**AZ1.39.1 VL** Germline IGKV4-1+Jk3.01 → IGKV2D

WT: DIVMTQSPDSLAVSLGERATINCKSSQSVFFRSNNKNCLAWYQQRPGQPPNLLIYWASSRES
LO: DIVMTQTPLSLSVTPGQPASISCKSSQSVFFRSNNKNILAWYLQKPGQPPQLLIYWASSRES

WT: GVPDRFSGSGSGTDFALTISSLQTEDVAVYYCQQYFGSPFTFGPGTKVDIK
LO: GVPDRFSGSGSGNDFTLKISRVEAEDVGVYYCQQYFGSPFTFGPGTKVDIK

**AZ1.39.1 VH** Germline VH3-21+JH4

WT: EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKG
LO: EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMSWVRQAPGKGLEWVSSISSSSSYIYYADSVKG

WT: RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGDGYNYYYFDYWGQGTLVTVSS
LO: RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGDAYNYYYFDYWGQGTLVTVSS

FIG. 1

| Construct [a] | IgG in sups (µg/ml) [b] |
|---|---|
| After LC-FR swapping | 140 |
| Before LC-FR swapping | 72 |

a: All constructs have the same heavy chain as G4A (AZ1.39.1 (WT) heavy chain with DG mutated to DA in CDR3H)

b: Average from two Octet-96 quants after 7 days in culture of 293F cells transfected with corresponding IgG vectors; cv% ~10%

**FIG. 2**

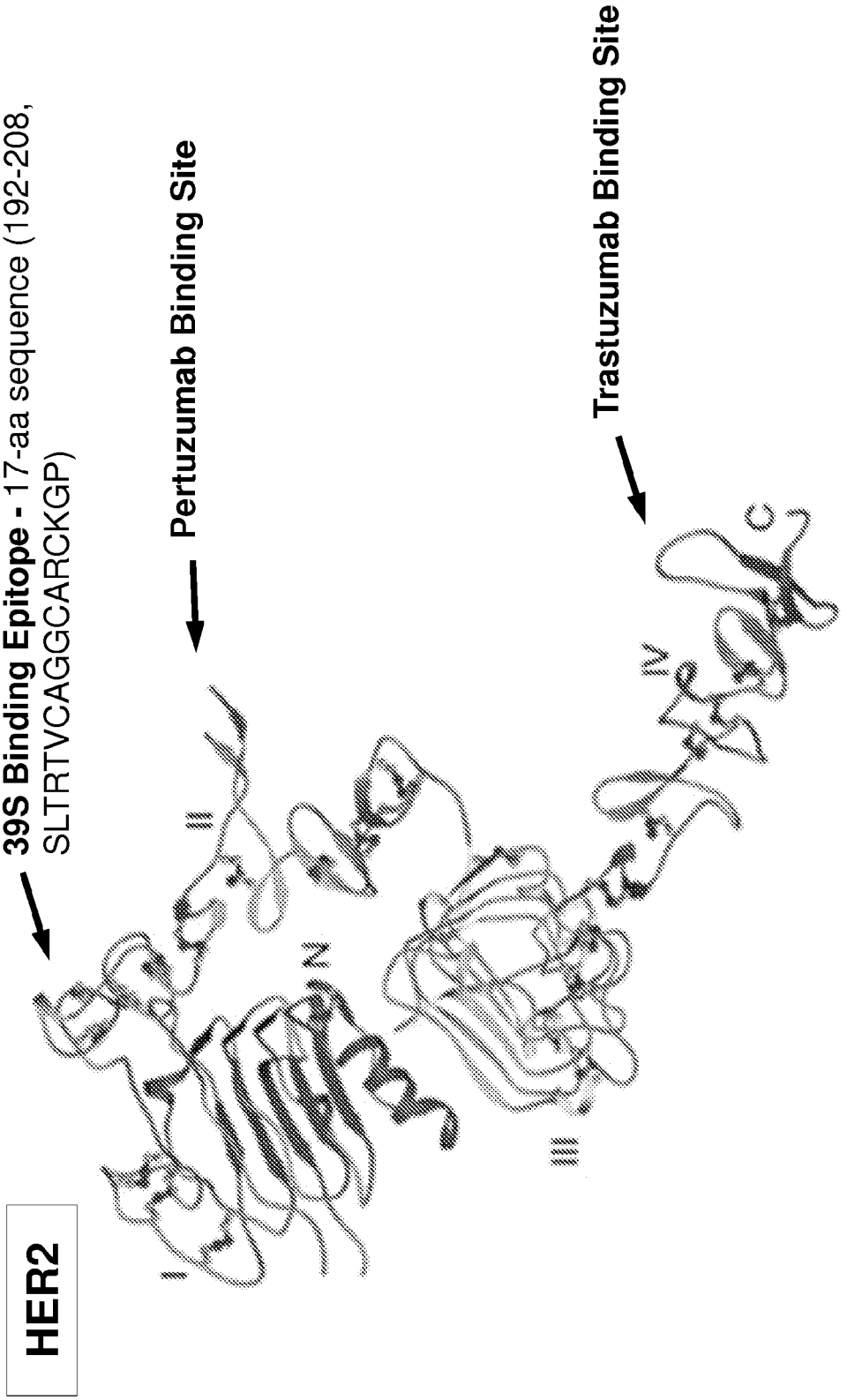

HER2
39S Binding Epitope - 17-aa sequence (192-208, SLTRTVCAGGCARCKGP)
Pertuzumab Binding Site
Trastuzumab Binding Site
I
II
III
IV
N
C

FIG. 3

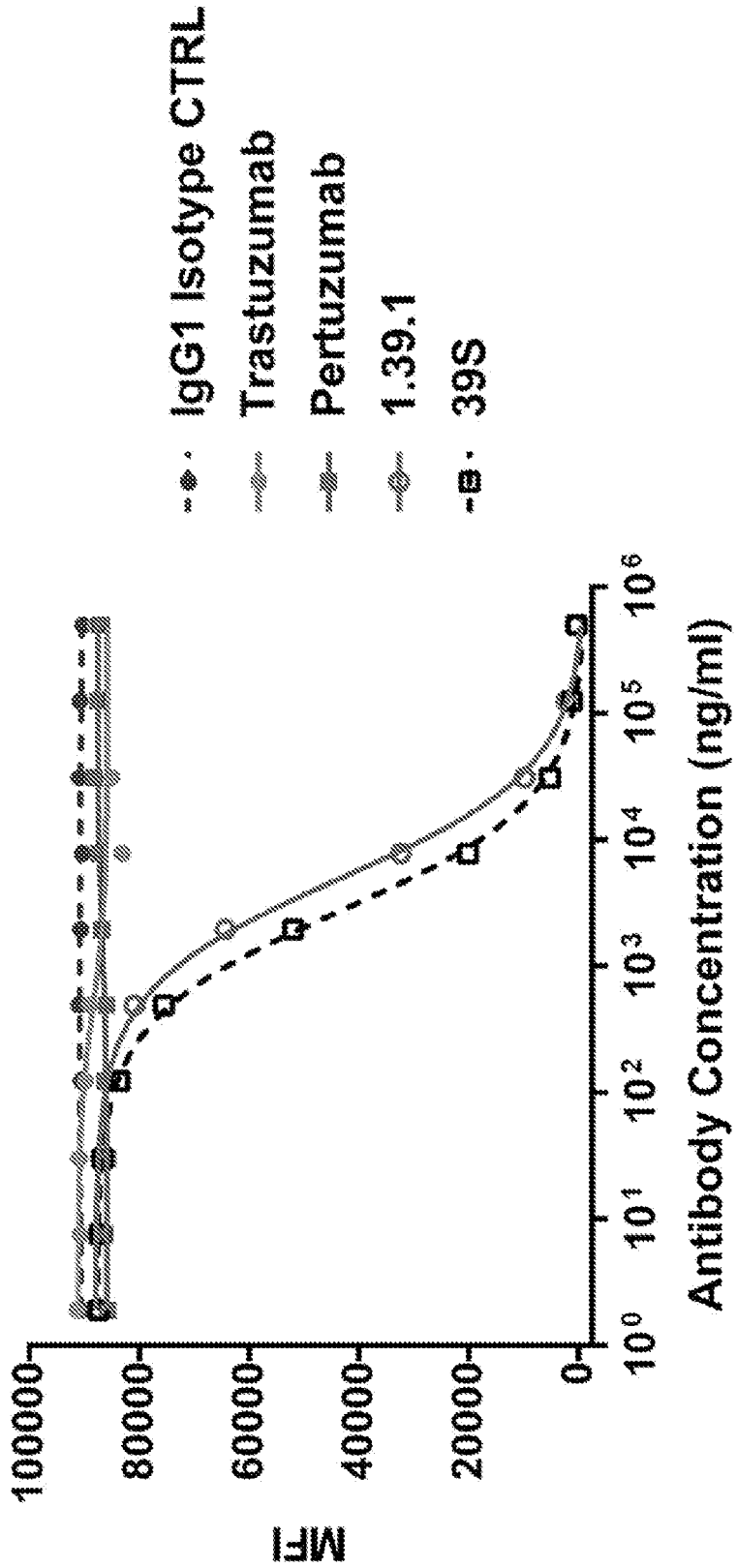

IgG1 Isotype CTRL
Trastuzumab
Pertuzumab
1.39.1
39S
MFI
100000
80000
60000
40000
20000
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Antibody Concentration (ng/ml)

FIG. 4

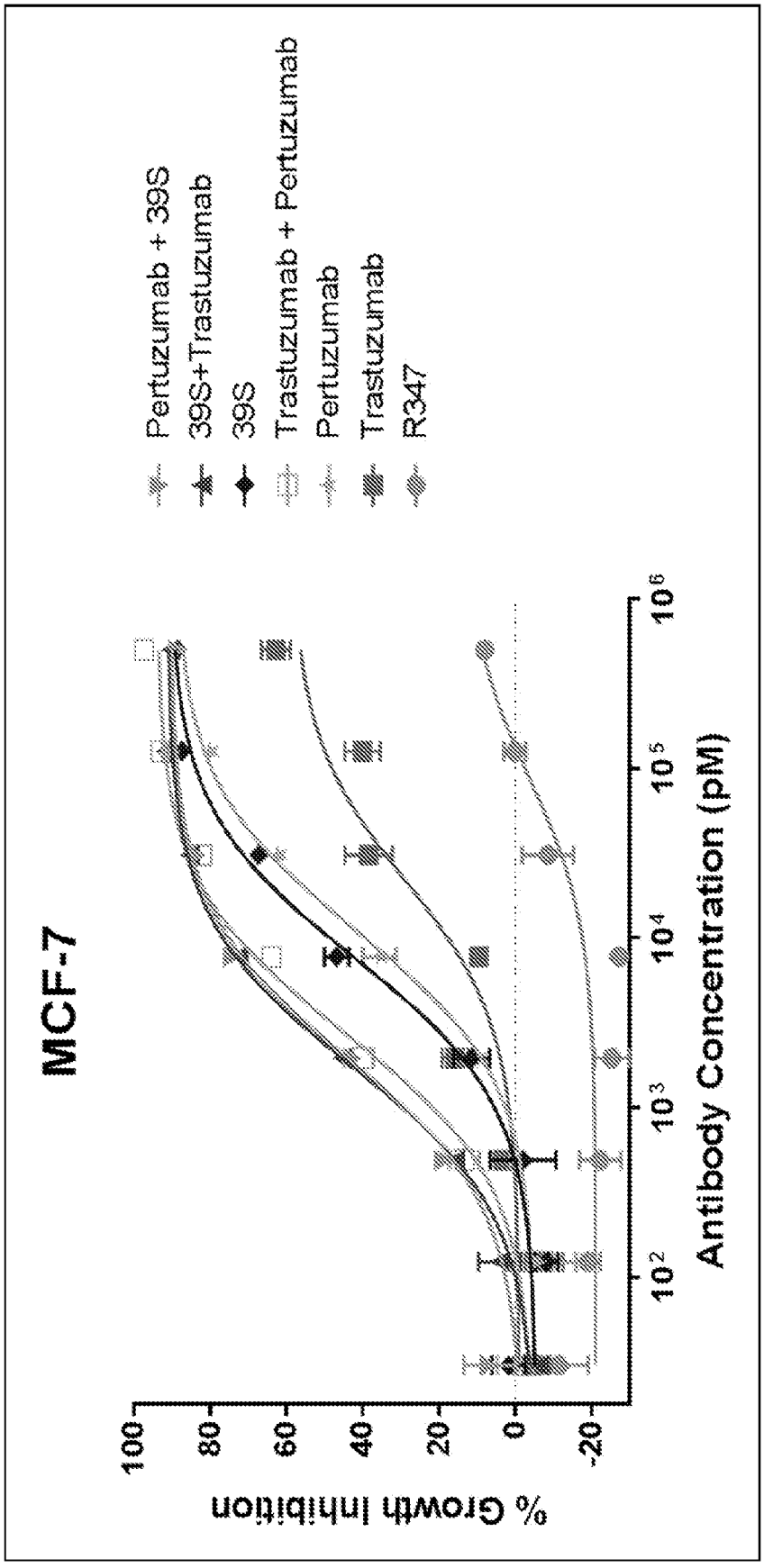

MCF-7
Pertuzumab + 39S
39S+Trastuzumab
39S
Trastuzumab + Pertuzumab
Pertuzumab
Trastuzumab
R347
% Growth Inhibition
100
80
60
40
20
0
-20
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Antibody Concentration (pM)

FIG. 5

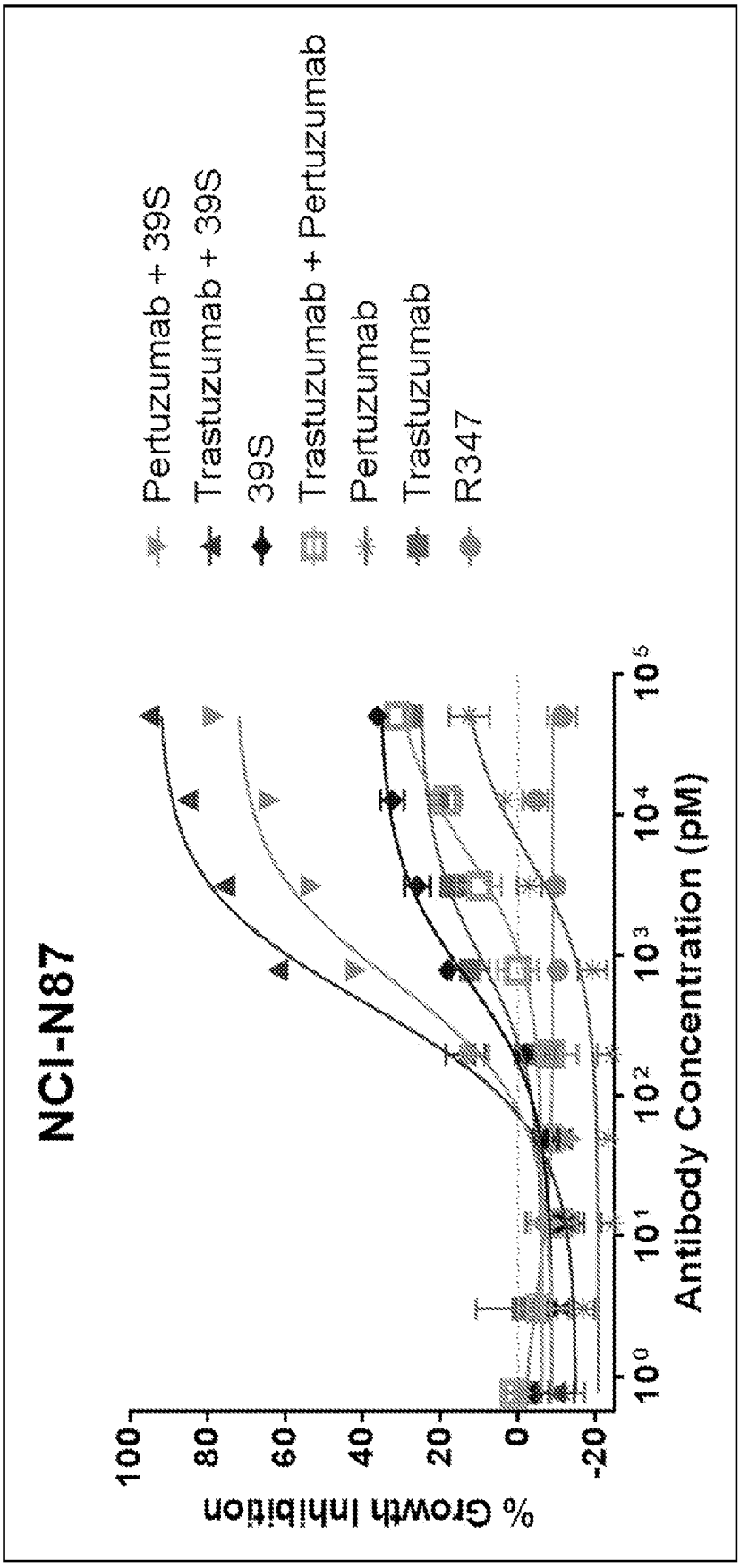

NCI-N87
% Growth Inhibition
100
80
60
40
20
0
-20
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
Antibody Concentration (pM)
Pertuzumab + 39S
Trastuzumab + 39S
39S
Trastuzumab + Pertuzumab
Pertuzumab
Trastuzumab
R347

