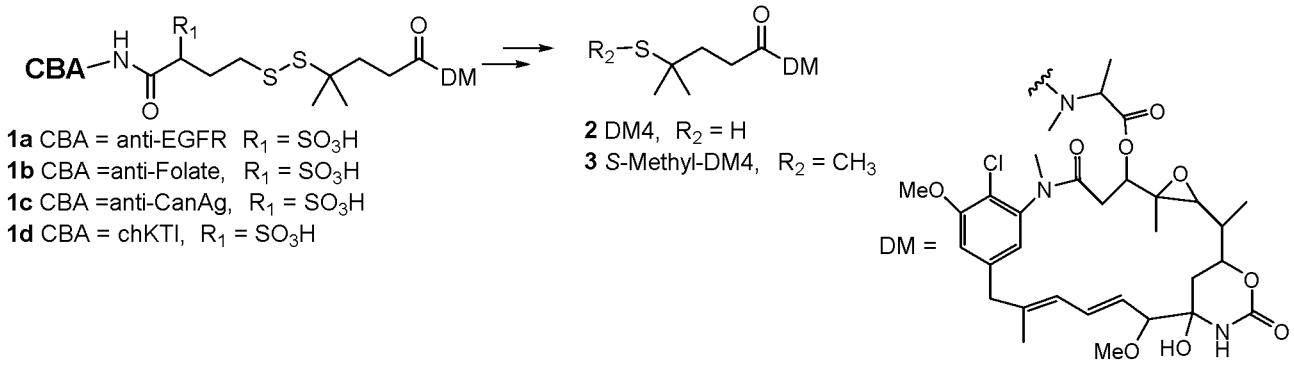

CBA
R1
S
S
O
DM
1a CBA = anti-EGFR R1 = SO3H
1b CBA =anti-Folate, R1 = SO3H
1c CBA =anti-CanAg, R1 = SO3H
1d CBA = chKTI, R1 = SO3H
R2–S
DM
2 DM4, R2 = H
3 S-Methyl-DM4, R2 = CH3
DM =
MeO
Cl
MeO
HO
N
H

FIG. 1

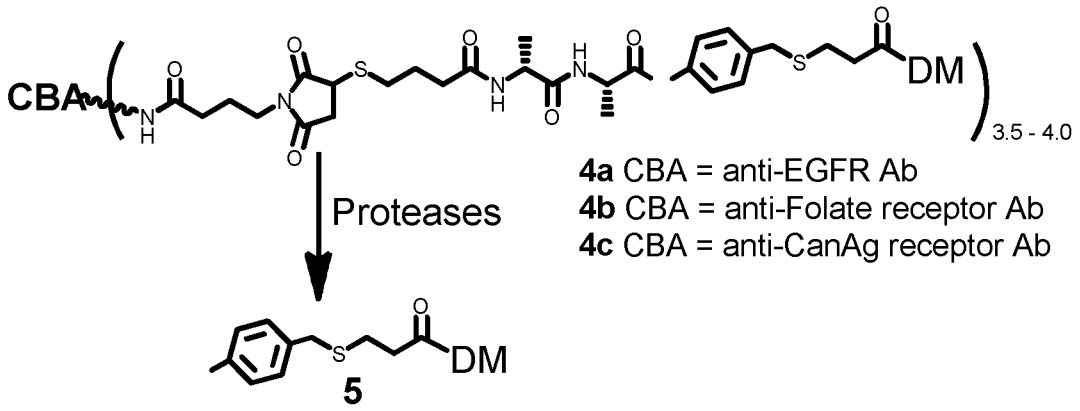

CBA
DM
3.5 - 4.0
Proteases
5
4a CBA = anti-EGFR Ab
4b CBA = anti-Folate receptor Ab
4c CBA = anti-CanAg receptor Ab

FIG. 2

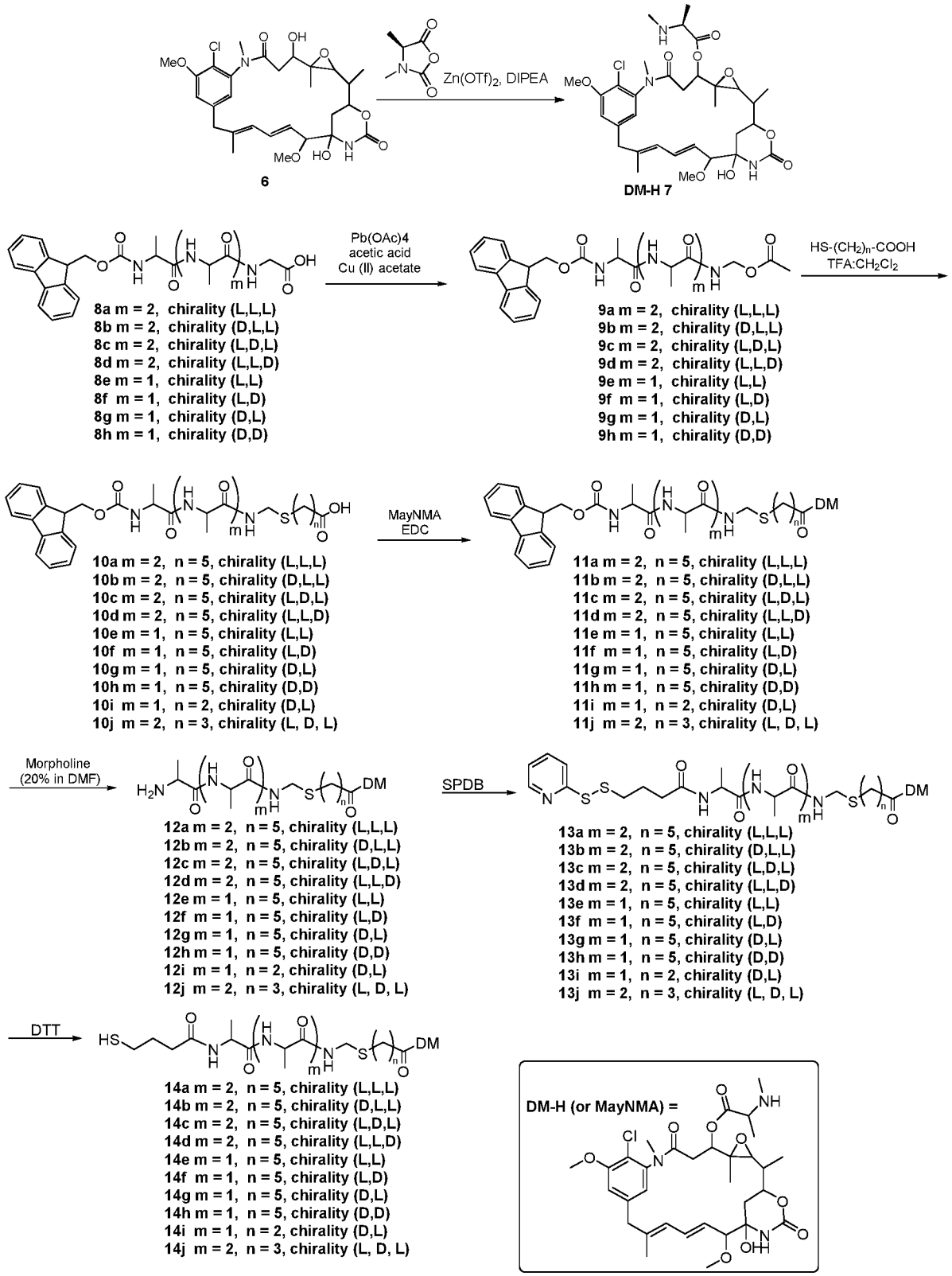
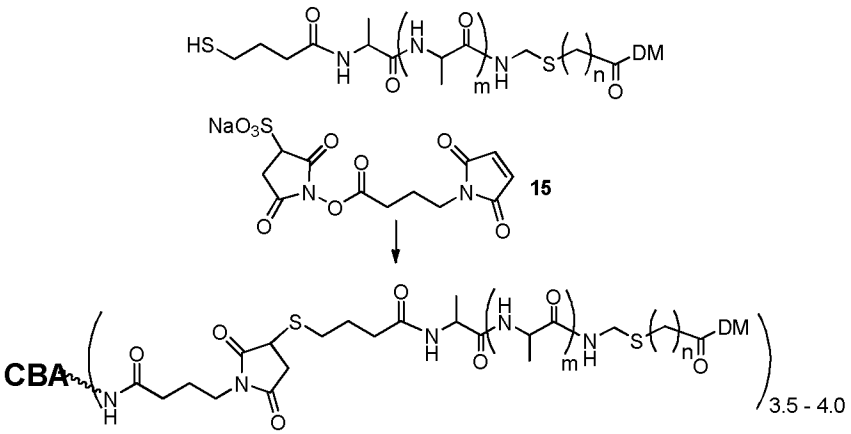

Zn(OTf)2, DIPEA
6
DM-H 7
Pb(OAc)4
acetic acid
Cu (II) acetate
HS-(CH2)n-COOH
TFA:CH2Cl2
8a m = 2, chirality (L,L,L)
8b m = 2, chirality (D,L,L)
8c m = 2, chirality (L,D,L)
8d m = 2, chirality (L,L,D)
8e m = 1, chirality (L,L)
8f m = 1, chirality (L,D)
8g m = 1, chirality (D,L)
8h m = 1, chirality (D,D)
9a m = 2, chirality (L,L,L)
9b m = 2, chirality (D,L,L)
9c m = 2, chirality (L,D,L)
9d m = 2, chirality (L,L,D)
9e m = 1, chirality (L,L)
9f m = 1, chirality (L,D)
9g m = 1, chirality (D,L)
9h m = 1, chirality (D,D)
MayNMA
EDC
10a m = 2, n = 5, chirality (L,L,L)
10b m = 2, n = 5, chirality (D,L,L)
10c m = 2, n = 5, chirality (L,D,L)
10d m = 2, n = 5, chirality (L,L,D)
10e m = 1, n = 5, chirality (L,L)
10f m = 1, n = 5, chirality (L,D)
10g m = 1, n = 5, chirality (D,L)
10h m = 1, n = 5, chirality (D,D)
10i m = 1, n = 2, chirality (D,L)
10j m = 2, n = 3, chirality (L, D, L)
11a m = 2, n = 5, chirality (L,L,L)
11b m = 2, n = 5, chirality (D,L,L)
11c m = 2, n = 5, chirality (L,D,L)
11d m = 2, n = 5, chirality (L,L,D)
11e m = 1, n = 5, chirality (L,L)
11f m = 1, n = 5, chirality (L,D)
11g m = 1, n = 5, chirality (D,L)
11h m = 1, n = 5, chirality (D,D)
11i m = 1, n = 2, chirality (D,L)
11j m = 2, n = 3, chirality (L, D, L)
Morpholine
(20% in DMF)
SPDB
12a m = 2, n = 5, chirality (L,L,L)
12b m = 2, n = 5, chirality (D,L,L)
12c m = 2, n = 5, chirality (L,D,L)
12d m = 2, n = 5, chirality (L,L,D)
12e m = 1, n = 5, chirality (L,L)
12f m = 1, n = 5, chirality (L,D)
12g m = 1, n = 5, chirality (D,L)
12h m = 1, n = 5, chirality (D,D)
12i m = 1, n = 2, chirality (D,L)
12j m = 2, n = 3, chirality (L, D, L)
13a m = 2, n = 5, chirality (L,L,L)
13b m = 2, n = 5, chirality (D,L,L)
13c m = 2, n = 5, chirality (L,D,L)
13d m = 2, n = 5, chirality (L,L,D)
13e m = 1, n = 5, chirality (L,L)
13f m = 1, n = 5, chirality (L,D)
13g m = 1, n = 5, chirality (D,L)
13h m = 1, n = 5, chirality (D,D)
13i m = 1, n = 2, chirality (D,L)
13j m = 2, n = 3, chirality (L, D, L)
DTT
14a m = 2, n = 5, chirality (L,L,L)
14b m = 2, n = 5, chirality (D,L,L)
14c m = 2, n = 5, chirality (L,D,L)
14d m = 2, n = 5, chirality (L,L,D)
14e m = 1, n = 5, chirality (L,L)
14f m = 1, n = 5, chirality (L,D)
14g m = 1, n = 5, chirality (D,L)
14h m = 1, n = 5, chirality (D,D)
14i m = 1, n = 2, chirality (D,L)
14j m = 2, n = 3, chirality (L, D, L)
DM-H (or MayNMA) =

