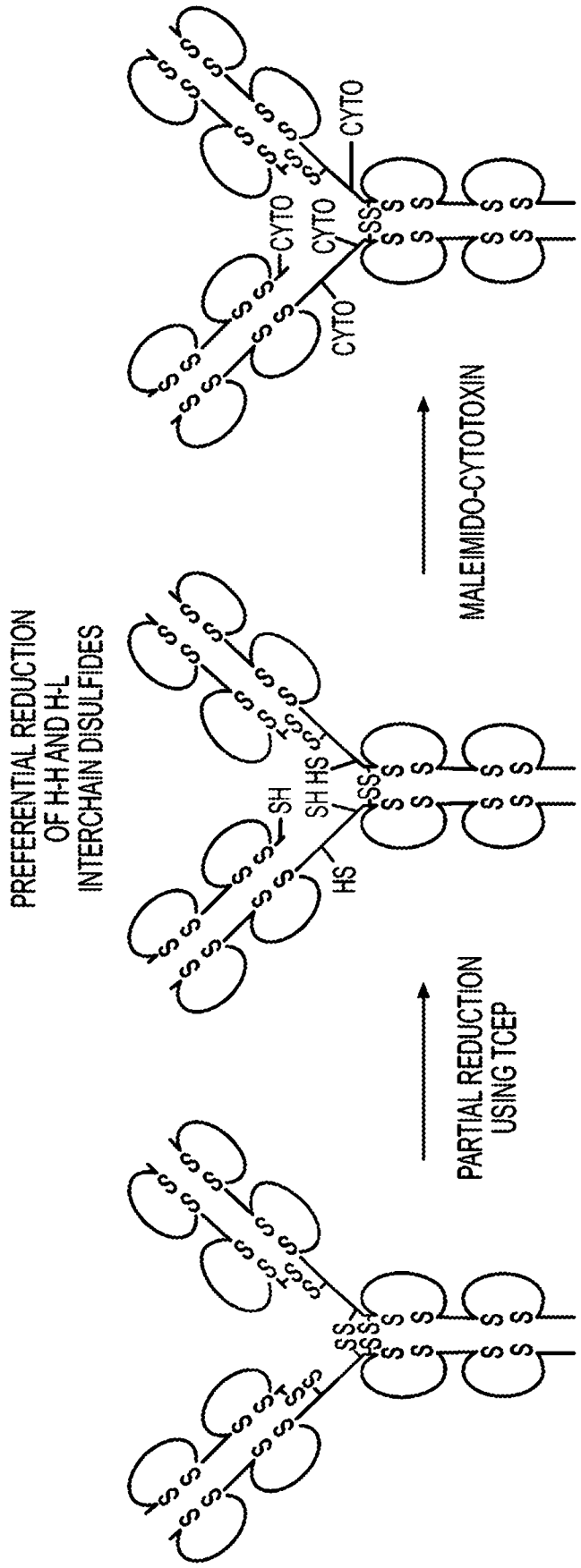
PREFERENTIAL REDUCTION
OF H-H AND H-L
INTERCHAIN DISULFIDES
PARTIAL REDUCTION
USING TCEP
MALEIMIDO-CYTOTOXIN
SH
SH HS
HS
CYTO
CYTO
CYTO
CYTO

FIG. 1

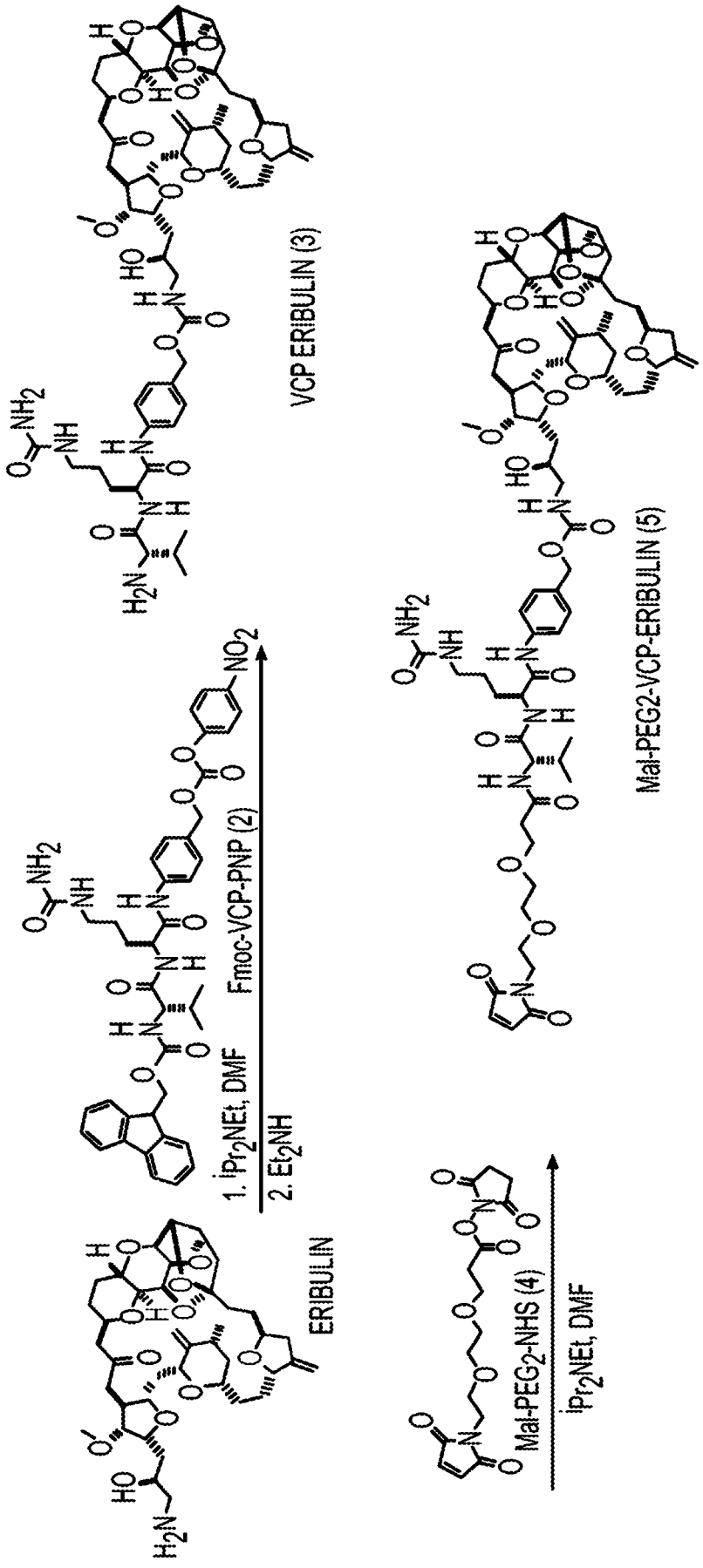
ERIBULIN
1. iPr2NEt, DMF
2. Et2NH
Fmoc-VCP-PNP (2)
VCP ERIBULIN (3)
Mal-PEG2-NHS (4)
iPr2NEt, DMF
Mal-PEG2-VCP-ERIBULIN (5)

FIG. 2

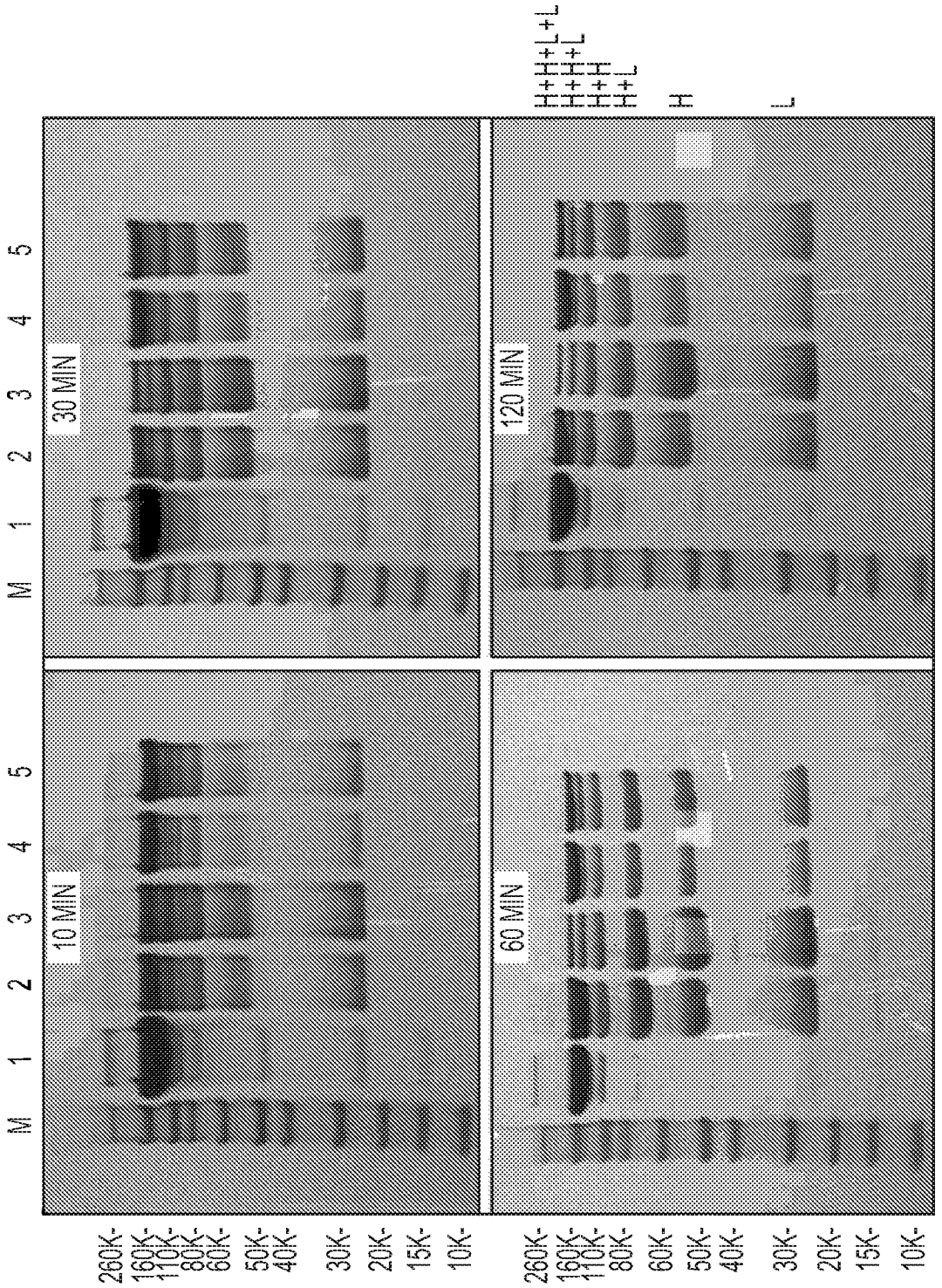
M 1 2 3 4 5
10 MIN
30 MIN
60 MIN
120 MIN
H+H+L+L
H+H+L
H+H
H+L
H
L
260K-
160K-
110K-
80K-
60K-
50K-
40K-
30K-
20K-
15K-
10K-

