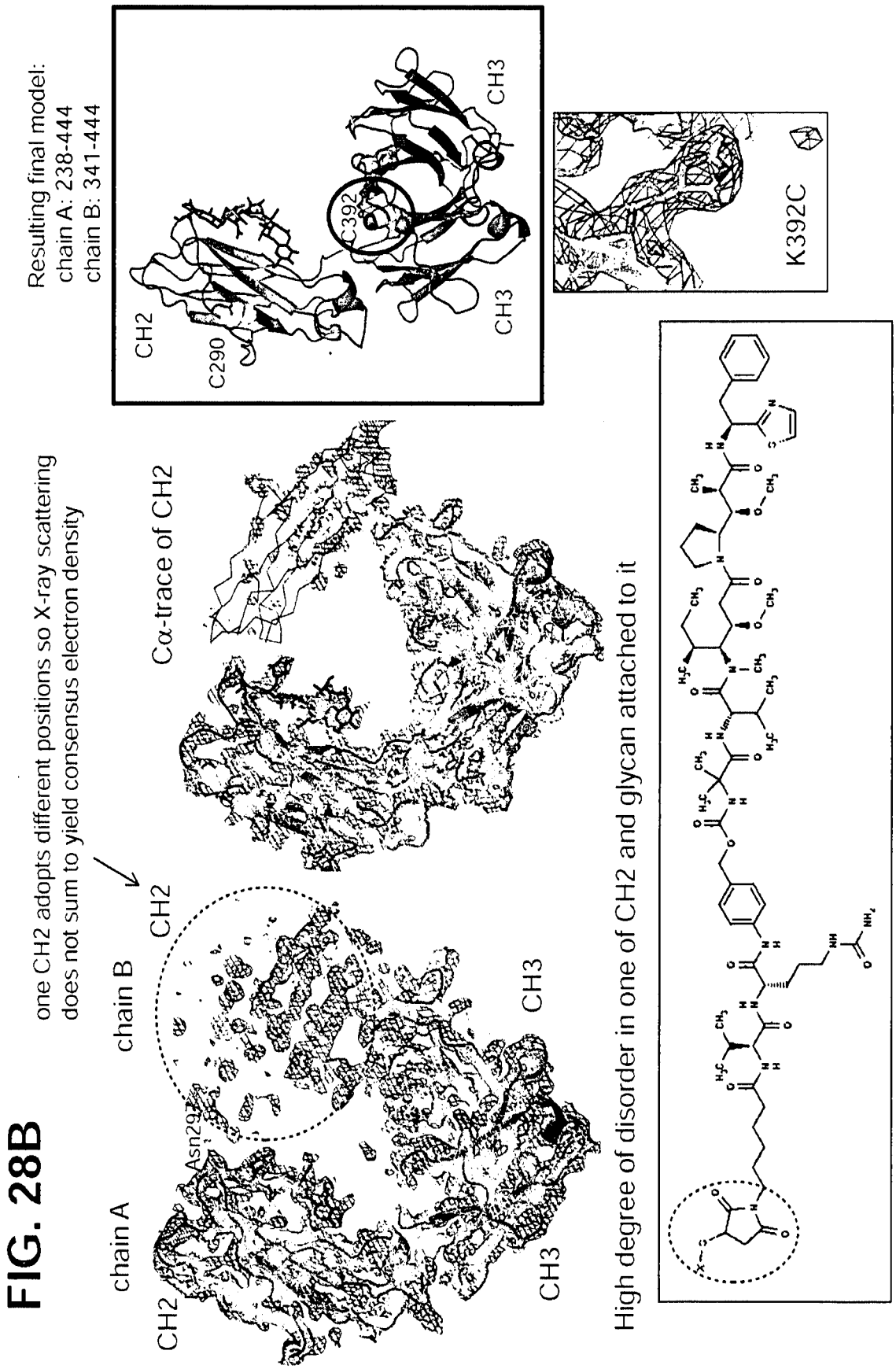

chain A
chain B
CH2
CH3
Asn297
one CH2 adopts different positions so X-ray scattering does not sum to yield consensus electron density
Cα-trace of CH2
Resulting final model:
chain A: 238-444
chain B: 341-444
C290
C392
K392C
High degree of disorder in one of CH2 and glycan attached to it

FIG. 28C

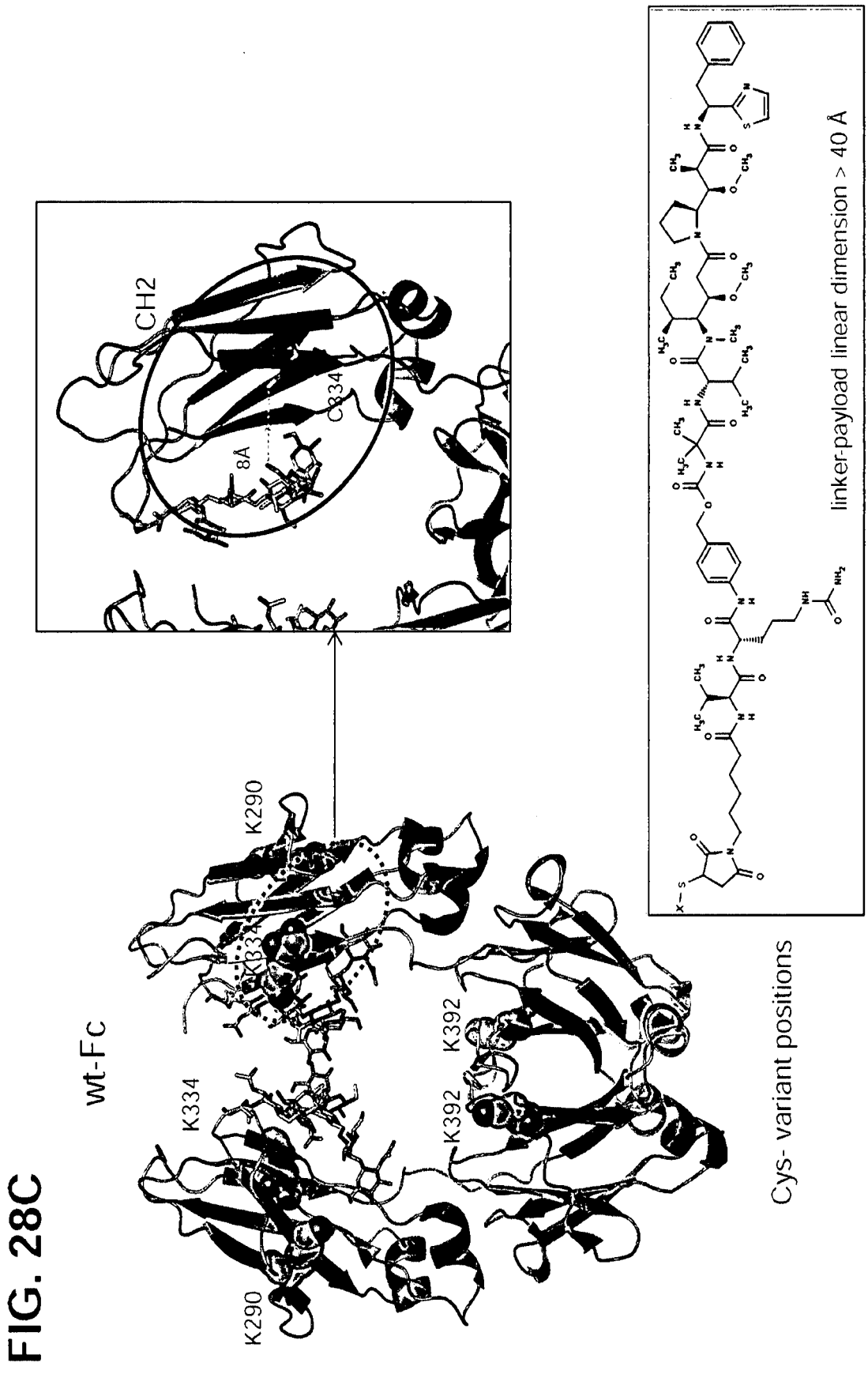

wt-Fc
K290
K334
K392
K290
K334
K392
CH2
8A
C334
Cys- variant positions
linker-payload linear dimension > 40 Å