FIG. 3

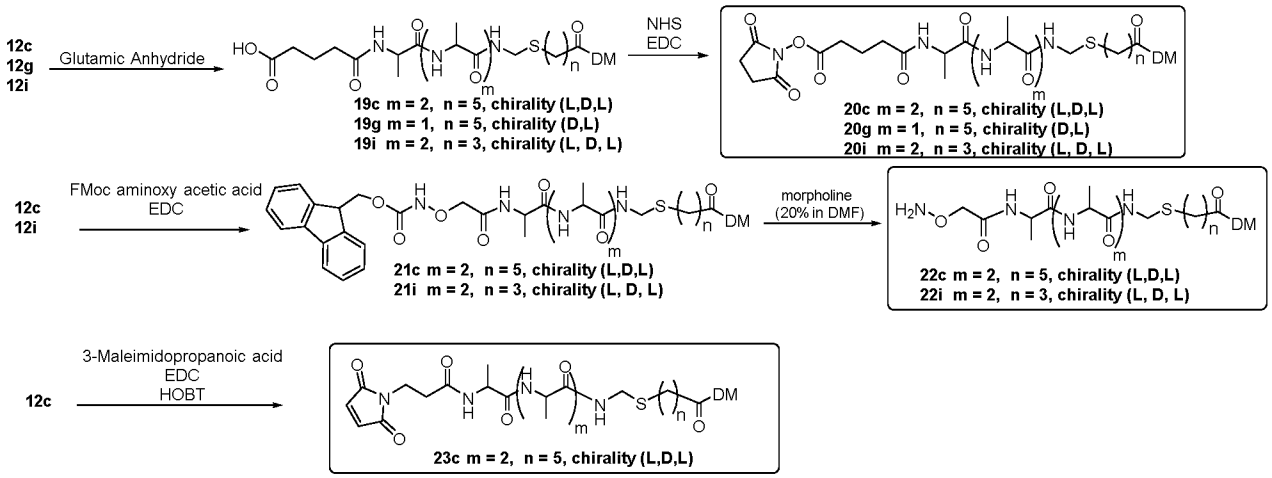


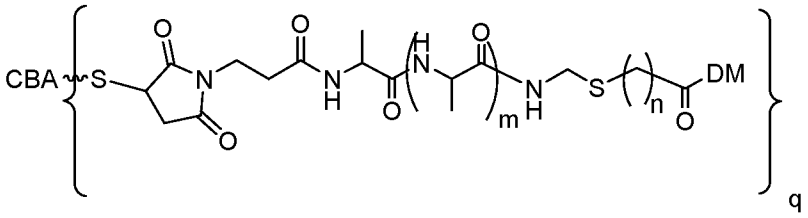
16a CBA = anti-EGFR Ab, m = 2, n = 5, chirality (L,L,L)
16b CBA = anti-EGFR Ab, m = 2, n = 5, chirality (D,L,L)
16c CBA = anti-EGFR Ab, m = 2, n = 5, chirality (L,D,L)
16d CBA = anti-EGFR Ab, m = 2, n = 5, chirality (L,L,D)
16e CBA = anti-EGFR Ab, m = 1, n = 5, chirality (L,L)
16f CBA = anti-EGFR Ab, m = 1, n = 5, chirality (L,D)
16g CBA = anti-EGFR Ab, m = 1, n = 5, chirality (D,L)
16h CBA = anti-EGFR Ab, m = 1, n = 2, chirality (D,L)
16i CBA = anti-EGFR Ab, m = 2, n = 3, chirality (L, D, L)

17a CBA = anti-Folate receptor Ab, m = 2, n = 5, chirality (L,L,L)
17b CBA = anti-Folate receptor Ab, m = 2, n = 5, chirality (D,L,L)
17c CBA = anti-Folate receptor Ab, m = 2, n = 5, chirality (L,D,L)
17d CBA = anti-Folate receptor Ab, m = 2, n = 5, chirality (L,L,D)
17e CBA = anti-Folate receptor Ab, m = 1, n = 5, chirality (L,L)
17f CBA = anti-Folate receptor Ab, m = 1, n = 5, chirality (L,D)
17g CBA = anti-Folate receptor Ab, m = 1, n = 5, chirality (D,L)
17h CBA = anti-Folatereceptor Ab, m = 1, n = 2, chirality (D,L)
17i CBA = anti-Folate receptor Ab, m = 2, n = 3, chirality (L, D, L)

18a CBA = anti-CanAg receptor Ab, m = 2, n = 5, chirality (L,L,L)
18b CBA = anti-CanAg receptor Ab, m = 2, n = 5, chirality (D,L,L)
18c CBA = anti-CanAg receptor Ab, m = 2, n = 5, chirality (L,D,L)
18d CBA = anti-CanAg receptor Ab, m = 2, n = 5, chirality (L,L,D)
18e CBA = anti-CanAg receptor Ab, m = 1, n = 5, chirality (L,L)
18f CBA = anti-CanAg receptor Ab, m = 1, n = 5, chirality (L,D)
18g CBA = anti-CanAg receptor Ab, m = 1, n = 5, chirality (D,L)
18h CBA = anti-CanAg receptor Ab, m = 1, n = 2, chirality (D,L)
18i CBA = anti-CanAg receptor Ab, m = 2, n = 3, chirality (L, D, L)

FIG. 4





26c CBA = anti-Folate receptor Ab, m = 2, n = 5, chirality (L,D,L)

FIG. 5

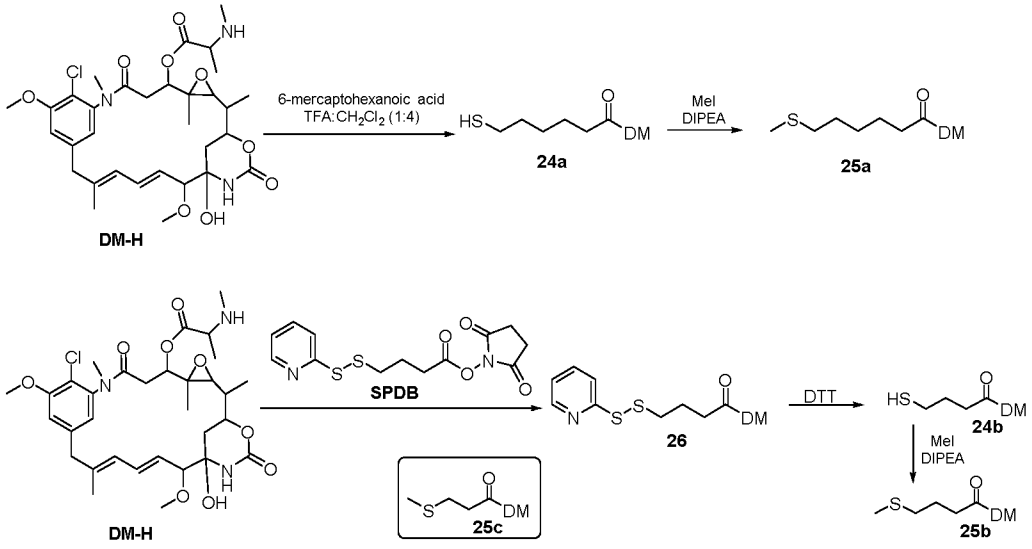

DM-H
6-mercaptohexanoic acid
TFA:CH2Cl2 (1:4)
24a
MeI
DIPEA
25a
SPDB
26
DTT
24b
25c
25b

FIG. 6

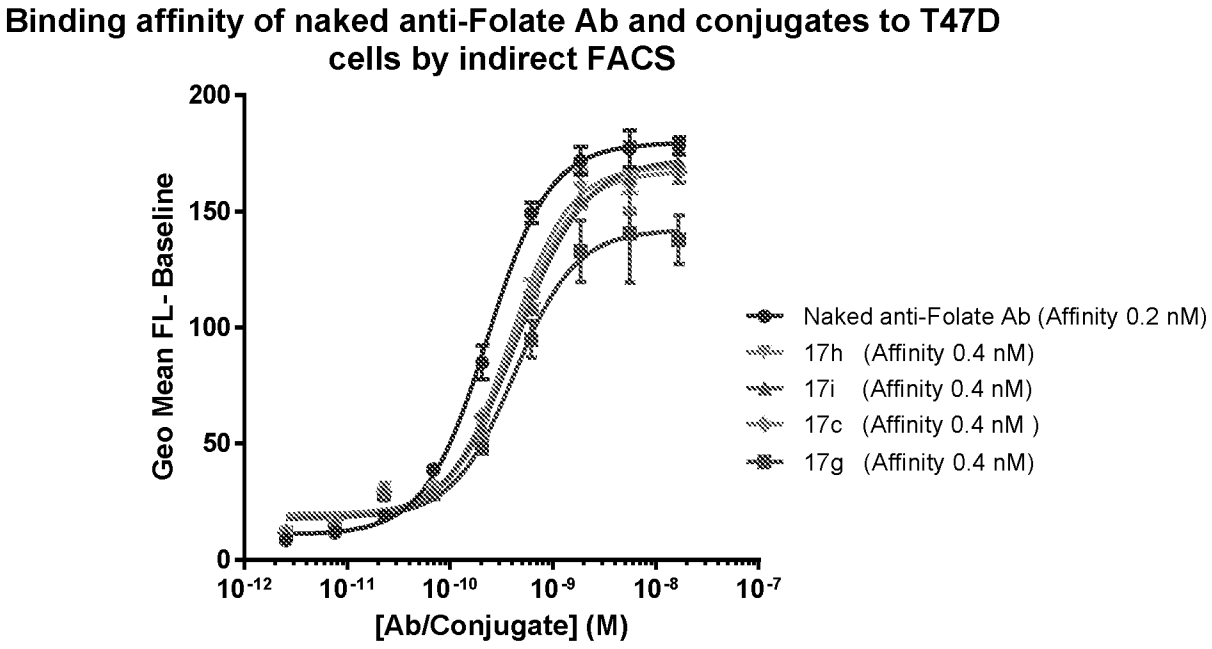

Binding affinity of naked anti-Folate Ab and conjugates to T47D cells by indirect FACS
Geo Mean FL- Baseline
200
150
100
50
0
10-12
10-11
10-10
10-9
10-8
10-7
[Ab/Conjugate] (M)
Naked anti-Folate Ab (Affinity 0.2 nM)
17h (Affinity 0.4 nM)
17i (Affinity 0.4 nM)
17c (Affinity 0.4 nM)
17g (Affinity 0.4 nM)