FIG. 3

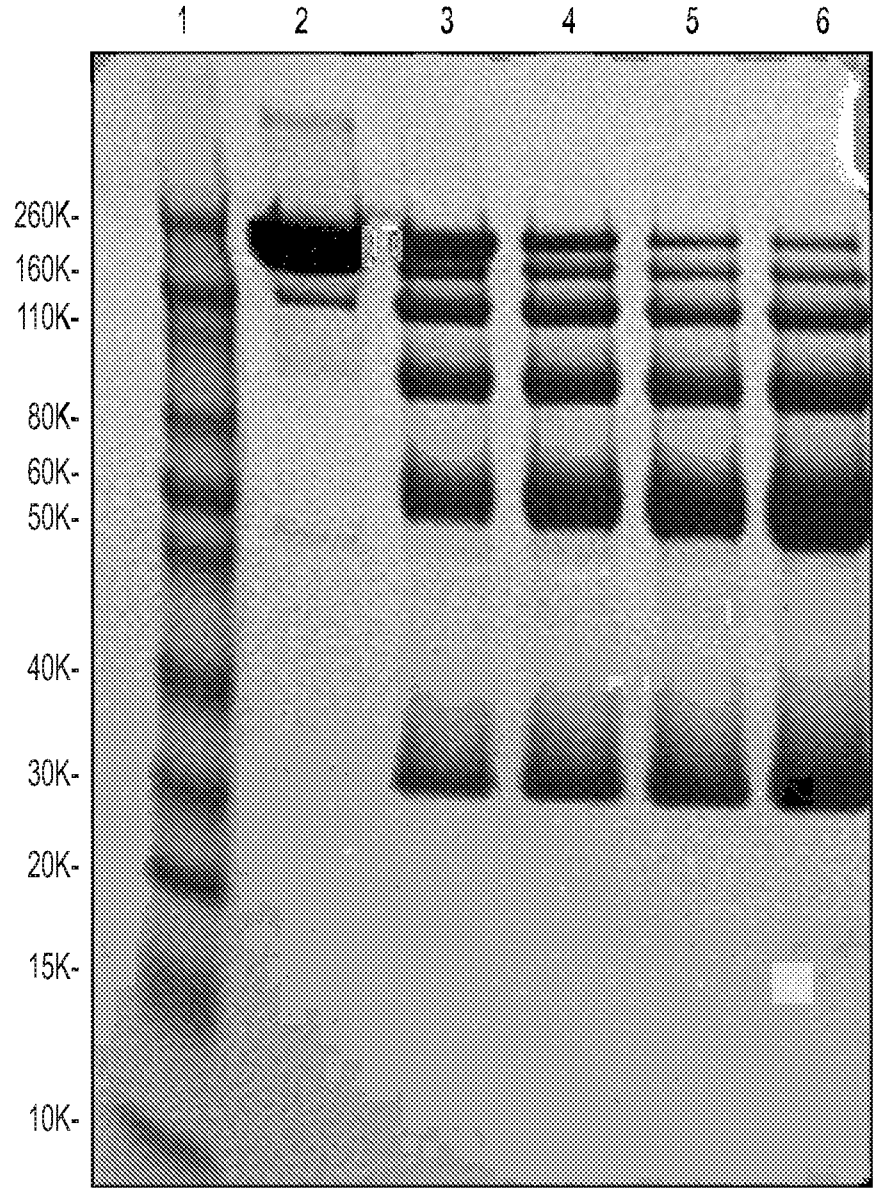

FIG. 4

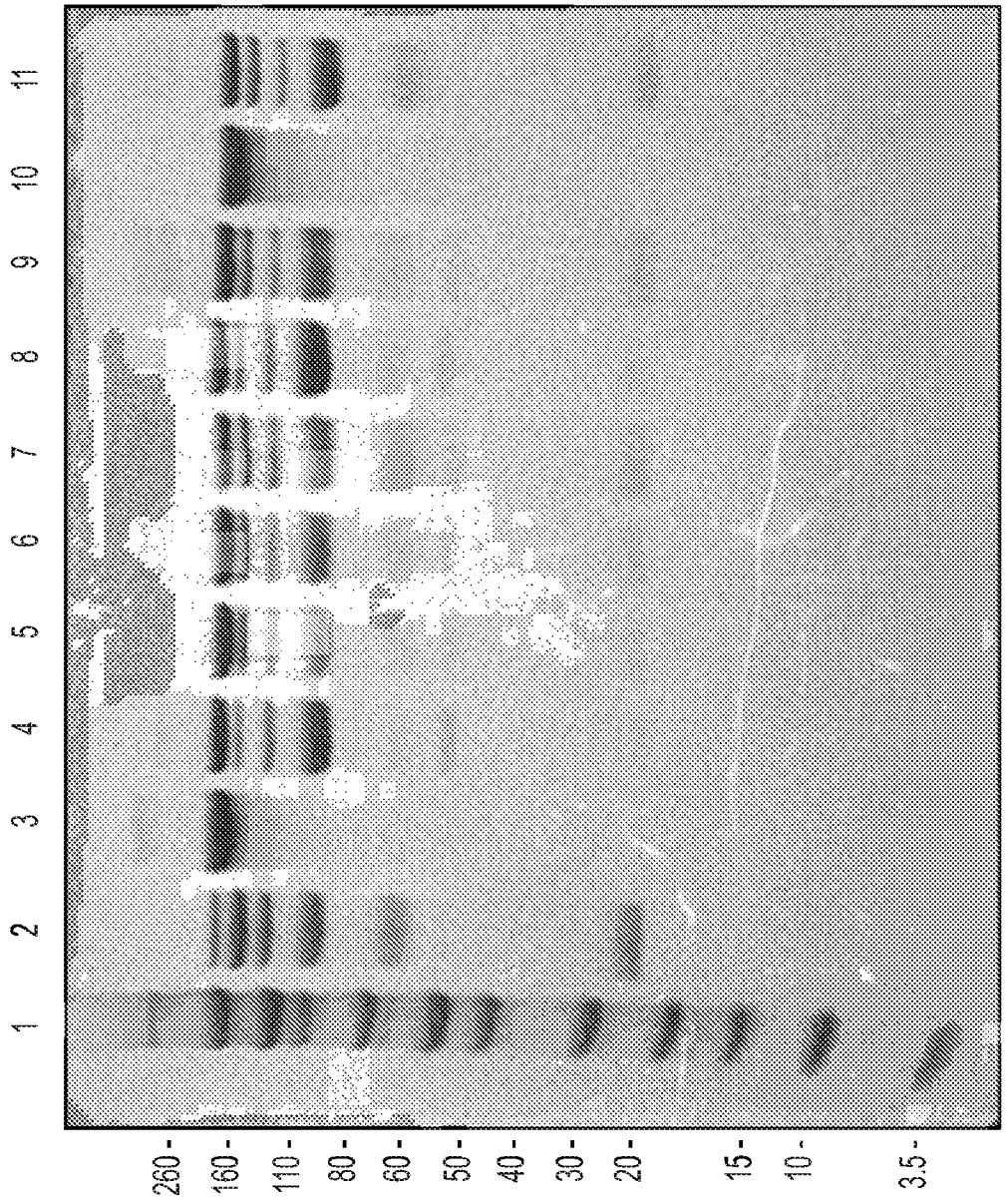

FIG. 5

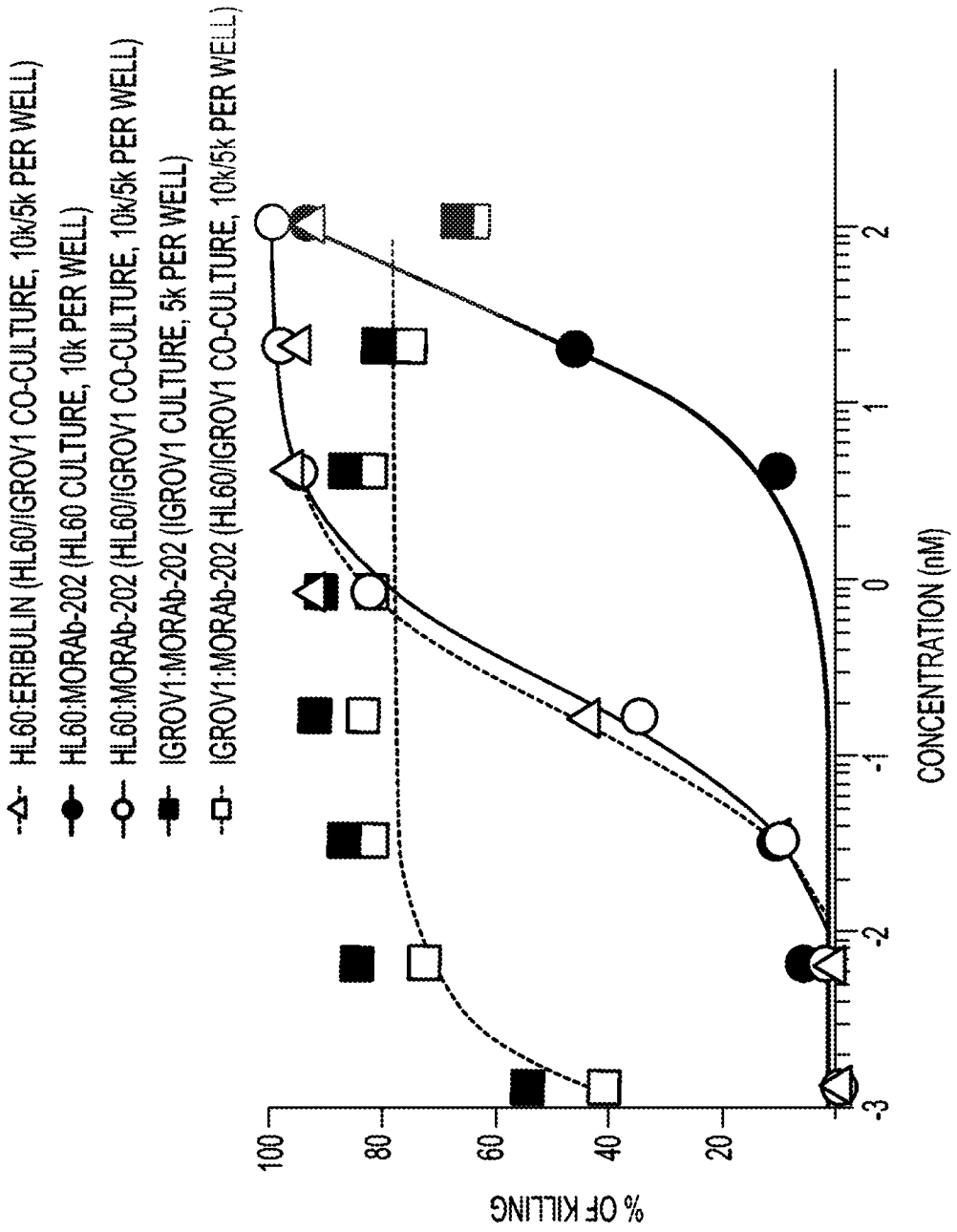
HL60:ERIBULIN (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
HL60:MORAb-202 (HL60 CULTURE, 10k PER WELL)
HL60:MORAb-202 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
IGROV1:MORAb-202 (IGROV1 CULTURE, 5k PER WELL)
IGROV1:MORAb-202 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
% OF KILLING
100
80
60
40
20
-3
-2
-1
0
1
2
CONCENTRATION (nM)

FIG. 6A

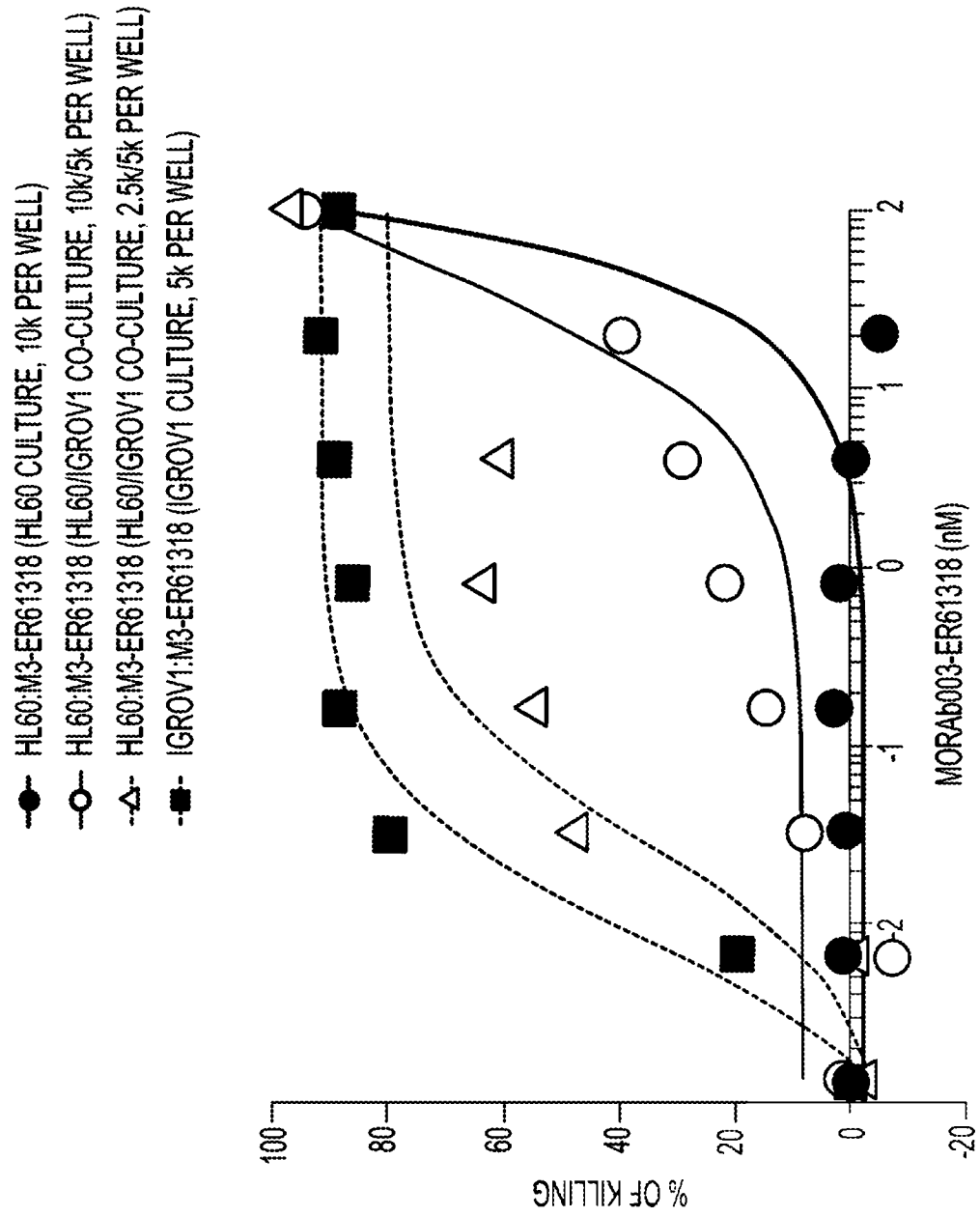
HL60:M3-ER61318 (HL60 CULTURE, 10k PER WELL)
HL60:M3-ER61318 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
HL60:M3-ER61318 (HL60/IGROV1 CO-CULTURE, 2.5k/5k PER WELL)
IGROV1:M3-ER61318 (IGROV1 CULTURE, 5k PER WELL)
% OF KILLING
100
80
60
40
20
0
-20
2
1
0
-1
-2
MORAb003-ER61318 (nM)

FIG. 6B

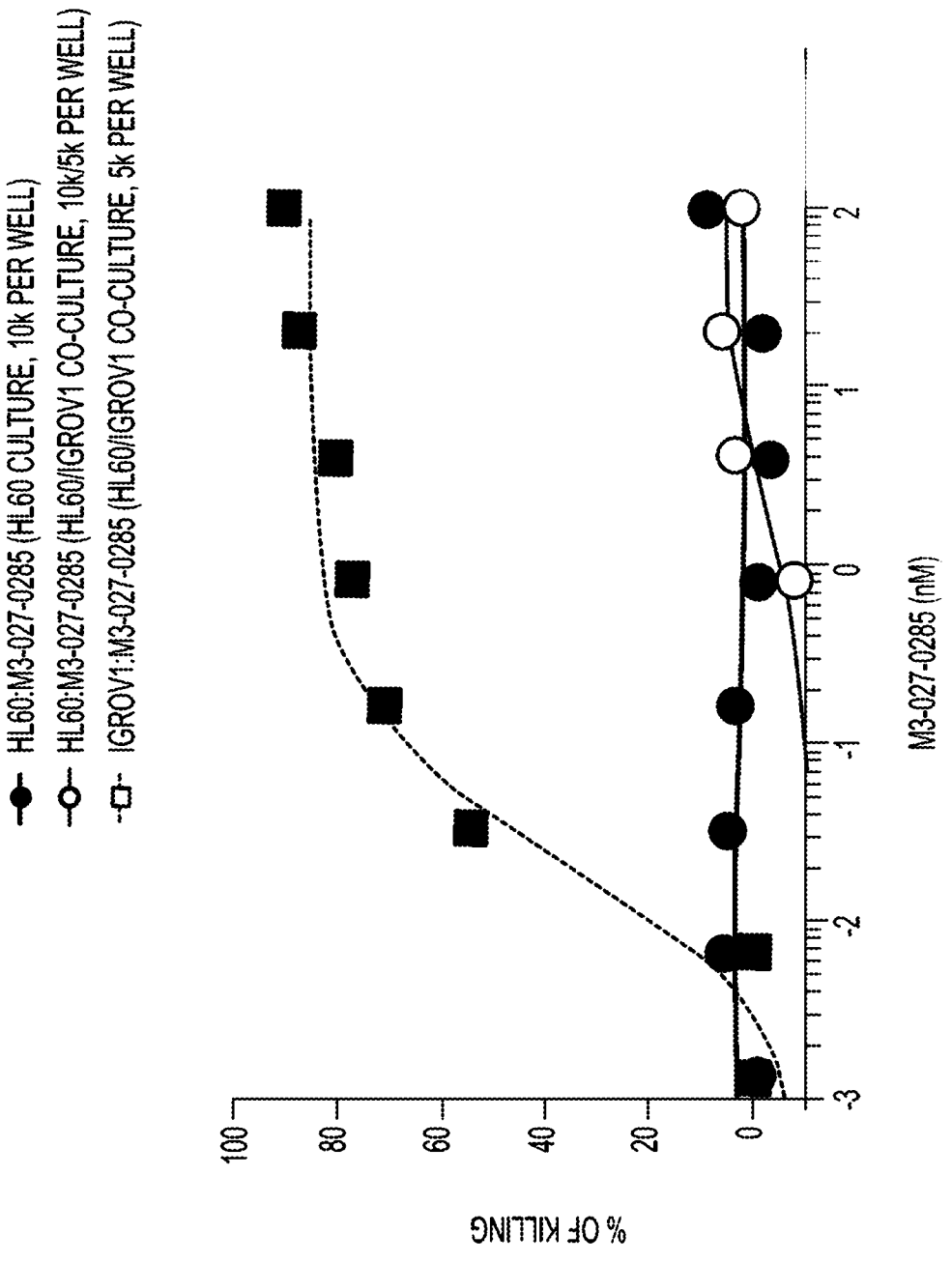
HL60:M3-027-0285 (HL60 CULTURE, 10k PER WELL)
HL60:M3-027-0285 (HL60/IGROV1 CO-CULTURE, 10k/5k PER WELL)
IGROV1:M3-027-0285 (HL60/IGROV1 CO-CULTURE, 5k PER WELL)
% OF KILLING
100
80
60
40
20
0
-3
-2
-1
0
1
2
M3-027-0285 (nM)