FIG. 29

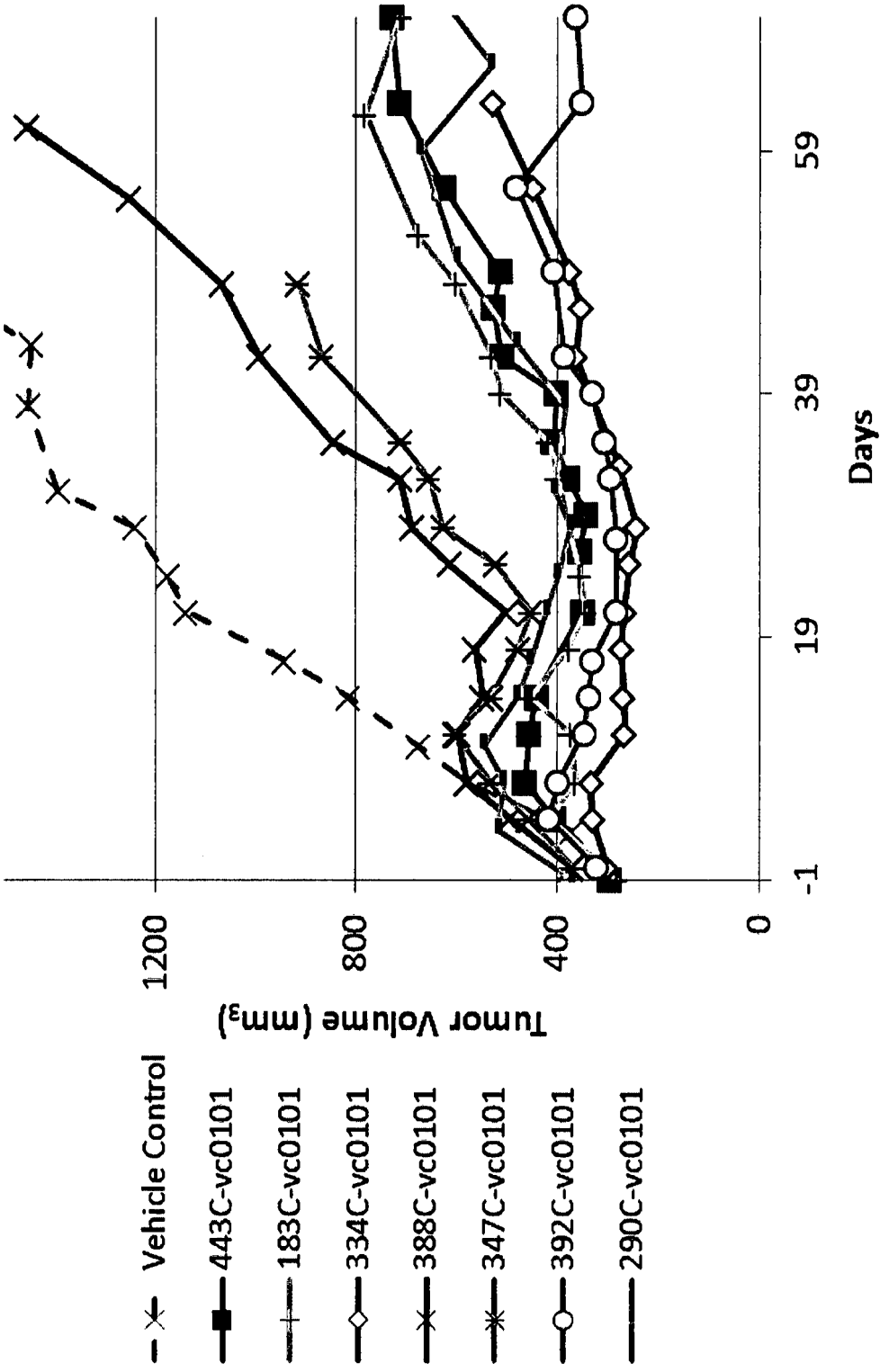
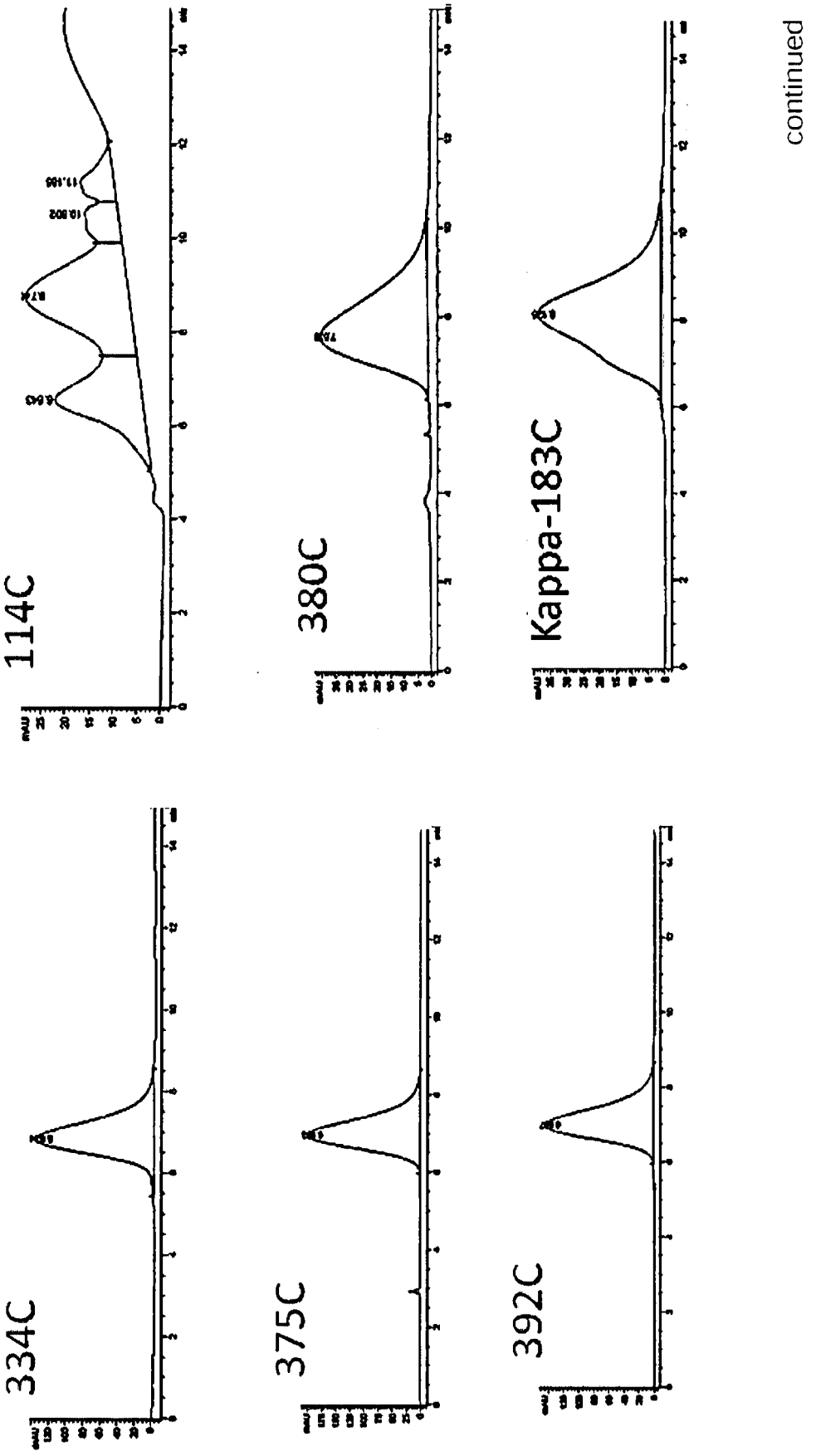

Tumor Volume (mm3)
1200
800
400
0
-1
19
39
59
Days
Vehicle Control
443C-vc0101
183C-vc0101
334C-vc0101
388C-vc0101
347C-vc0101
392C-vc0101
290C-vc0101

FIG. 30



continued

FIG. 30 continued

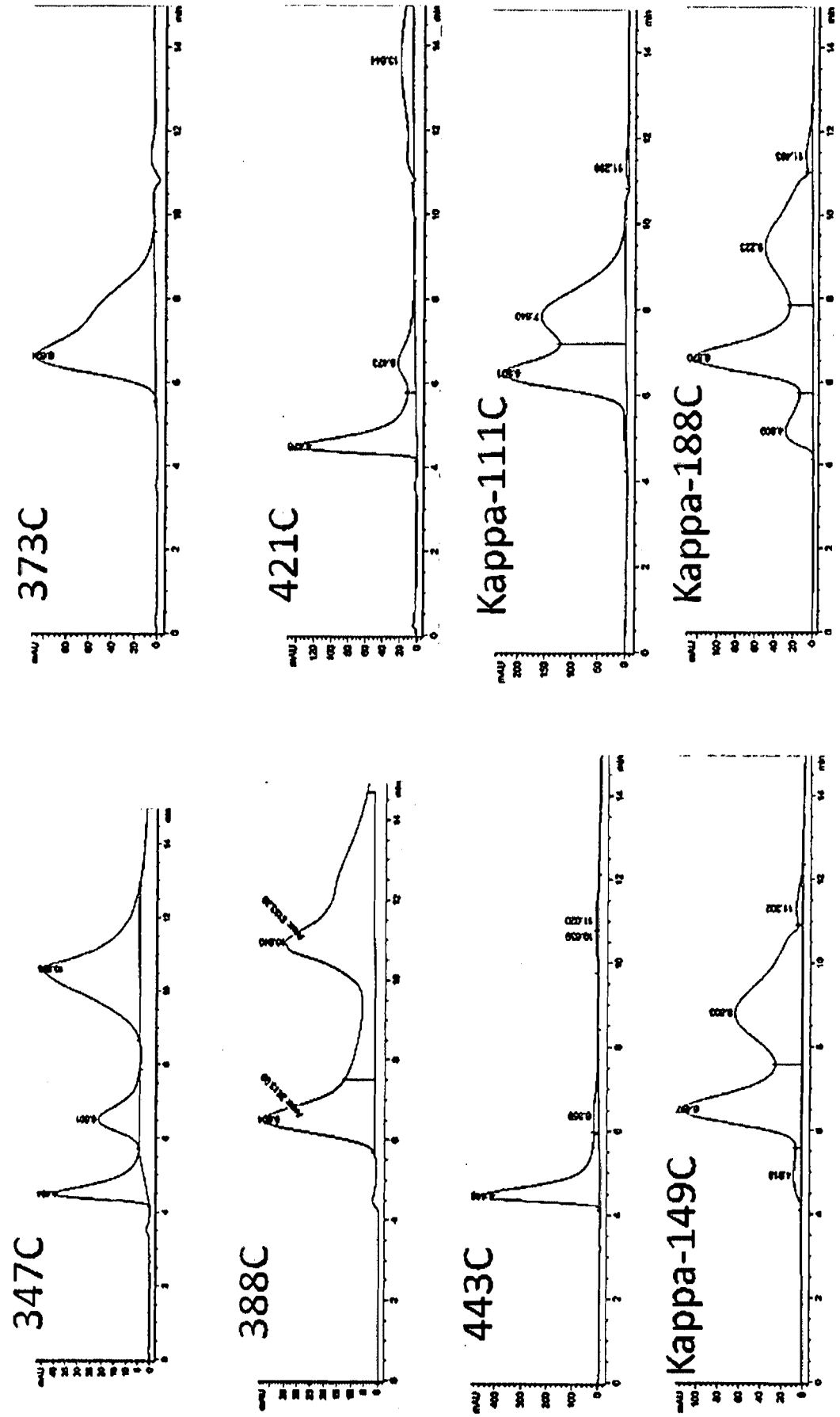


FIG. 31

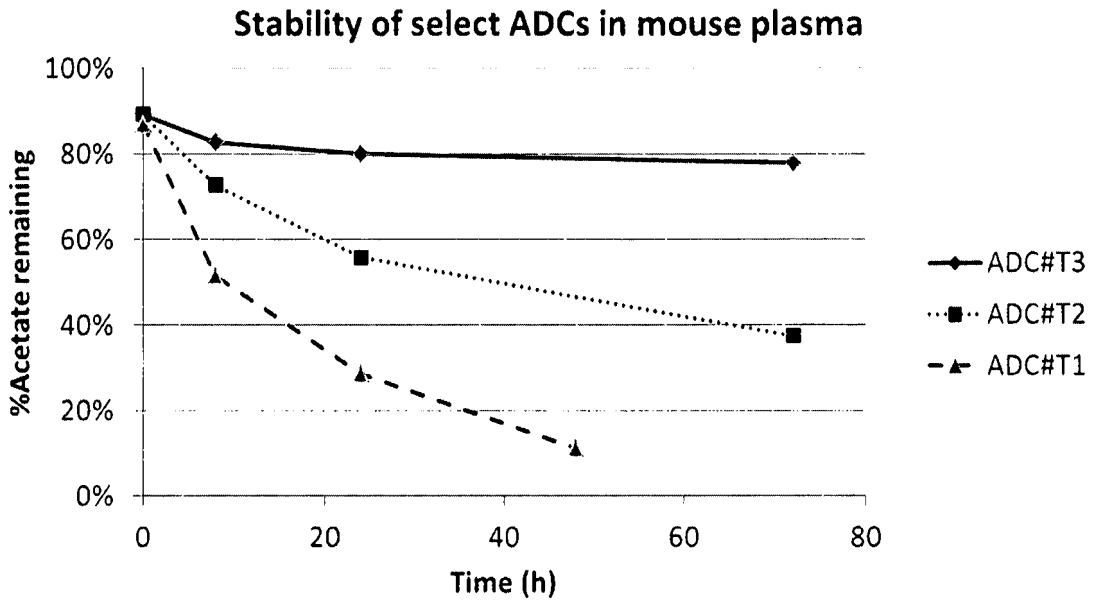

Stability of select ADCs in mouse plasma
100%
80%
60%
40%
20%
0%
%Acetate remaining
0
20
40
60
80
Time (h)
ADC#T3
ADC#T2
ADC#T1