FIG. 6

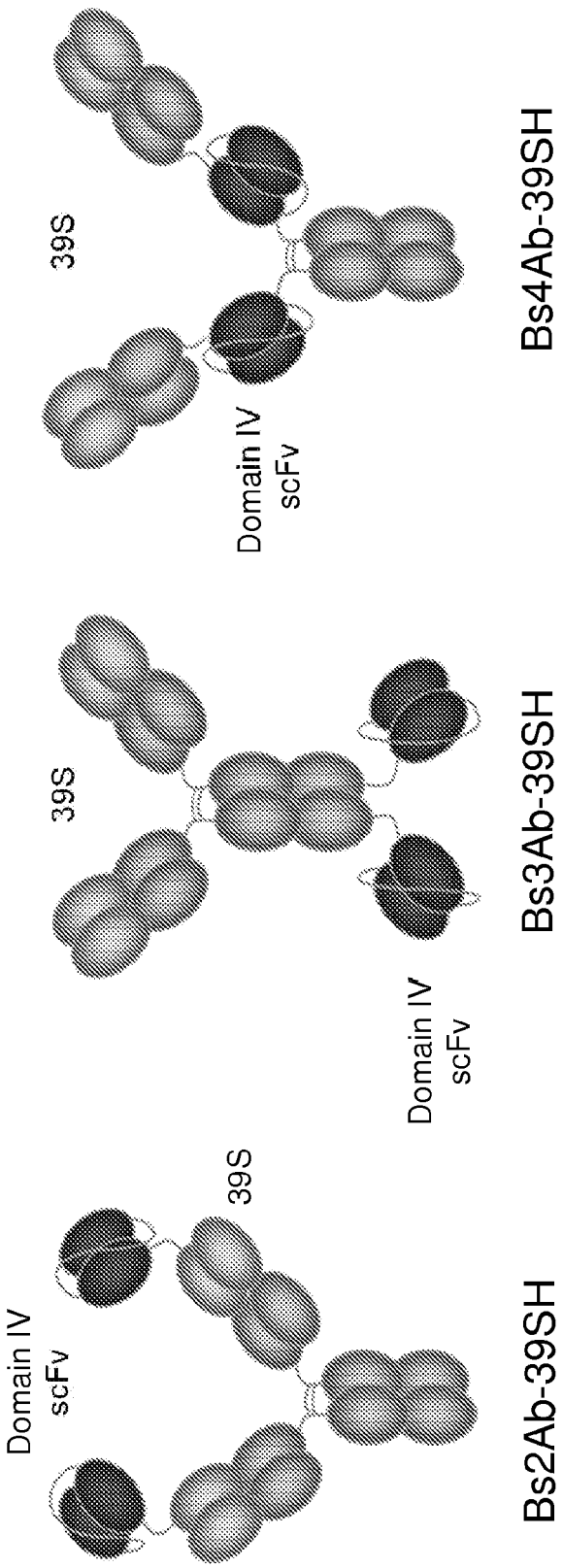

A
Domain IV
scFv
39S
Bs2Ab-39SH
B
39S
Domain IV
scFv
Bs3Ab-39SH
C
39S
Domain IV
scFv
Bs4Ab-39SH

FIG. 7

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFA
TYYCQQHYTTPPTFGCGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAP
GKCLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSGGGGS
GGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMSWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDN
AKNSLYLQMNSLRAEDTAVYYCARGGDAYNYYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE***X1***LGG
P***X2****VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALP**X3**P**X4**EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL**X5**LSPGK

| EU# | | | |
|---|---|---|---|
| 234 | X1 = L for WT; | L for ADCC; | F for ADC “F”,“FC”,”FCC” |
| 239 | X2 = S for WT; | S or D for ADCC; | C for ADC “FC”,”FCC” |
| 239i | * = absent for WT; | absent for ADCC; | C for ADC 239-ins “C” |
| 330 | X3 = A for WT; | L or A for ADCC; | A for ADC |
| 332 | X4 = I for WT; | E for ADCC; | I for ADC |
| 442 | X5 = S for WT; | S for ADCC; | C for ADC “FCC” and “CC” |

**FIG. 8A**

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMSWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMN
SLRAEDTAVYYCARGGDAYNYYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE***X1***LGGP***X2****VFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
**X3**P**X4**EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSL**X5**LSPGKGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDVNTA
VAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGCGTKVEIKGGGGSGGGG
SGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKCLEWVARIYPTNGYTRYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

| EU# | | | |
|---|---|---|---|
| 234 | X1 = L for WT; | L for ADCC; | F for ADC “F”,“FC”,”FCC” |
| 239 | X2 = S for WT; | S or D for ADCC; | C for ADC “FC”,”FCC” |
| 239i | * = absent for WT; | absent for ADCC; | C for ADC 239-ins “C” |
| 330 | X3 = A for WT; | L or A for ADCC; | A for ADC |
| 332 | X4 = I for WT; | E for ADCC; | I for ADC |
| 442 | X5 = S for WT; | S for ADCC; | C for ADC “FCC” and “CC” |

**FIG. 8B**

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMSWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMN
SLRAEDTAVYYCARGGDAYNYYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGKTGGGGSGGGGSDIQMTQSPSSLSASV
GDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFG
CGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKCLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSSGGGGSGGGGSCPPCPAPE**X**
***1***LGGP***X2****VFLFPPKPKDILMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALP**X3**P**X4**EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL**X5**LSPGK

| EU# | | | |
|---|---|---|---|
| 234 | X1 = L for WT; | L for ADCC; | F for ADC “F”,“FC”,”FCC” |
| 239 | X2 = S for WT; | S or D for ADCC; | C for ADC “FC”,”FCC” |
| 239i | * = absent for WT; | absent for ADCC; | C for ADC 239-ins “C” |
| 330 | X3 = A for WT; | L or A for ADCC; | A for ADC |
| 332 | X4 = I for WT; | E for ADCC; | I for ADC |
| 442 | X5 = S for WT; | S for ADCC; | C for ADC “FCC” and “CC” |

**FIG. 8C**

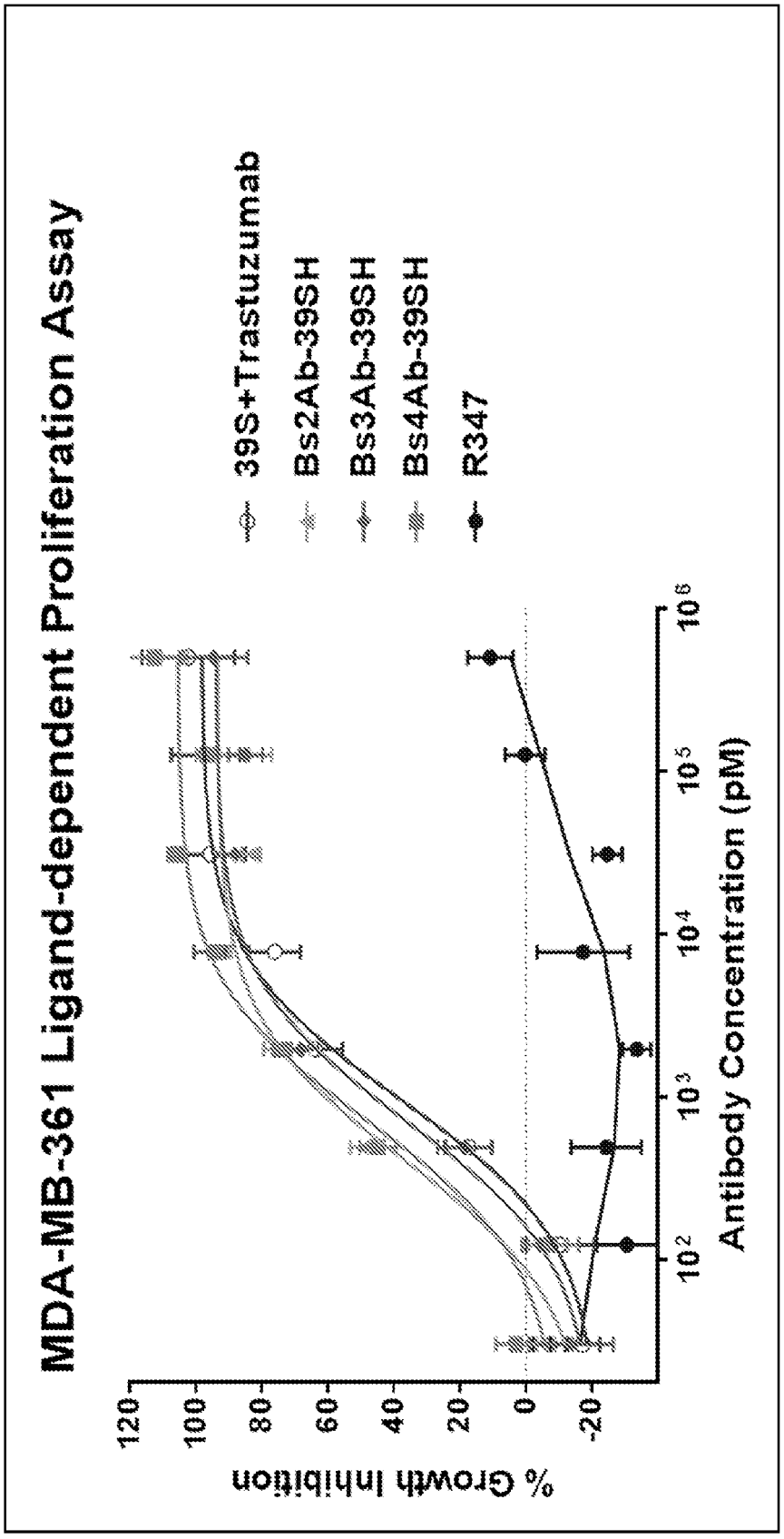

MDA-MB-361 Ligand-dependent Proliferation Assay
39S+Trastuzumab
Bs2Ab-39SH
Bs3Ab-39SH
Bs4Ab-39SH
R347
% Growth Inhibition
120
100
80
60
40
20
0
-20
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Antibody Concentration (pM)

FIG. 9A

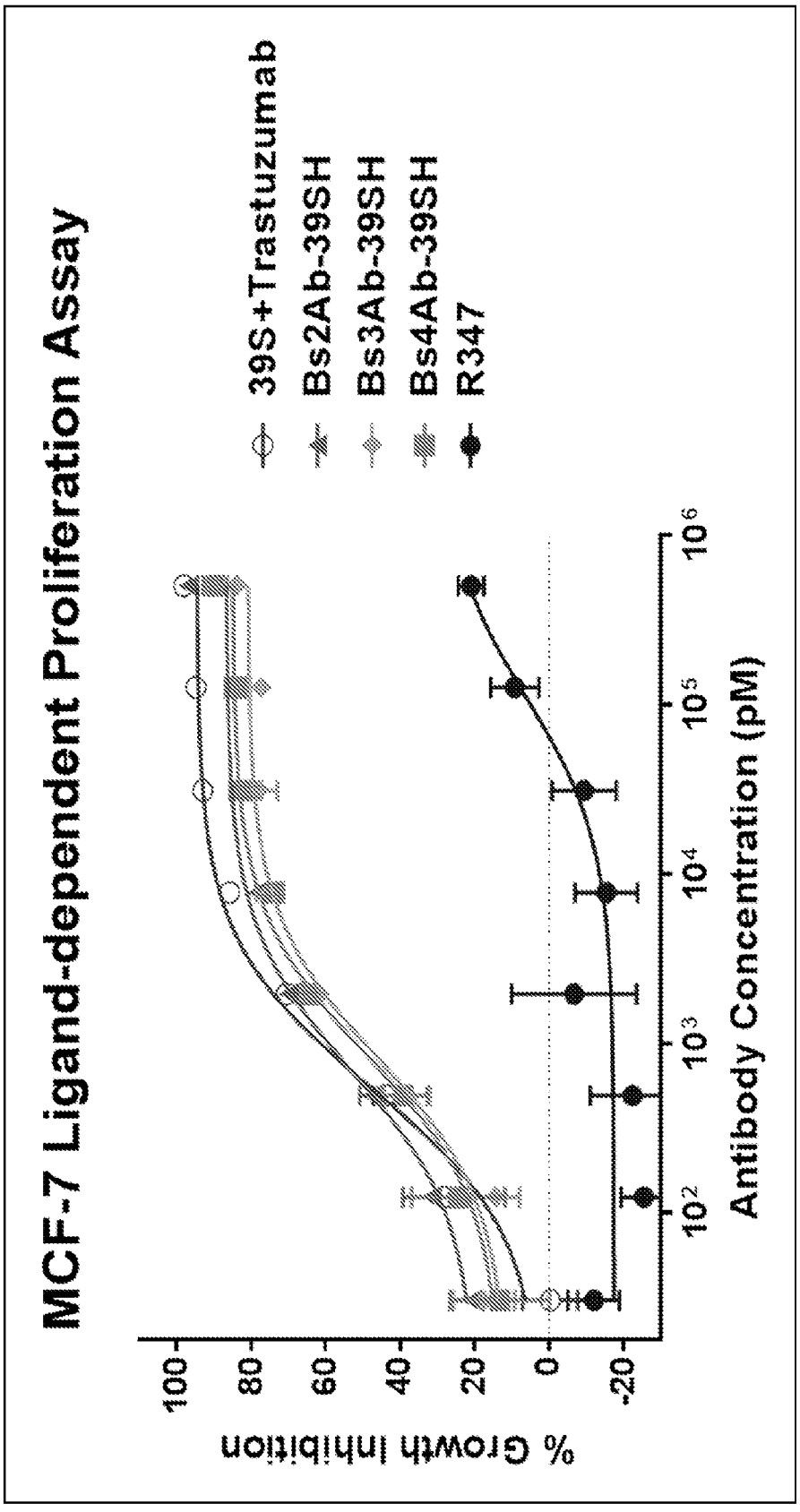

MCF-7 Ligand-dependent Proliferation Assay
39S+Trastuzumab
Bs2Ab-39SH
Bs3Ab-39SH
Bs4Ab-39SH
R347
% Growth Inhibition
100
80
60
40
20
0
-20
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Antibody Concentration (pM)