FIG. 6C

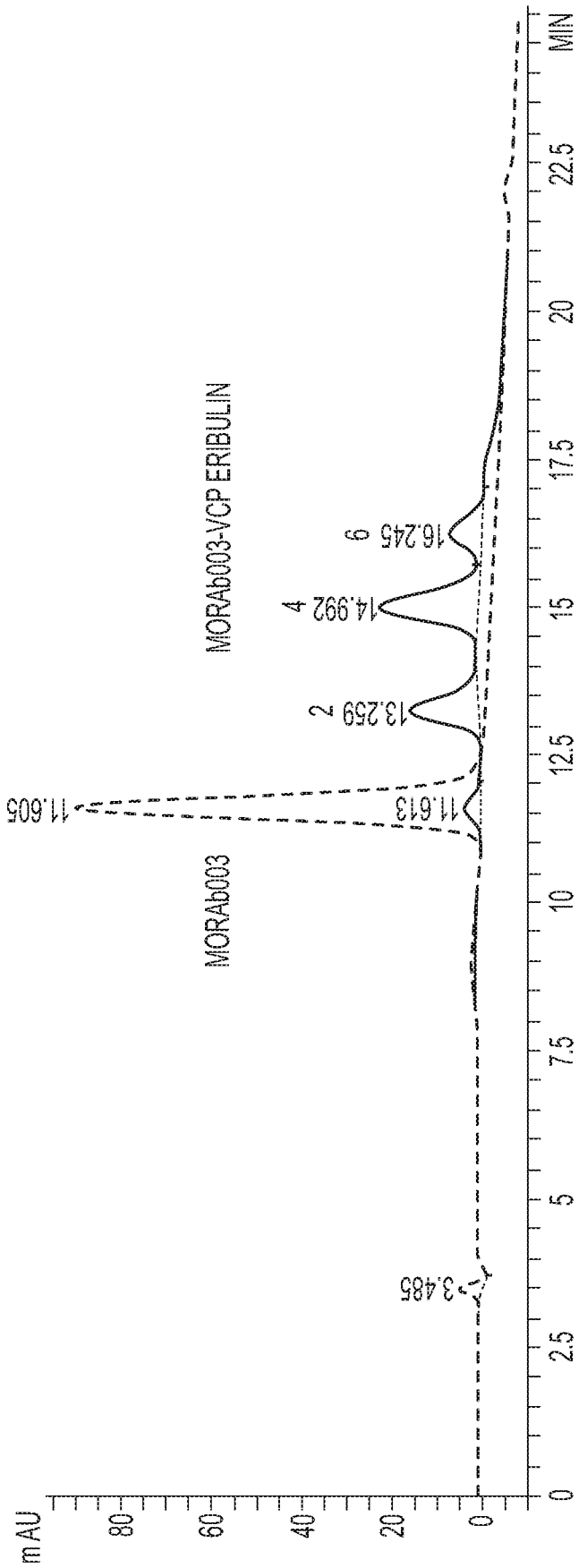
mAU
80
60
40
20
0
0
2.5
5
7.5
10
12.5
15
17.5
20
22.5
MIN
MORAb003-VCP ERIBULIN
MORAb003
3.485
11.605
11.613
13.259
14.992
16.245
2
4
6
9

FIG. 7A

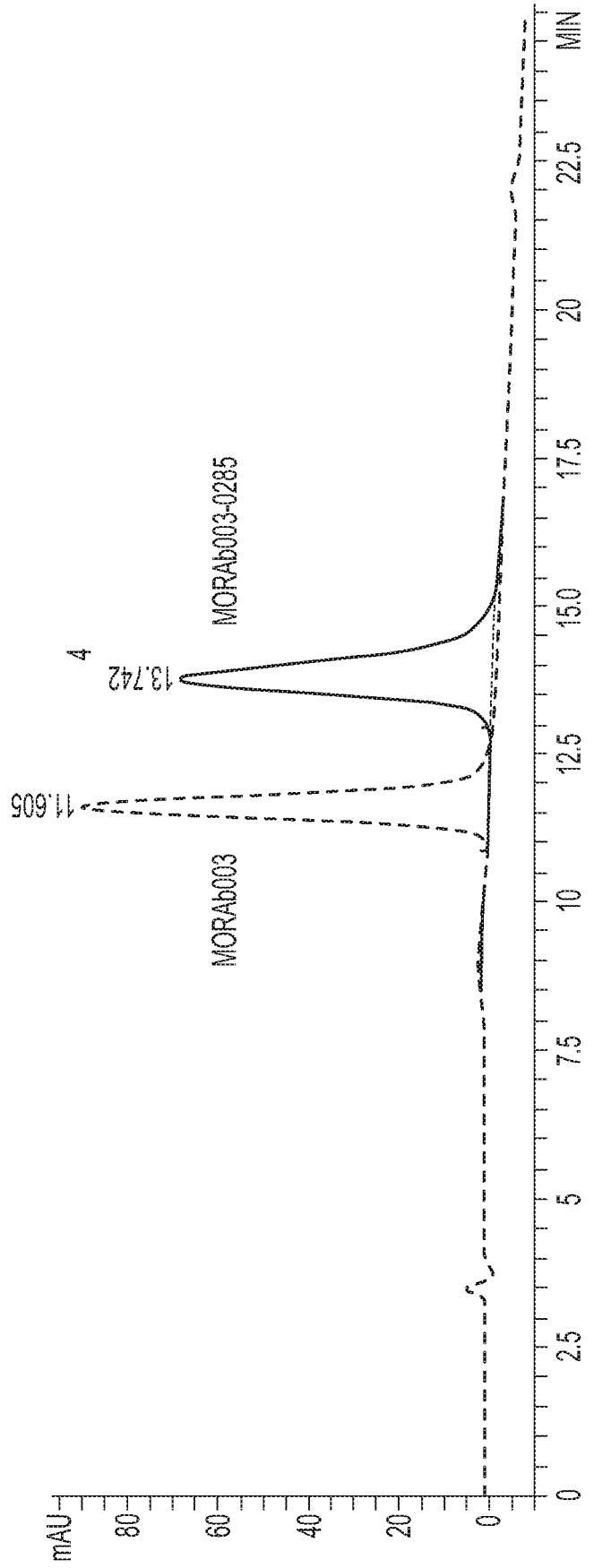
mAU
80
60
40
20
0
0
2.5
5
7.5
10
12.5
15.0
17.5
20
22.5
MIN
MORAb003
11.605
4
13.742
MORAb003-0285

FIG. 7B

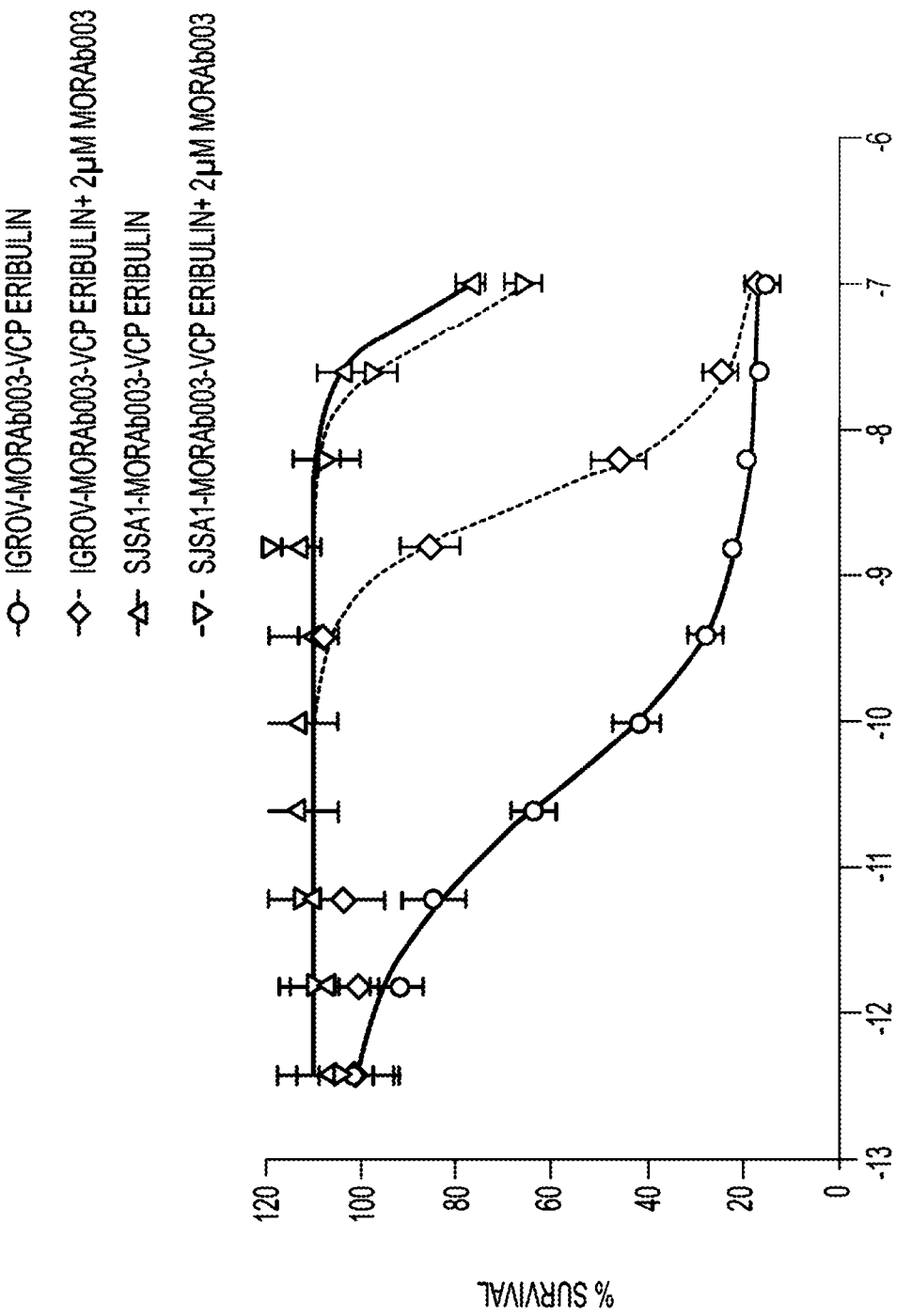
IGROV-MORAb003-VCP ERIBULIN
IGROV-MORAb003-VCP ERIBULIN+ 2µM MORAb003
SJSA1-MORAb003-VCP ERIBULIN
SJSA1-MORAb003-VCP ERIBULIN+ 2µM MORAb003
% SURVIVAL
120
100
80
60
40
20
0
-6
-7
-8
-9
-10
-11
-12
-13
CONJUGATE CONCENTRATION, LOG (M)

FIG. 8

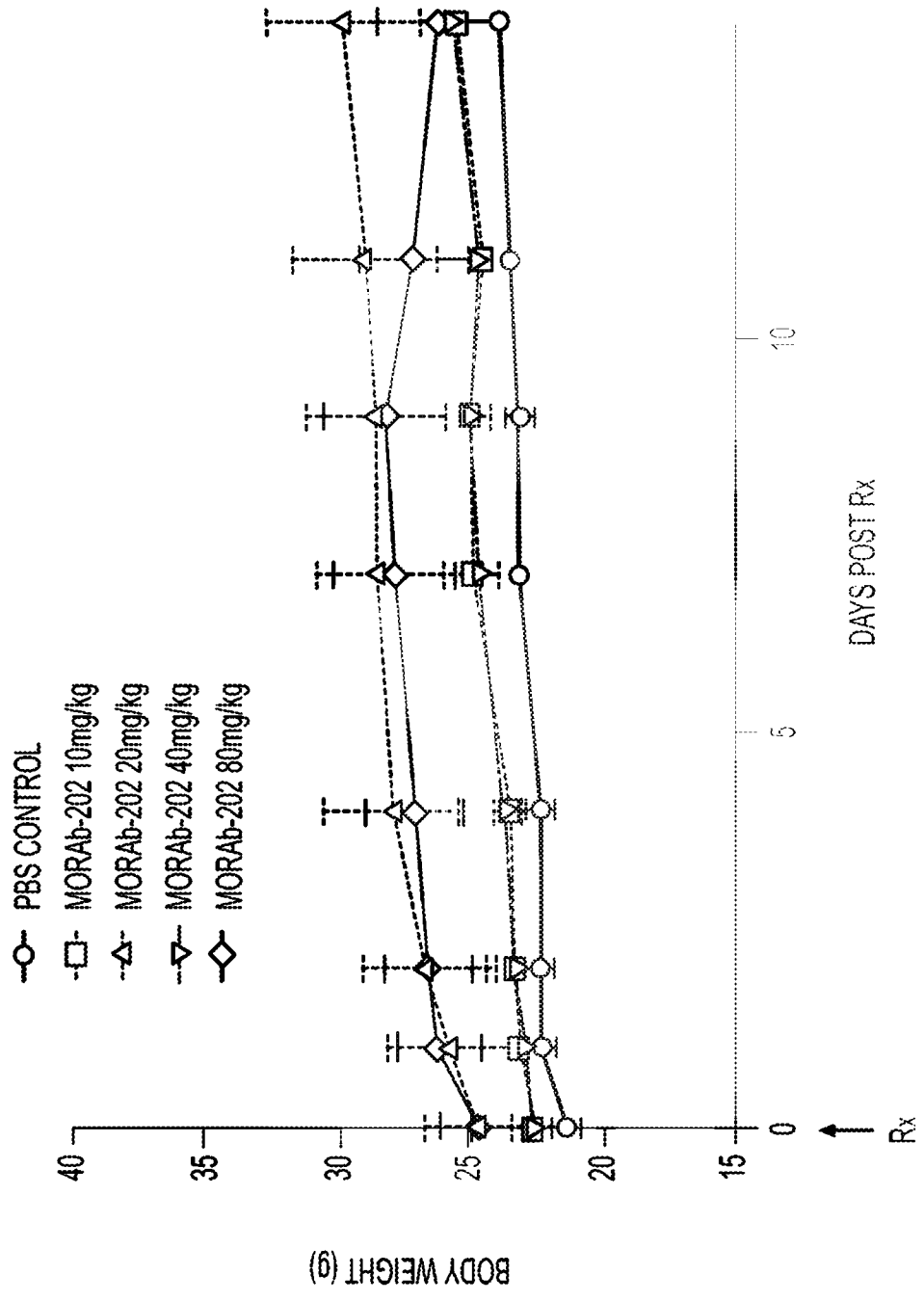
PBS CONTROL
MORAb-202 10mg/kg
MORAb-202 20mg/kg
MORAb-202 40mg/kg
MORAb-202 80mg/kg
BODY WEIGHT (g)
40
35
30
25
20
15
0
5
10
Rx
DAYS POST Rx