FIG. 32

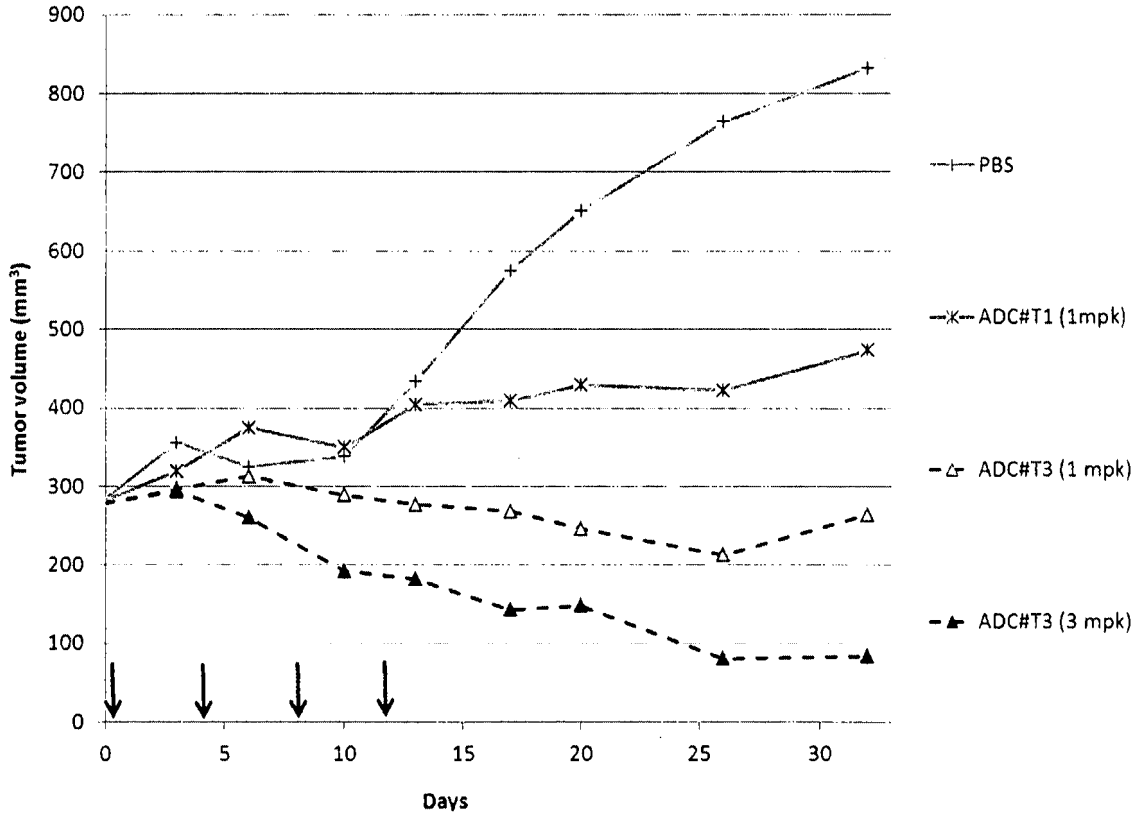

900
800
700
600
500
400
300
200
100
0
Tumor volume (mm³)
0
5
10
15
20
25
30
Days
PBS
ADC#T1 (1mpk)
ADC#T3 (1 mpk)
ADC#T3 (3 mpk)