FIG. 7

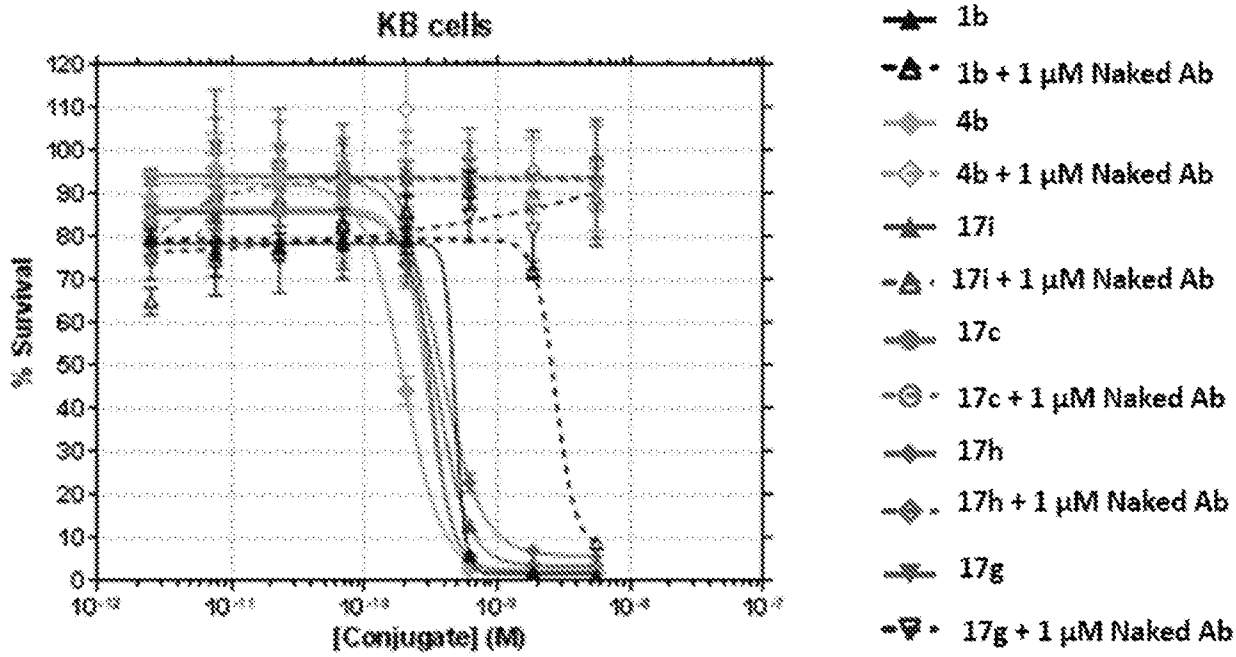

KB cells
% Survival
[Conjugate] (M)
1b
1b + 1 µM Naked Ab
4b
4b + 1 µM Naked Ab
17i
17i + 1 µM Naked Ab
17c
17c + 1 µM Naked Ab
17h
17h + 1 µM Naked Ab
17g
17g + 1 µM Naked Ab

FIG. 8

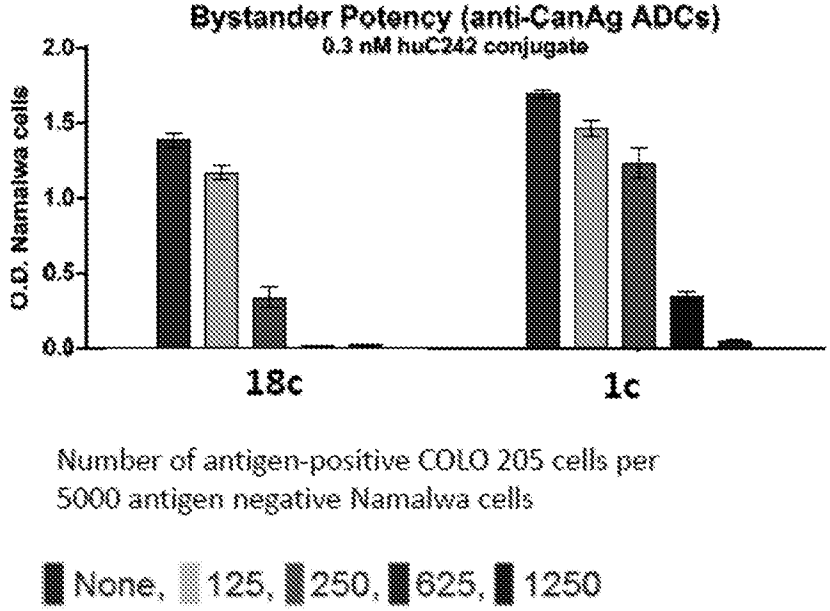

Bystander Potency (anti-CanAg ADCs)
0.3 nM huC242 conjugate
O.D. Namalwa cells
2.0
1.5
1.0
0.5
0.0
18c
1c
Number of antigen-positive COLO 205 cells per 5000 antigen negative Namalwa cells
None, 125, 250, 625, 1250

FIG. 9A

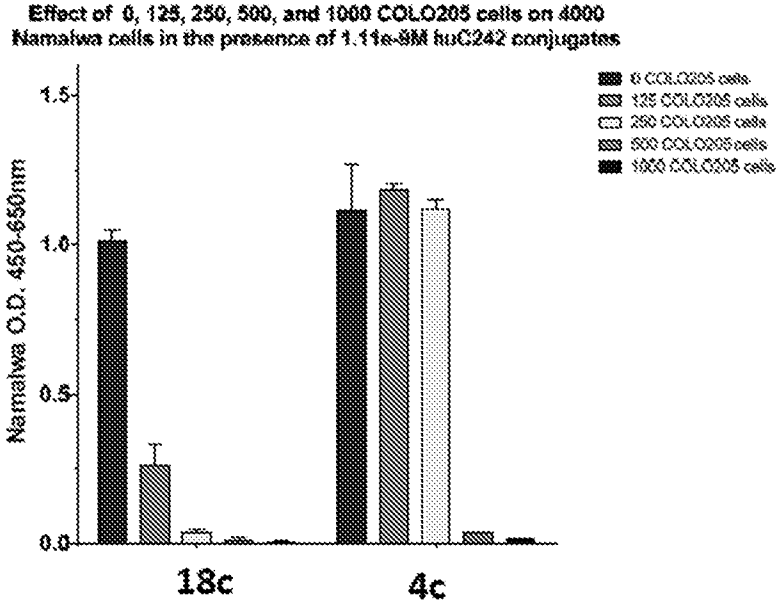

Effect of 0, 125, 250, 500, and 1000 COLO205 cells on 4000 Namalwa cells in the presence of 1.11e-9M huC242 conjugates
Namalwa O.D. 450-650nm
1.5
1.0
0.5
0.0
18c
4c

FIG. 9B

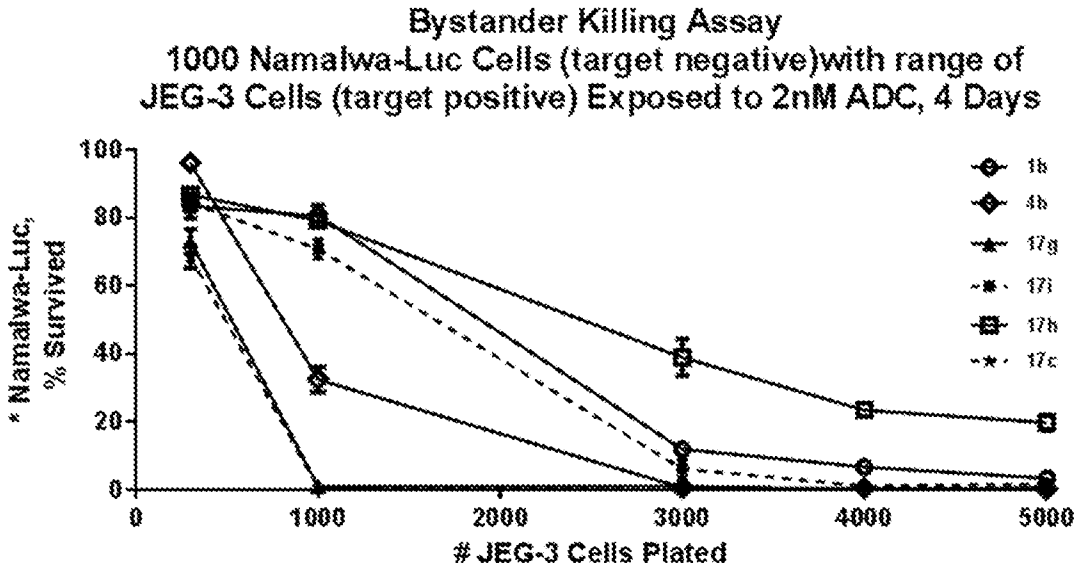


FIG. 9C

Bystander Killing Assay
1,500 MCF-7 Cells (target negative) with range of
HCC827 cells (target positive) exposed to ADCs for 5 days

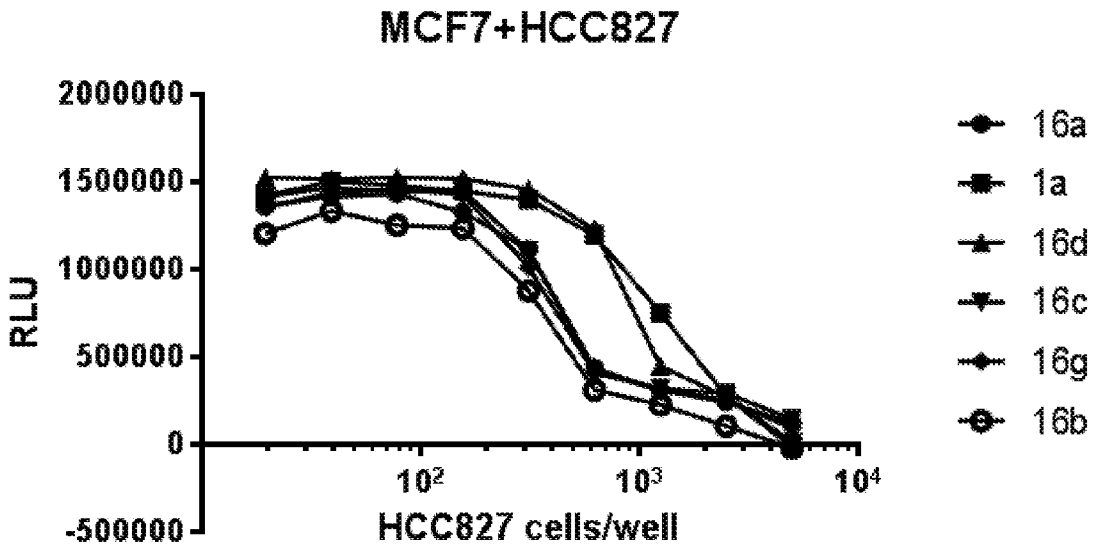


FIG. 9D

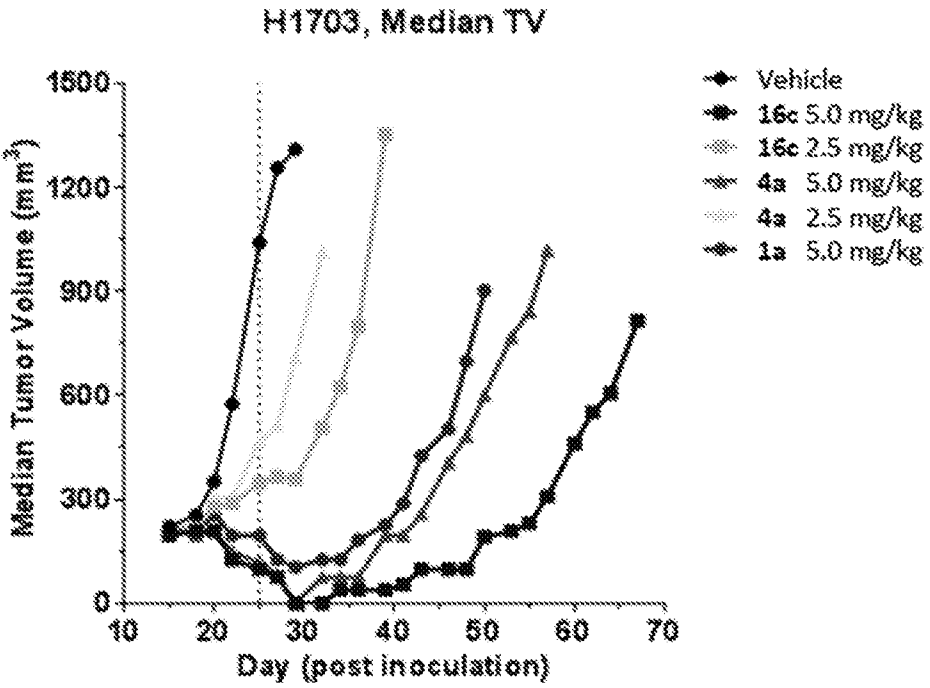

H1703, Median TV
Median Tumor Volume (mm³)
1500
1200
900
600
300
0
10
20
30
40
50
60
70
Day (post inoculation)
Vehicle
16c 5.0 mg/kg
16c 2.5 mg/kg
4a 5.0 mg/kg
4a 2.5 mg/kg
1a 5.0 mg/kg