FIG. 9

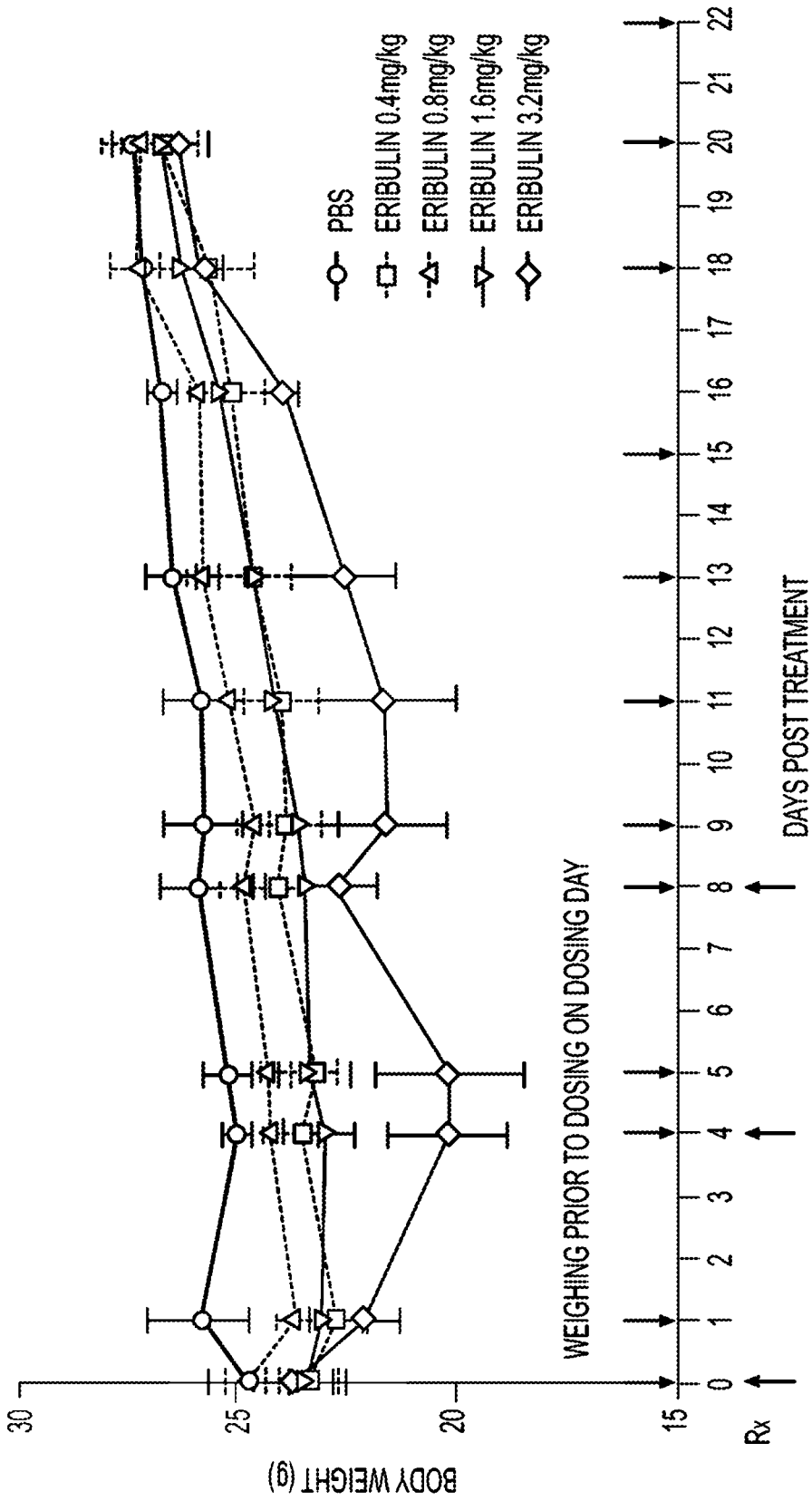
BODY WEIGHT (g)
30
25
20
15
PBS
ERIBULIN 0.4mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg
ERIBULIN 3.2mg/kg
WEIGHING PRIOR TO DOSING ON DOSING DAY
DAYS POST TREATMENT
Rx
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

FIG. 10

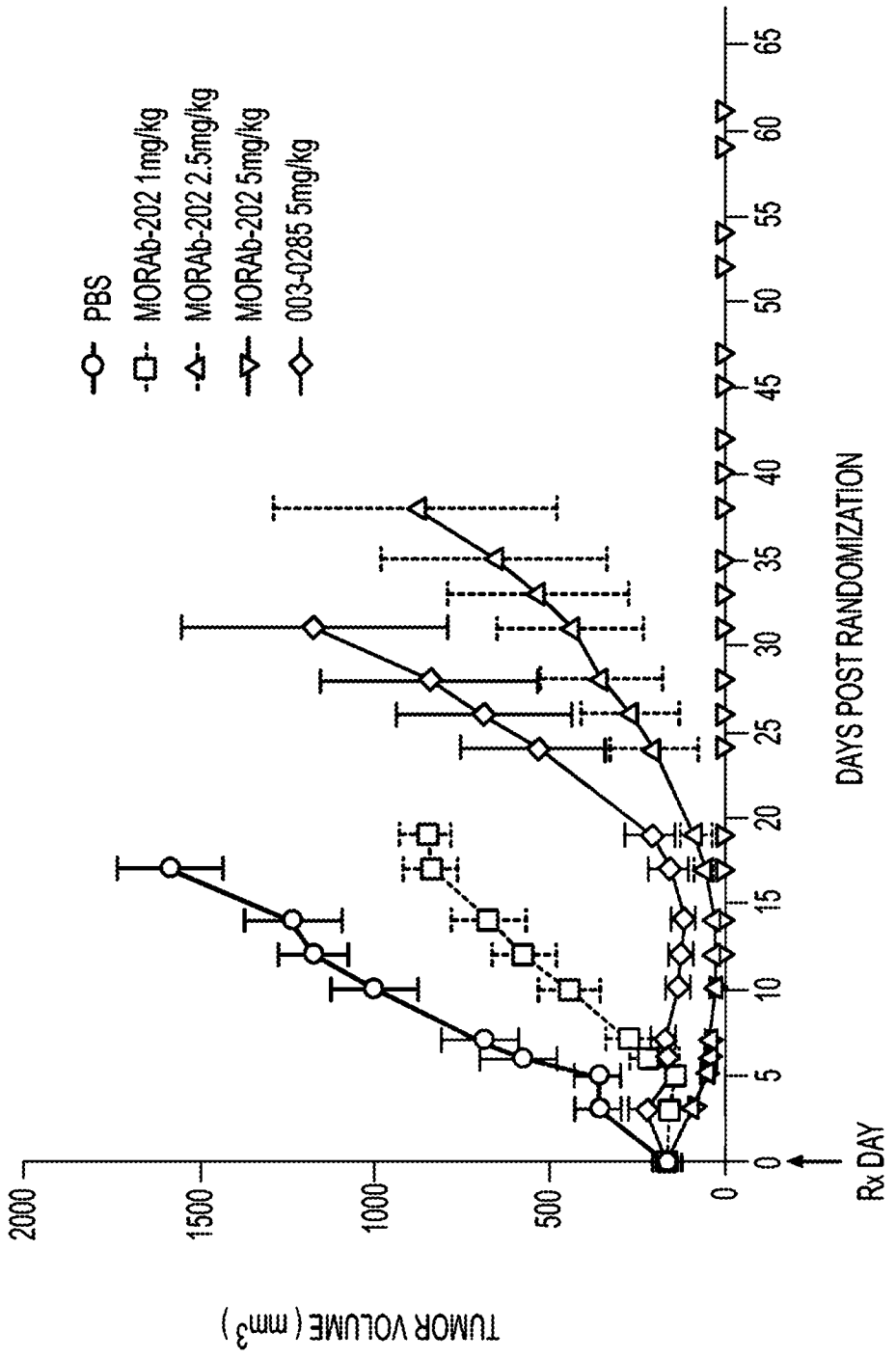
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
003-0285 5mg/kg
TUMOR VOLUME (mm3)
2000
1500
1000
500
0
Rx DAY
0
5
10
15
20
25
30
35
40
45
50
55
60
65
DAYS POST RANDOMIZATION

FIG. 11

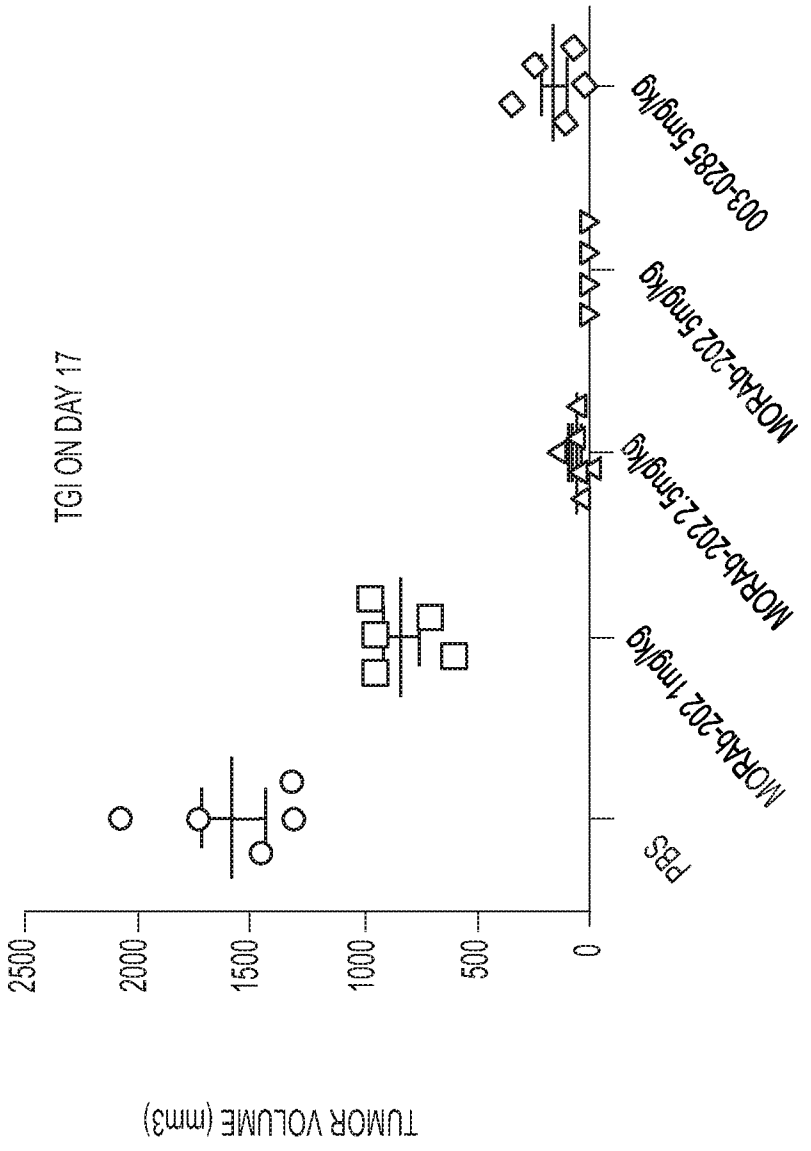
TUMOR VOLUME (mm3)
2500
2000
1500
1000
500
0
TGI ON DAY 17
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
003-0285 5mg/kg

FIG. 12

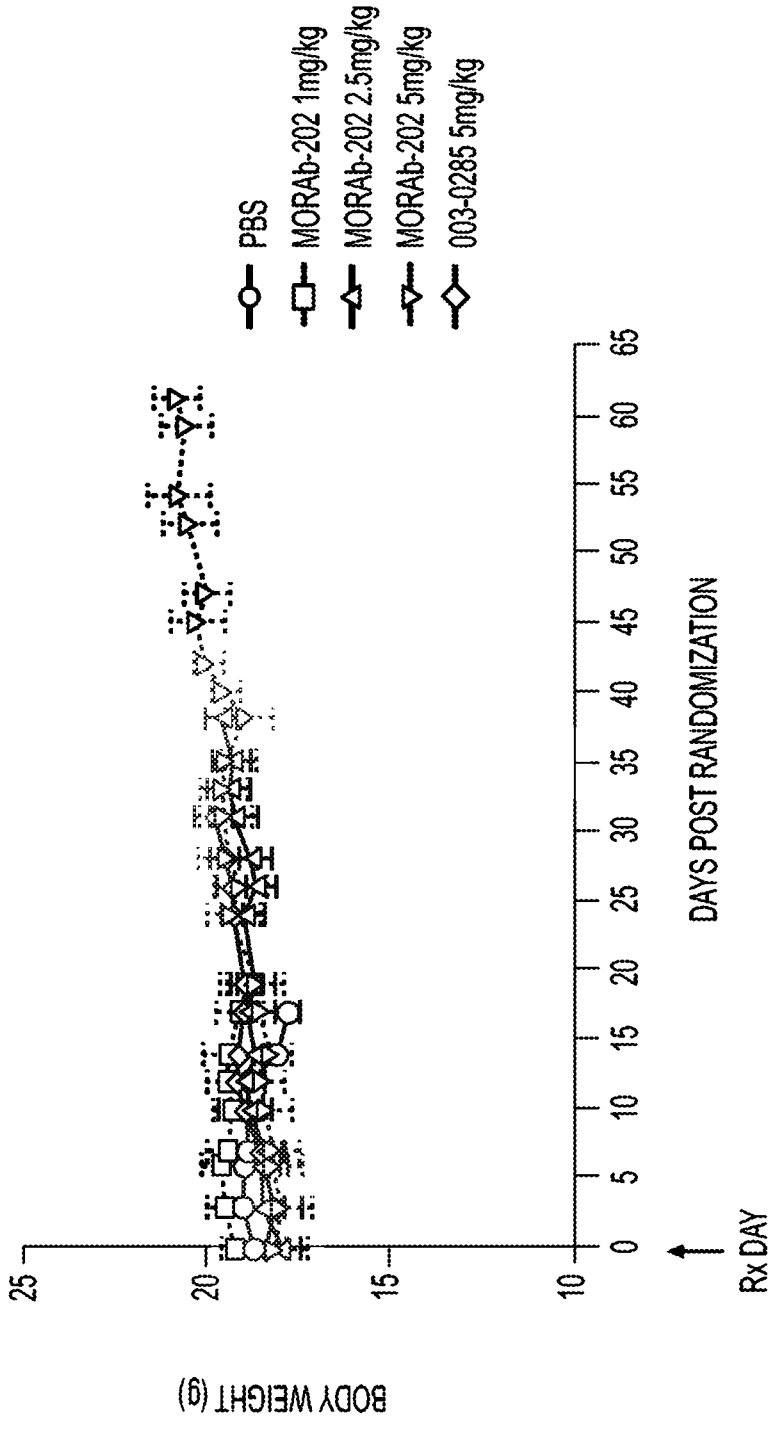
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
003-0285 5mg/kg
BODY WEIGHT (g)
25
20
15
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
DAYS POST RANDOMIZATION
Rx DAY