FIG. 33

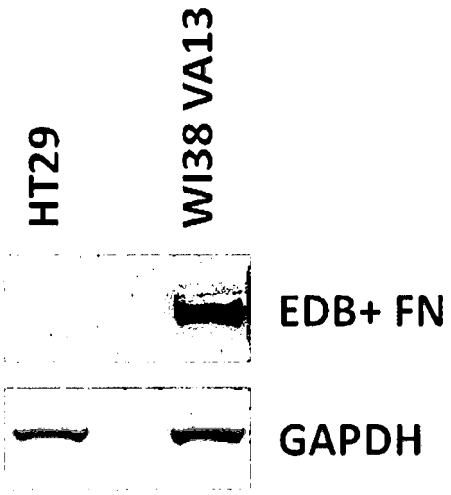


FIG. 34A

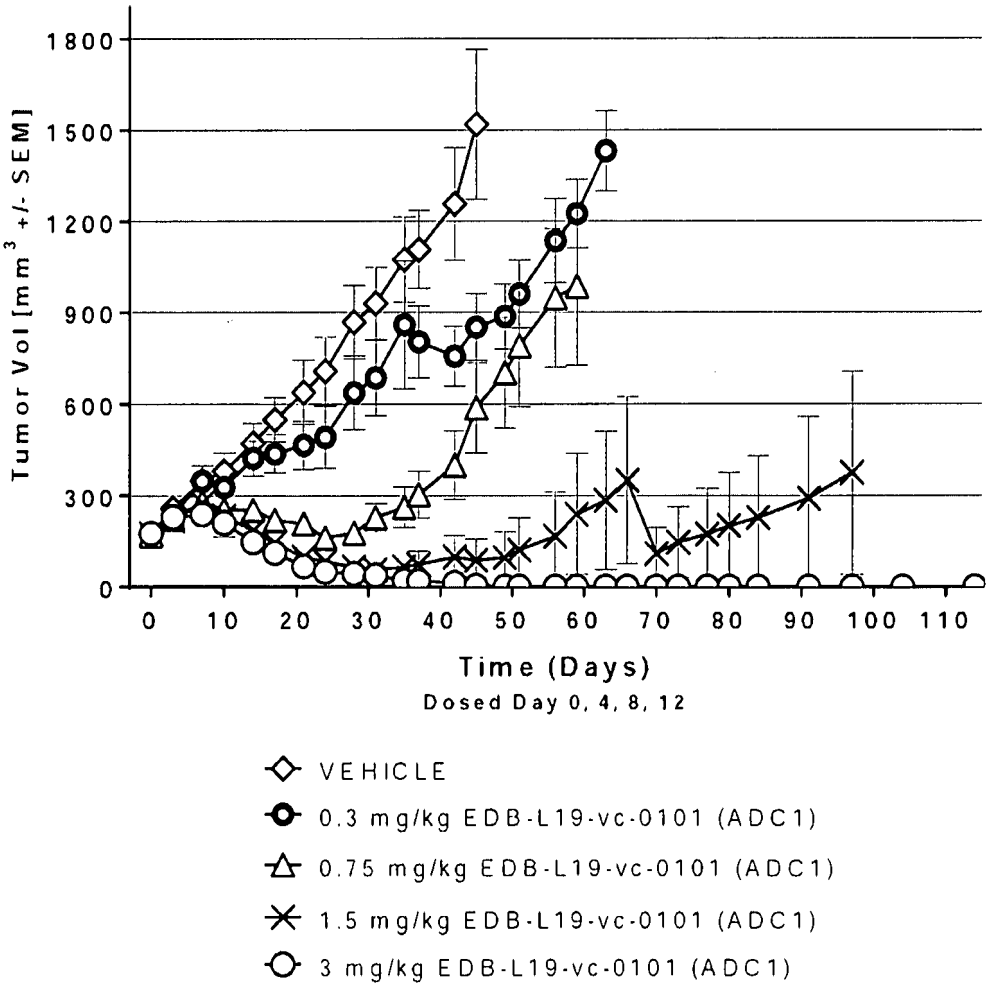


FIG. 34B

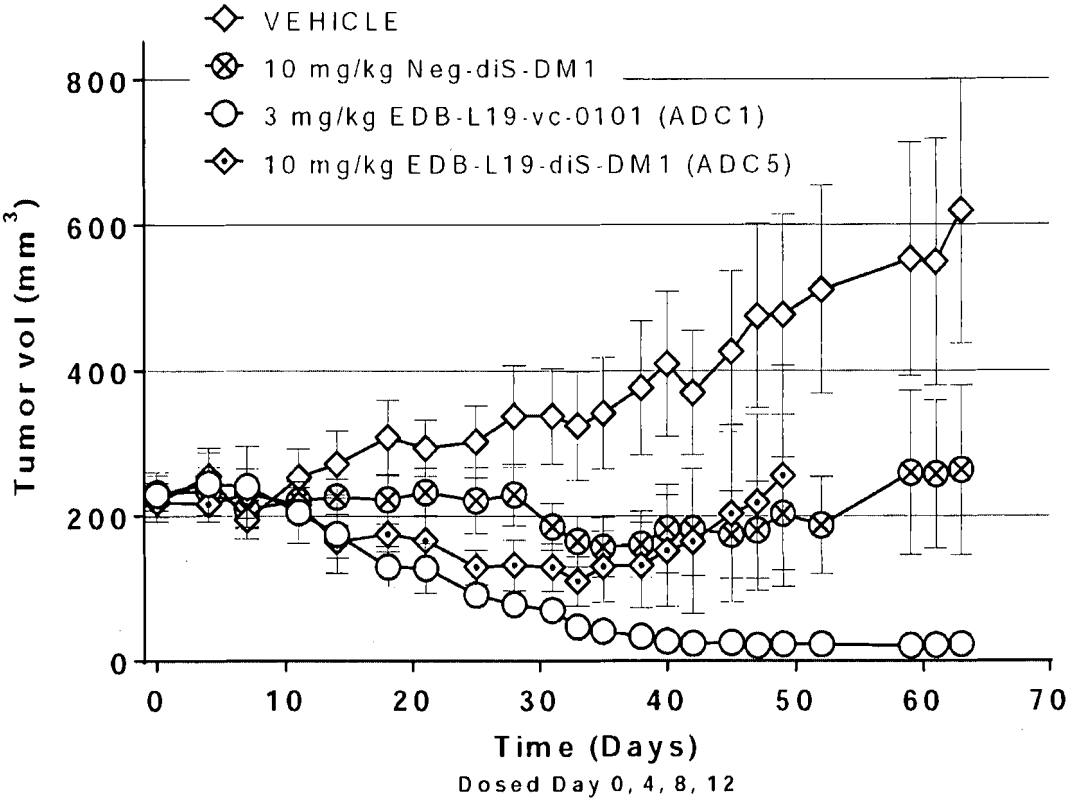


FIG. 34C

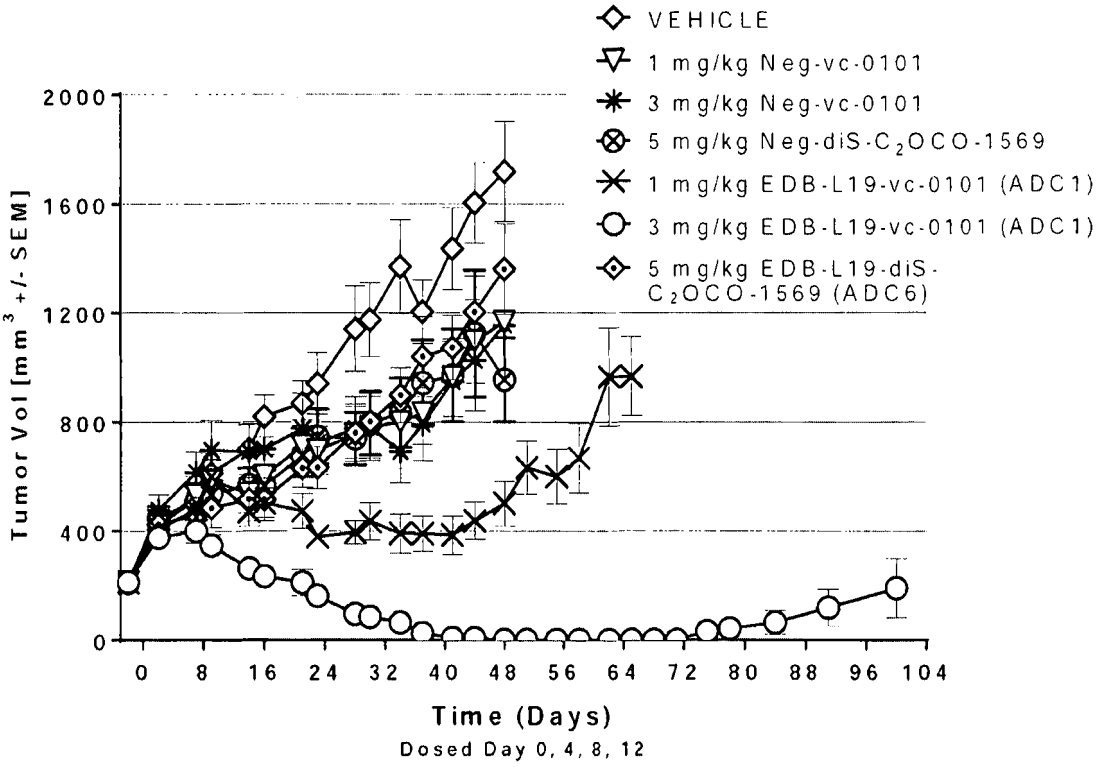


FIG. 34D

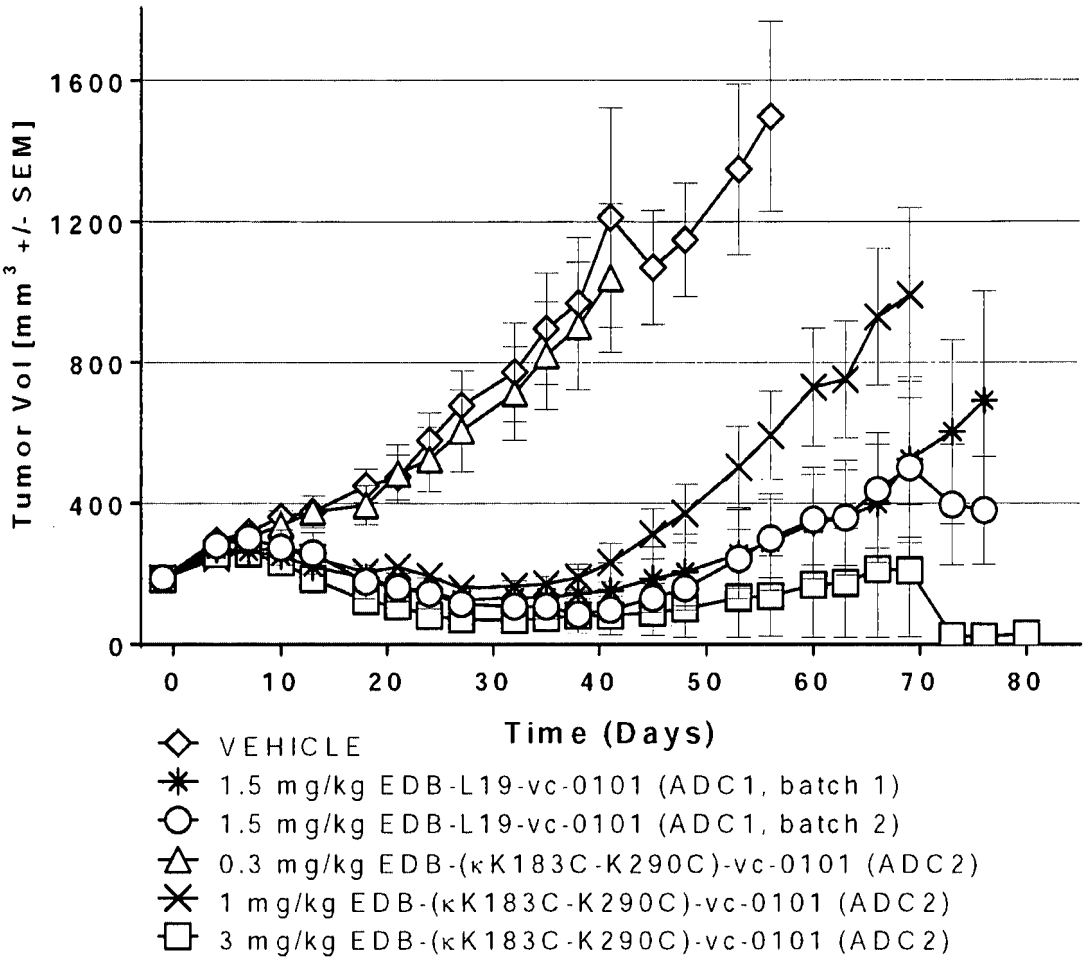

Tumor Vol [mm3 +/- SEM]
1600
1200
800
400
0
0
10
20
30
40
50
60
70
80
Time (Days)
VEHICLE
1.5 mg/kg EDB-L19-vc-0101 (ADC1, batch 1)
1.5 mg/kg EDB-L19-vc-0101 (ADC1, batch 2)
0.3 mg/kg EDB-(κK183C-K290C)-vc-0101 (ADC2)
1 mg/kg EDB-(κK183C-K290C)-vc-0101 (ADC2)
3 mg/kg EDB-(κK183C-K290C)-vc-0101 (ADC2)