FIG. 10

Efficacy of ADCs against CanAg+ HT-29 xenografts at 5 mg/kg doses

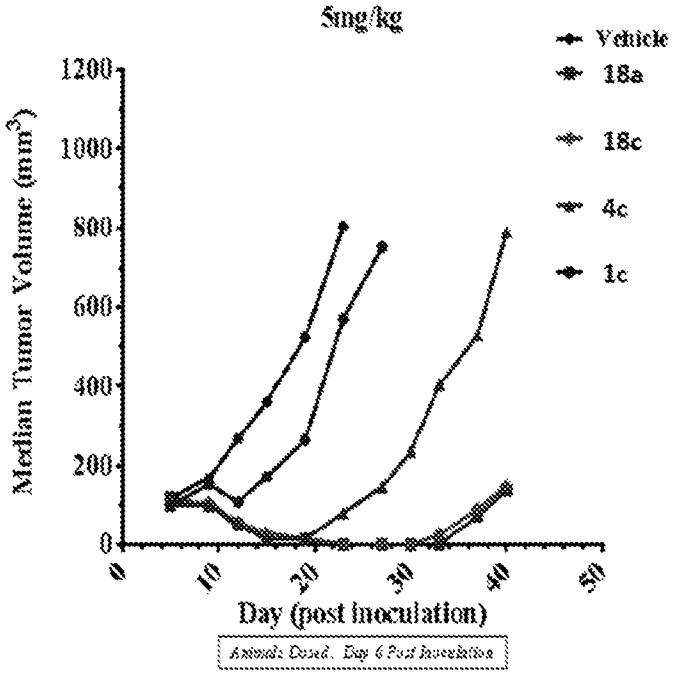


FIG. 11A

Efficacy of ADCs against CanAg+ HT-29 xenografts at 2.5 mg/kg doses

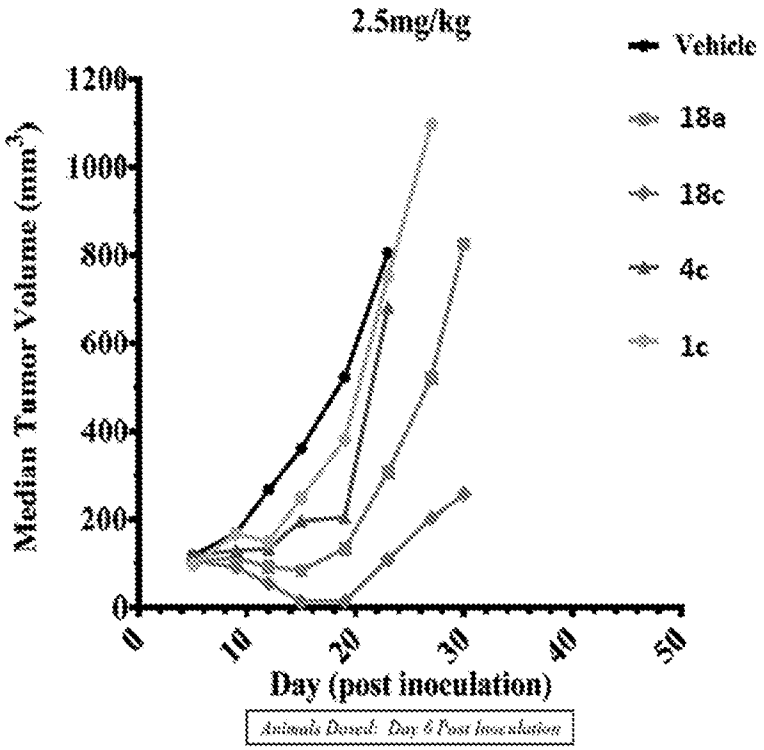


FIG. 11B

Efficacy of ADCs against NCI-H2110 folate receptor positive xenografts at 3 mg/kg doses

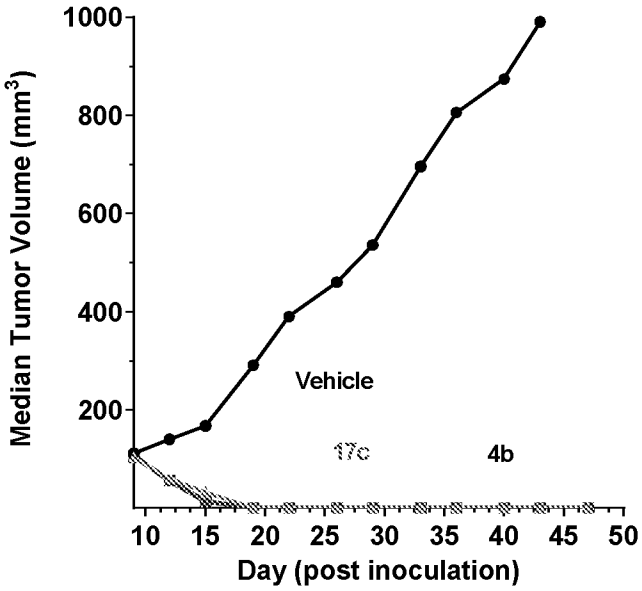


FIG. 12

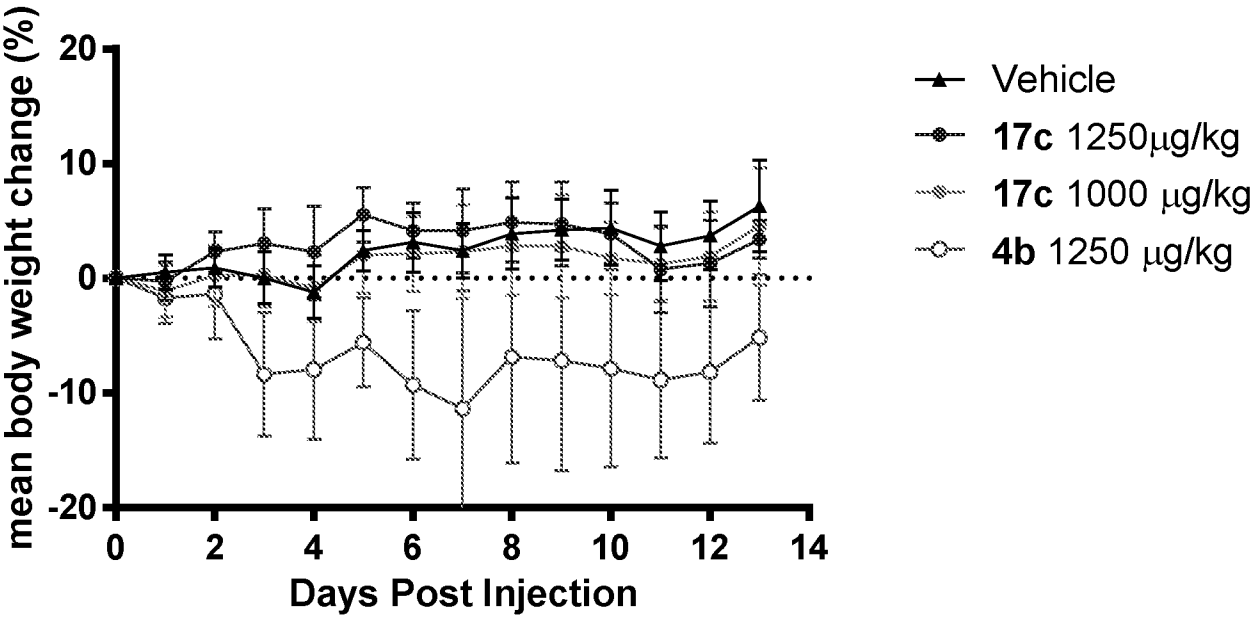

mean body weight change (%)
20
10
0
-10
-20
0
2
4
6
8
10
12
14
Days Post Injection
Vehicle
17c 1250μg/kg
17c 1000 μg/kg
4b 1250 μg/kg

FIG. 13

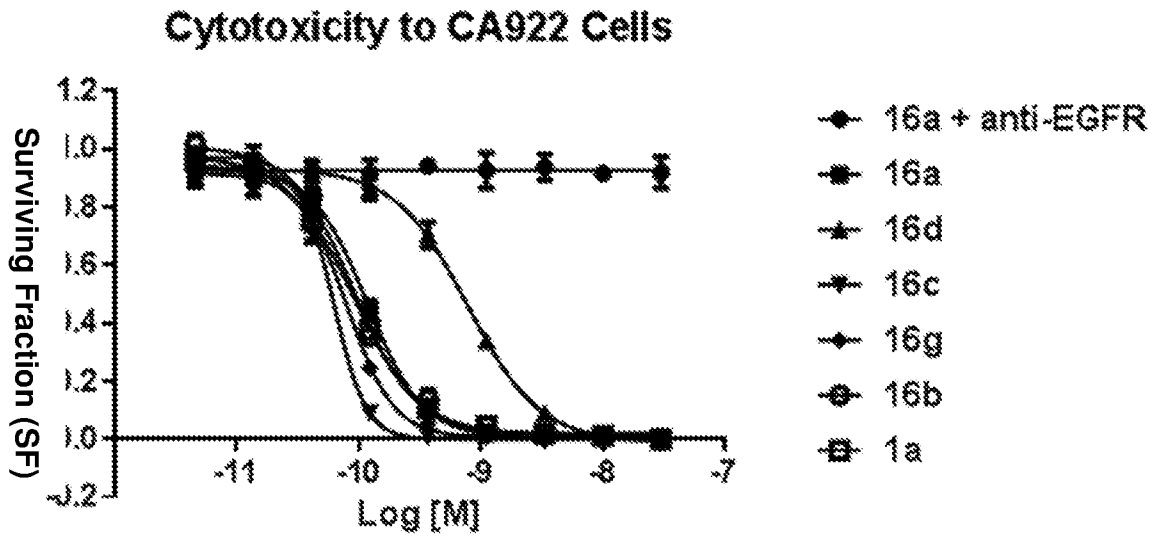

Cytotoxicity to CA922 Cells
Surviving Fraction (SF)
1.2
1.0
.8
.6
.4
.2
.0
-.2
-11
-10
-9
-8
-7
Log [M]
16a + anti-EGFR
16a
16d
16c
16g
16b
1a