FIG. 13

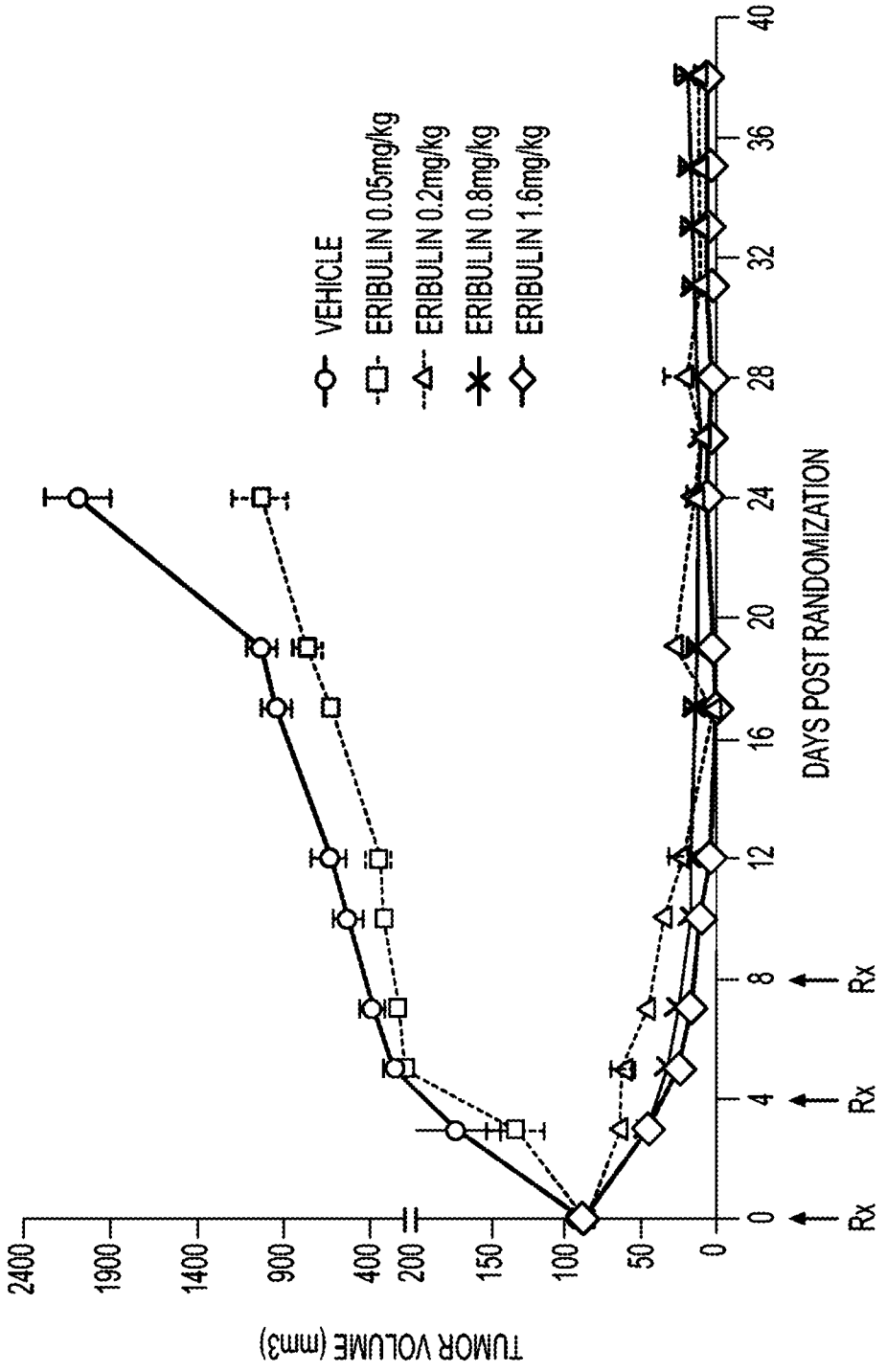
VEHICLE
ERIBULIN 0.05mg/kg
ERIBULIN 0.2mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg
TUMOR VOLUME (mm3)
2400
1900
1400
900
400
200
150
100
50
0
0
4
8
12
16
20
24
28
32
36
40
Rx
Rx
Rx
DAYS POST RANDOMIZATION

FIG. 14

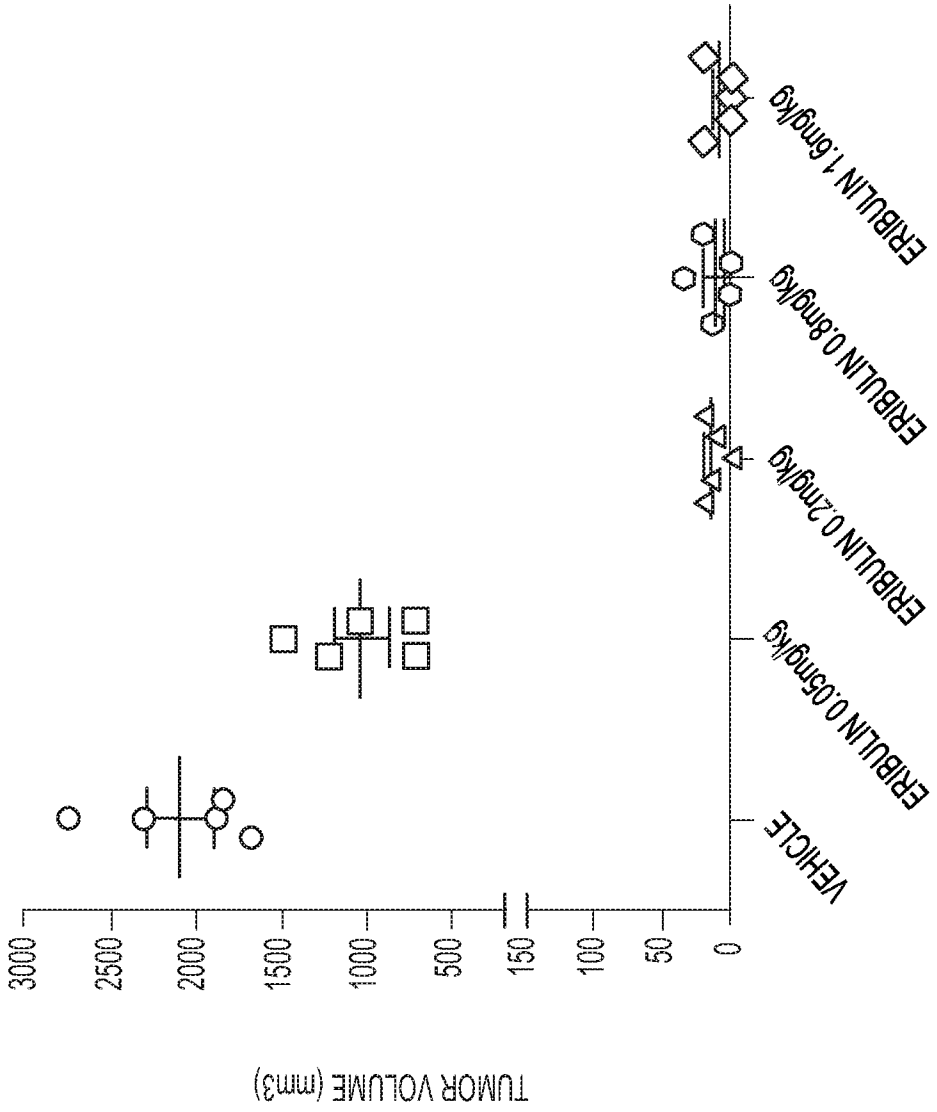
TUMOR VOLUME (mm3)
3000
2500
2000
1500
1000
500
150
100
50
0
VEHICLE
ERIBULIN 0.05mg/kg
ERIBULIN 0.2mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg

FIG. 15

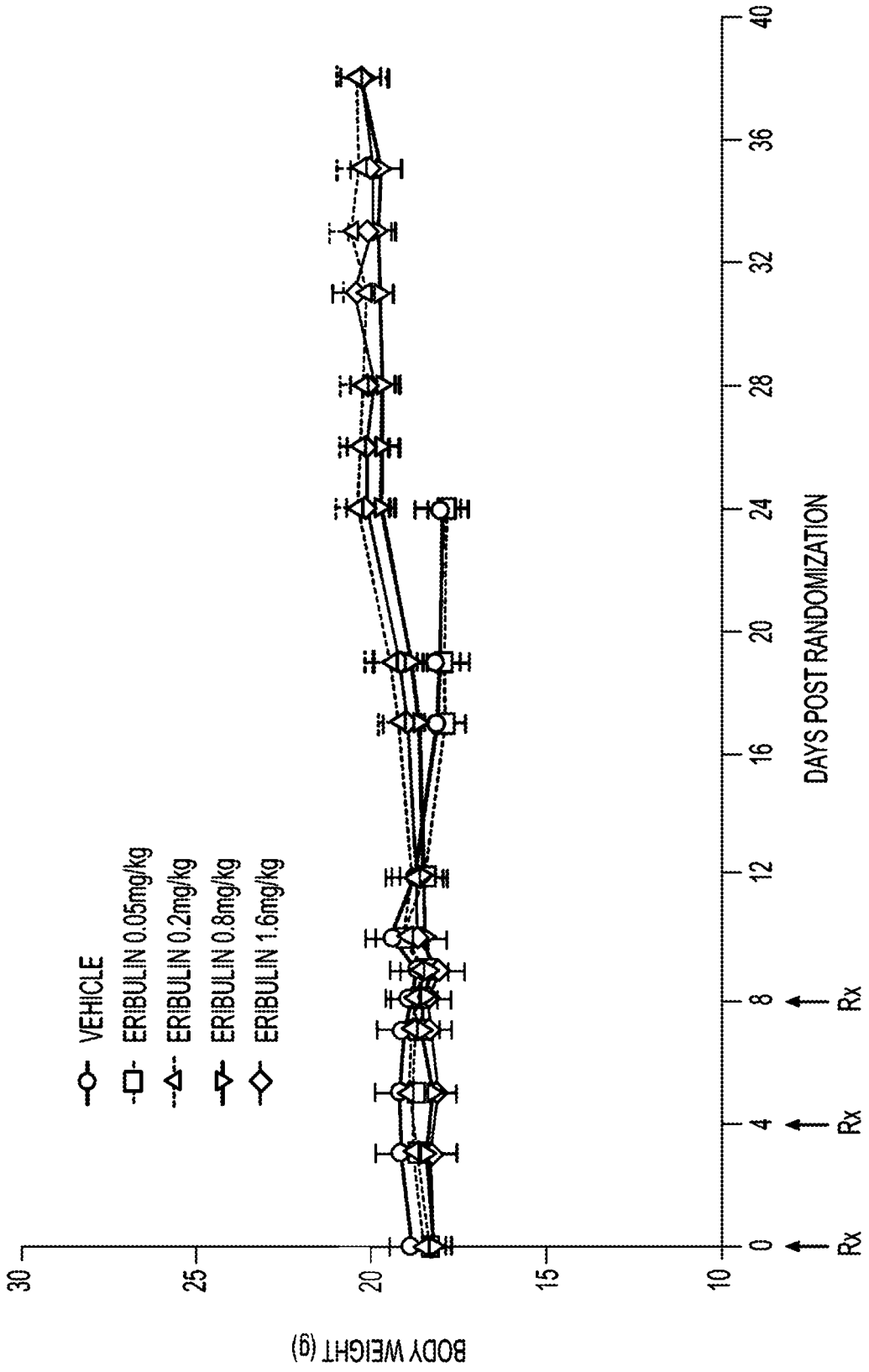
VEHICLE
ERIBULIN 0.05mg/kg
ERIBULIN 0.2mg/kg
ERIBULIN 0.8mg/kg
ERIBULIN 1.6mg/kg
BODY WEIGHT (g)
30
25
20
15
10
0
4
8
12
16
20
24
28
32
36
40
Rx
Rx
Rx
DAYS POST RANDOMIZATION

FIG. 16

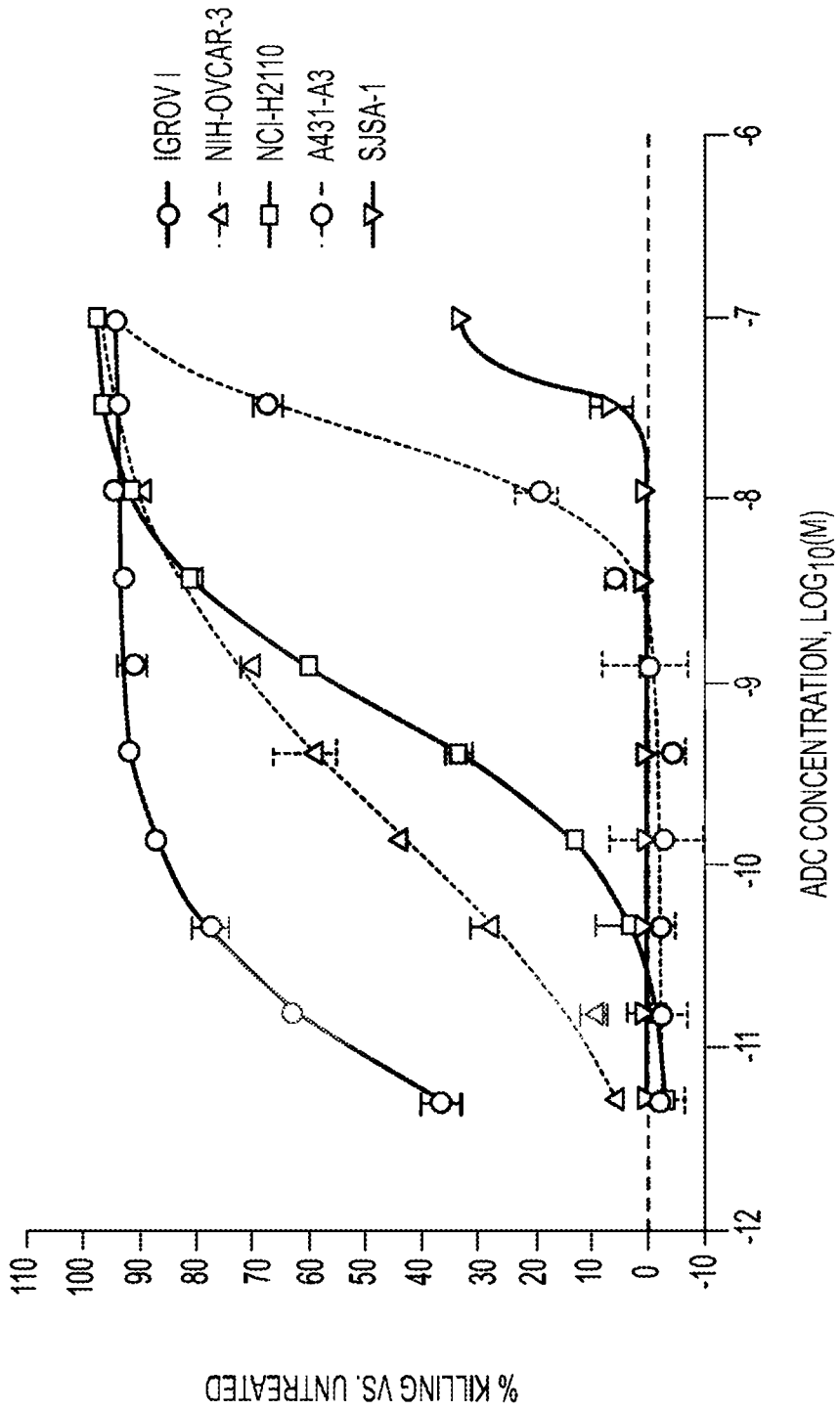
IGROV I
NIH-OVCAR-3
NCI-H2110
A431-A3
SJSA-1
% KILLING VS. UNTREATED
110
100
90
80
70
60
50
40
30
20
10
0
-10
-12
-11
-10
-9
-8
-7
-6
ADC CONCENTRATION, LOG10(M)