FIG. 9B

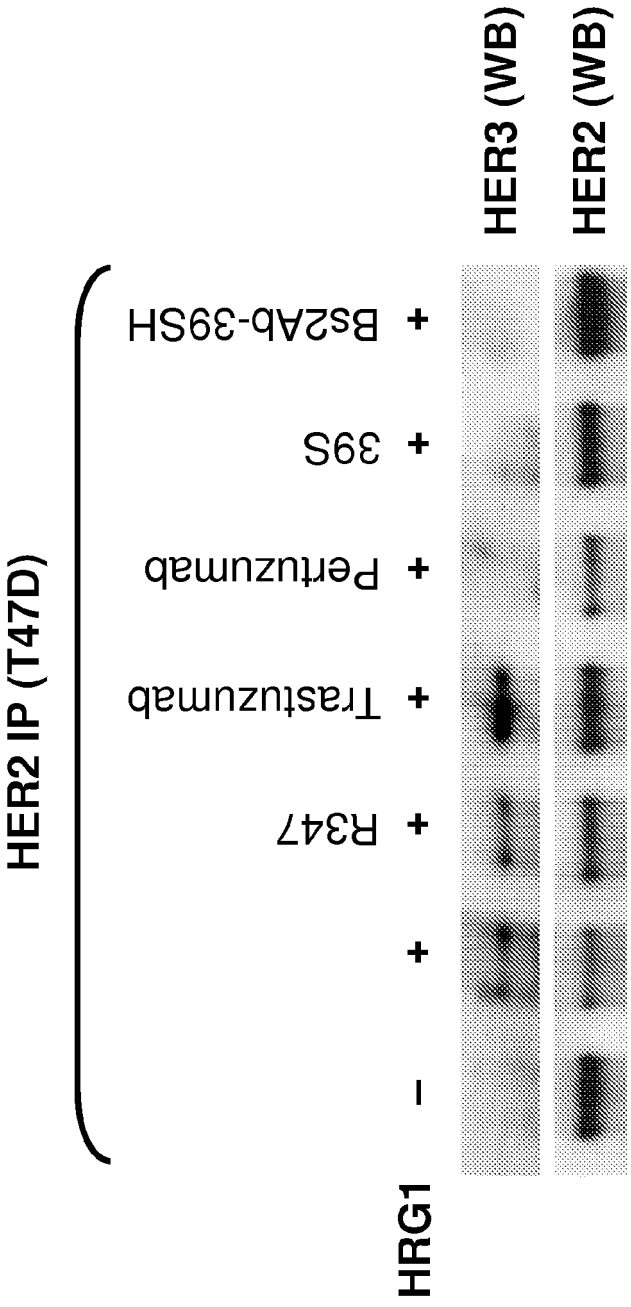

HER3 (WB)
HER2 (WB)
HRG1
-
+
+
R347
+
Trastuzumab
+
Pertuzumab
+
39S
+
BsAb-39SH
HER2 IP (T47D)

FIG. 10

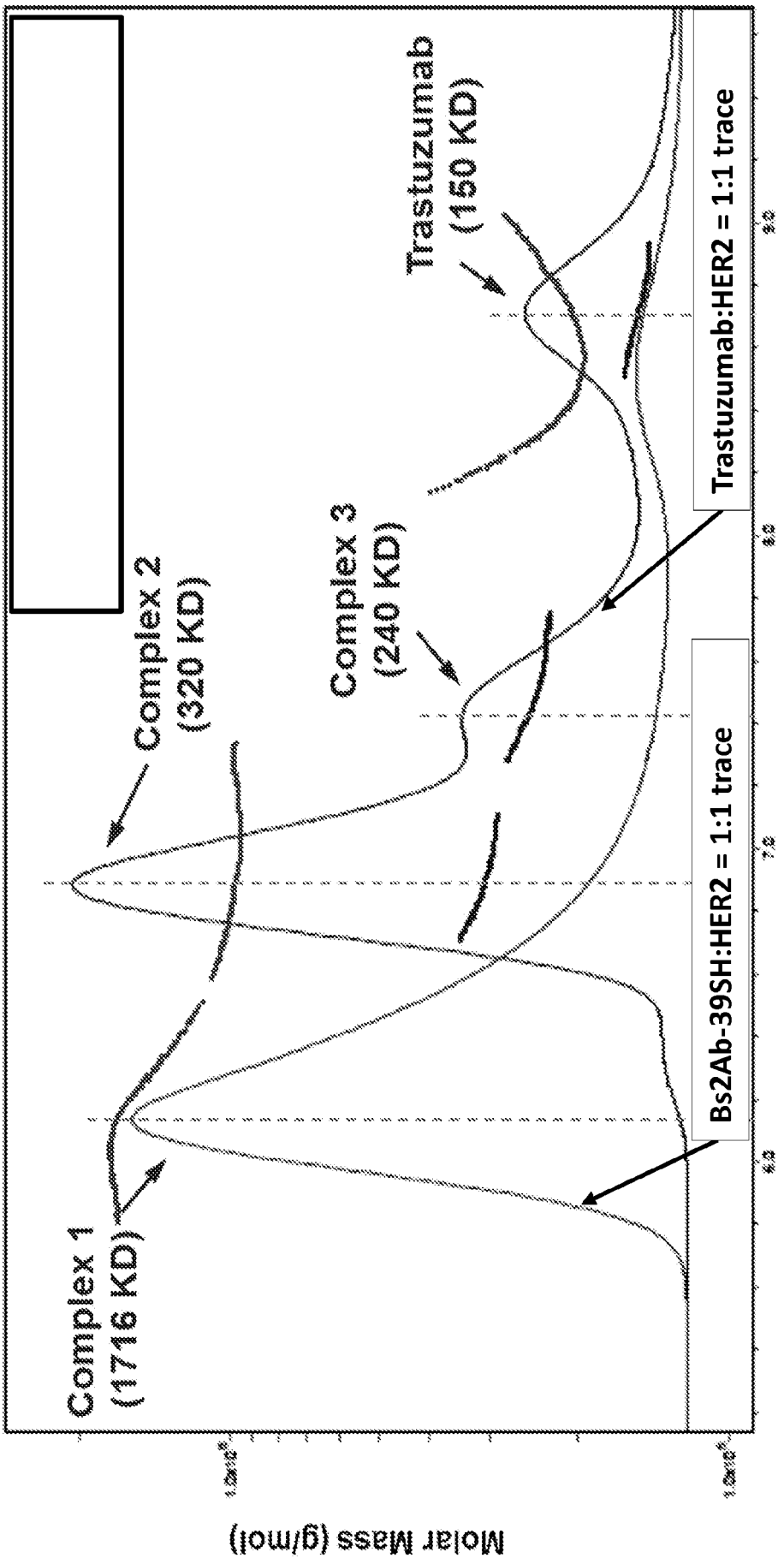

Molar Mass (g/mol)
Time (min)
Complex 1
(1716 KD)
Complex 2
(320 KD)
Complex 3
(240 KD)
Trastuzumab
(150 KD)
Bs2Ab-39SH:HER2 = 1:1 trace
Trastuzumab:HER2 = 1:1 trace

FIG. 11

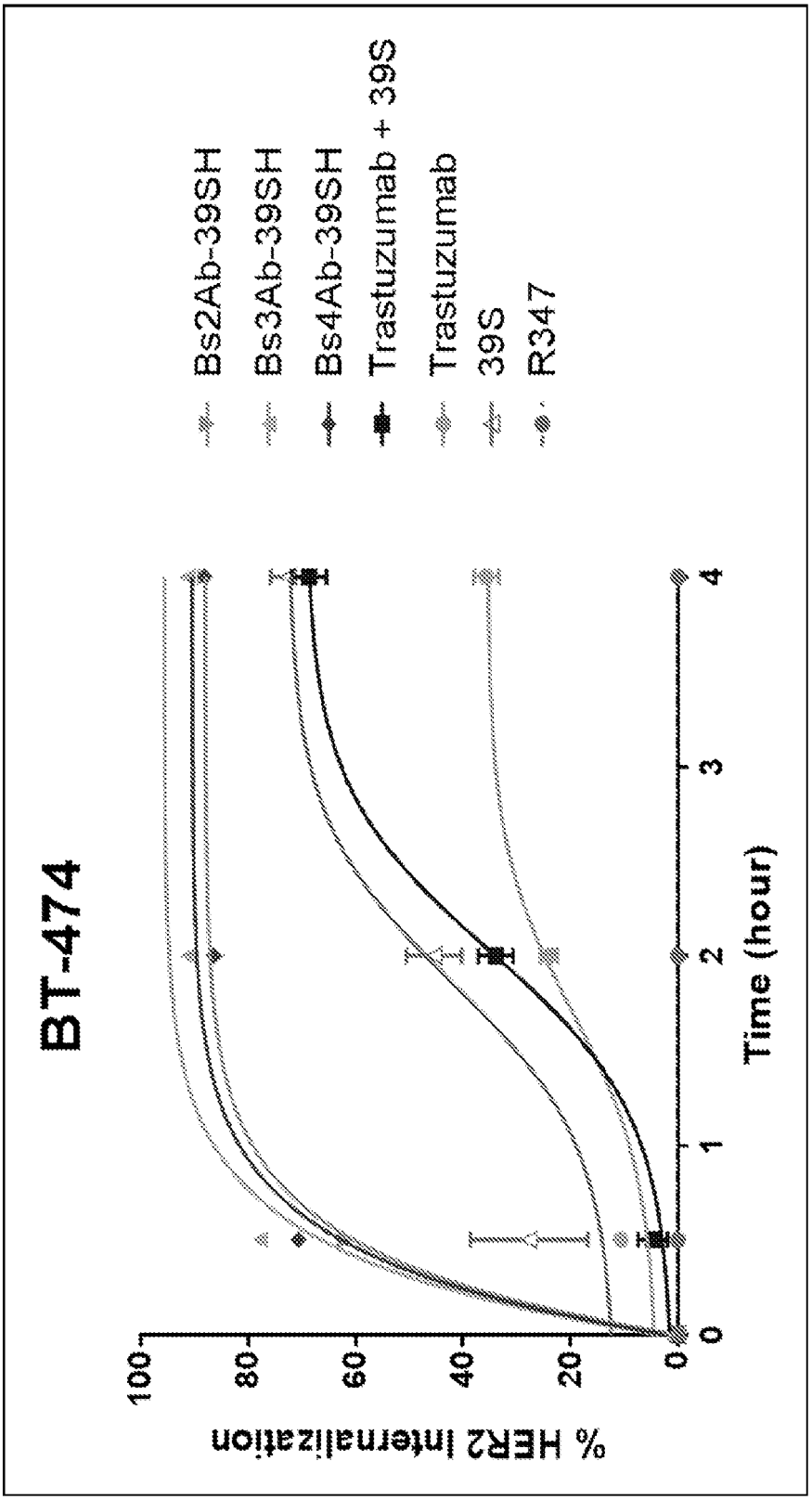

BT-474
% HER2 Internalization
Time (hour)
0
20
40
60
80
100
0
1
2
3
4
Bs2Ab-39SH
Bs3Ab-39SH
Bs4Ab-39SH
Trastuzumab + 39S
Trastuzumab
39S
R347

FIG. 12

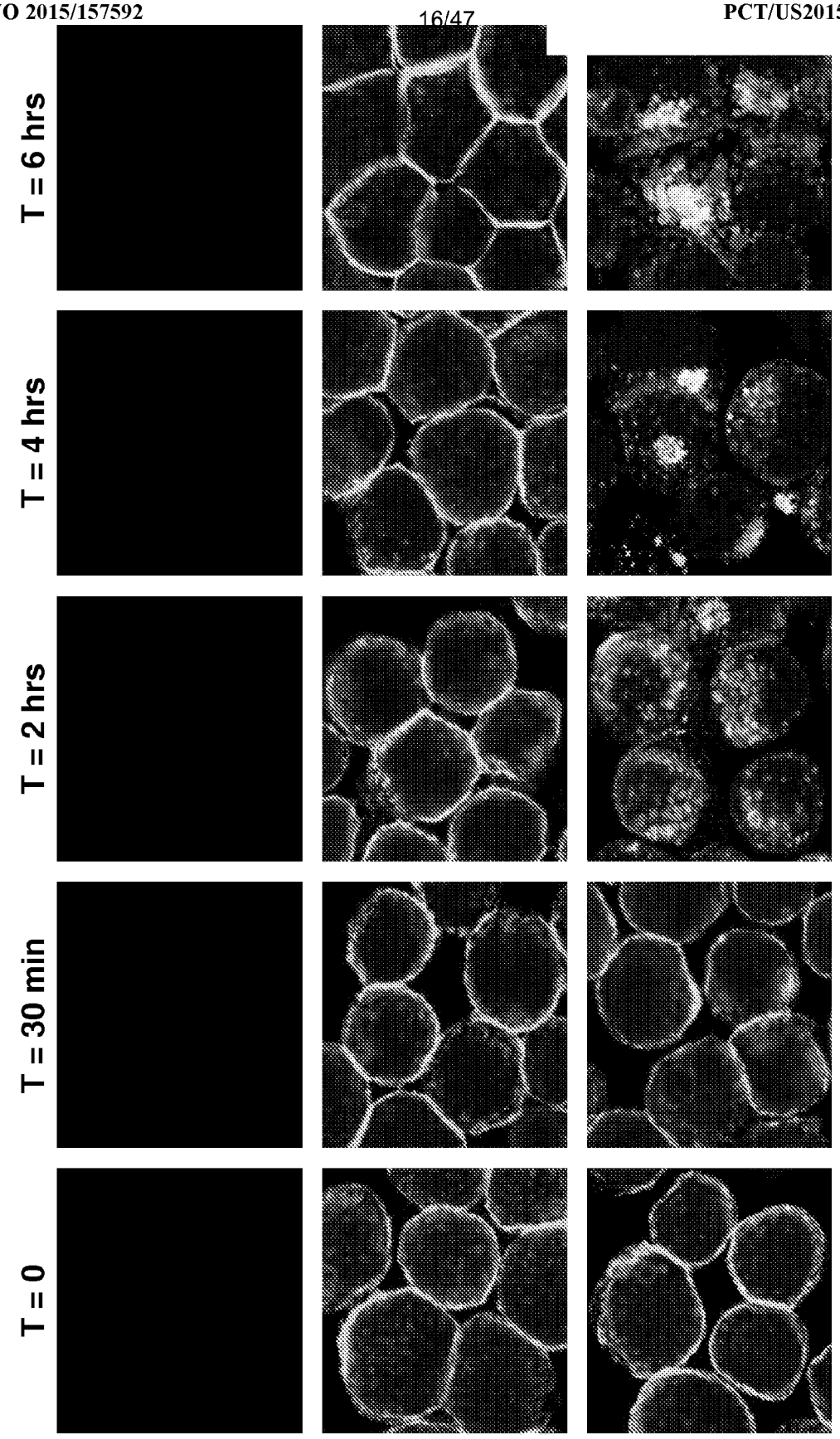

T = 0
T = 30 min
T = 2 hrs
T = 4 hrs
T = 6 hrs
R347
Trastuzumab
Bs2Ab-39SH (Unarmed)