FIG. 14

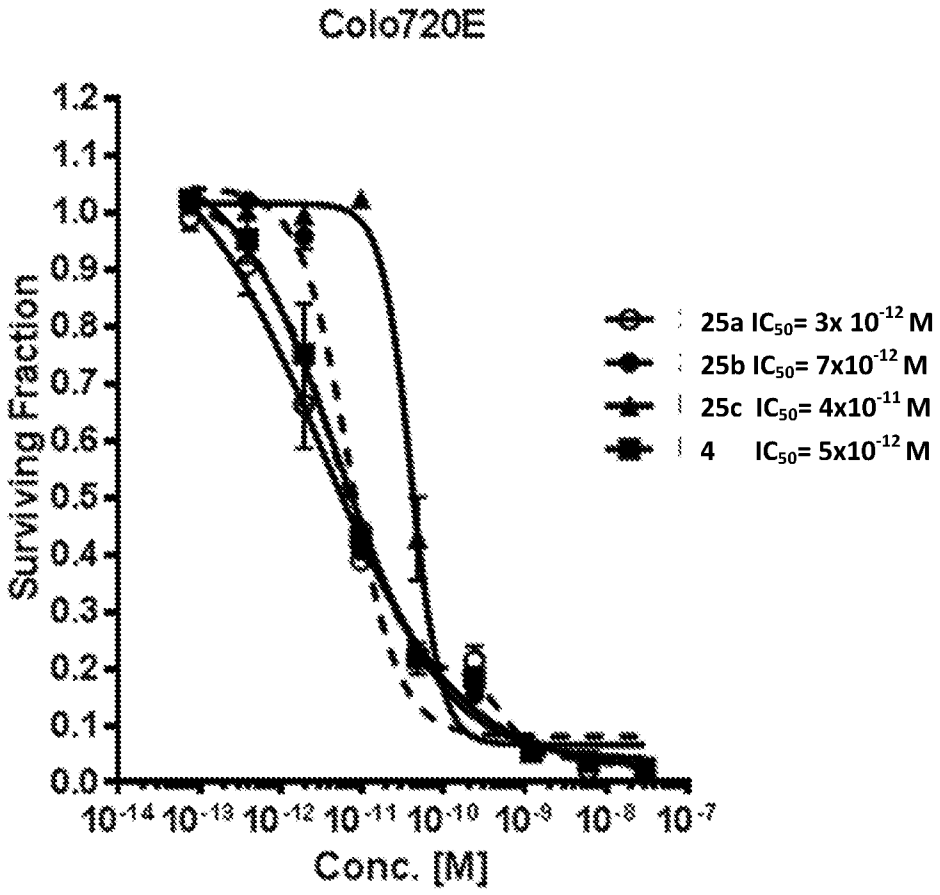

Colo720E
Surviving Fraction
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10-14 10-13 10-12 10-11 10-10 10-9 10-8 10-7
Conc. [M]
25a IC50= 3x 10-12 M
25b IC50= 7x10-12 M
25c IC50= 4x10-11 M
4 IC50= 5x10-12 M

FIG. 15

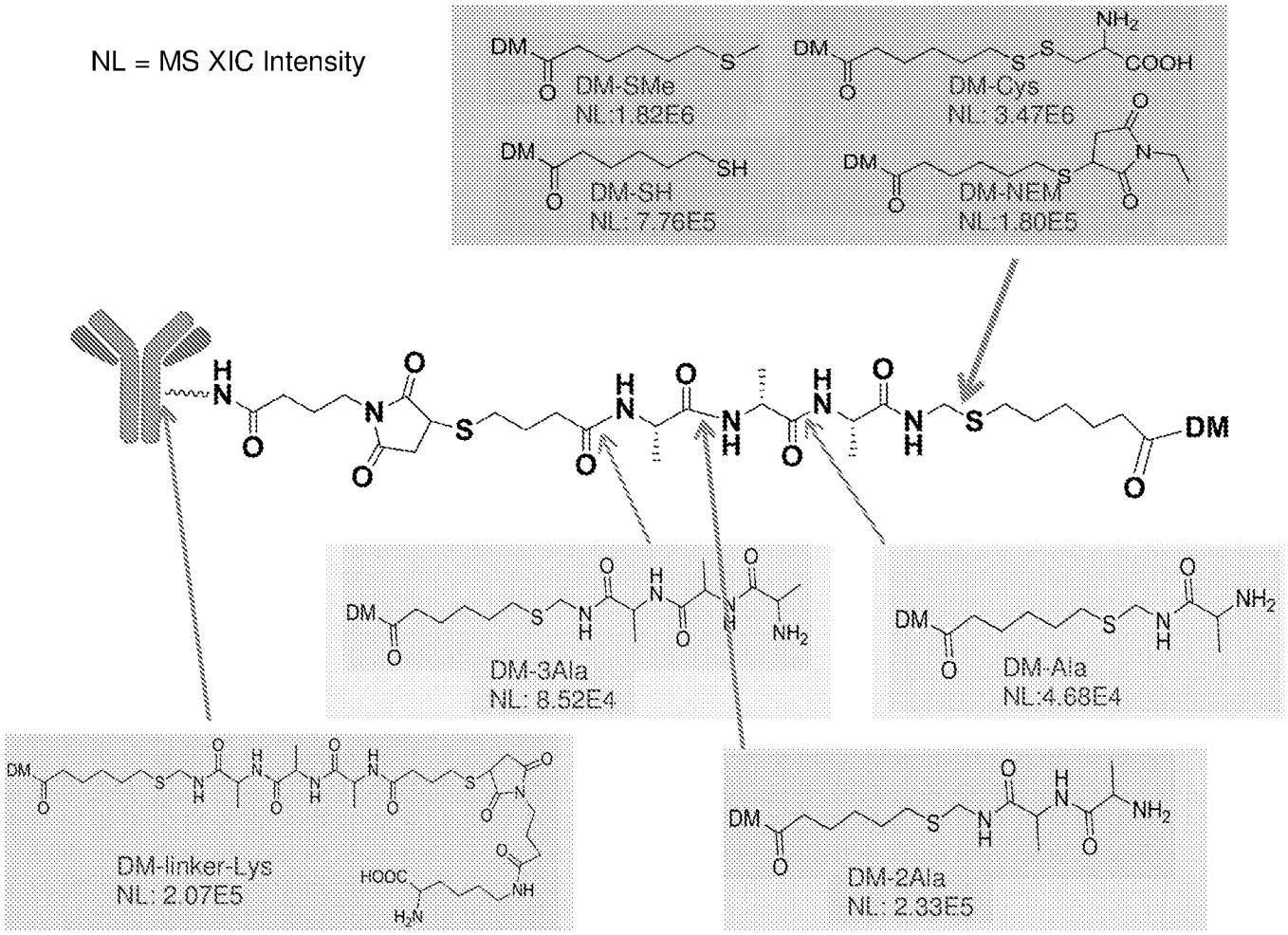

NL = MS XIC Intensity
DM-SMe
NL: 1.82E6
DM-Cys
NL: 3.47E6
DM-SH
NL: 7.76E5
DM-NEM
NL: 1.80E5
DM-3Ala
NL: 8.52E4
DM-Ala
NL:4.68E4
DM-linker-Lys
NL: 2.07E5
DM-2Ala
NL: 2.33E5

FIG. 16

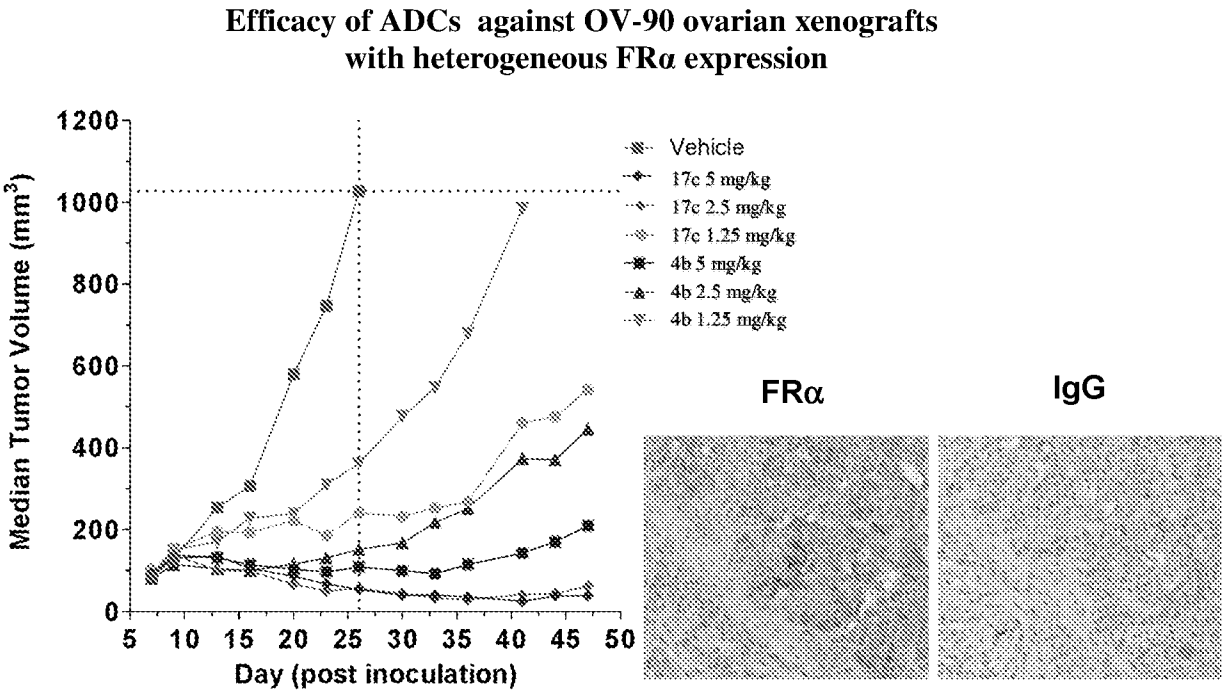

Efficacy of ADCs against OV-90 ovarian xenografts
with heterogeneous FRα expression
Median Tumor Volume (mm3)
1200
1000
800
600
400
200
0
5 10 15 20 25 30 35 40 45 50
Day (post inoculation)
Vehicle
17c 5 mg/kg
17c 2.5 mg/kg
17c 1.25 mg/kg
4b 5 mg/kg
4b 2.5 mg/kg
4b 1.25 mg/kg
FRα
IgG

FIG. 17A

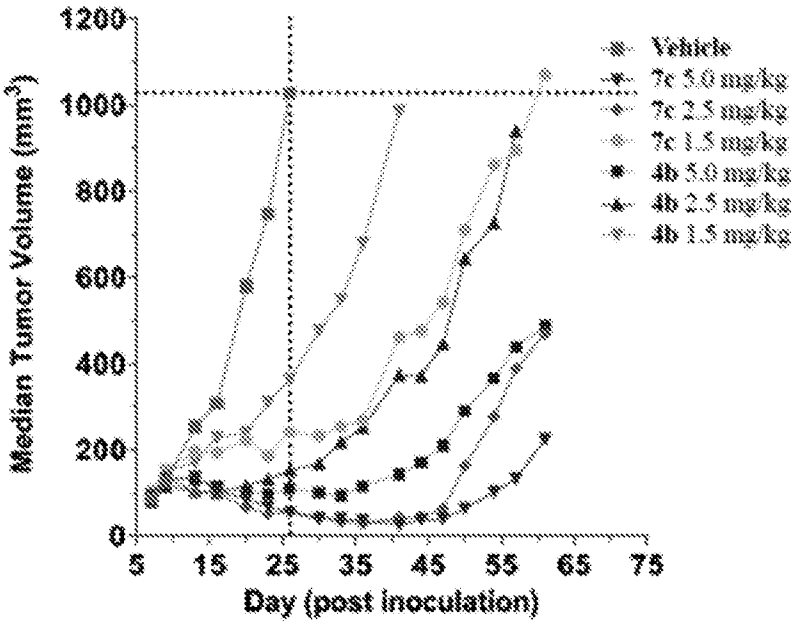

Median Tumor Volume (mm3)
1200
1000
800
600
400
200
0
5
15
25
35
45
55
65
75
Day (post inoculation)
Vehicle
7c 5.0 mg/kg
7c 2.5 mg/kg
7c 1.5 mg/kg
4b 5.0 mg/kg
4b 2.5 mg/kg
4b 1.5 mg/kg