FIG. 17

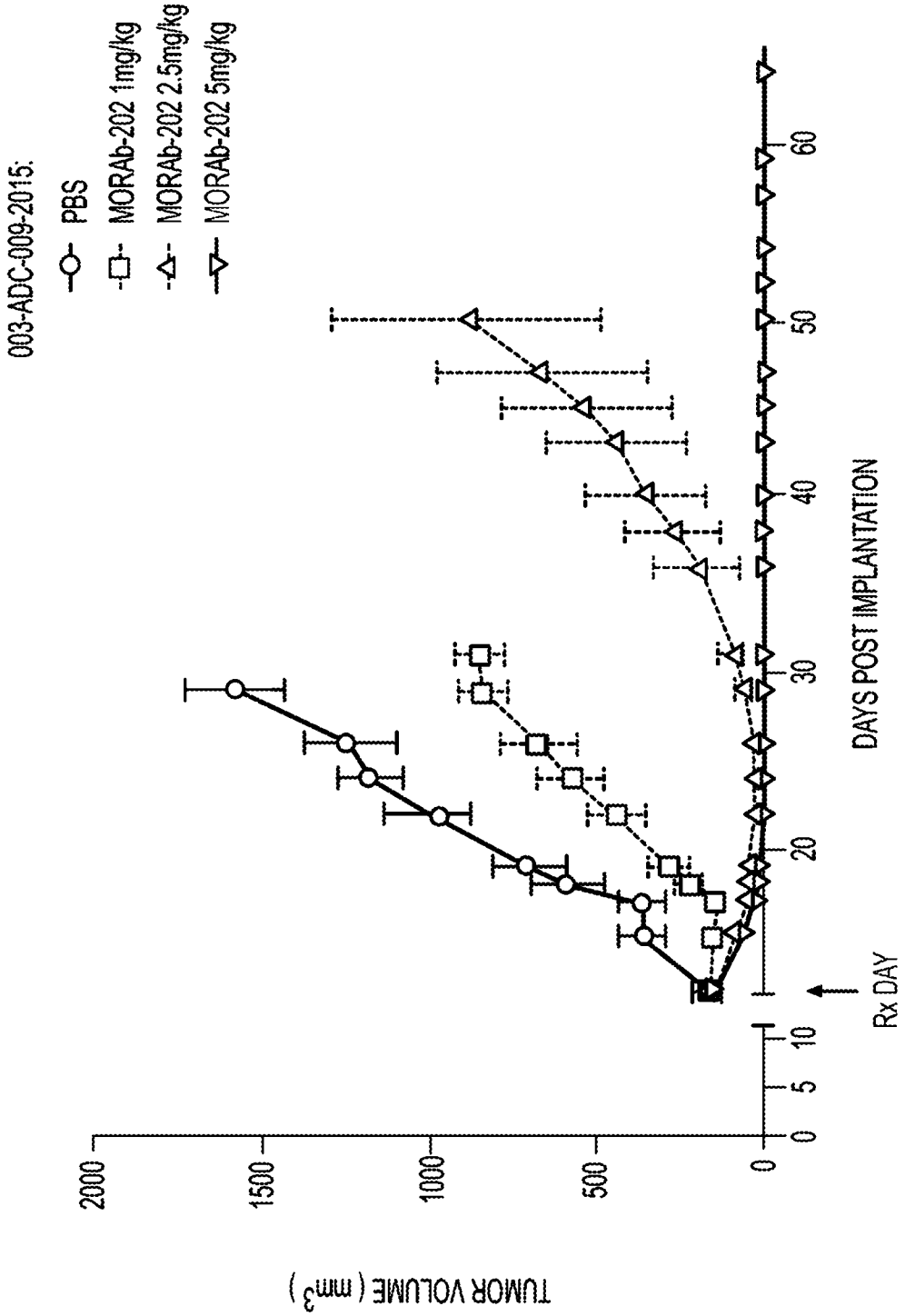
003-ADC-009-2015:
PBS
MORAb-202 1mg/kg
MORAb-202 2.5mg/kg
MORAb-202 5mg/kg
TUMOR VOLUME (mm3)
2000
1500
1000
500
0
0
5
10
20
30
40
50
60
Rx DAY
DAYS POST IMPLANTATION

FIG. 18

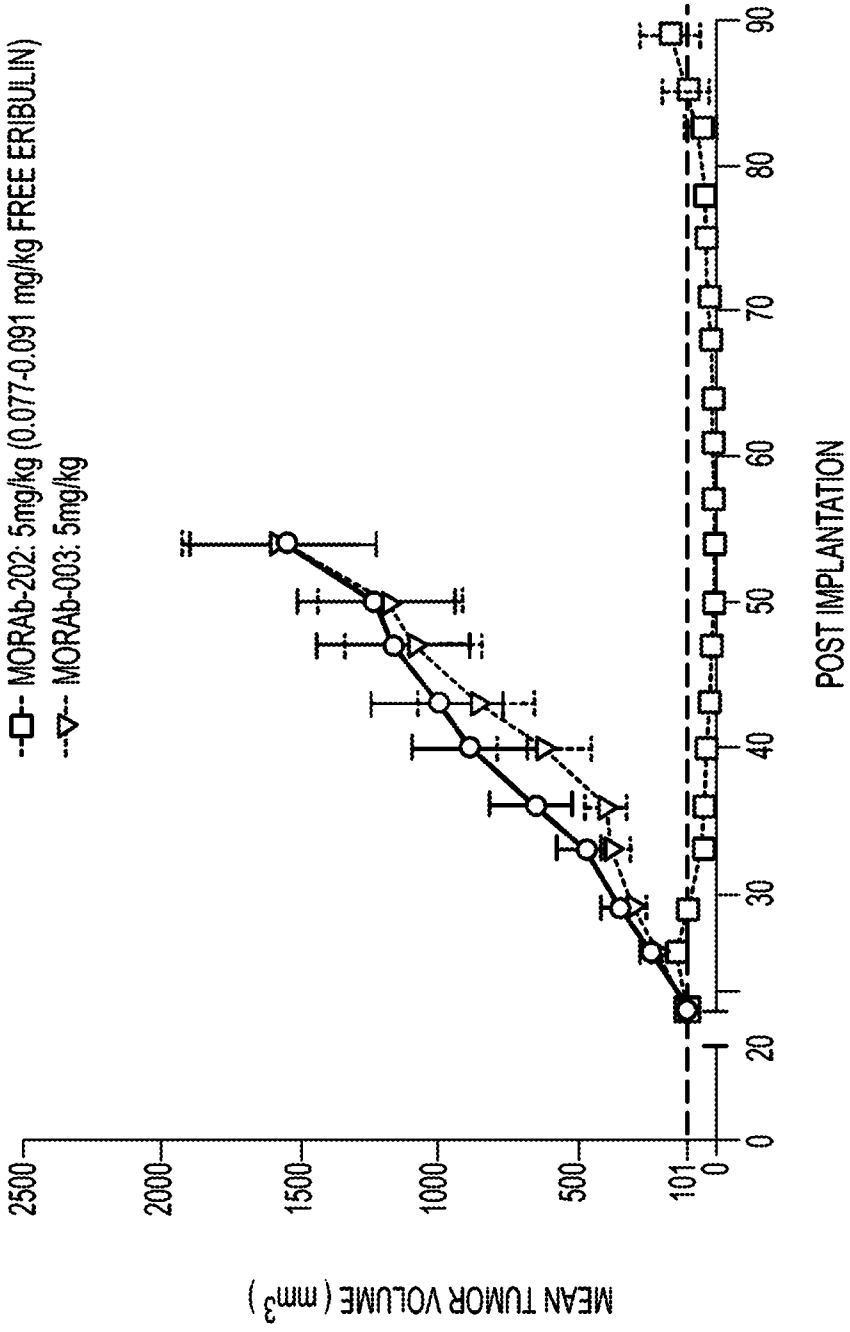
MORAb-202: 5mg/kg (0.077-0.091 mg/kg FREE ERIBULIN)
MORAb-003: 5mg/kg
MEAN TUMOR VOLUME (mm^3)
2500
2000
1500
1000
500
101
0
0
20
30
40
50
60
70
80
90
POST IMPLANTATION

FIG. 19A

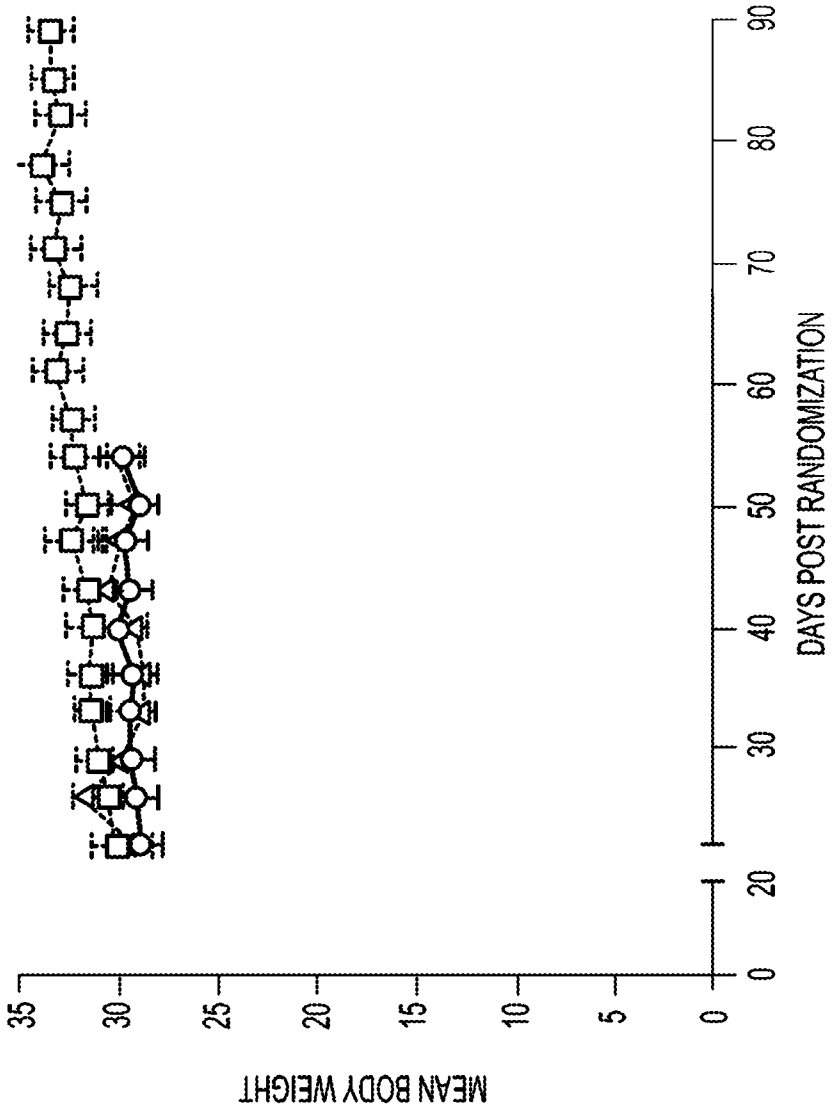
MEAN BODY WEIGHT
35
30
25
20
15
10
5
0
0
20
30
40
50
60
70
80
90
DAYS POST RANDOMIZATION

FIG. 19B

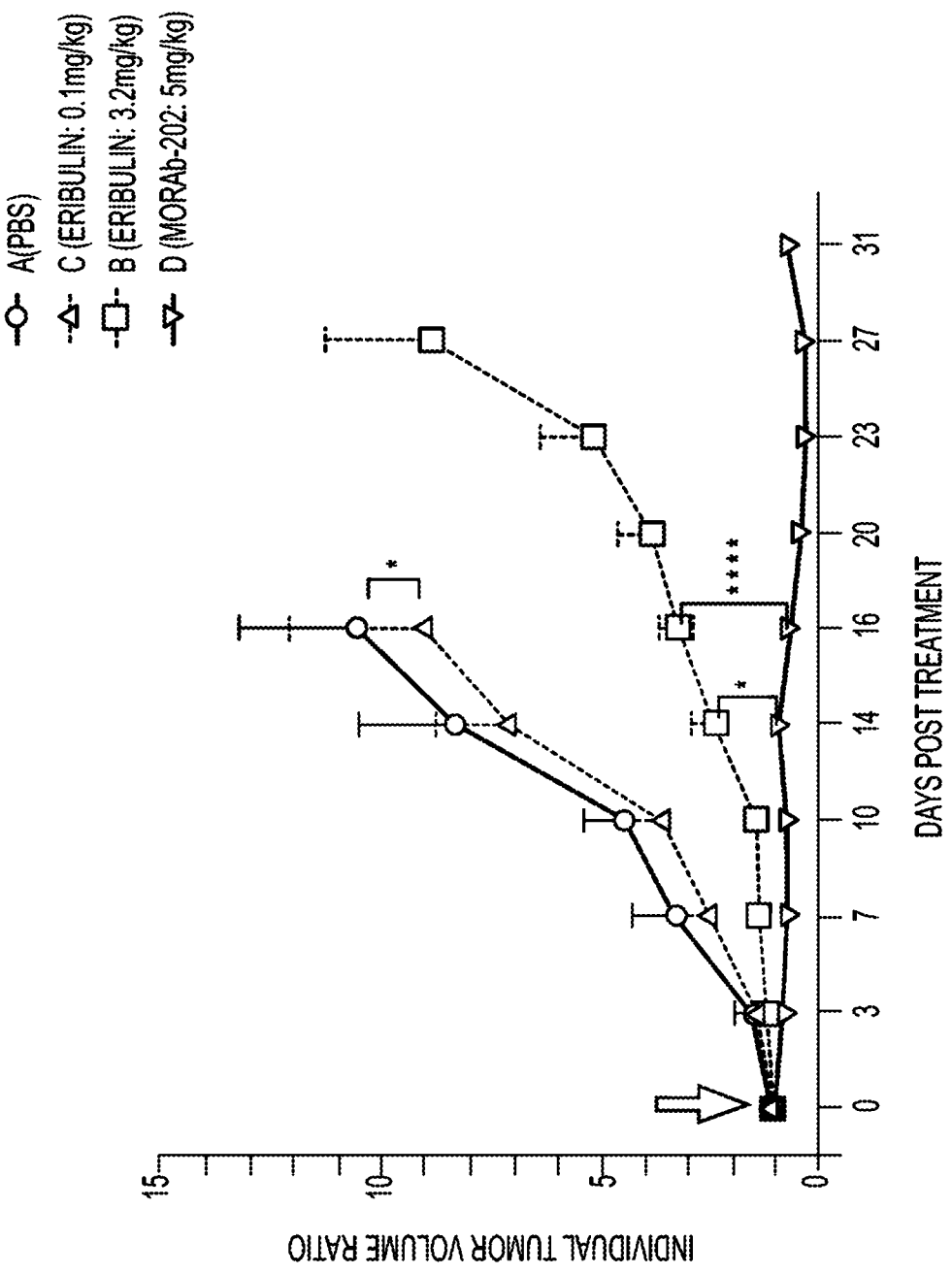
A(PBS)
C (ERIBULIN: 0.1mg/kg)
B (ERIBULIN: 3.2mg/kg)
D (MORAb-202: 5mg/kg)
INDIVIDUAL TUMOR VOLUME RATIO
DAYS POST TREATMENT
0
5
10
15
0
3
7
10
14
16
20
23
27
31
*
*
****