FIG. 34E

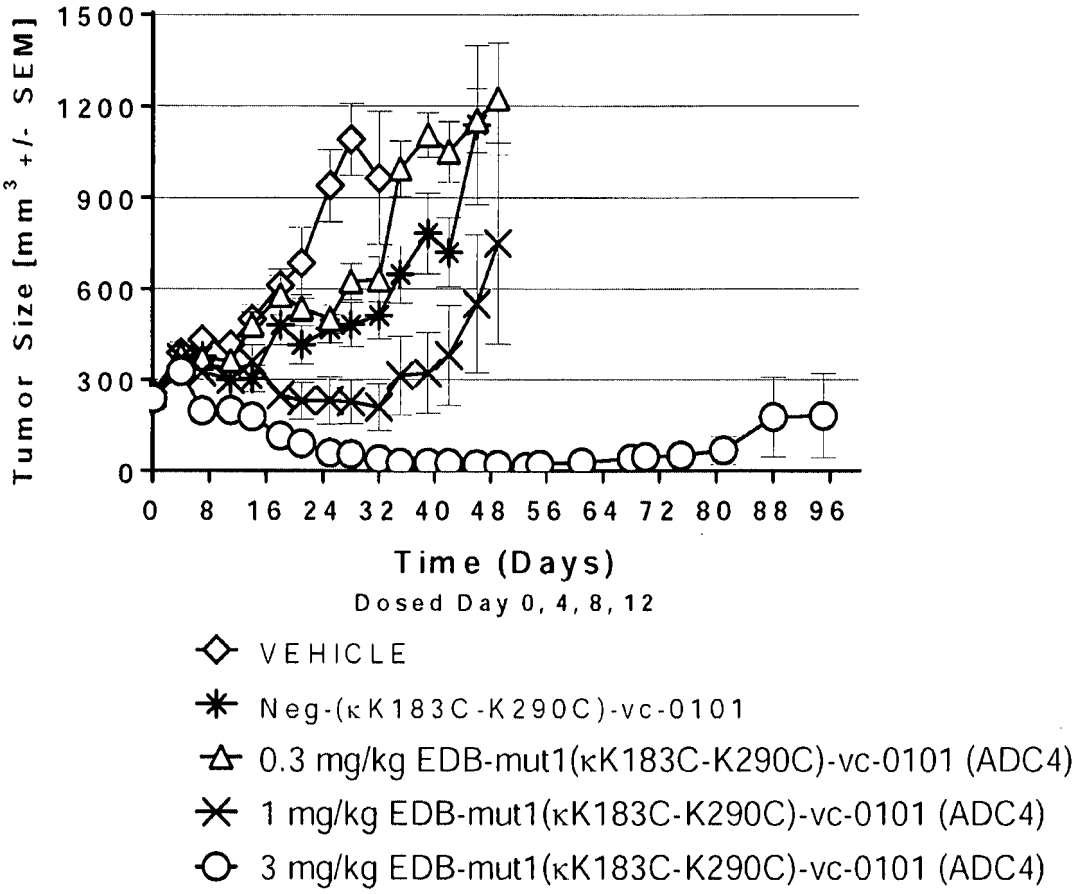


FIG. 34F

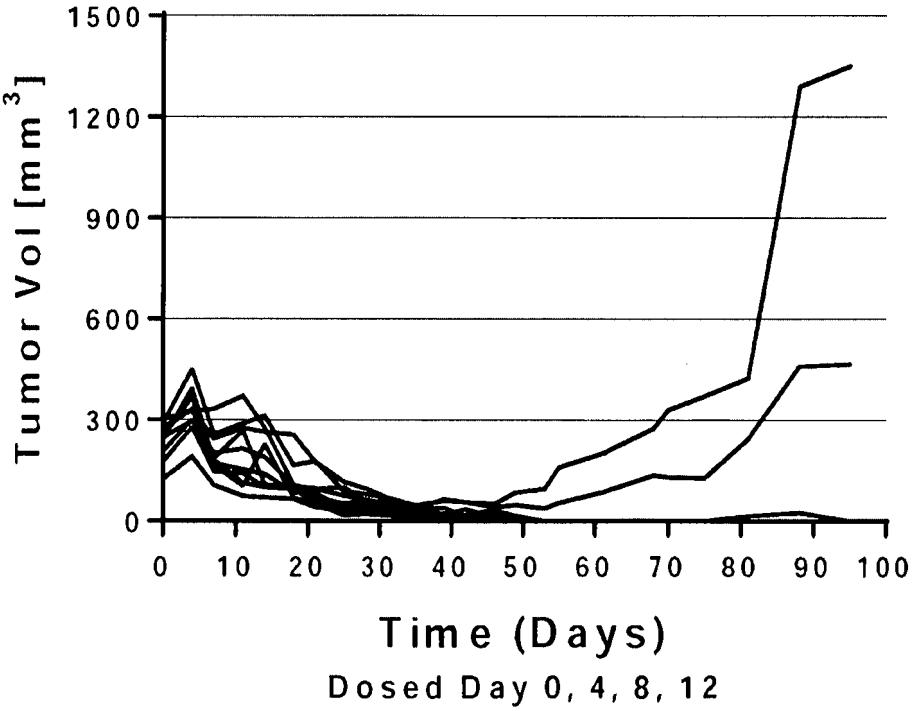


FIG. 35A

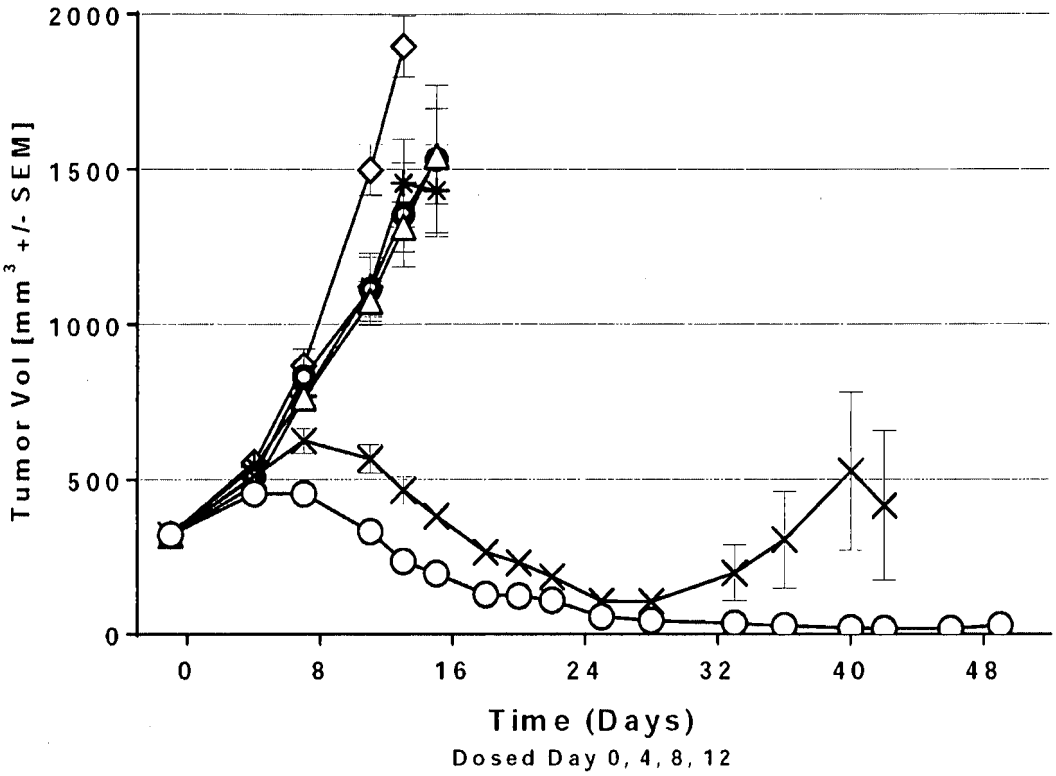

2000
1500
1000
500
0
Tumor Vol [mm3 +/- SEM]
0
8
16
24
32
40
48
Time (Days)
Dosed Day 0, 4, 8, 12
VEHICLE
3 mg/kg Neg-vc-0101
0.3 mg/kg EDB-L19-vc-0101 (ADC1)
0.75 mg/kg EDB-L19-vc-0101 (ADC1)
1.5 mg/kg EDB-L19-vc-0101 (ADC1)
3 mg/kg EDB-L19-vc-0101 (ADC1)