FIG. 17B

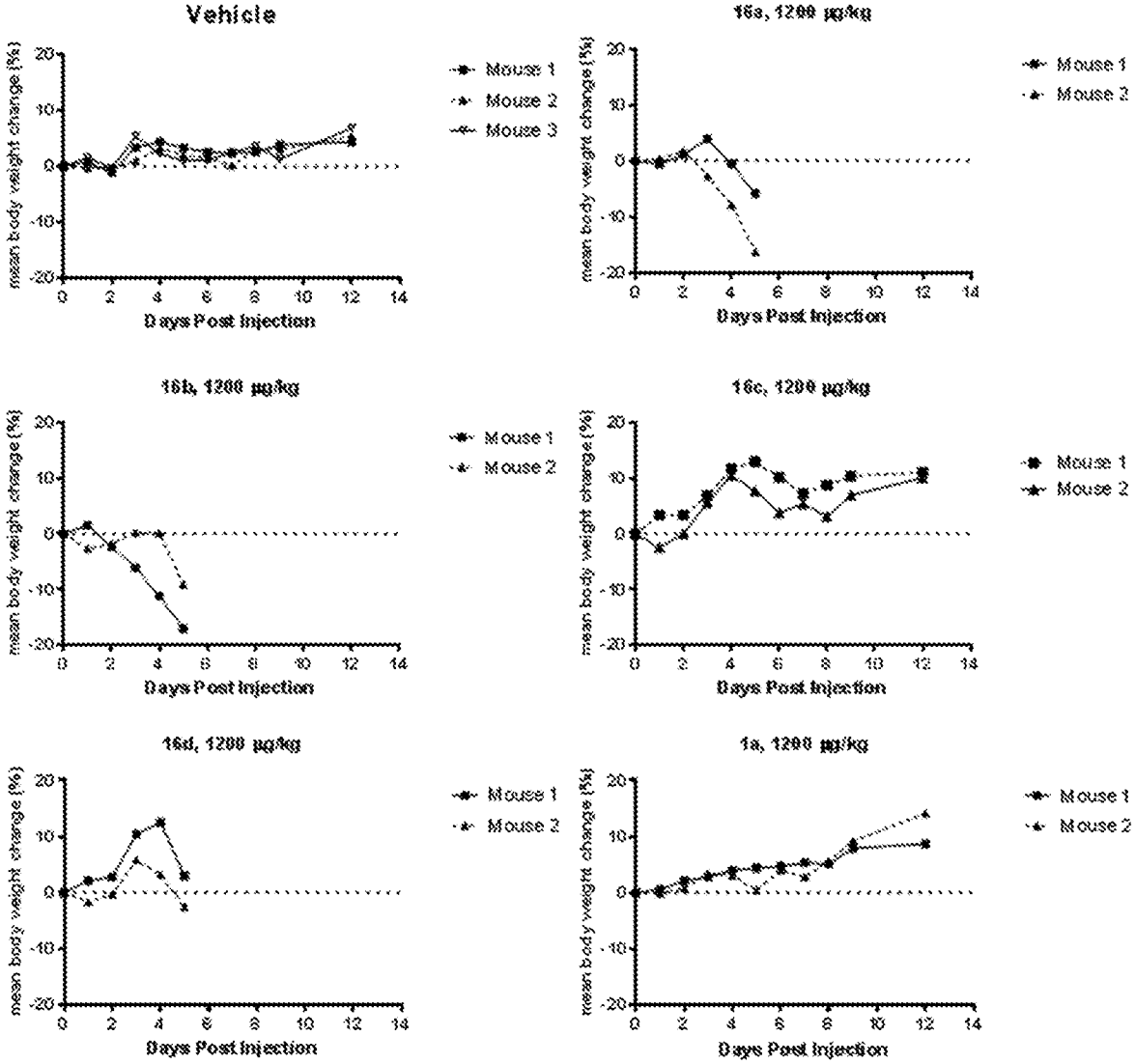

Vehicle
16a, 1200 µg/kg
16b, 1200 µg/kg
16c, 1200 µg/kg
16d, 1200 µg/kg
1a, 1200 µg/kg
mean body weight change (%)
Days Post Injection
Mouse 1
Mouse 2
Mouse 3

FIG. 18

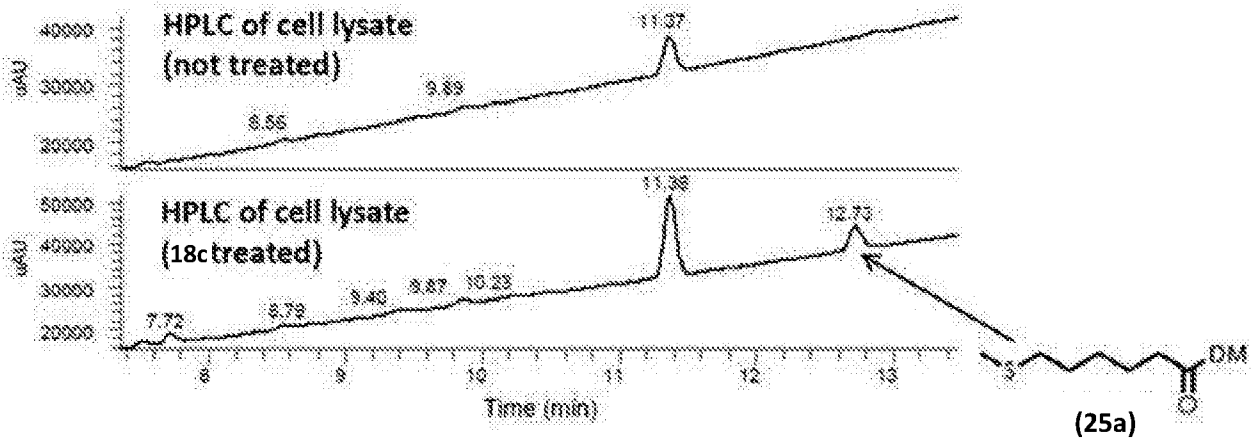

HPLC of cell lysate
(not treated)
11.37
9.89
8.56
HPLC of cell lysate
(18ctreated)
11.38
12.73
9.87
10.23
9.46
8.73
7.72
Time (min)
DM
(25a)

FIG. 19

FIG. 20

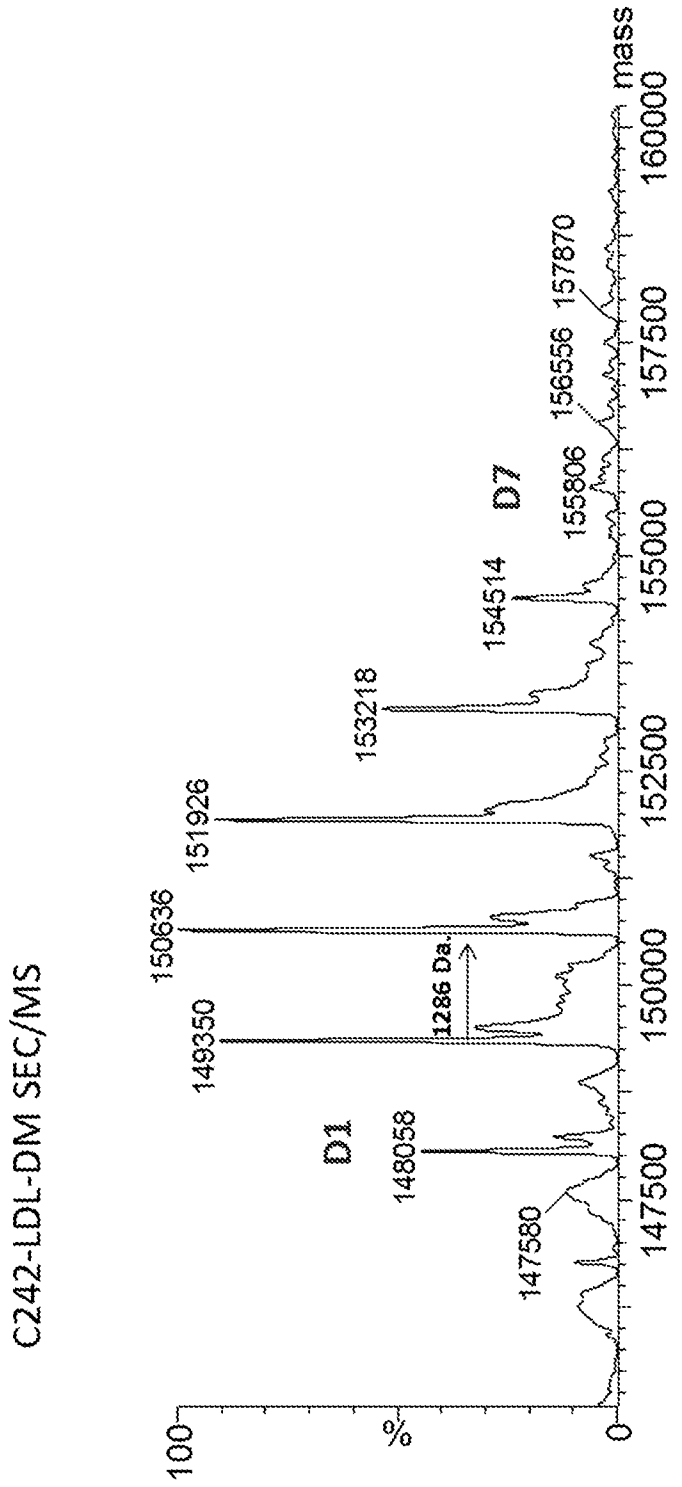
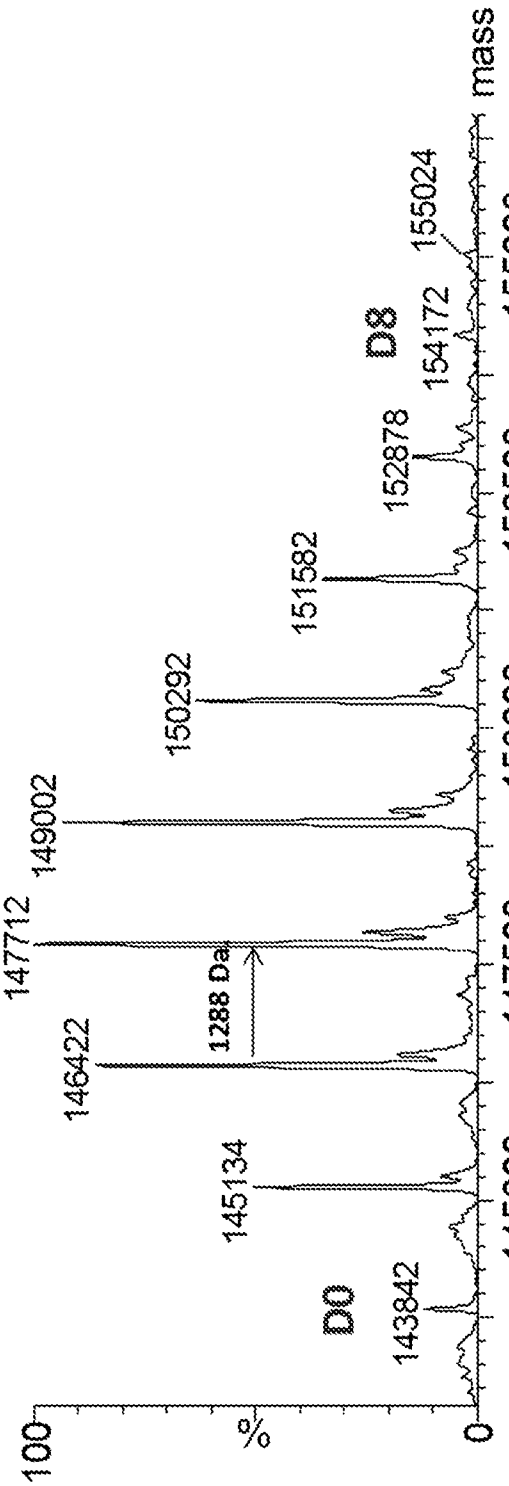