FIG. 20A

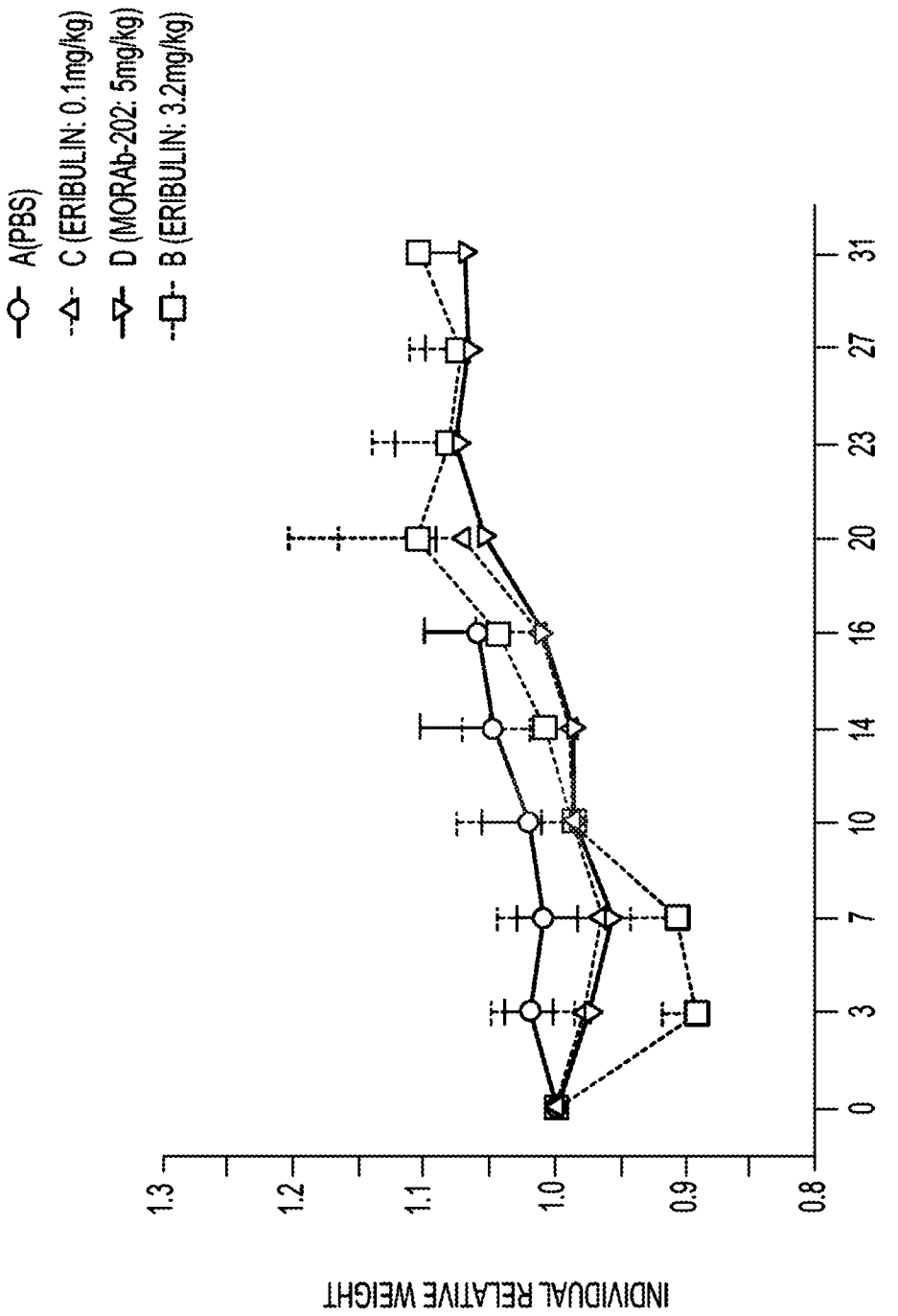
A(PBS)
C (ERIBULIN: 0.1mg/kg)
D (MORAb-202: 5mg/kg)
B (ERIBULIN: 3.2mg/kg)
INDIVIDUAL RELATIVE WEIGHT
1.3
1.2
1.1
1.0
0.9
0.8
0
3
7
10
14
16
20
23
27
31
DAYS POST TREATMENT

FIG. 20B

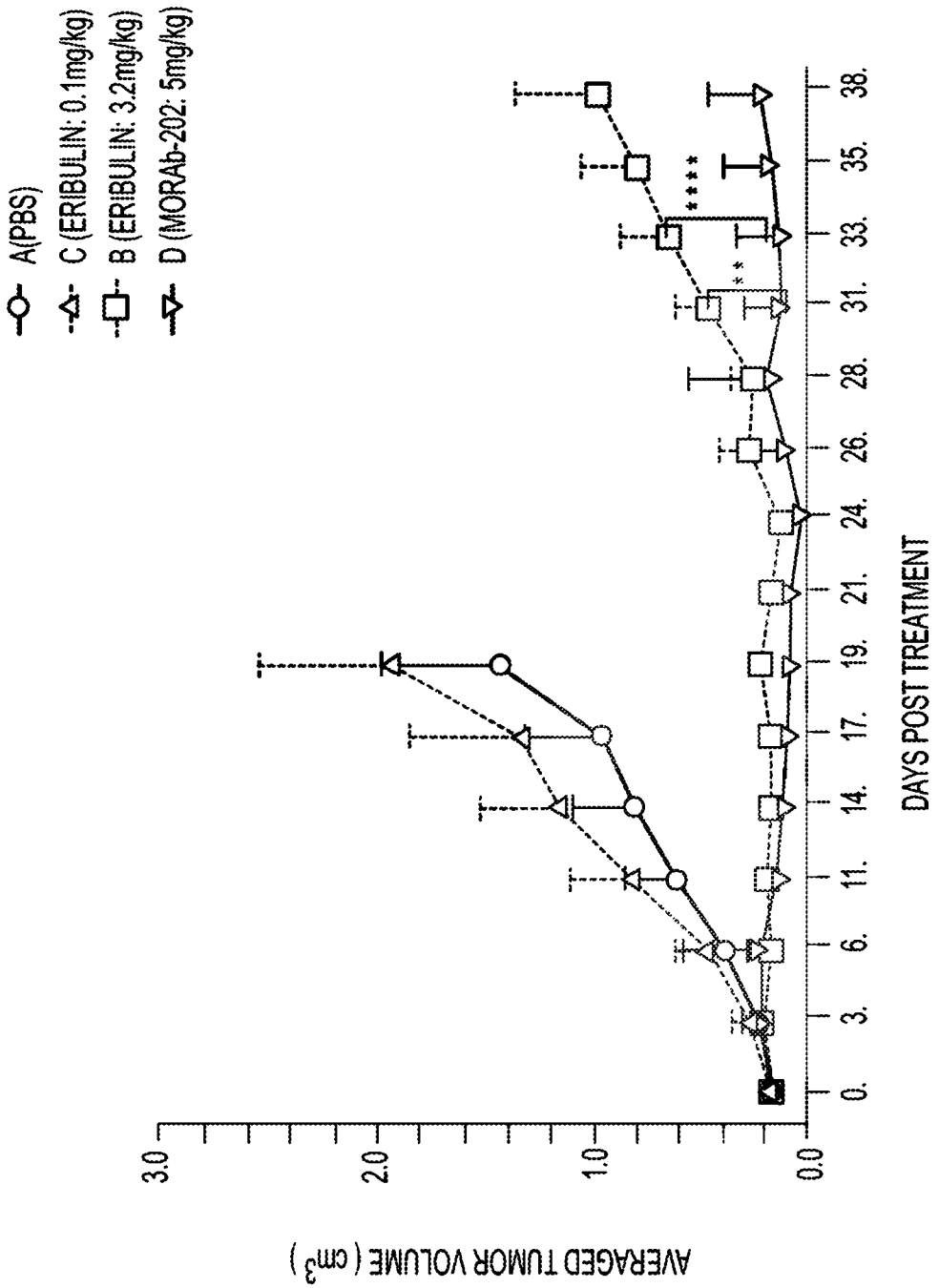
A(PBS)
C (ERIBULIN: 0.1mg/kg)
B (ERIBULIN: 3.2mg/kg)
D (MORAb-202: 5mg/kg)
AVERAGED TUMOR VOLUME (cm3)
3.0
2.0
1.0
0.0
0.
3.
6.
11.
14.
17.
19.
21.
24.
26.
28.
31.
33.
35.
38.
DAYS POST TREATMENT
**
****

FIG. 20C

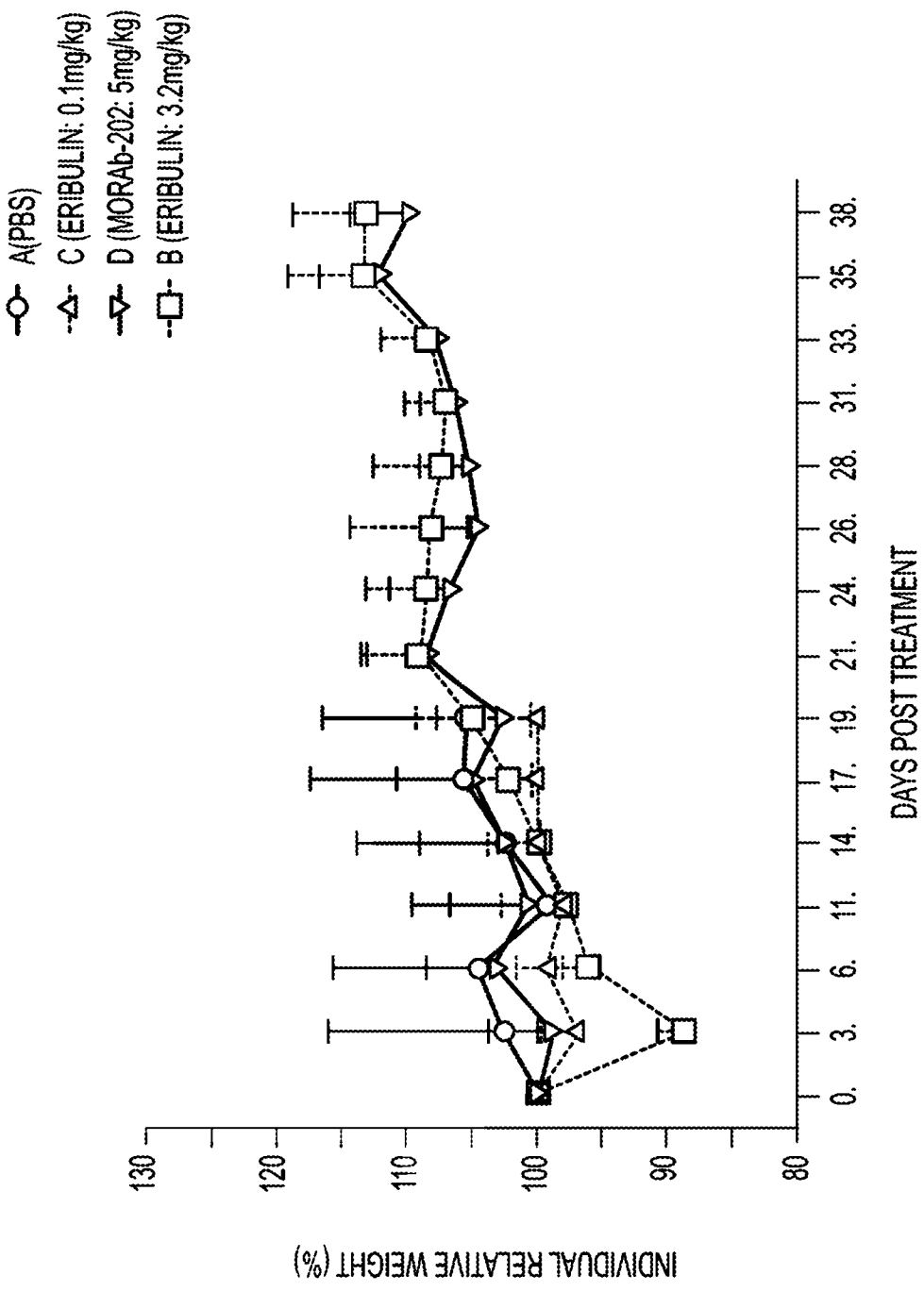
A(PBS)
C (ERIBULIN: 0.1mg/kg)
D (MORAb-202: 5mg/kg)
B (ERIBULIN: 3.2mg/kg)
INDIVIDUAL RELATIVE WEIGHT (%)
130
120
110
100
90
80
0.
3.
6.
11.
14.
17.
19.
21.
24.
26.
28.
31.
33.
35.
38.
DAYS POST TREATMENT