FIG. 13

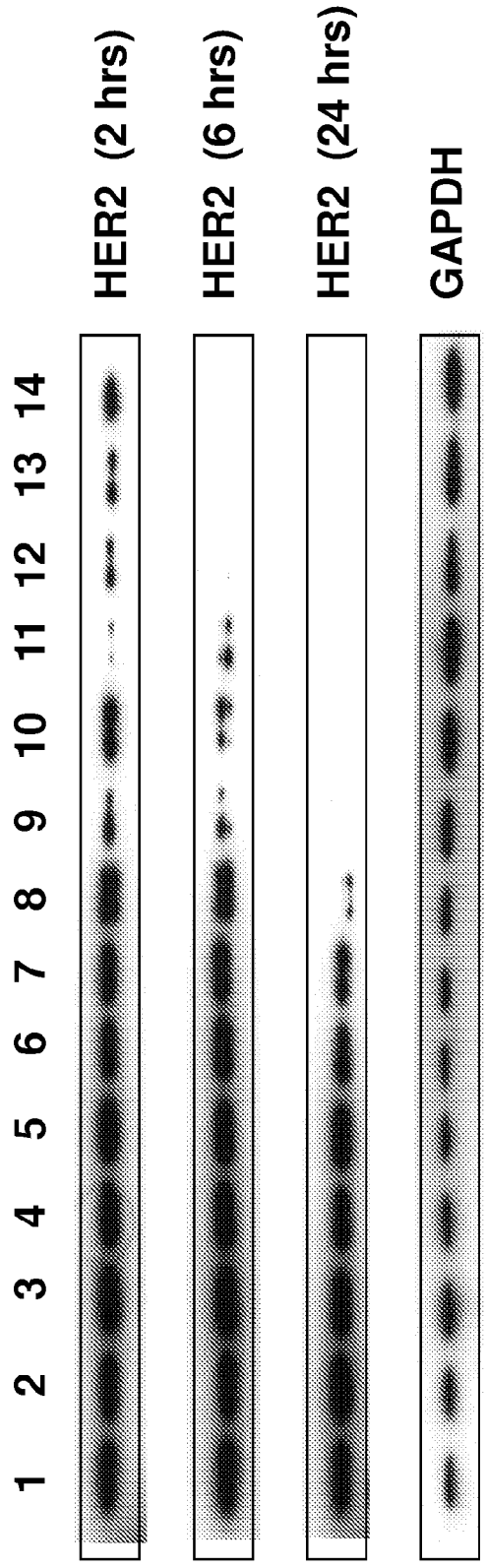



FIG. 14

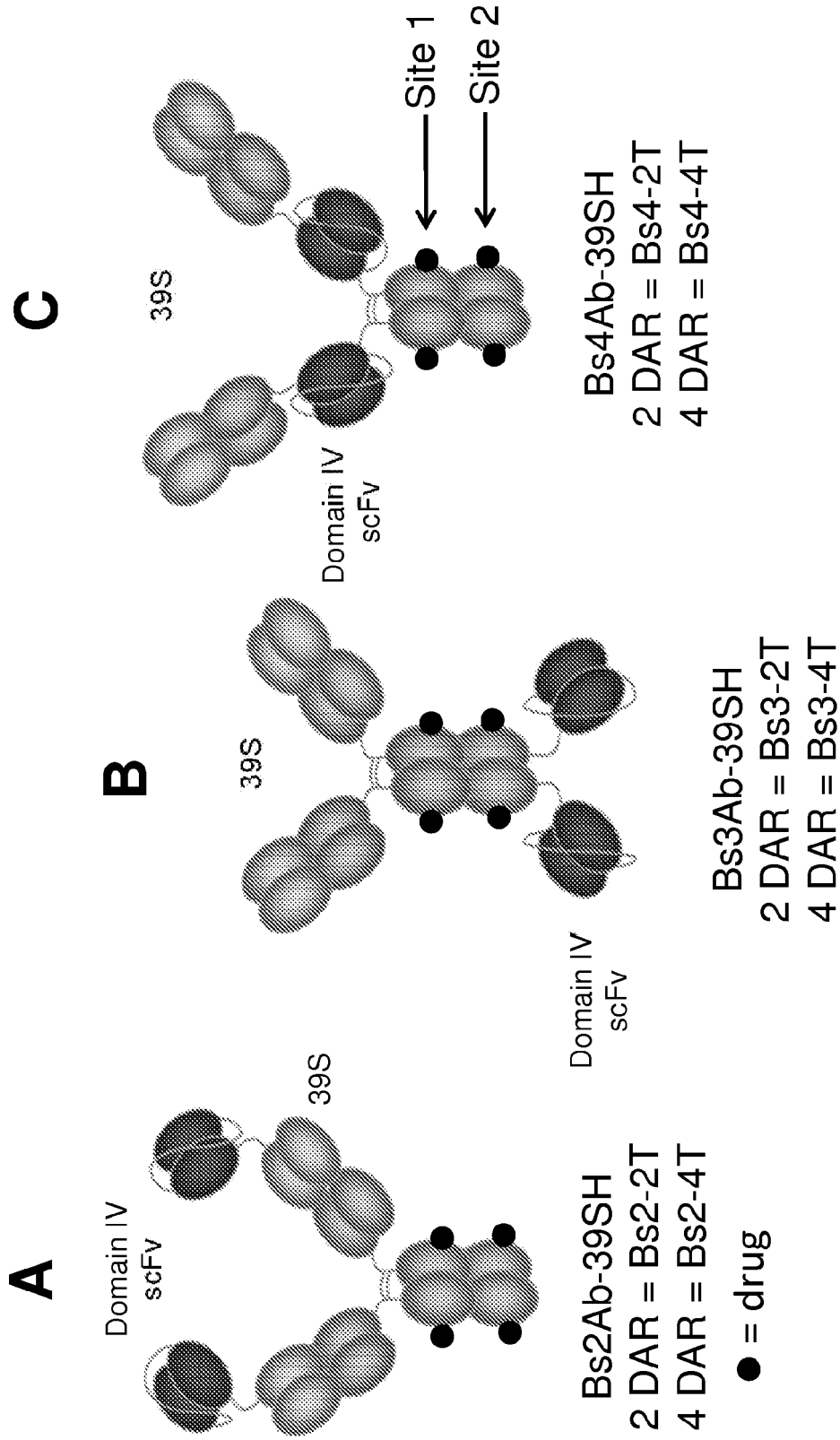

A
Domain IV
scFv
39S
Bs2Ab-39SH
2 DAR = Bs2-2T
4 DAR = Bs2-4T
● = drug
B
39S
Domain IV
scFv
Bs3Ab-39SH
2 DAR = Bs3-2T
4 DAR = Bs3-4T
C
39S
Site 1
Site 2
Domain IV
scFv
Bs4Ab-39SH
2 DAR = Bs4-2T
4 DAR = Bs4-4T

FIG. 15

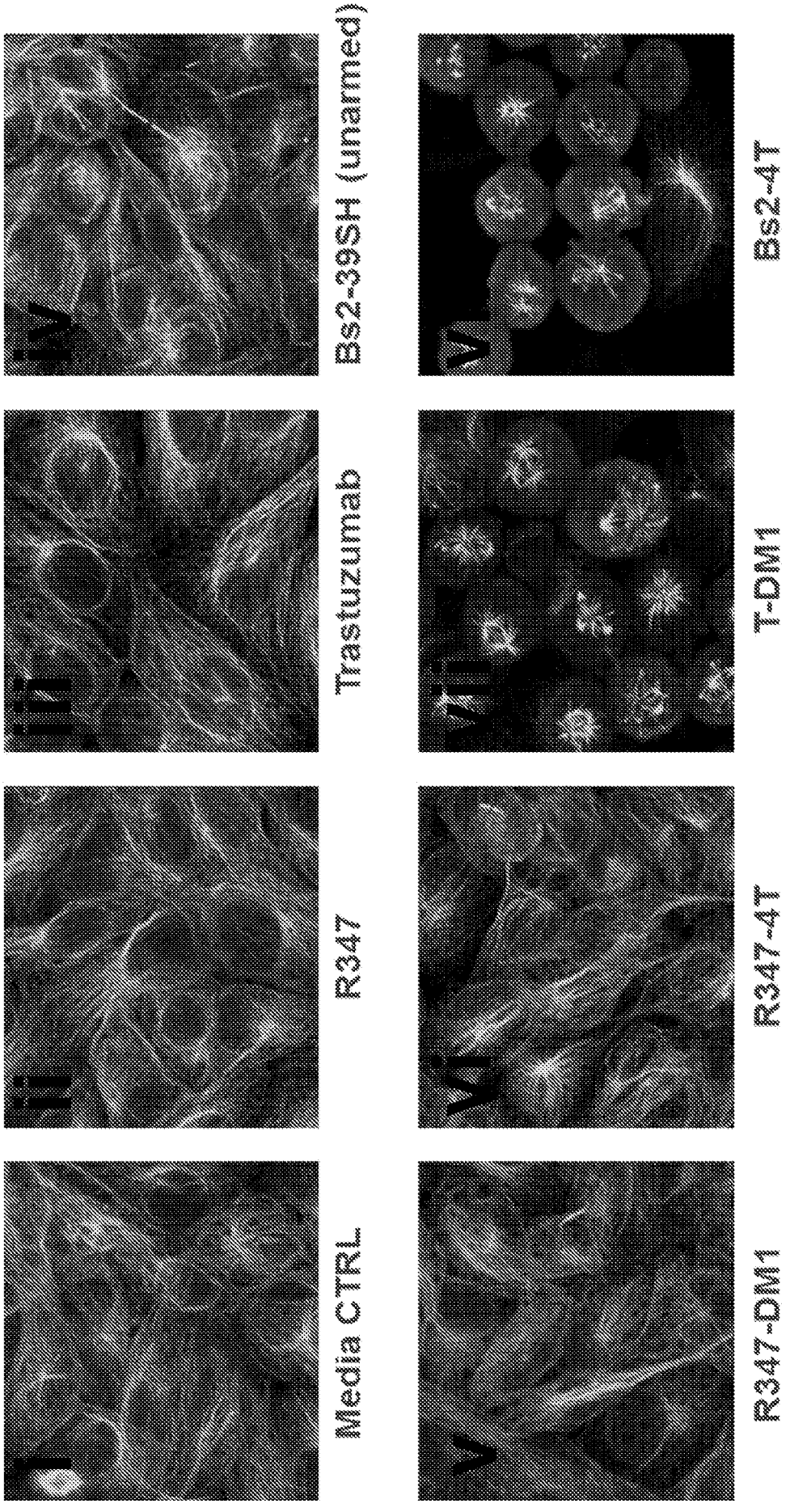

I
Media CTRL
II
R347
III
Trastuzumab
IV
Bs2-39SH (unarmed)
V
R347-DM1
VI
R347-4T
VII
T-DM1
VIII
Bs2-4T

FIG. 16A

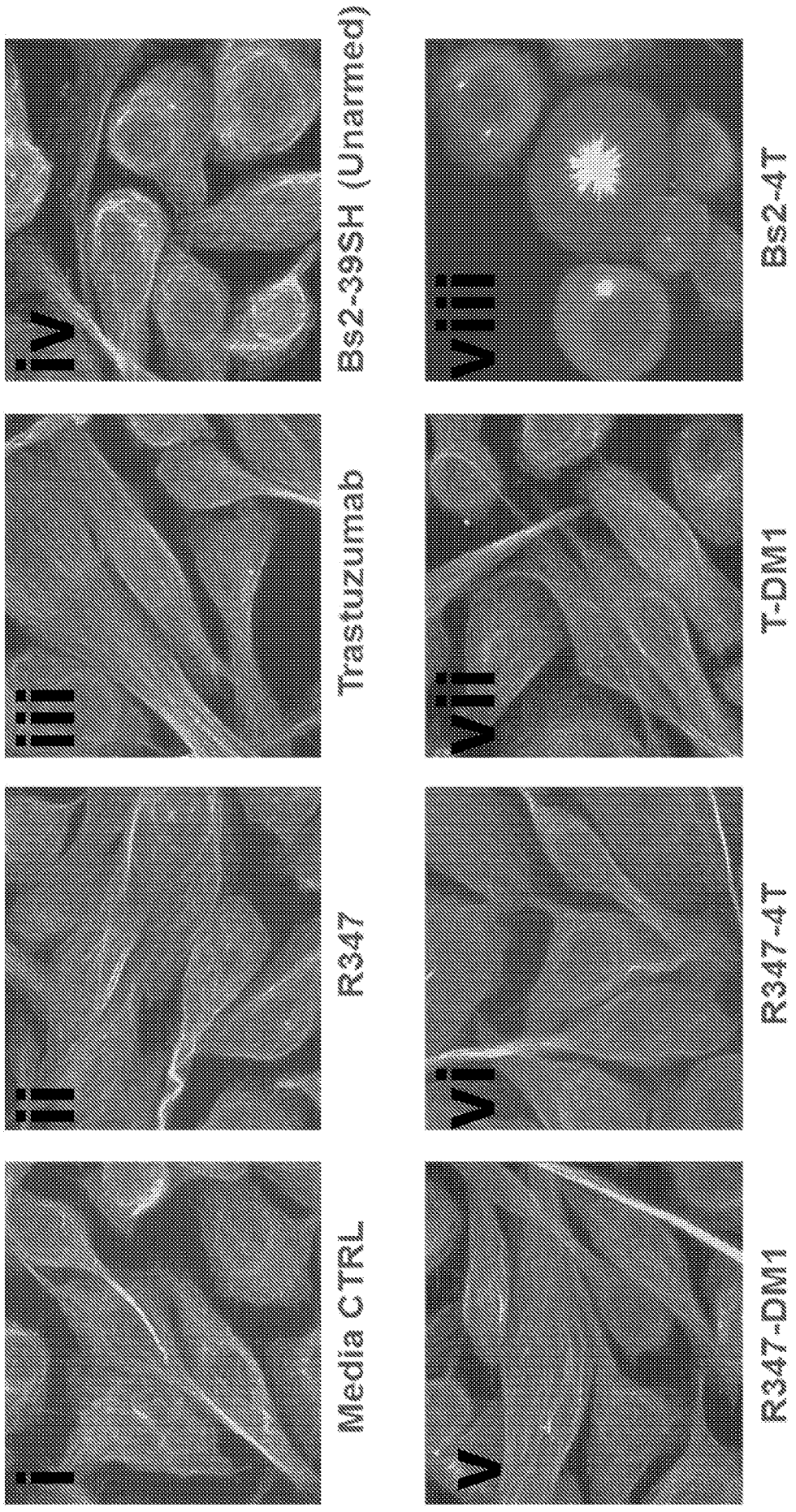

i
Media CTRL
ii
R347
iii
Trastuzumab
iv
Bs2-39SH (Unarmed)
v
R347-DM1
vi
R347-4T
vii
T-DM1
viii
Bs2-4T

FIG. 16B

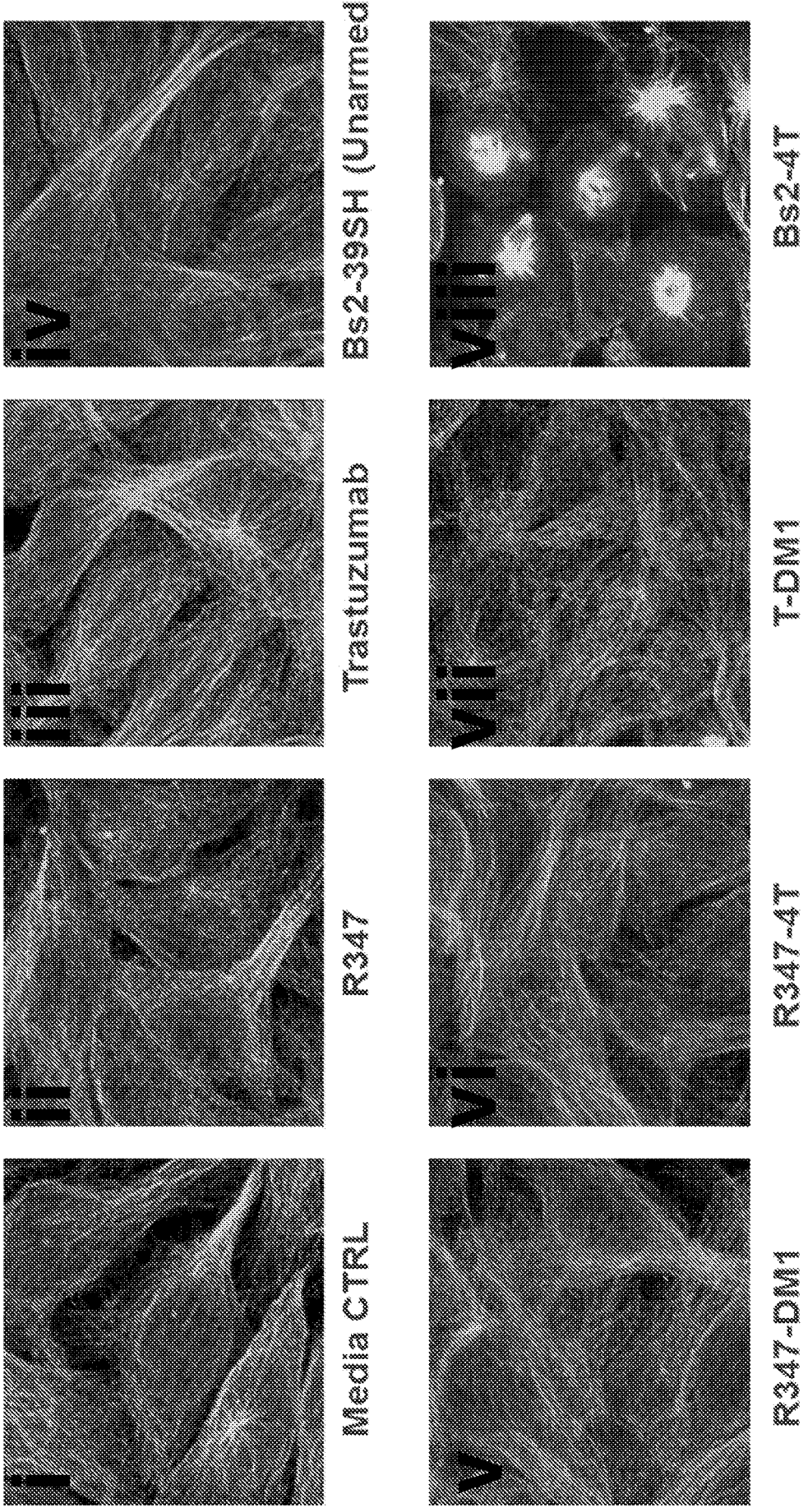

i
Media CTRL
ii
R347
iii
Trastuzumab
iv
Bs2-39SH (Unarmed)
v
R347-DM1
vi
R347-4T
vii
T-DM1
viii
Bs2-4T