FIG. 35B

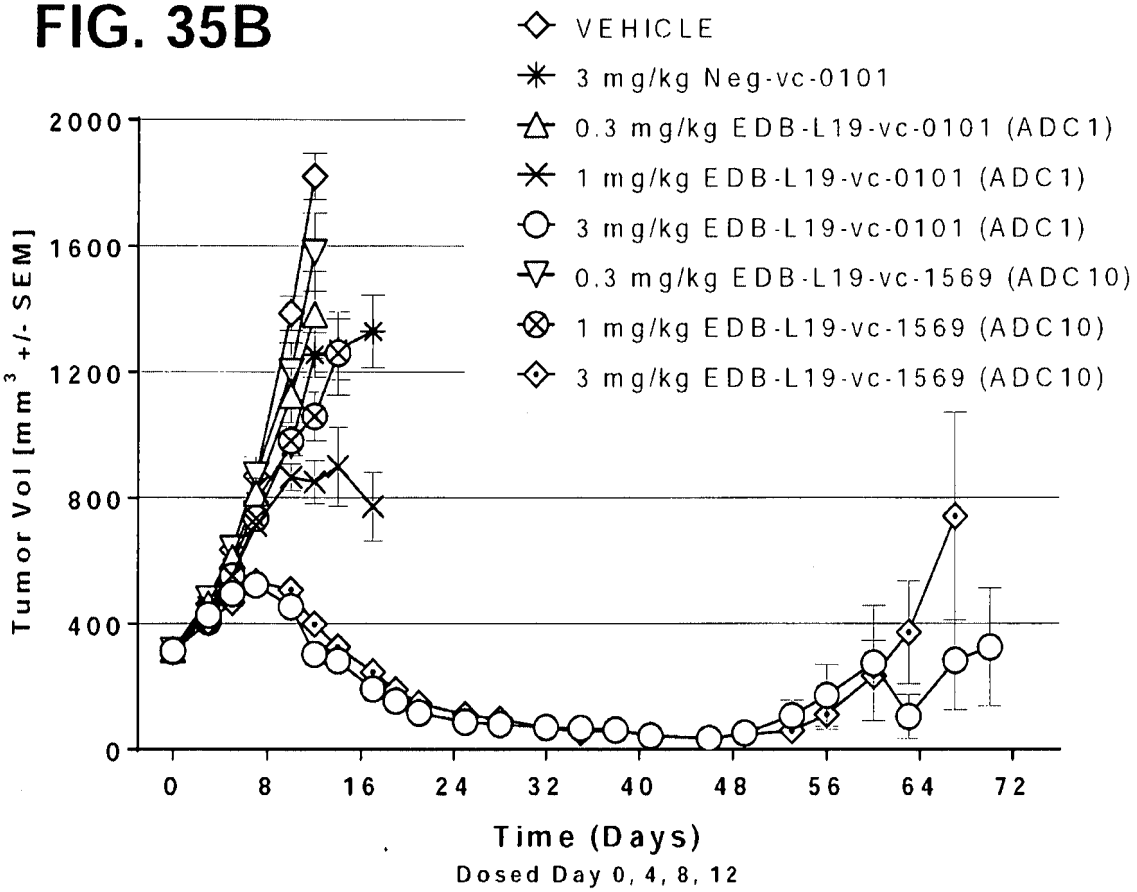


FIG. 35C

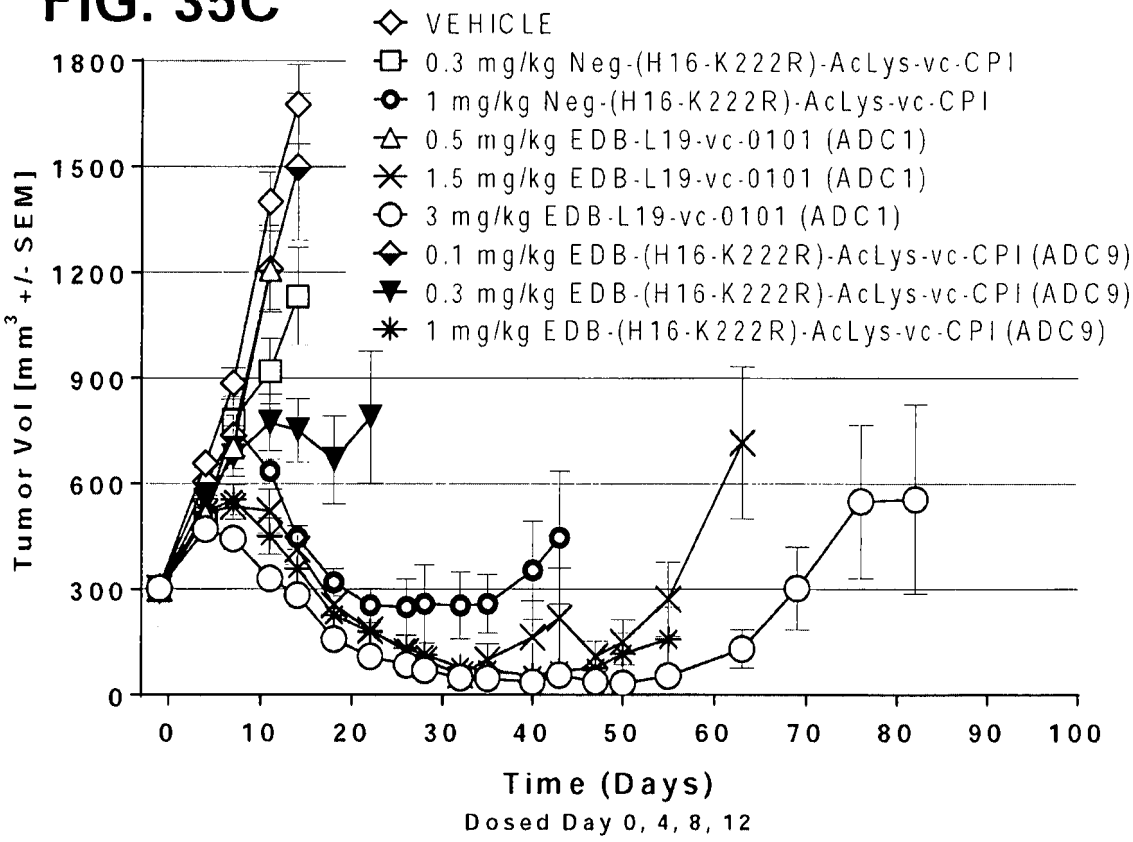


FIG. 35D

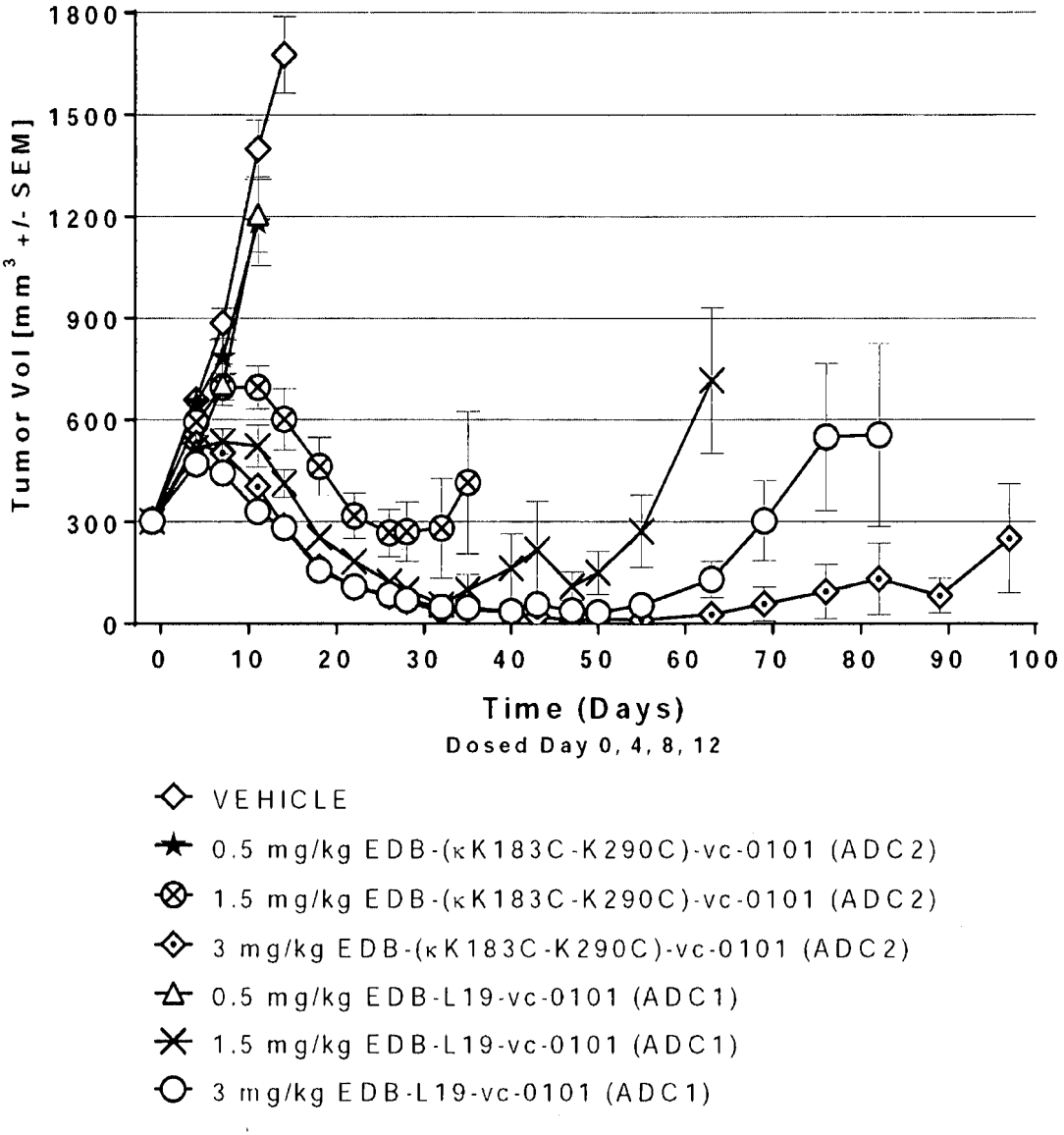

1800
1500
1200
900
600
300
0
Tumor Vol [mm3 +/- SEM]
0
10
20
30
40
50
60
70
80
90
100
Time (Days)
Dosed Day 0, 4, 8, 12
VEHICLE
0.5 mg/kg EDB-(κK183C-K290C)-vc-0101 (ADC2)
1.5 mg/kg EDB-(κK183C-K290C)-vc-0101 (ADC2)
3 mg/kg EDB-(κK183C-K290C)-vc-0101 (ADC2)
0.5 mg/kg EDB-L19-vc-0101 (ADC1)
1.5 mg/kg EDB-L19-vc-0101 (ADC1)
3 mg/kg EDB-L19-vc-0101 (ADC1)

FIG. 35E

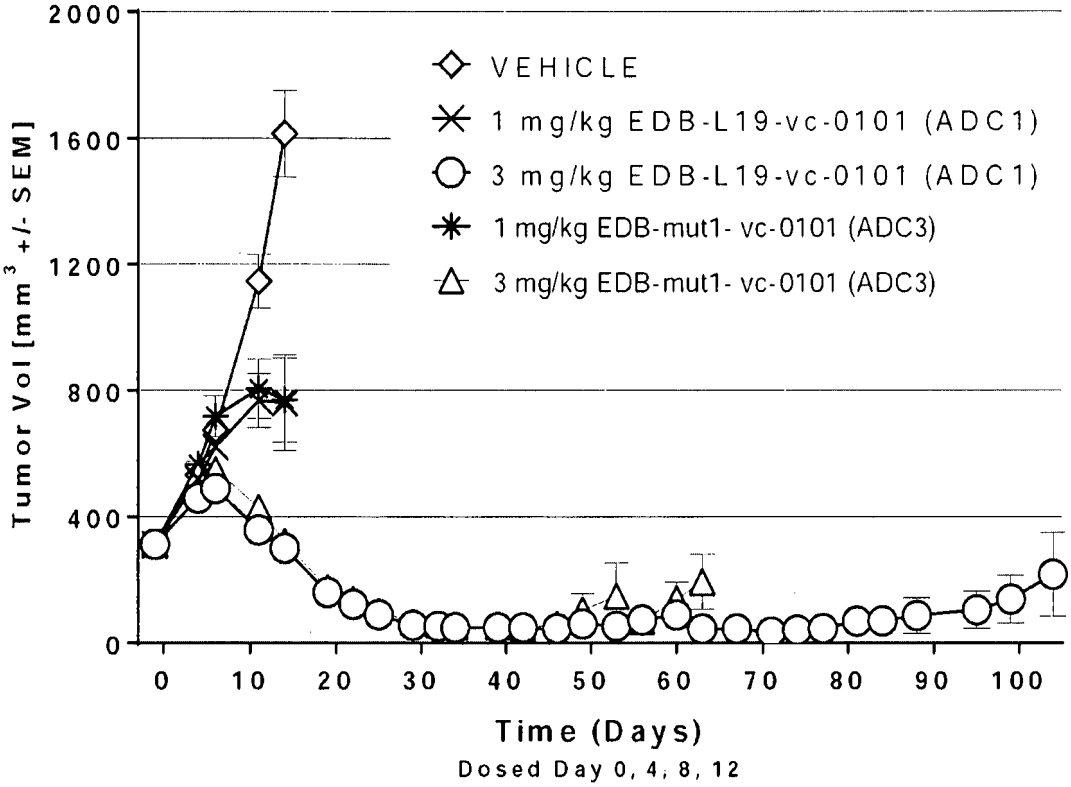


FIG. 35F

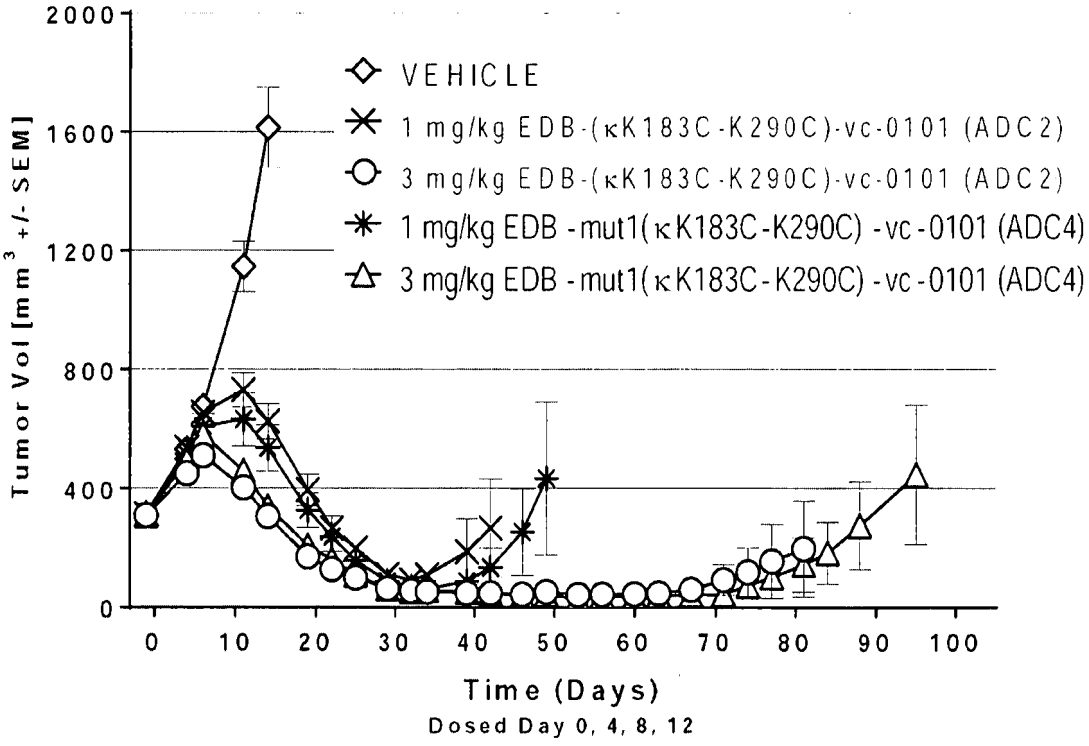


FIG. 36

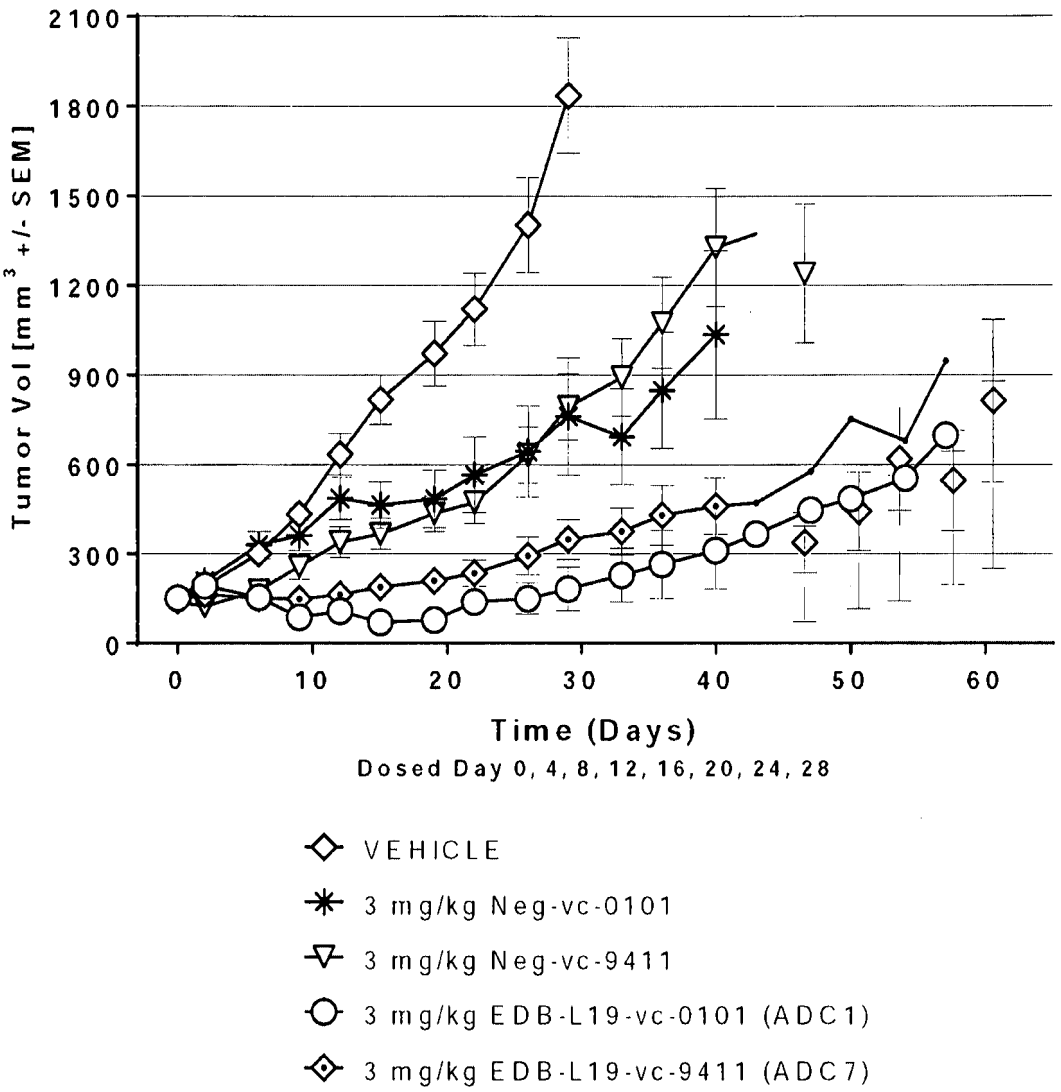

2100
1800
1500
1200
900
600
300
0
Tumor Vol [mm3 +/- SEM]
0
10
20
30
40
50
60
Time (Days)
Dosed Day 0, 4, 8, 12, 16, 20, 24, 28
VEHICLE
3 mg/kg Neg-vc-0101
3 mg/kg Neg-vc-9411
3 mg/kg EDB-L19-vc-0101 (ADC1)
3 mg/kg EDB-L19-vc-9411 (ADC7)