FIG. 20D

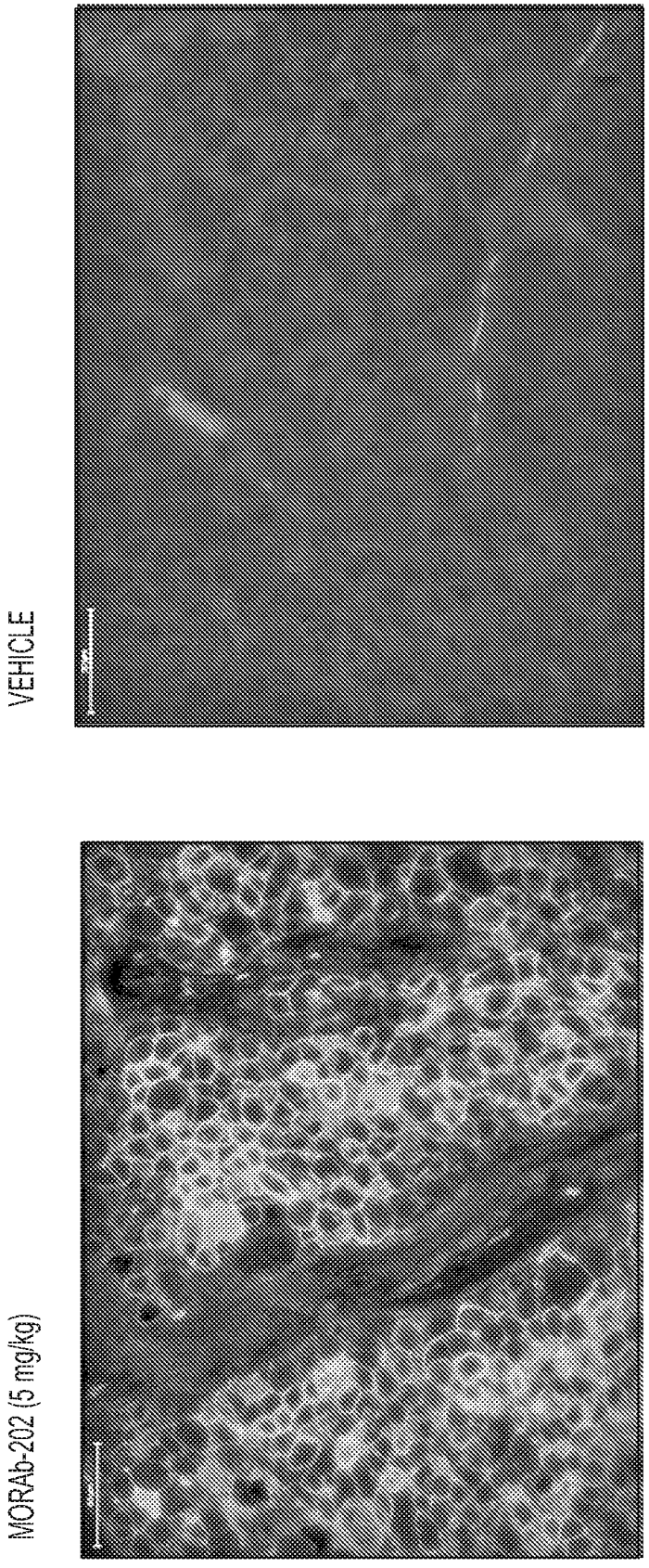

FIG. 21A

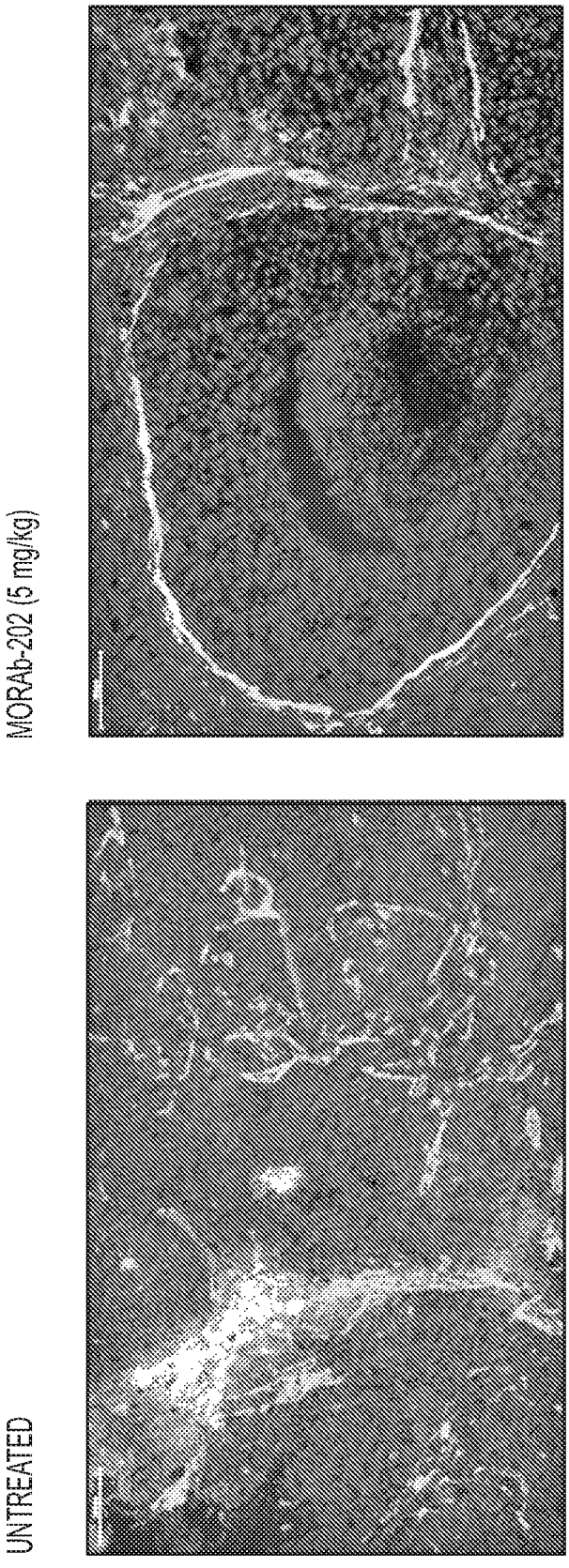

FIG. 21B

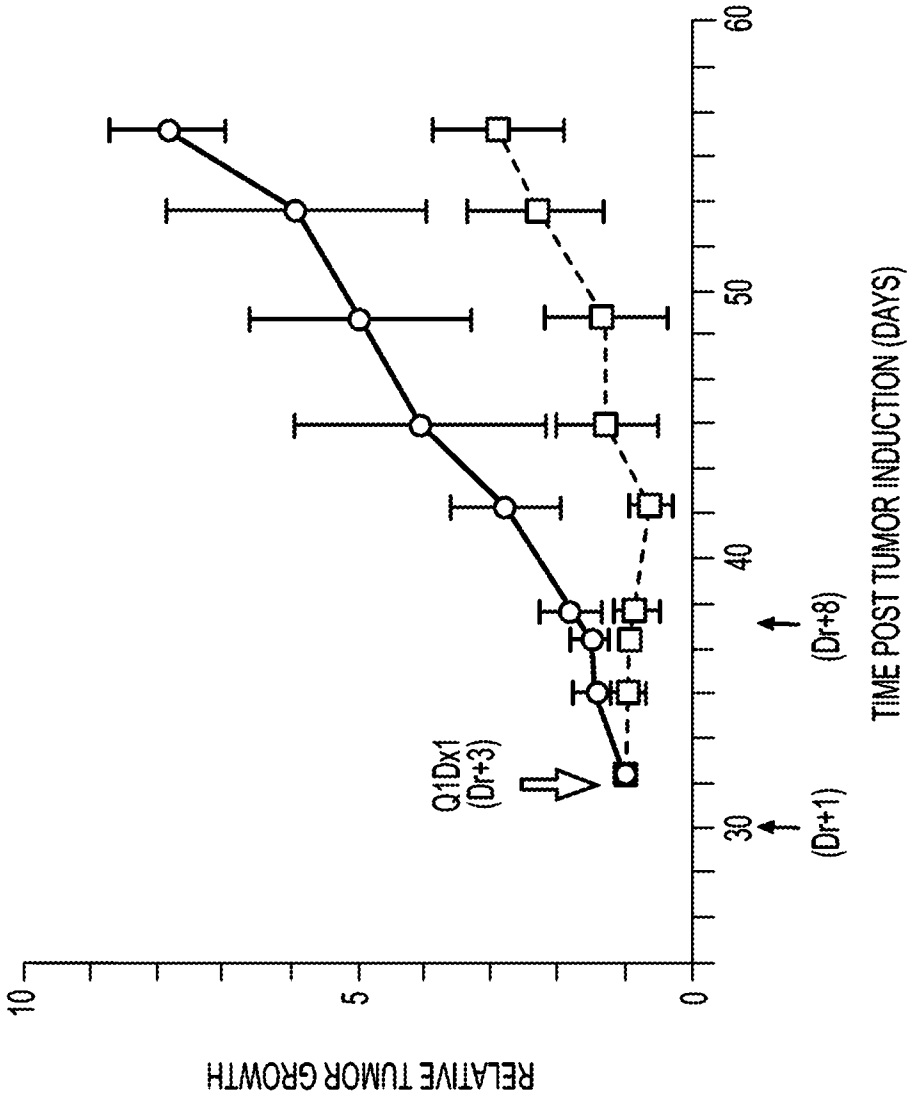
RELATIVE TUMOR GROWTH
10
5
0
60
50
40
30
Q1Dx1
(Dr+3)
(Dr+1)
(Dr+8)
TIME POST TUMOR INDUCTION (DAYS)

FIG. 21C

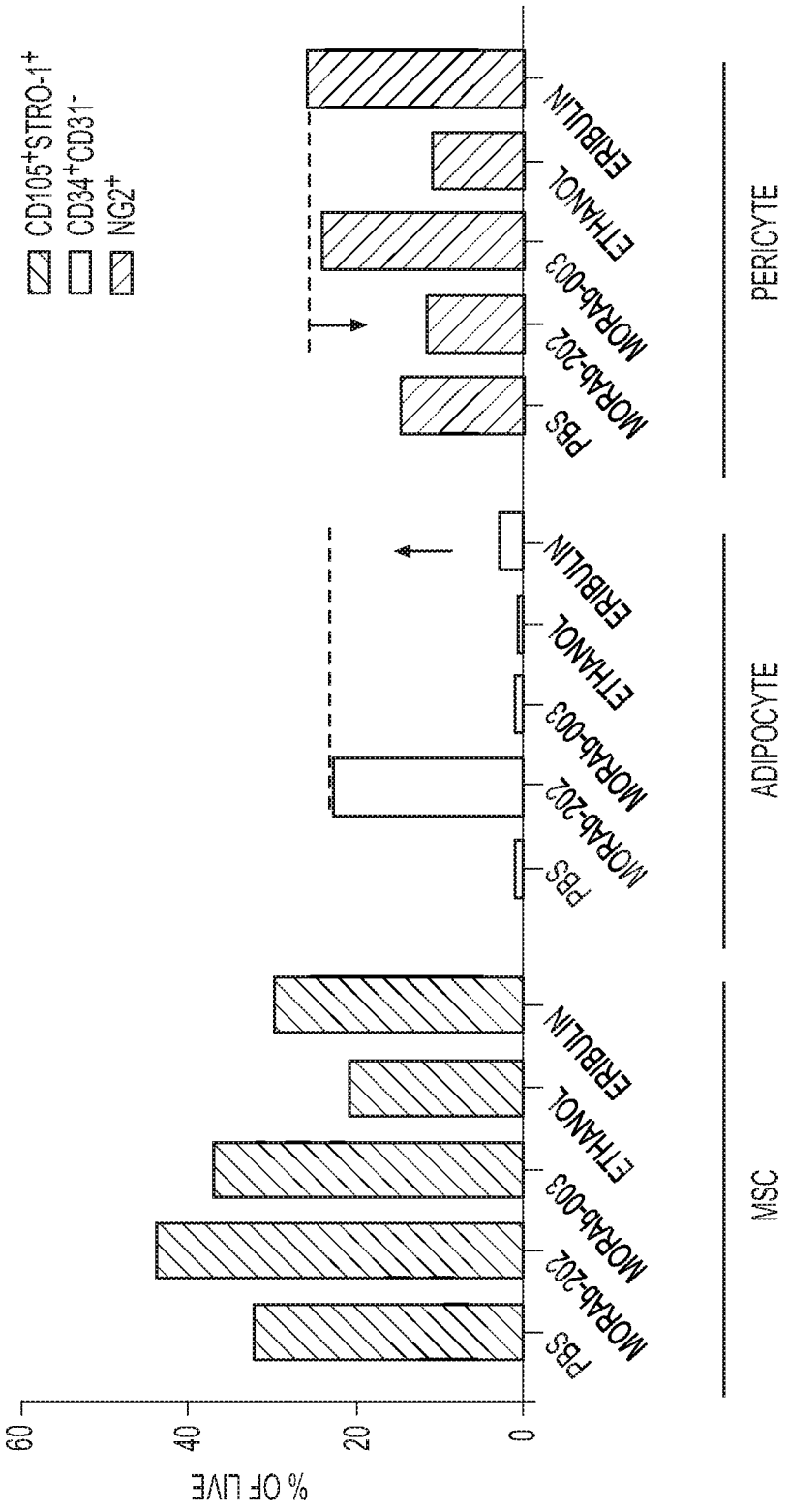
CD105+STRO-1+
CD34+CD31-
NG2+
% OF LIVE
60
40
20
0
PBS
MORAb-202
MORAb-003
ETHANOL
ERIBULIN
MSC
ADIPOCYTE
PERICYTE

FIG. 22

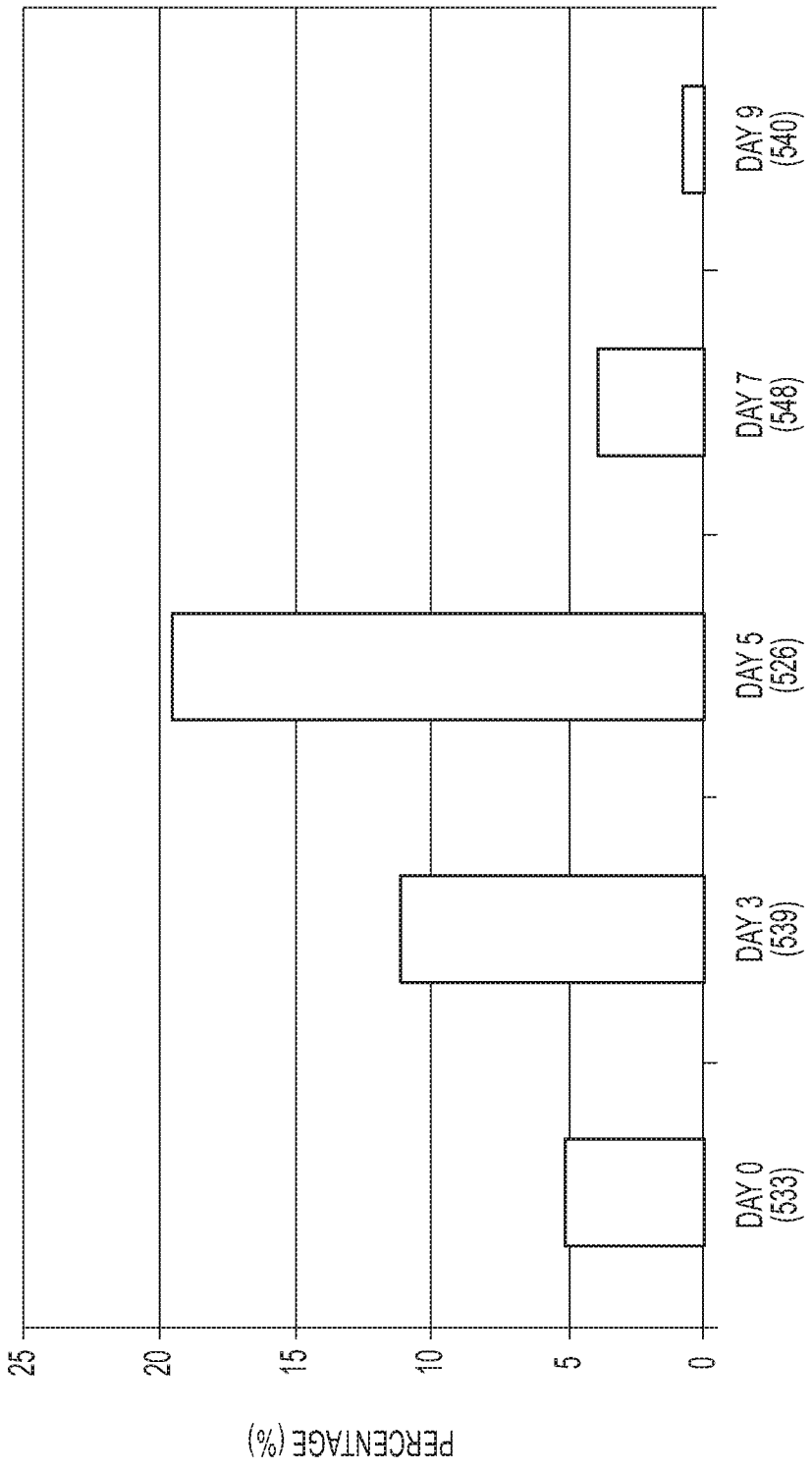
25
20
15
10
5
0
PERCENTAGE (%)
DAY 0 (533)
DAY 3 (539)
DAY 5 (526)
DAY 7 (548)
DAY 9 (540)

FIG. 23