FIG. 16C

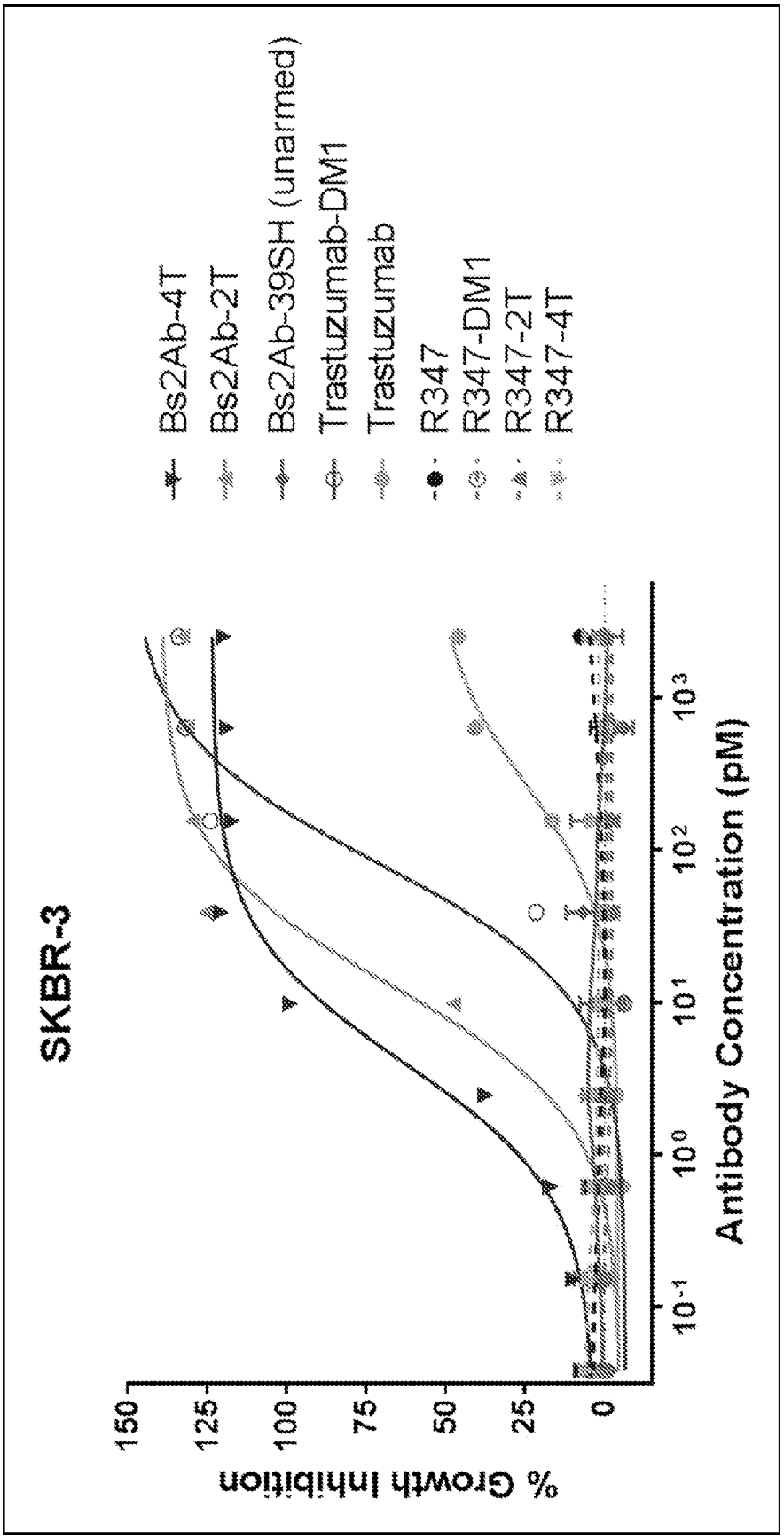

SKBR-3
% Growth Inhibition
150
125
100
75
50
25
0
$10^{-1}$
$10^{0}$
$10^{1}$
$10^{2}$
$10^{3}$
Antibody Concentration (pM)
Bs2Ab-4T
Bs2Ab-2T
Bs2Ab-39SH (unarmed)
Trastuzumab-DM1
Trastuzumab
R347
R347-DM1
R347-2T
R347-4T

FIG. 17A

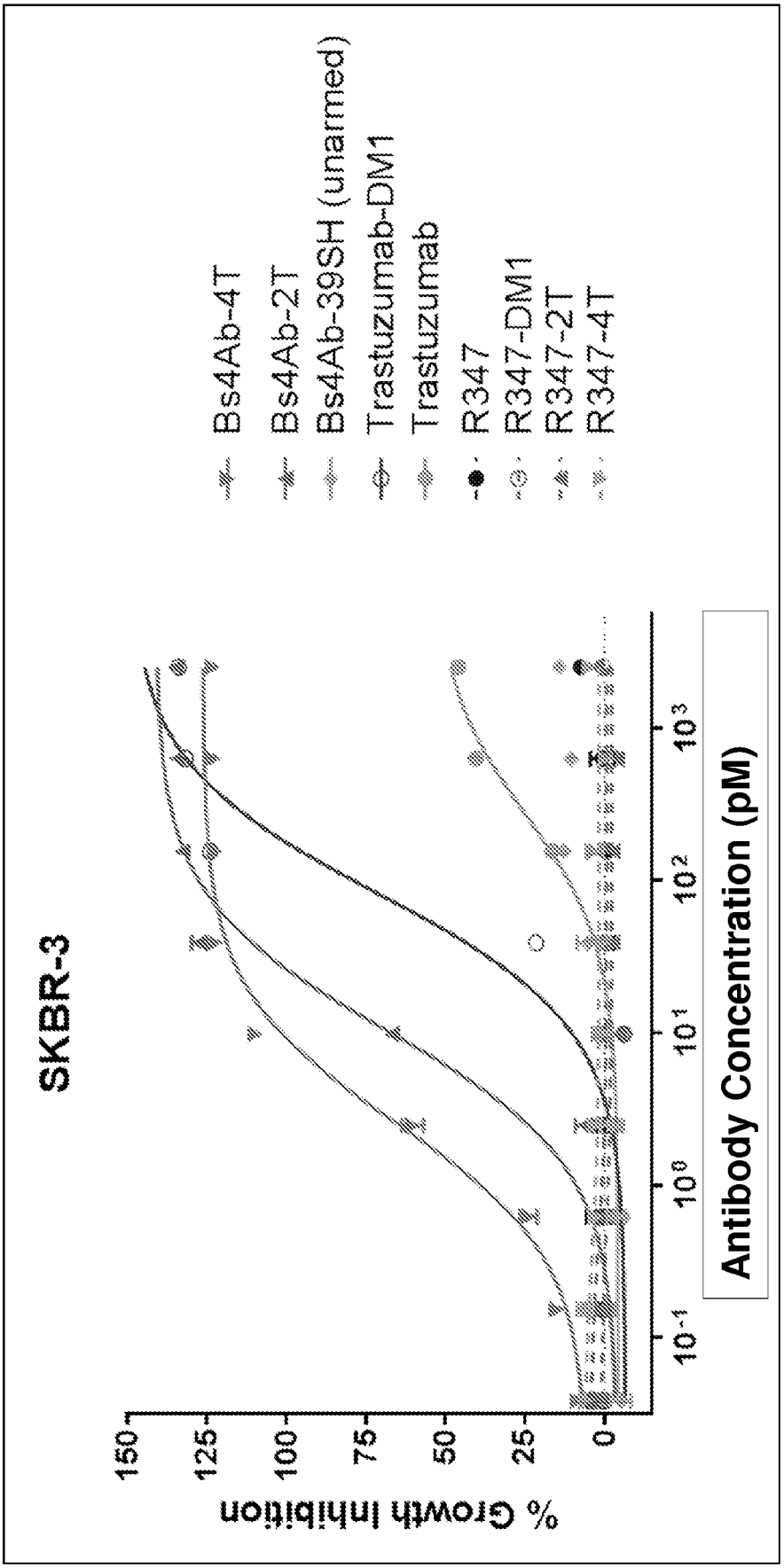

SKBR-3
% Growth Inhibition
150
125
100
75
50
25
0
$10^{-1}$
$10^{0}$
$10^{1}$
$10^{2}$
$10^{3}$
Antibody Concentration (pM)
Bs4Ab-4T
Bs4Ab-2T
Bs4Ab-39SH (unarmed)
Trastuzumab-DM1
Trastuzumab
R347
R347-DM1
R347-2T
R347-4T

FIG. 17B

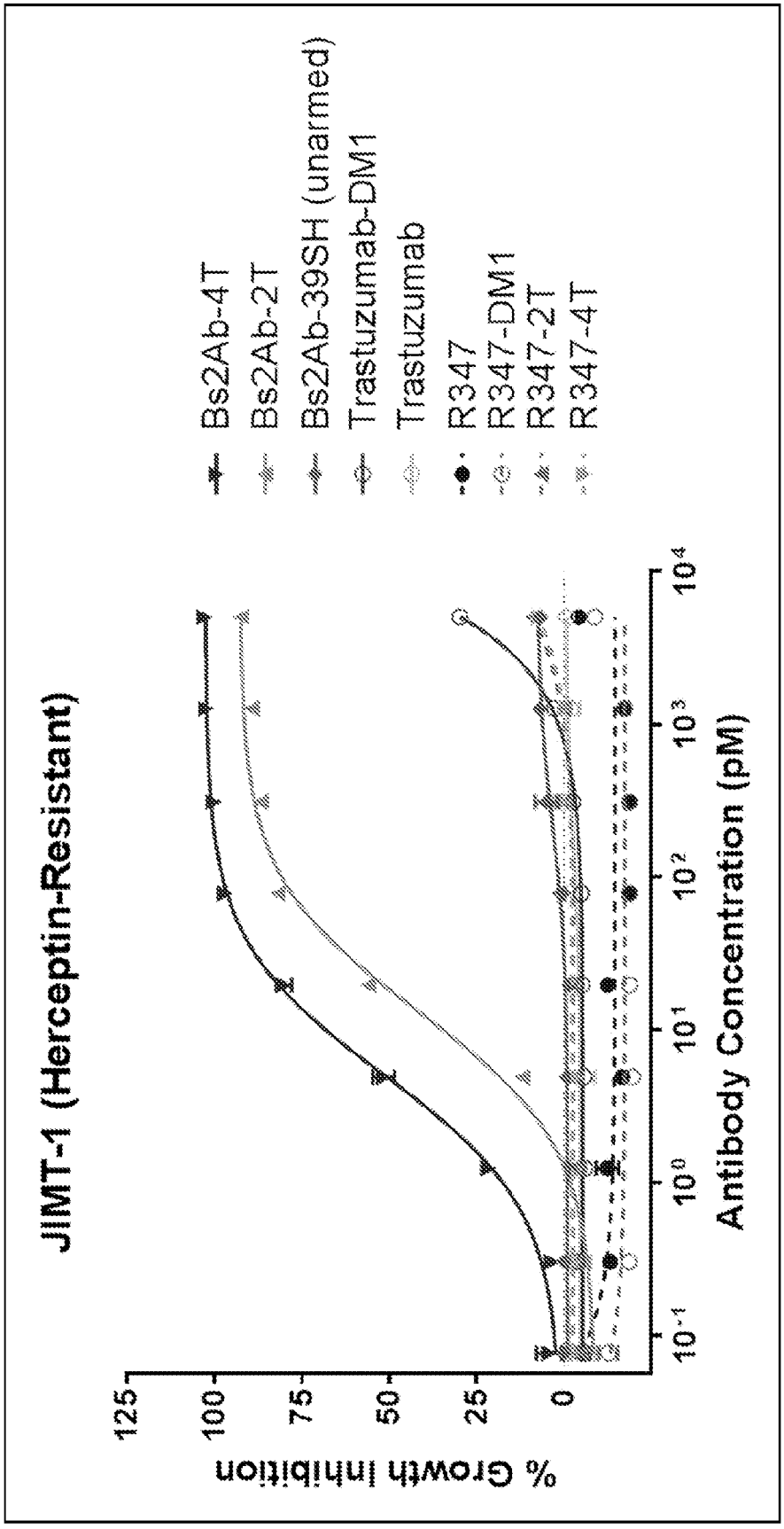

JIMT-1 (Herceptin-Resistant)
% Growth Inhibition
125
100
75
50
25
0
$10^{-1}$
$10^{0}$
$10^{1}$
$10^{2}$
$10^{3}$
$10^{4}$
Antibody Concentration (pM)
Bs2Ab-4T
Bs2Ab-2T
Bs2Ab-39SH (unarmed)
Trastuzumab-DM1
Trastuzumab
R347
R347-DM1
R347-2T
R347-4T