C242-LDL-DM SEC/MS
100
%
0
147580
148058
D1
149350
1286 Da.
150636
151926
153218
154514
D7
155806
156556
157870
147500
150000
152500
155000
157500
160000
mass

FIG. 21


ML66-LDL-DM SEC/MS
100
%
0
D0
143842
145134
146422
1288 Da
147712
149002
150292
151582
D8
152878
154172
155024
145000
147500
150000
152500
155000
mass

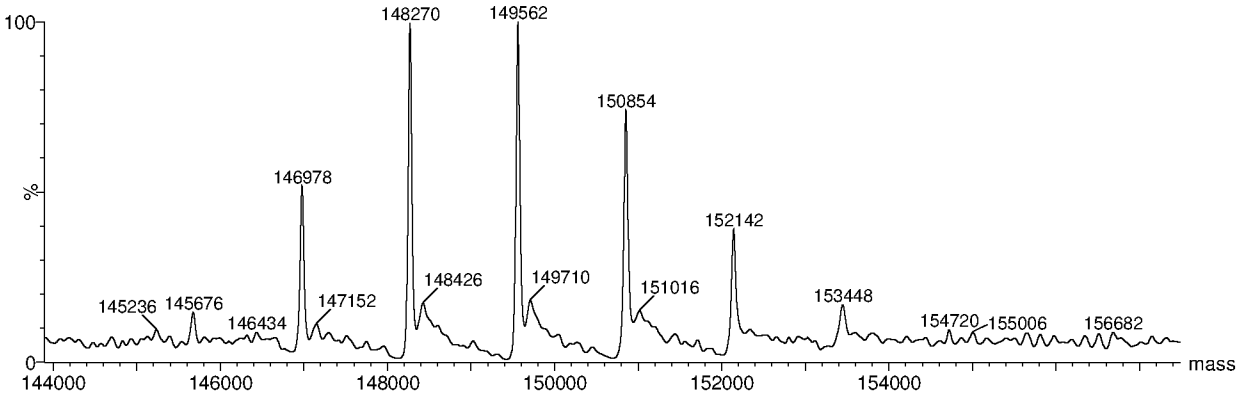

100
%
0
148270
149562
150854
146978
152142
148426
149710
151016
145236
145676
147152
153448
146434
154720
155006
156682
144000
146000
148000
150000
152000
154000
mass

FIG. 22

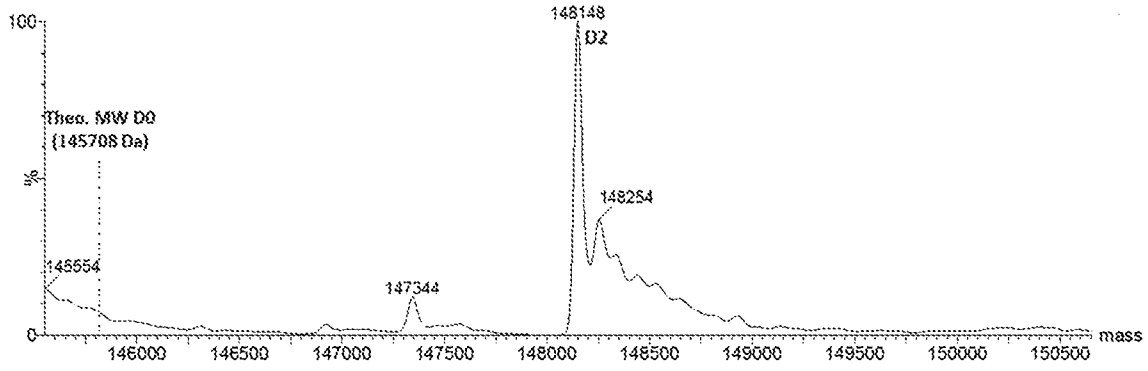

100
%
0
Theo. MW D0
(145708 Da)
145554
147344
148148
D2
148254
146000
146500
147000
147500
148000
148500
149000
149500
150000
150500
mass