FIG. 37A

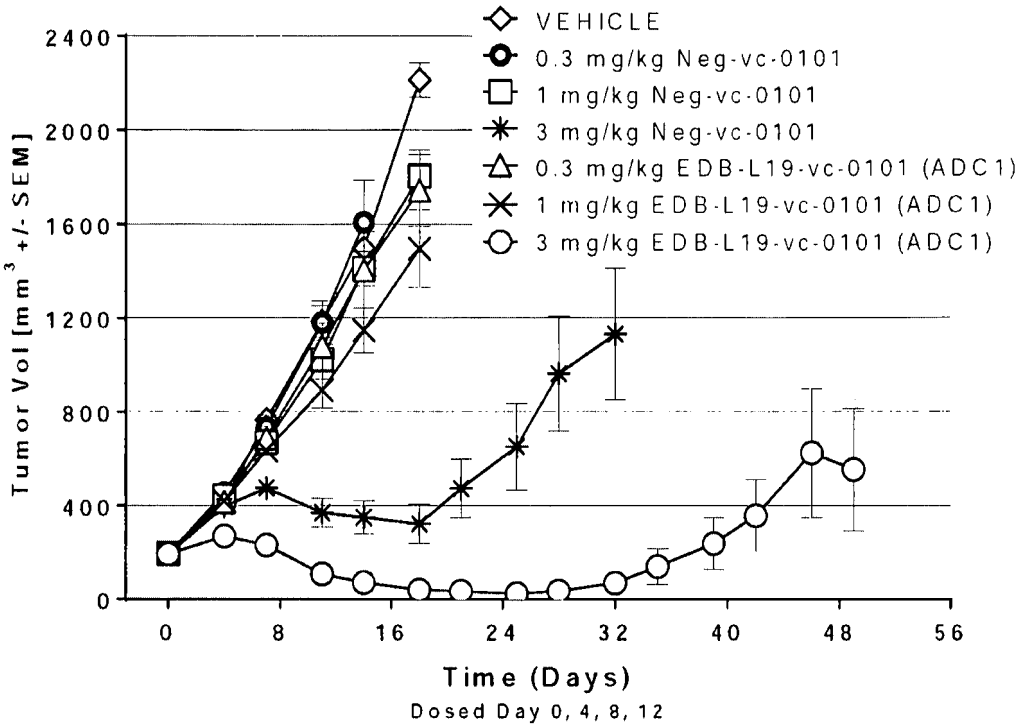


FIG. 37B

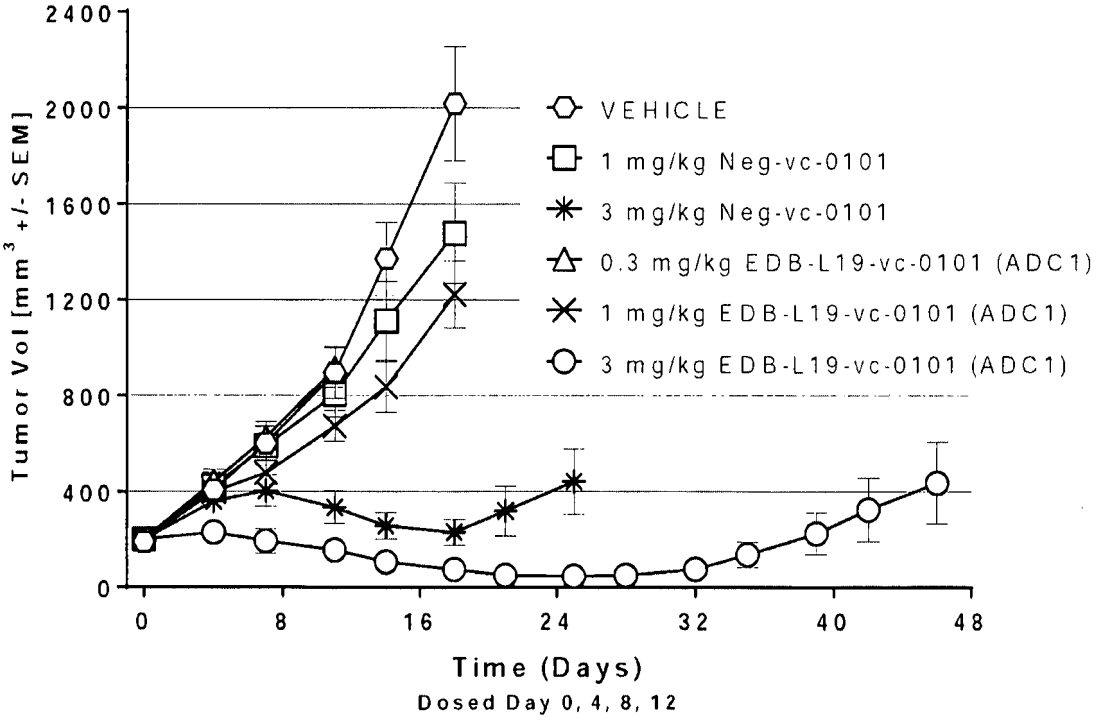


FIG. 38

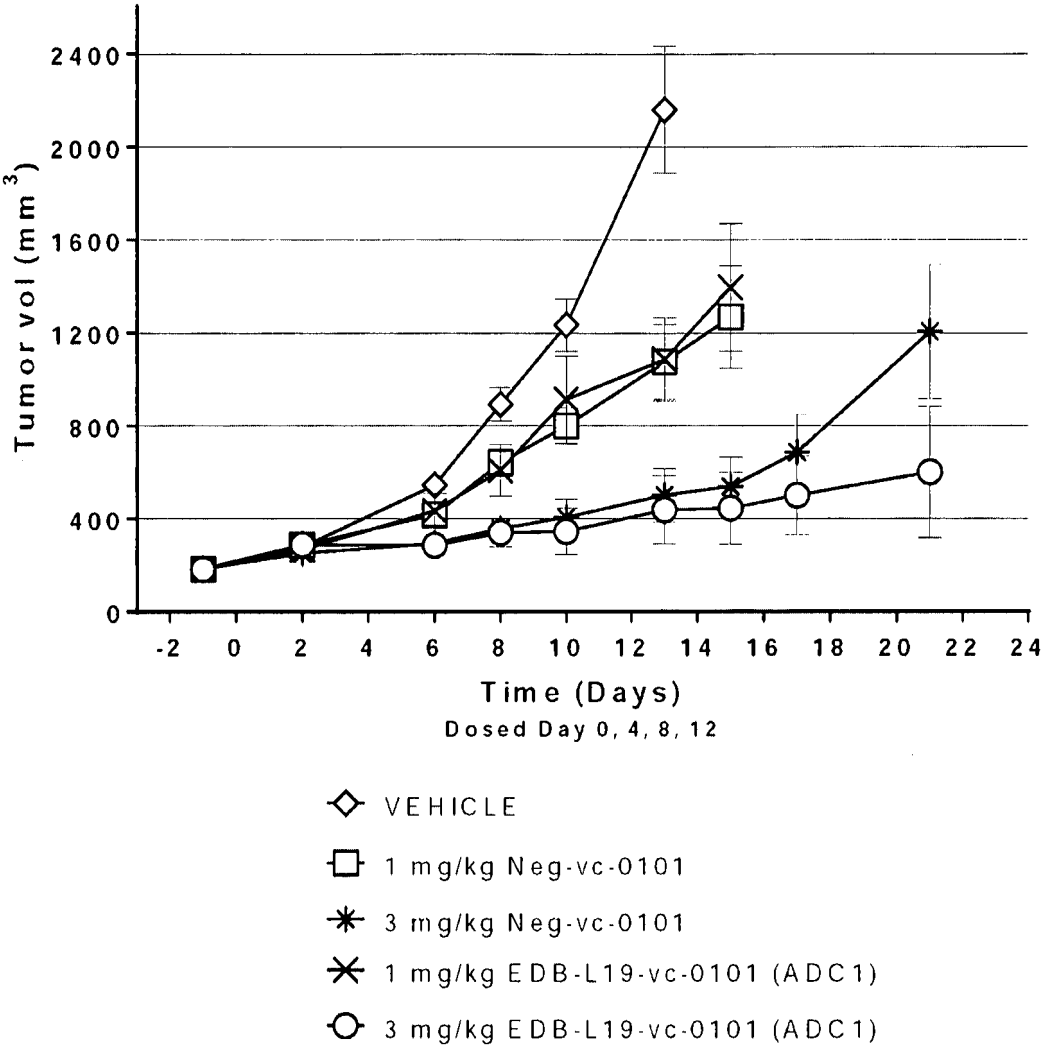

Tumor vol (mm3)
2400
2000
1600
1200
800
400
0
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (Days)
Dosed Day 0, 4, 8, 12
VEHICLE
1 mg/kg Neg-vc-0101
3 mg/kg Neg-vc-0101
1 mg/kg EDB-L19-vc-0101 (ADC1)
3 mg/kg EDB-L19-vc-0101 (ADC1)