FIG. 18A

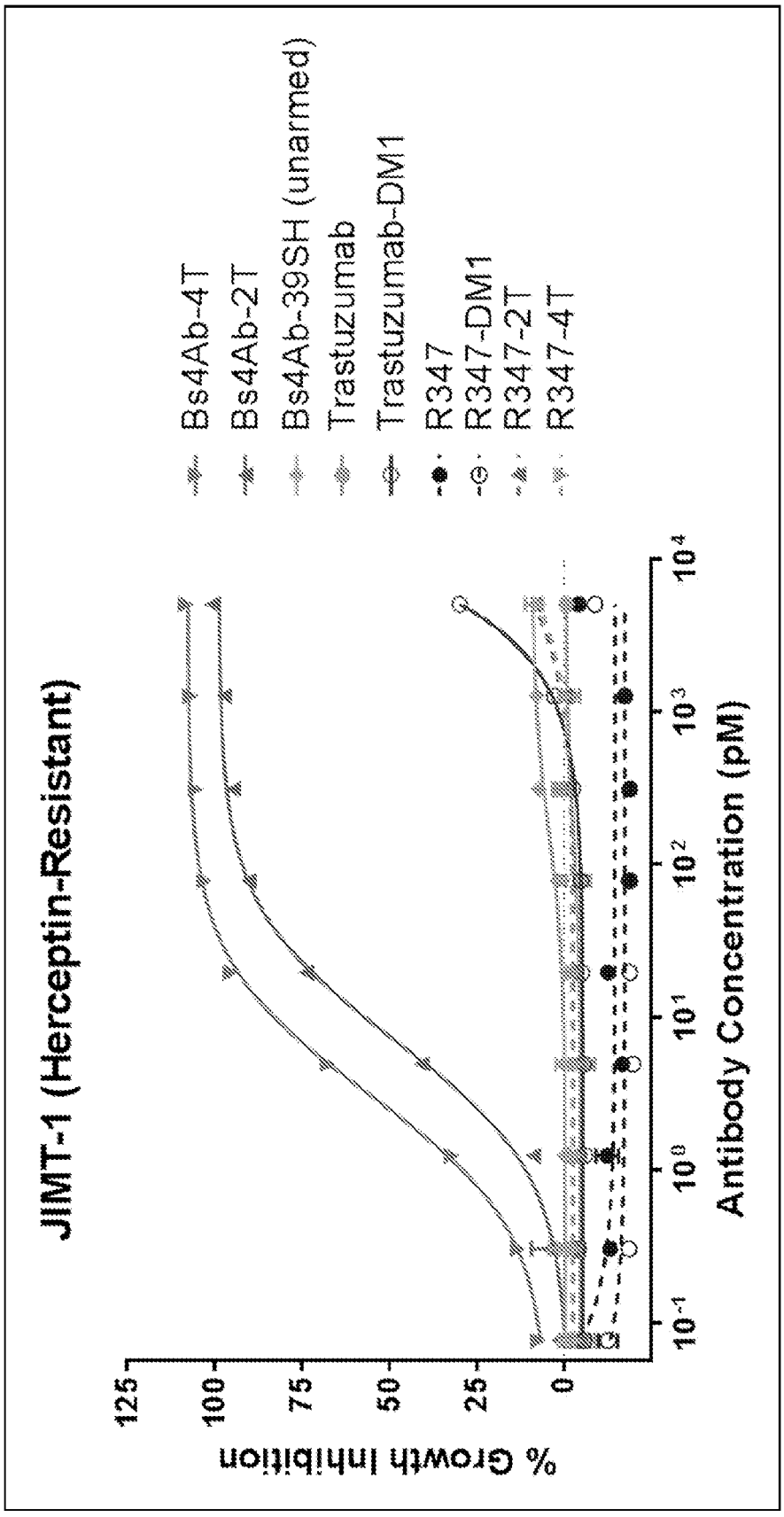

JIMT-1 (Herceptin-Resistant)
% Growth Inhibition
125
100
75
50
25
0
$10^{-1}$
$10^{0}$
$10^{1}$
$10^{2}$
$10^{3}$
$10^{4}$
Antibody Concentration (pM)
Bs4Ab-4T
Bs4Ab-2T
Bs4Ab-39SH (unarmed)
Trastuzumab
Trastuzumab-DM1
R347
R347-DM1
R347-2T
R347-4T

FIG. 18B

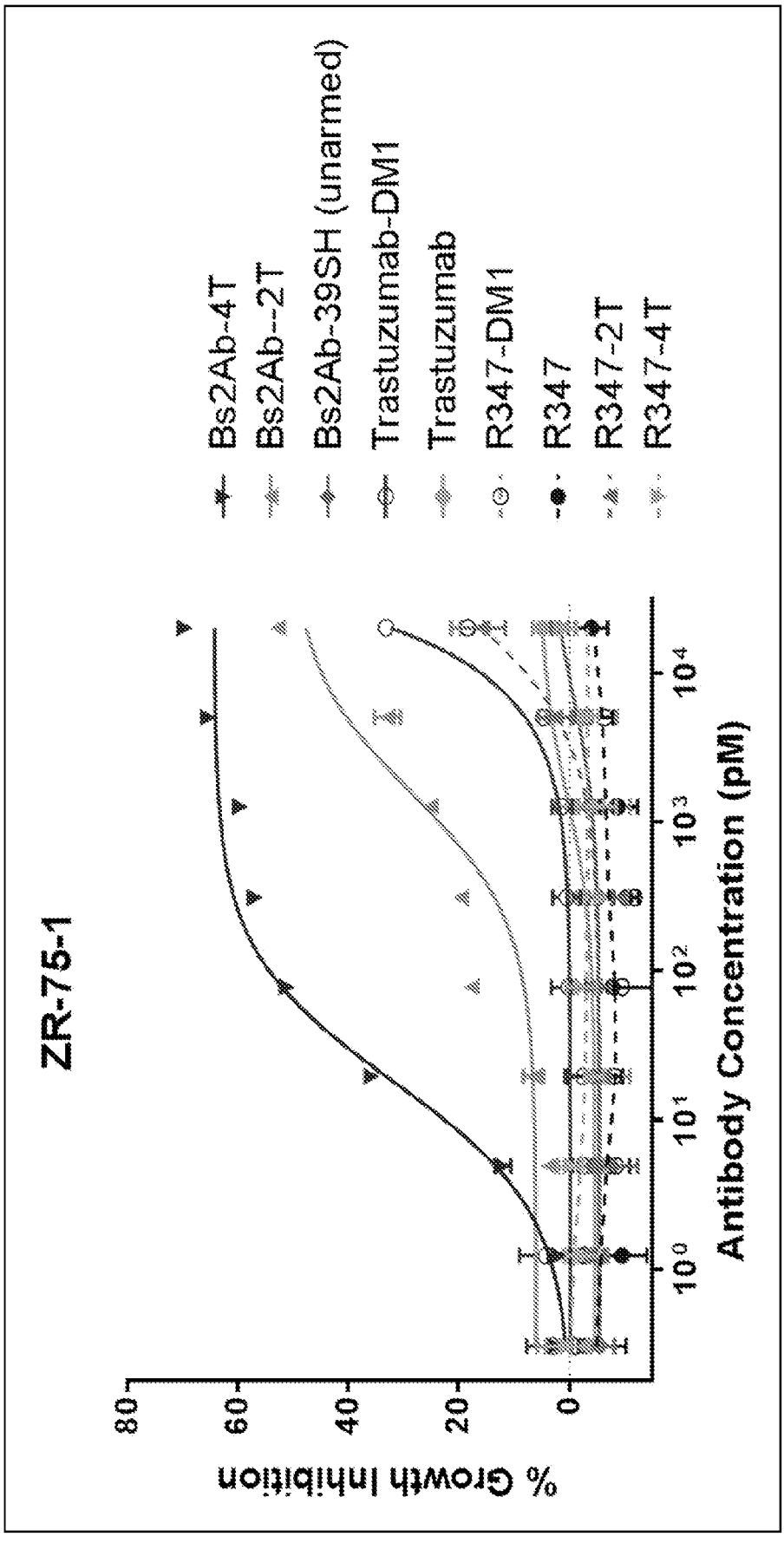

ZR-75-1
% Growth Inhibition
80
60
40
20
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
Antibody Concentration (pM)
Bs2Ab-4T
Bs2Ab--2T
Bs2Ab-39SH (unarmed)
Trastuzumab-DM1
Trastuzumab
R347-DM1
R347
R347-2T
R347-4T

FIG. 19A

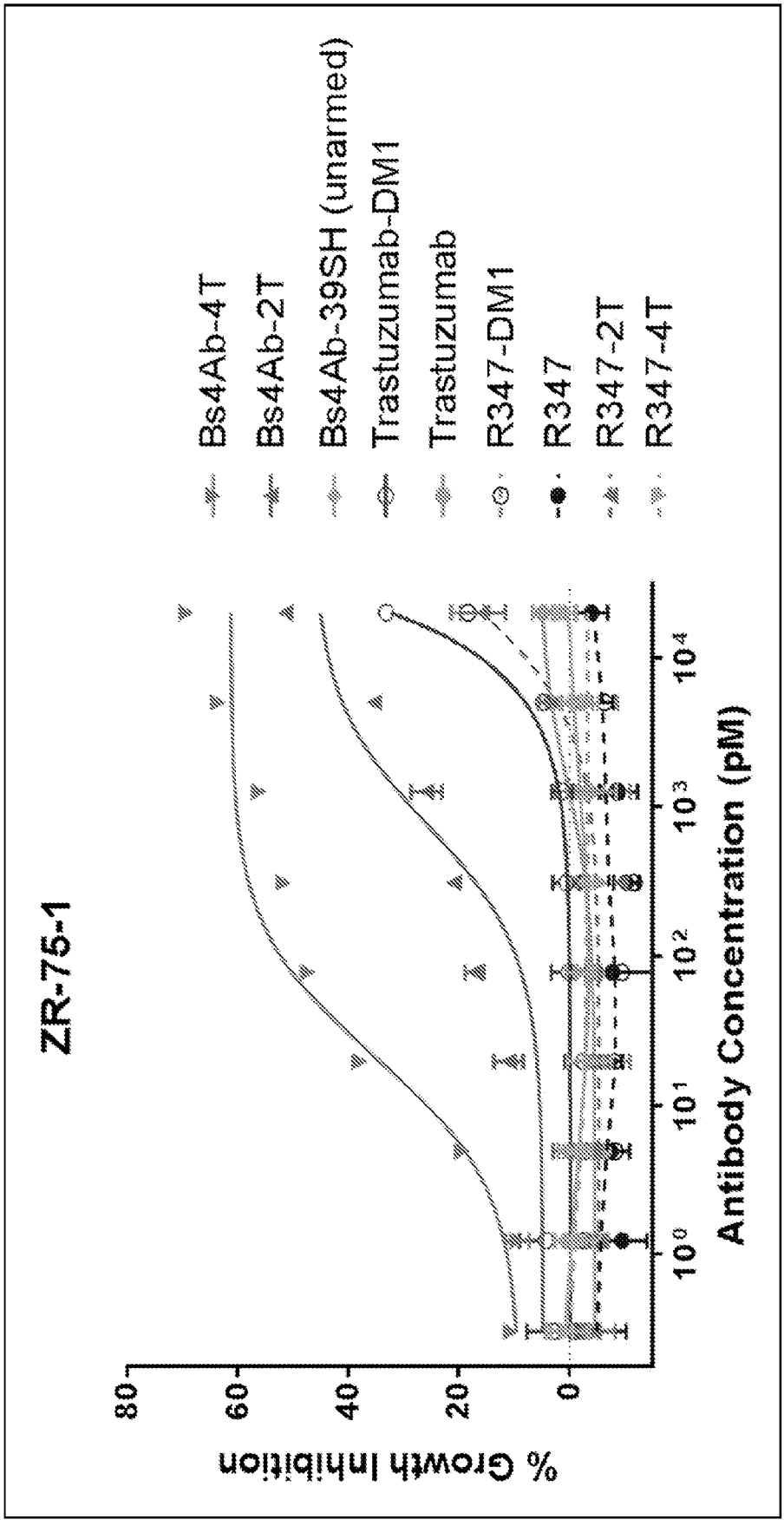

ZR-75-1
% Growth Inhibition
Antibody Concentration (pM)
Bs4Ab-4T
Bs4Ab-2T
Bs4Ab-39SH (unarmed)
Trastuzumab-DM1
Trastuzumab
R347-DM1
R347
R347-2T
R347-4T

FIG. 19B

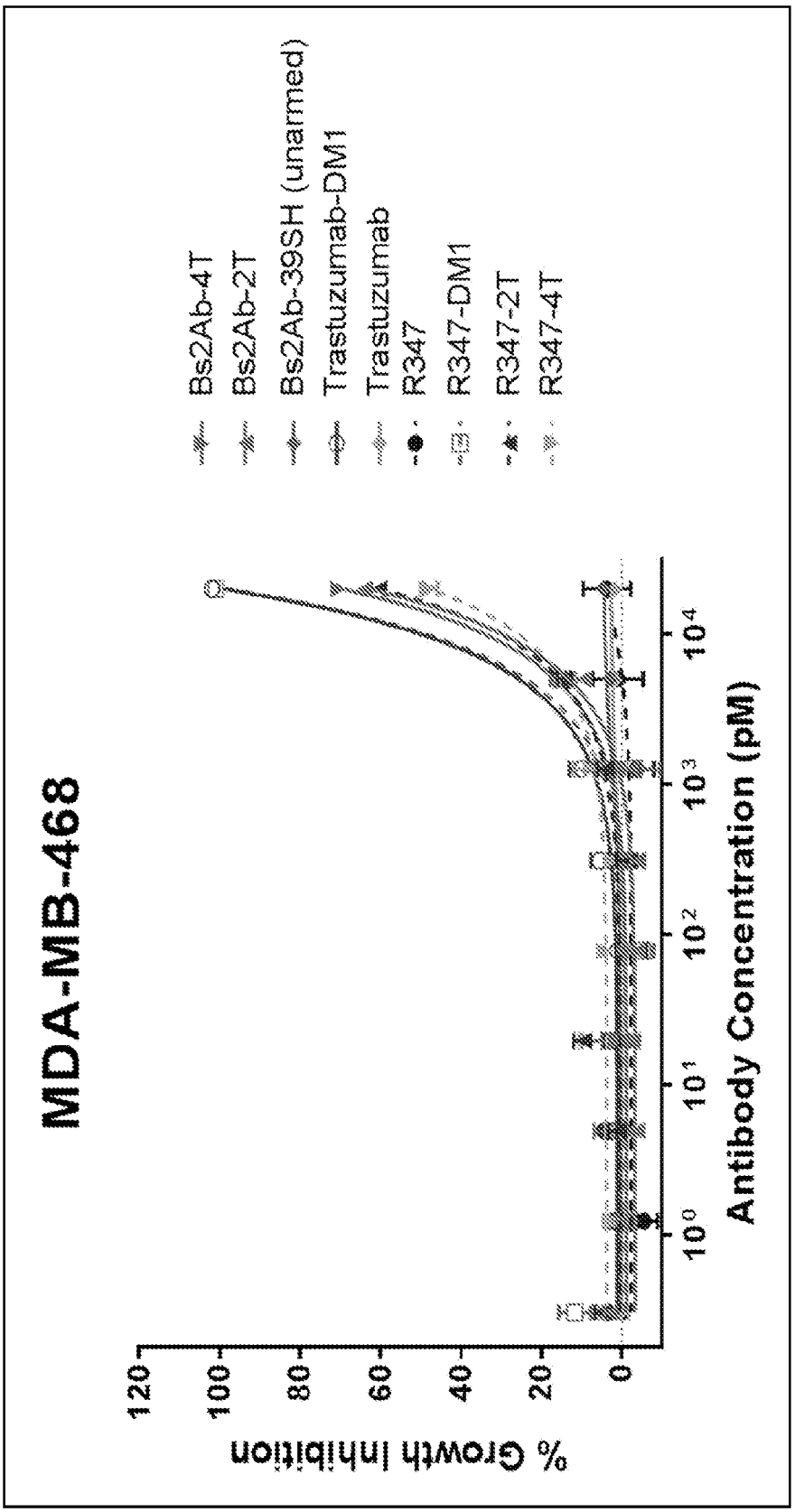

MDA-MB-468
% Growth Inhibition
120
100
80
60
40
20
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
Antibody Concentration (pM)
Bs2Ab-4T
Bs2Ab-2T
Bs2Ab-39SH (unarmed)
Trastuzumab-DM1
Trastuzumab
R347
R347-DM1
R347-2T
R347-4T