FIG. 23

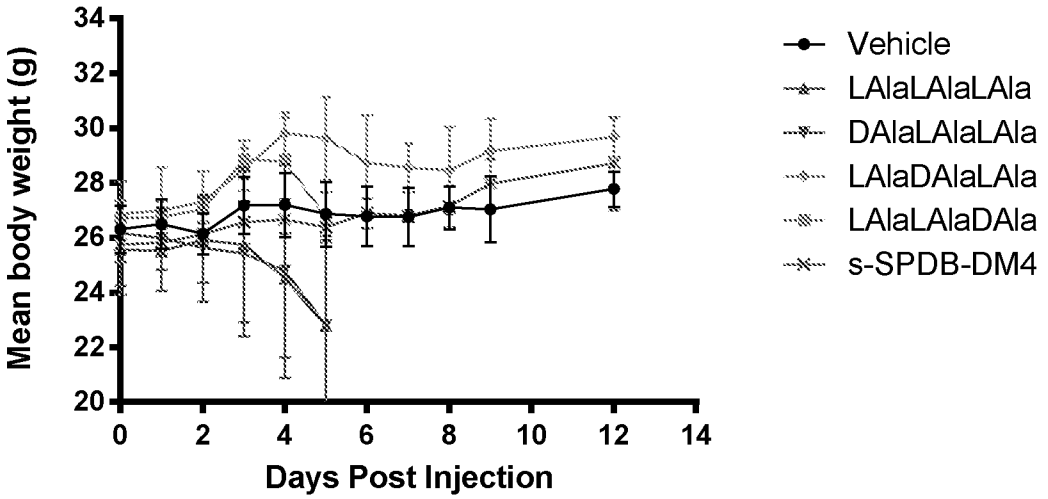


FIG. 24A

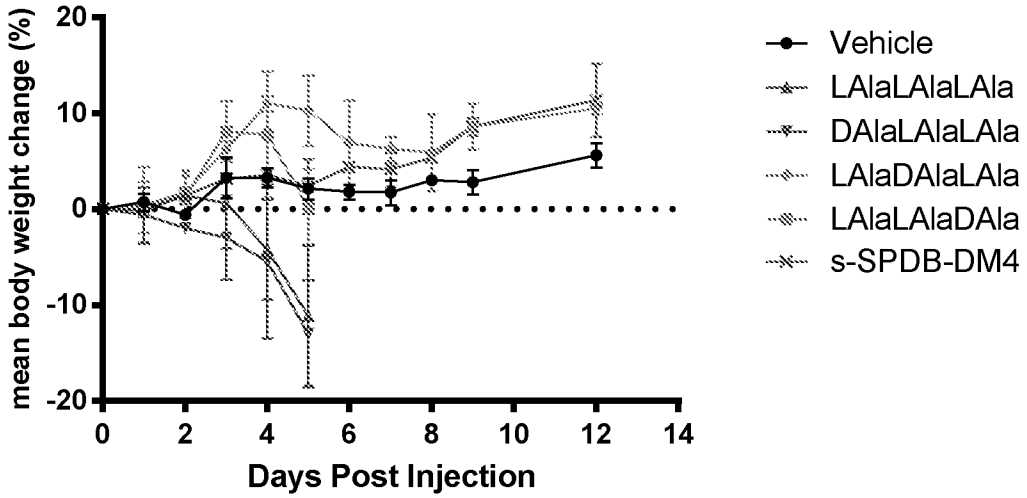


FIG. 24B

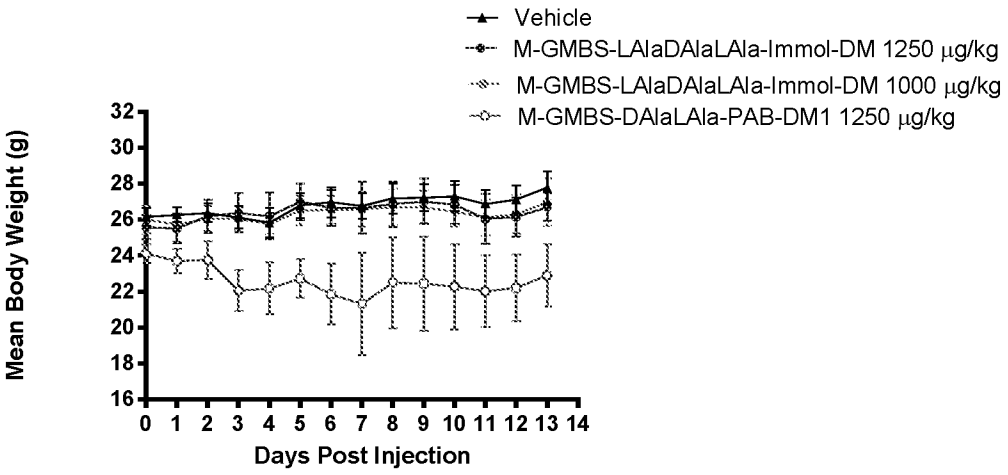


FIG. 25A

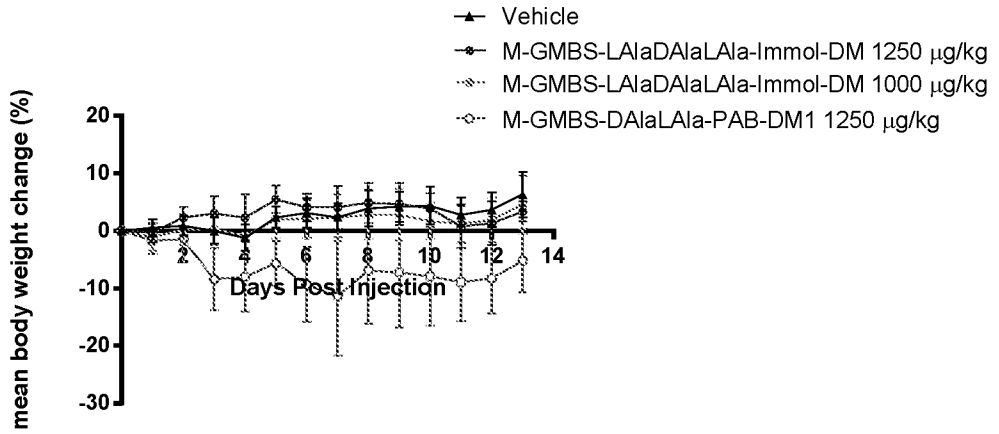


FIG. 25B

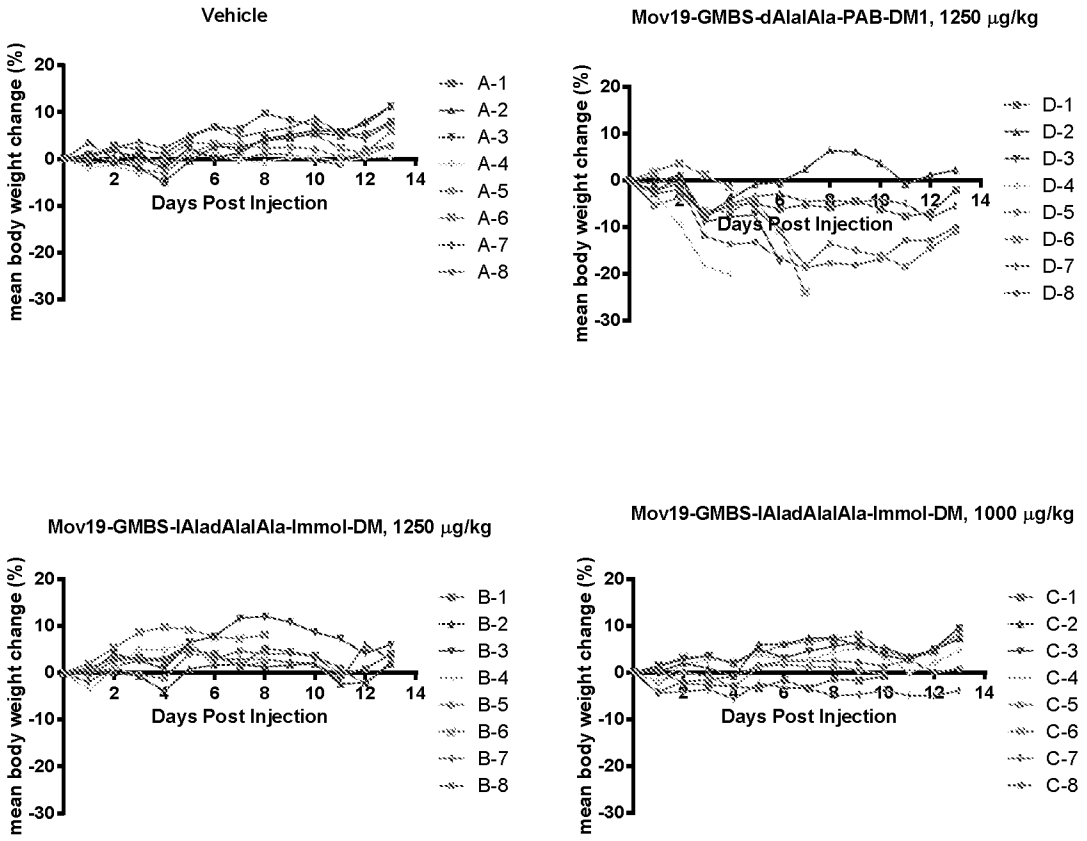

Vehicle
mean body weight change (%)
Days Post Injection
A-1
A-2
A-3
A-4
A-5
A-6
A-7
A-8
Mov19-GMBS-dAlalAla-PAB-DM1, 1250 μg/kg
D-1
D-2
D-3
D-4
D-5
D-6
D-7
D-8
Mov19-GMBS-lAladAlalAla-Immol-DM, 1250 μg/kg
B-1
B-2
B-3
B-4
B-5
B-6
B-7
B-8
Mov19-GMBS-lAladAlalAla-Immol-DM, 1000 μg/kg
C-1
C-2
C-3
C-4
C-5
C-6
C-7
C-8

FIG. 25C

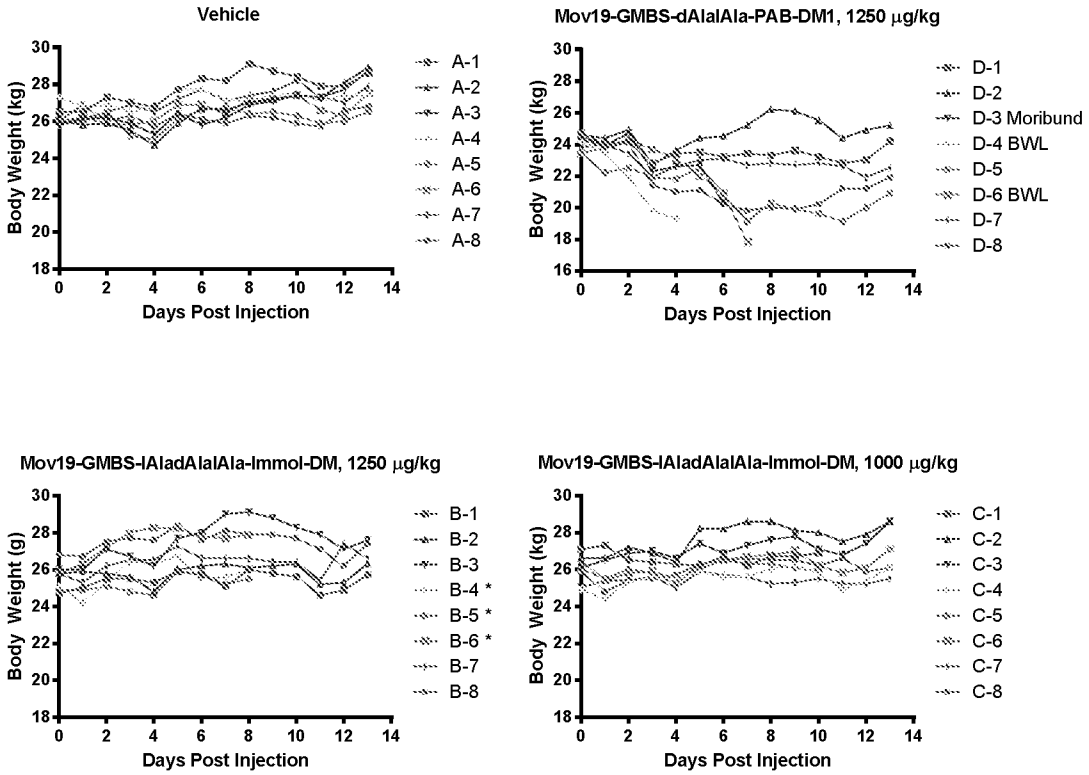
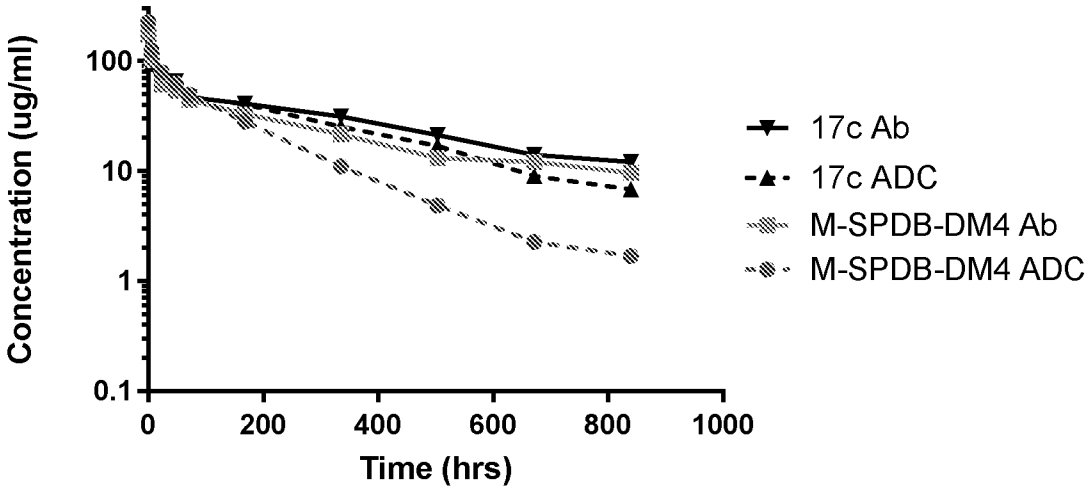
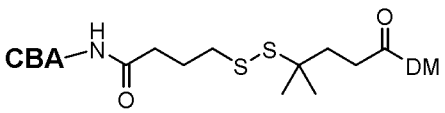

Vehicle
Body Weight (kg)
Days Post Injection
A-1
A-2
A-3
A-4
A-5
A-6
A-7
A-8
Mov19-GMBS-dAlalAla-PAB-DM1, 1250 μg/kg
Body Weight (kg)
Days Post Injection
D-1
D-2
D-3 Moribund
D-4 BWL
D-5
D-6 BWL
D-7
D-8
Mov19-GMBS-lAladAlalAla-Immol-DM, 1250 μg/kg
Body Weight (g)
Days Post Injection
B-1
B-2
B-3
B-4 *
B-5 *
B-6 *
B-7
B-8
Mov19-GMBS-lAladAlalAla-Immol-DM, 1000 μg/kg
Body Weight (kg)
Days Post Injection
C-1
C-2
C-3
C-4
C-5
C-6
C-7
C-8

FIG. 25D

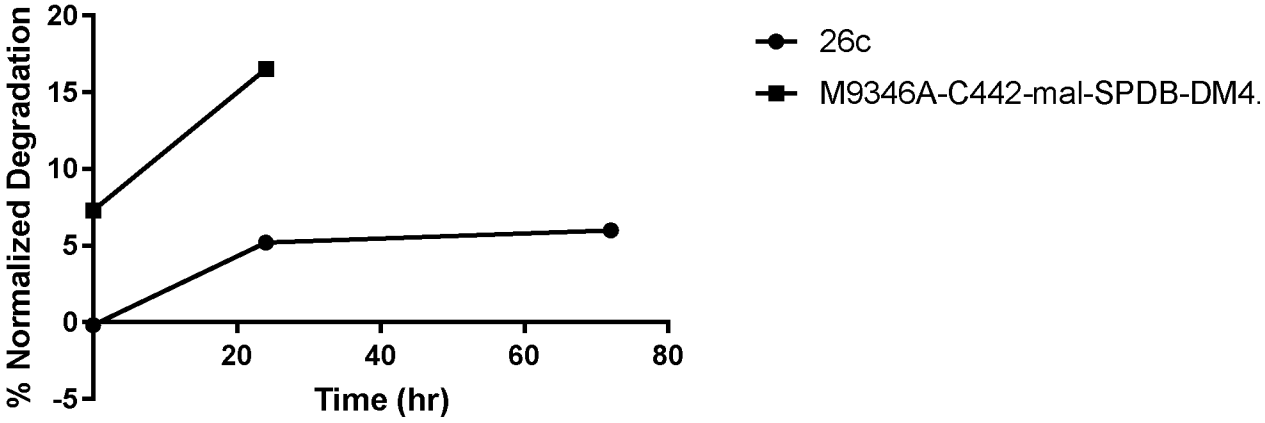


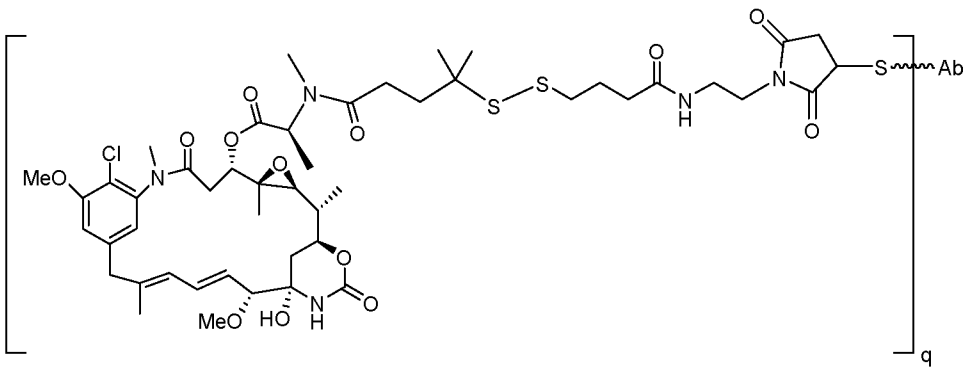


CBA =anti-folate receptor antibody

M-SPDB-DM4

FIG. 26





Ab = ant-folate receptor antibody M9346A-C442
M9346A-C442-mal-SPDB-DM4

FIG. 27