FIG. 39A

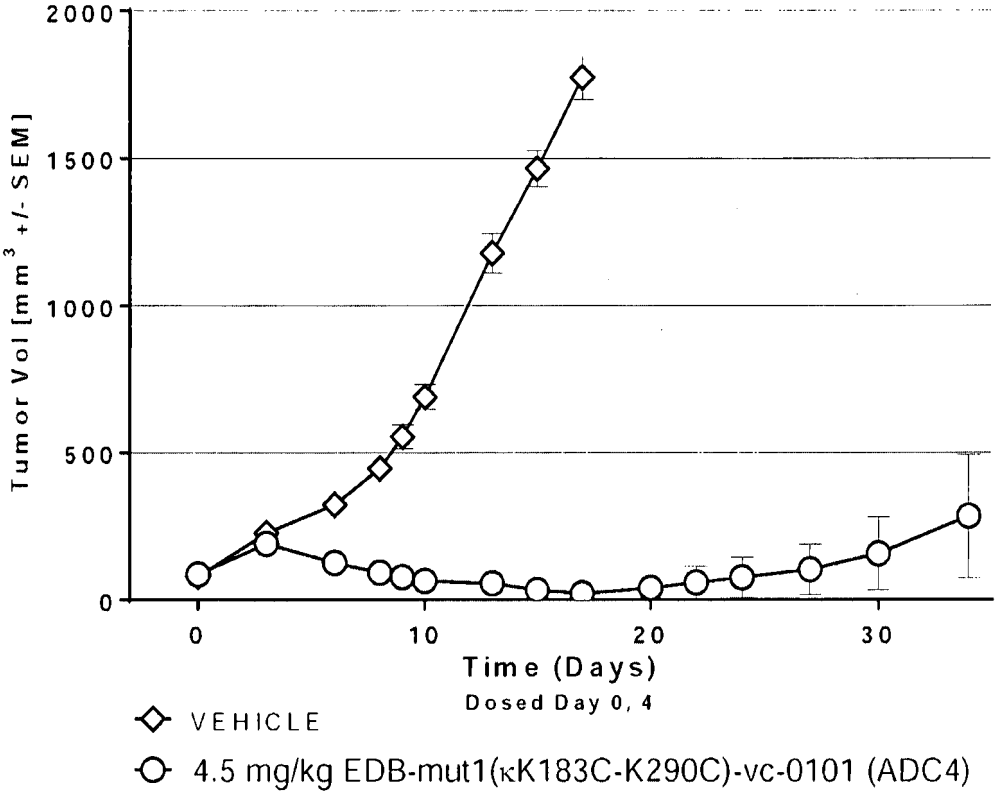


FIG. 39B

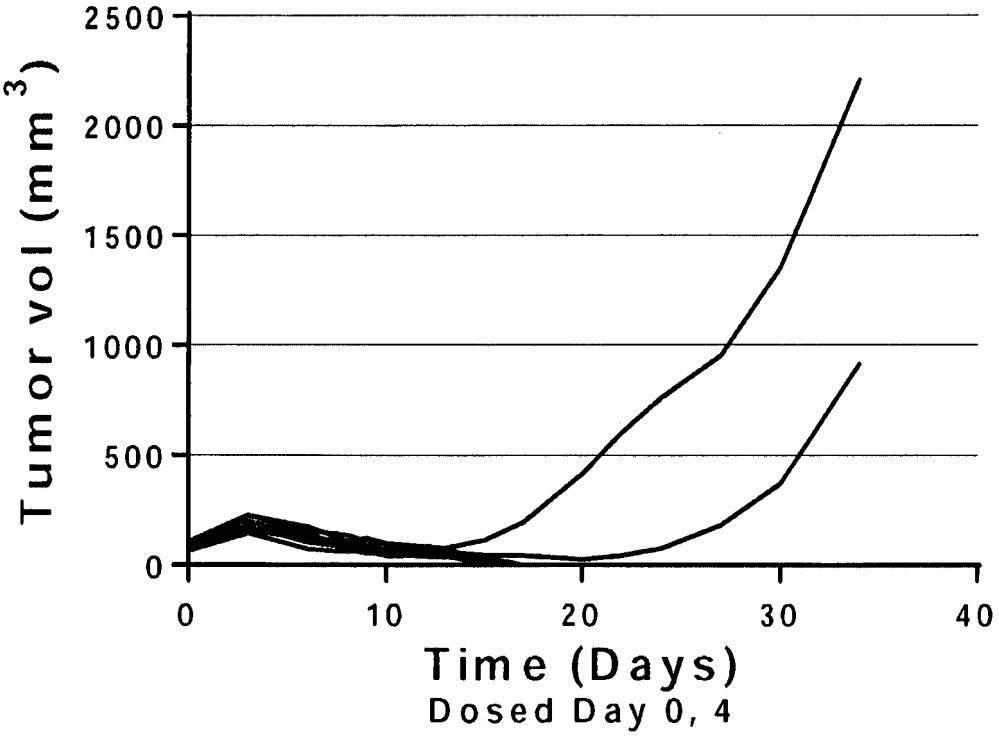


FIG. 40

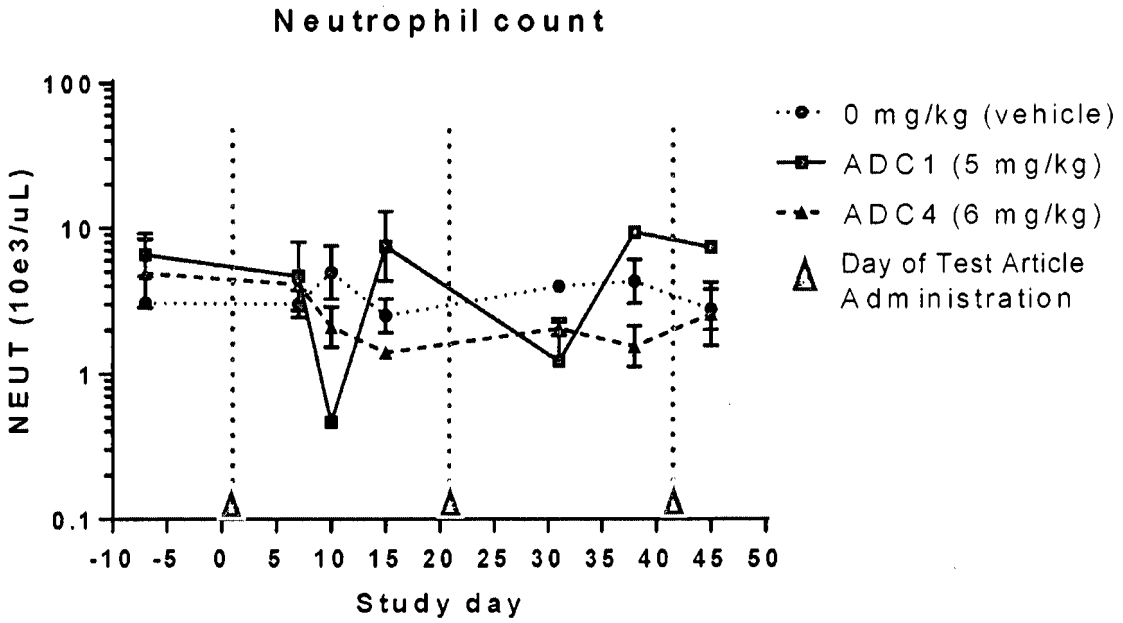

Neutrophil count
NEUT (10e3/uL)
100
10
1
0.1
-10 -5 0 5 10 15 20 25 30 35 40 45 50
Study day
0 mg/kg (vehicle)
ADC1 (5 mg/kg)
ADC4 (6 mg/kg)
Day of Test Article Administration

FIG. 41

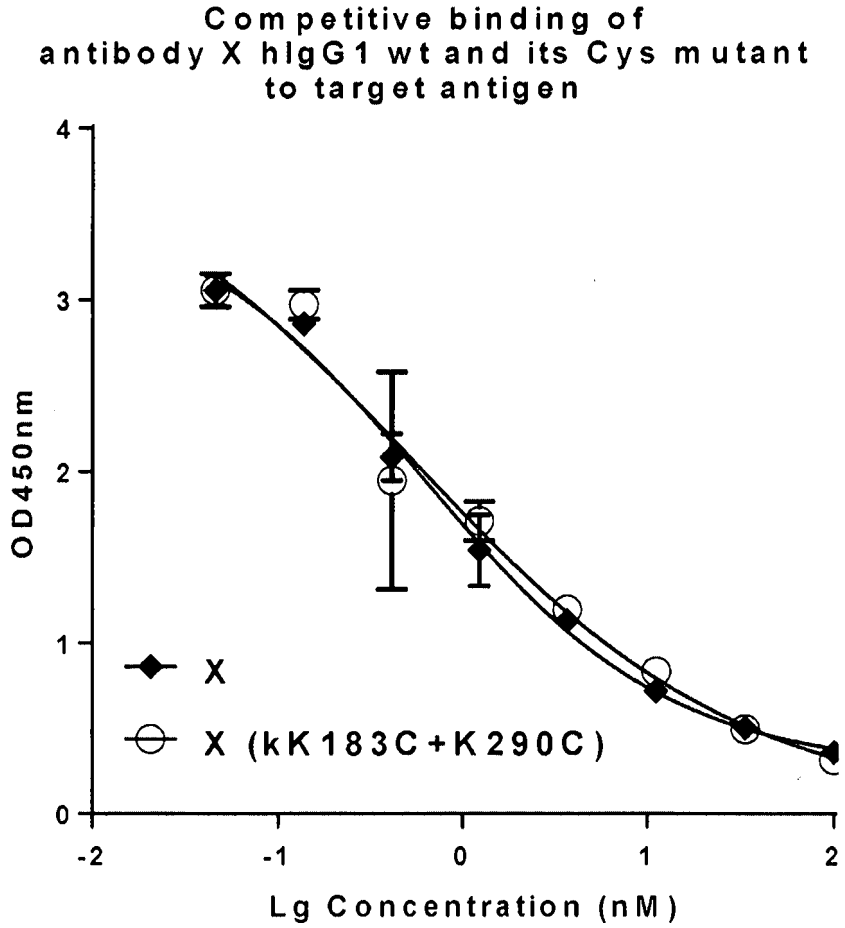
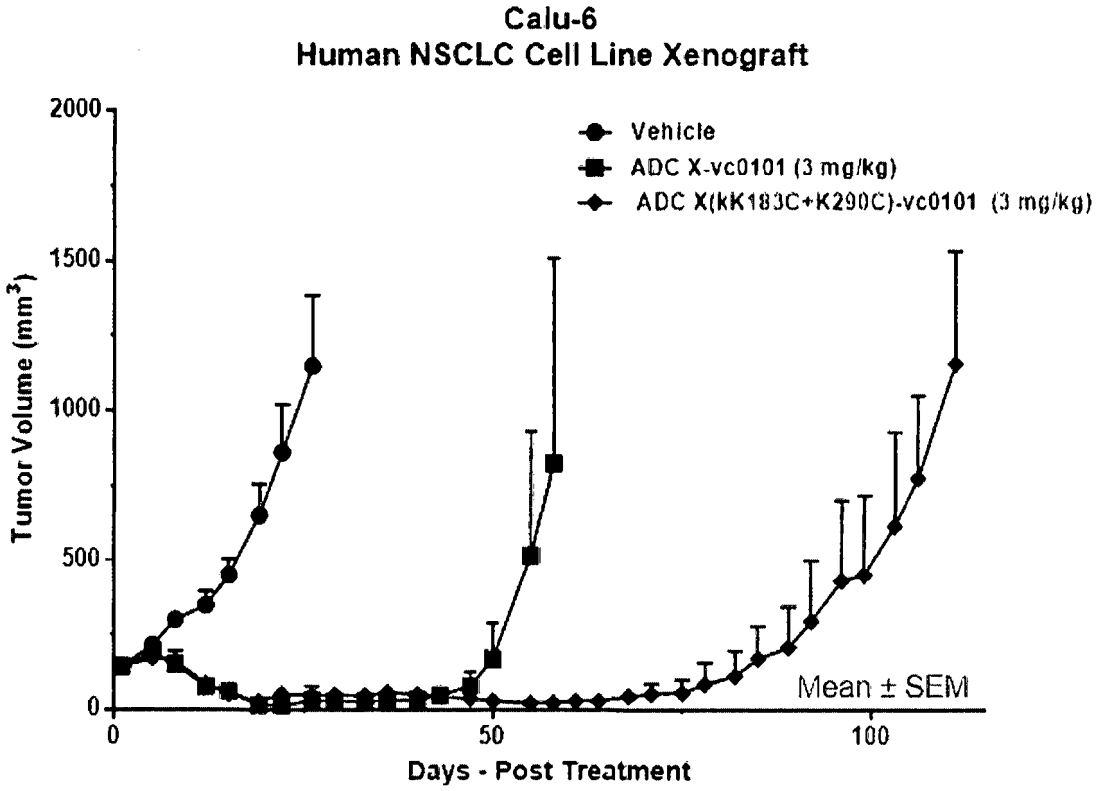

Competitive binding of antibody X hIgG1 wt and its Cys mutant to target antigen
OD450nm
4
3
2
1
0
-2 -1 0 1 2
Lg Concentration (nM)
X
X (kK183C+K290C)

FIG. 42


Calu-6
Human NSCLC Cell Line Xenograft
Vehicle
ADC X-vc0101 (3 mg/kg)
ADC X(kK183C+K290C)-vc0101 (3 mg/kg)
Tumor Volume (mm3)
2000
1500
1000
500
0
0
50
100
Days - Post Treatment
Mean ± SEM