FIG. 20

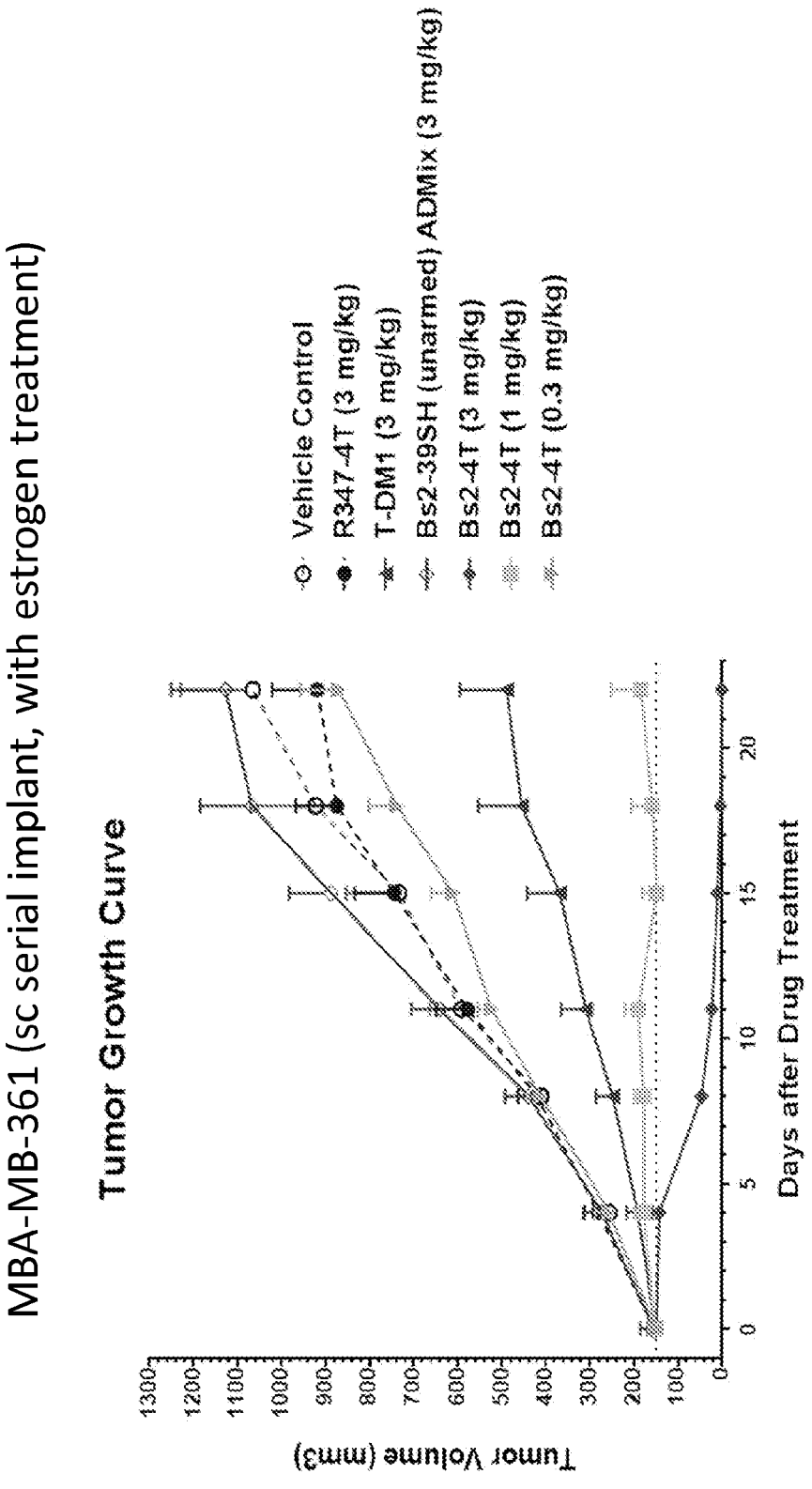

MBA-MB-361 (sc serial implant, with estrogen treatment)
Tumor Growth Curve
Tumor Volume (mm3)
Days after Drug Treatment
Vehicle Control
R347-4T (3 mg/kg)
T-DM1 (3 mg/kg)
Bs2-39SH (unarmed) ADMix (3 mg/kg)
Bs2-4T (3 mg/kg)
Bs2-4T (1 mg/kg)
Bs2-4T (0.3 mg/kg)

FIG. 21A

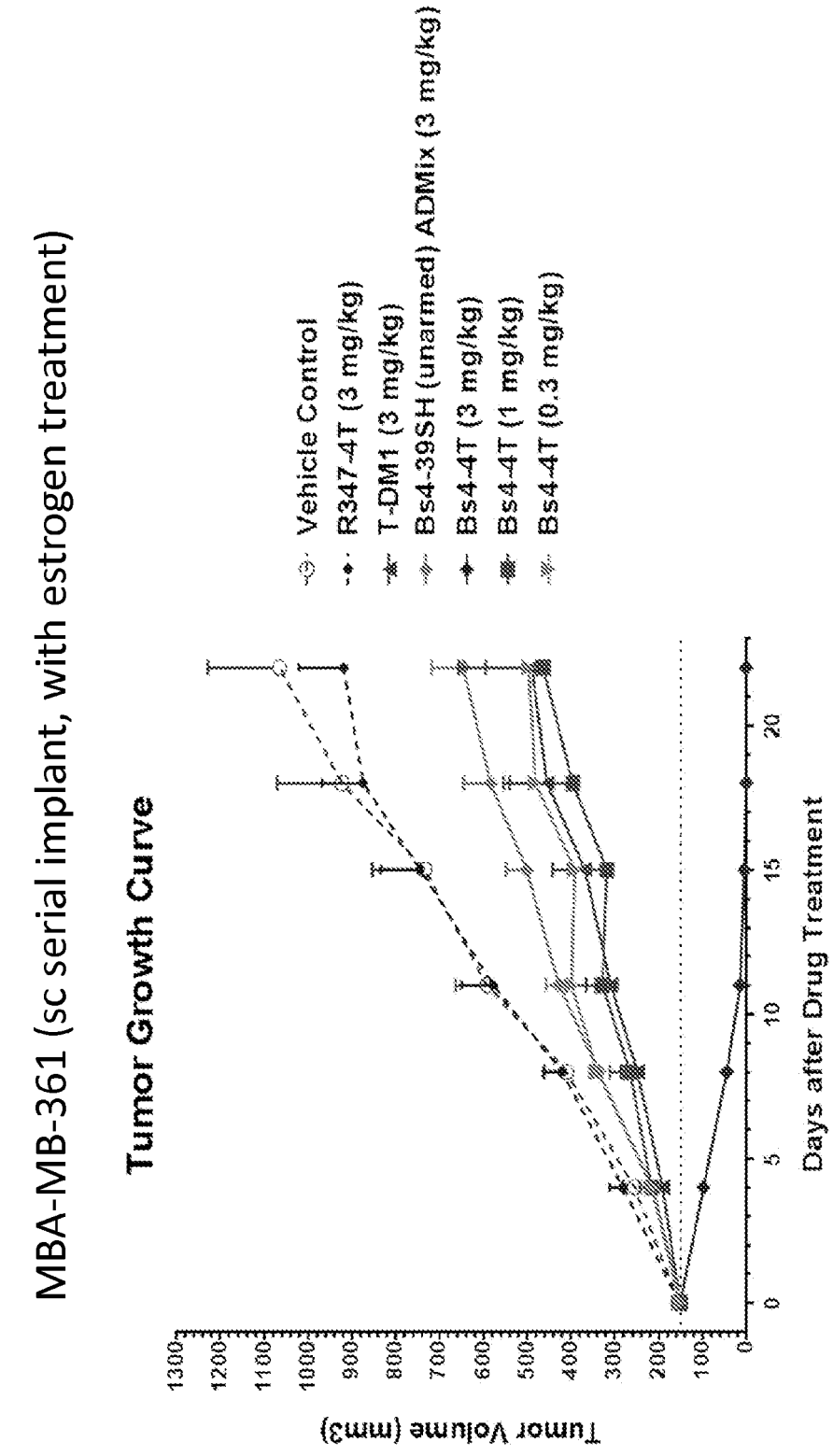

MBA-MB-361 (sc serial implant, with estrogen treatment)
Tumor Growth Curve
Vehicle Control
R347-4T (3 mg/kg)
T-DM1 (3 mg/kg)
Bs4-39SH (unarmed) ADMix (3 mg/kg)
Bs4-4T (3 mg/kg)
Bs4-4T (1 mg/kg)
Bs4-4T (0.3 mg/kg)
Tumor Volume (mm3)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
Days after Drug Treatment

FIG. 21B

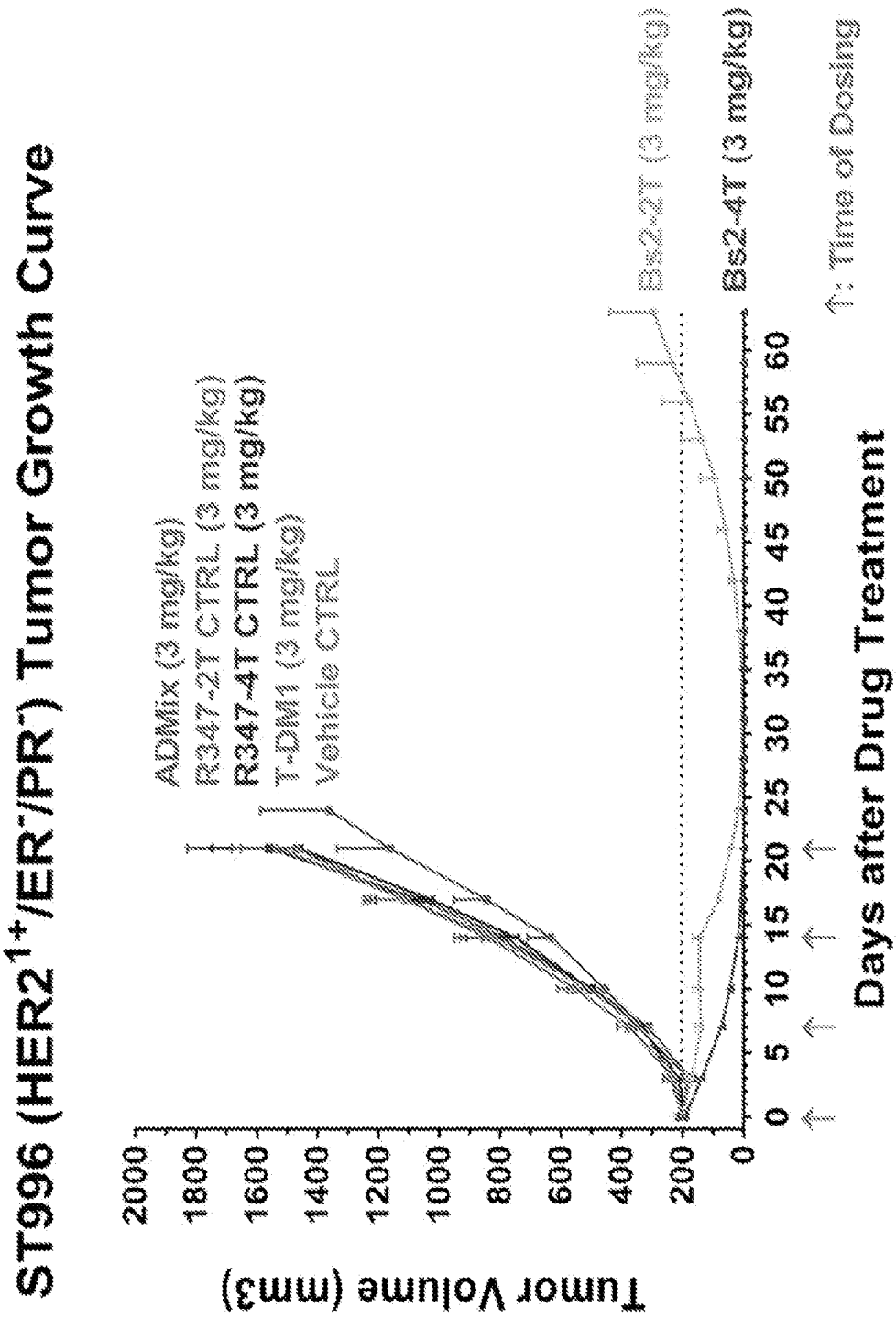

ST996 (HER2$^{1+}$/ER$^{-}$/PR$^{-}$) Tumor Growth Curve
Tumor Volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Days after Drug Treatment
ADMix (3 mg/kg)
R347-2T CTRL (3 mg/kg)
R347-4T CTRL (3 mg/kg)
T-DM1 (3 mg/kg)
Vehicle CTRL
Bs2-2T (3 mg/kg)
Bs2-4T (3 mg/kg)
↑: Time of Dosing

FIG. 22

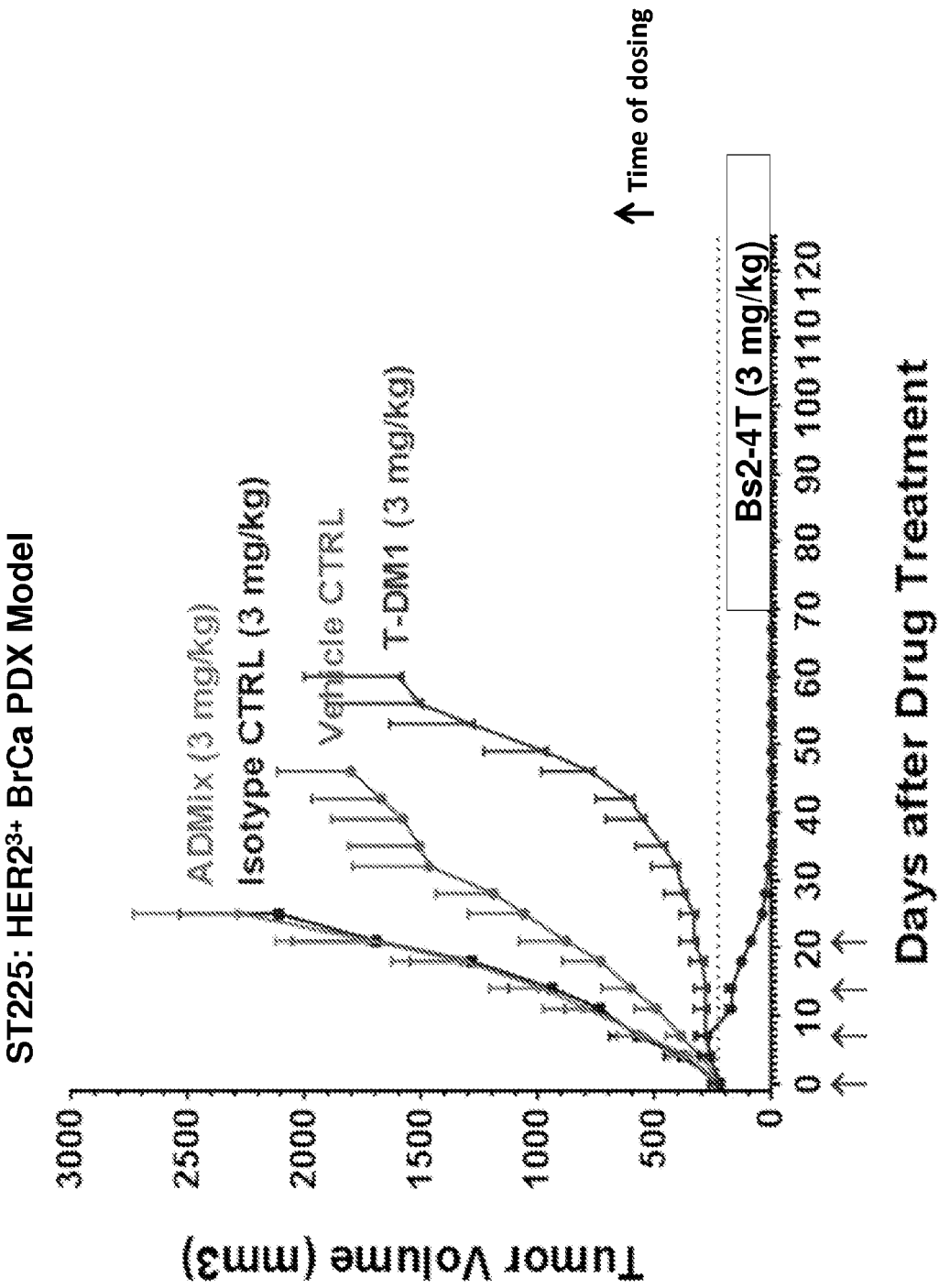

ST225: HER2$^{3+}$ BrCa PDX Model
Tumor Volume (mm3)
3000
2500
2000
1500
1000
500
0
ADMIx (3 mg/kg)
Isotype CTRL (3 mg/kg)
Vehicle CTRL
T-DM1 (3 mg/kg)
Bs2-4T (3 mg/kg)
Time of dosing
0 10 20 30 40 50 60 70 80 90 100 110 120
Days after Drug Treatment

FIG. 23

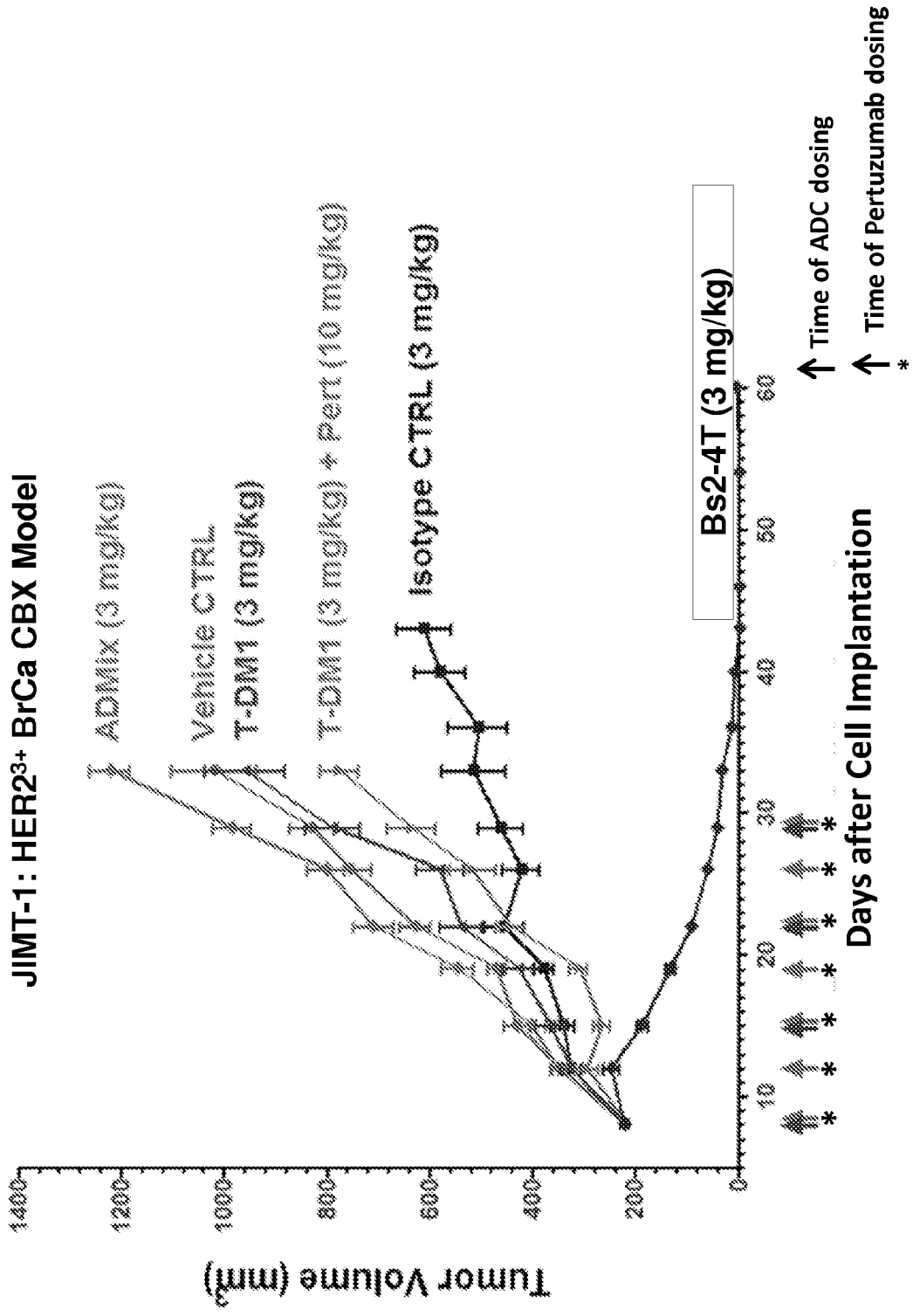

JIMT-1: HER2$^{3+}$ BrCa CBX Model
ADMix (3 mg/kg)
Vehicle CTRL
T-DM1 (3 mg/kg)
T-DM1 (3 mg/kg) + Pert (10 mg/kg)
Isotype CTRL (3 mg/kg)
Bs2-4T (3 mg/kg)
Tumor Volume (mm$^3$)
Days after Cell Implantation
Time of ADC dosing
Time of Pertuzumab dosing
*

FIG. 24

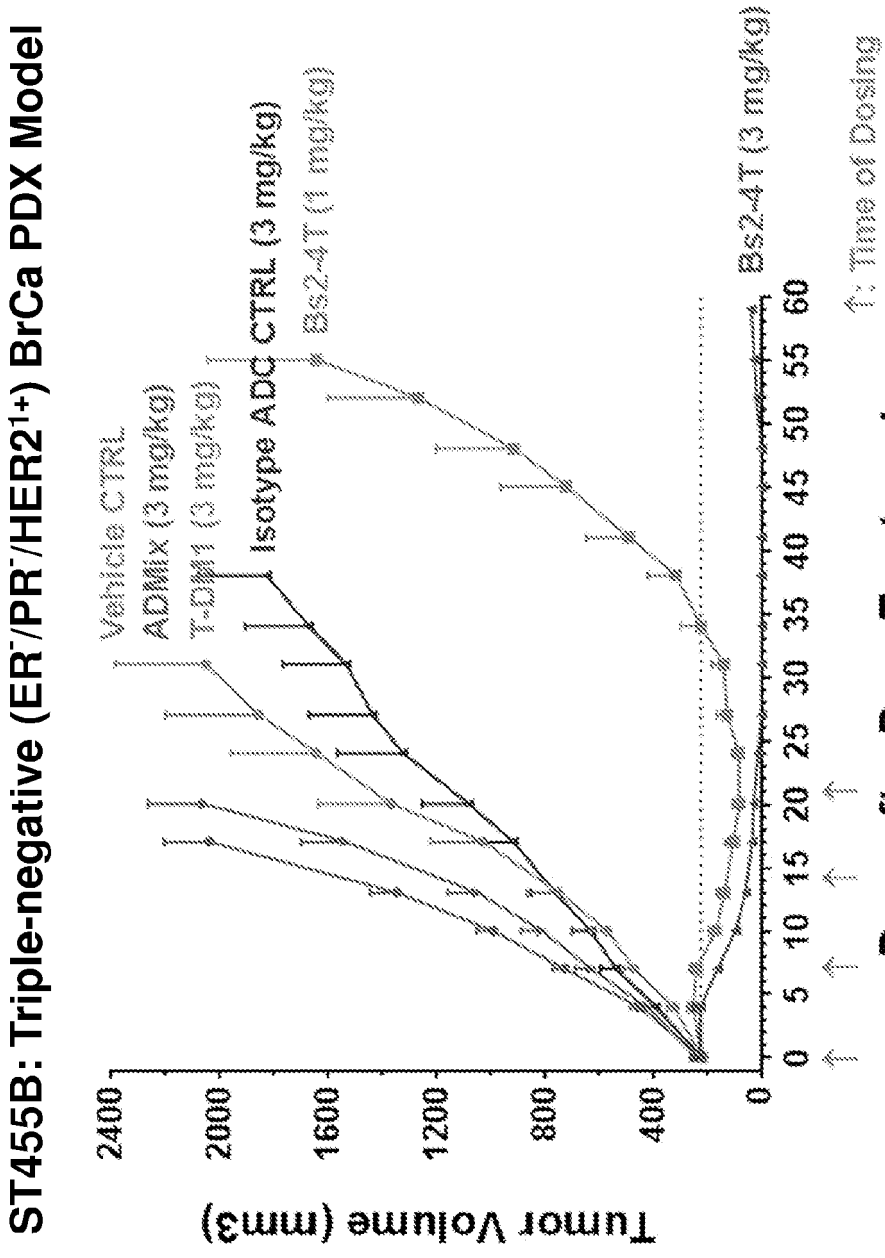

ST455B: Triple-negative (ER-/PR-/HER2+) BrCa PDX Model
Tumor Volume (mm3)
2400
2000
1600
1200
800
400
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Days after Drug Treatment
Vehicle CTRL
ADMix (3 mg/kg)
T-DM1 (3 mg/kg)
Isotype ADC CTRL (3 mg/kg)
Bs2-4T (1 mg/kg)
Bs2-4T (3 mg/kg)
↑: Time of Dosing

FIG. 25

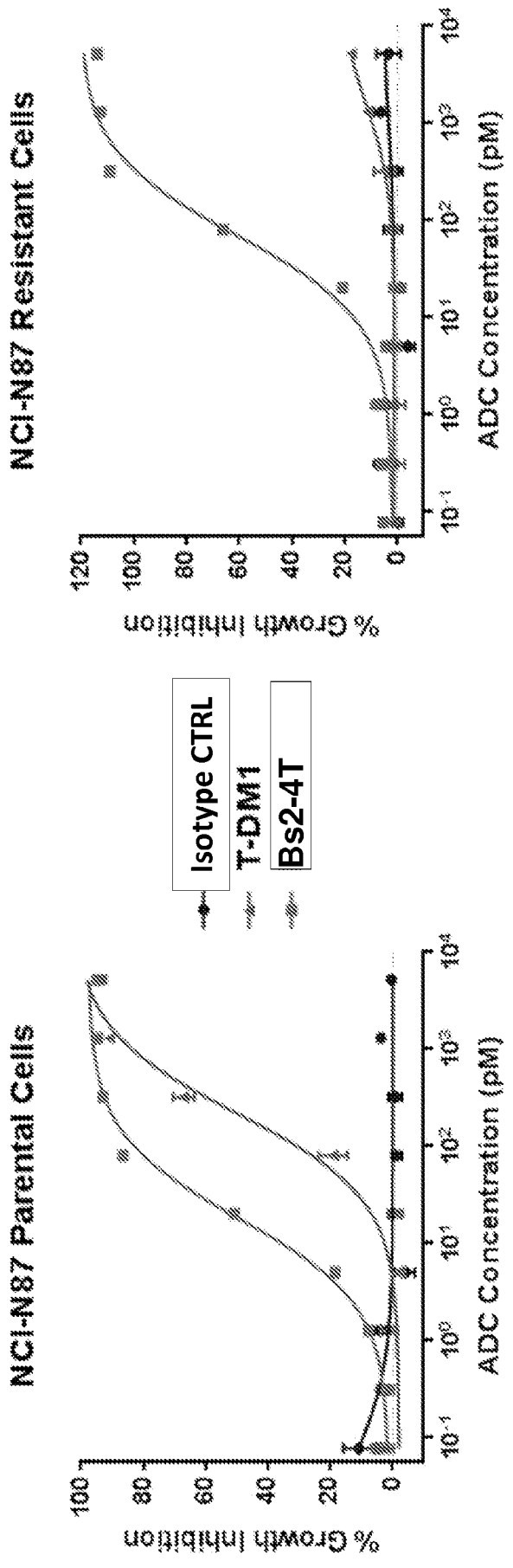

NCI-N87 Parental Cells
NCI-N87 Resistant Cells
% Growth Inhibition
ADC Concentration (pM)
Isotype CTRL
T-DM1
Bs2-4T

FIG. 26A

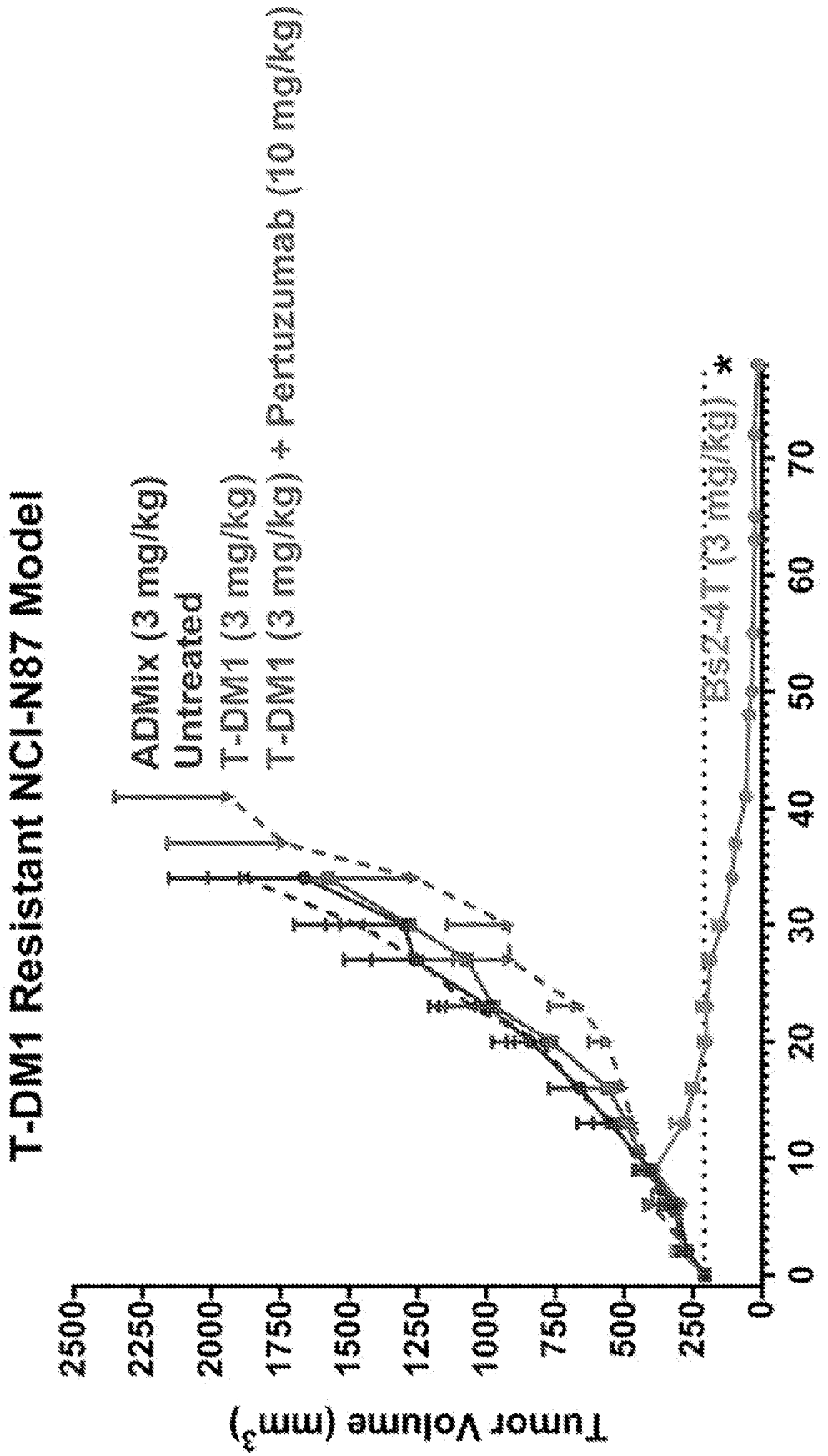

T-DM1 Resistant NCI-N87 Model
ADMix (3 mg/kg)
Untreated
T-DM1 (3 mg/kg)
T-DM1 (3 mg/kg) + Pertuzumab (10 mg/kg)
BS2-4T (3 mg/kg) **
Tumor Volume (mm3)
Days after Drug Treatment
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0
10
20
30
40
50
60
70

FIG. 26B

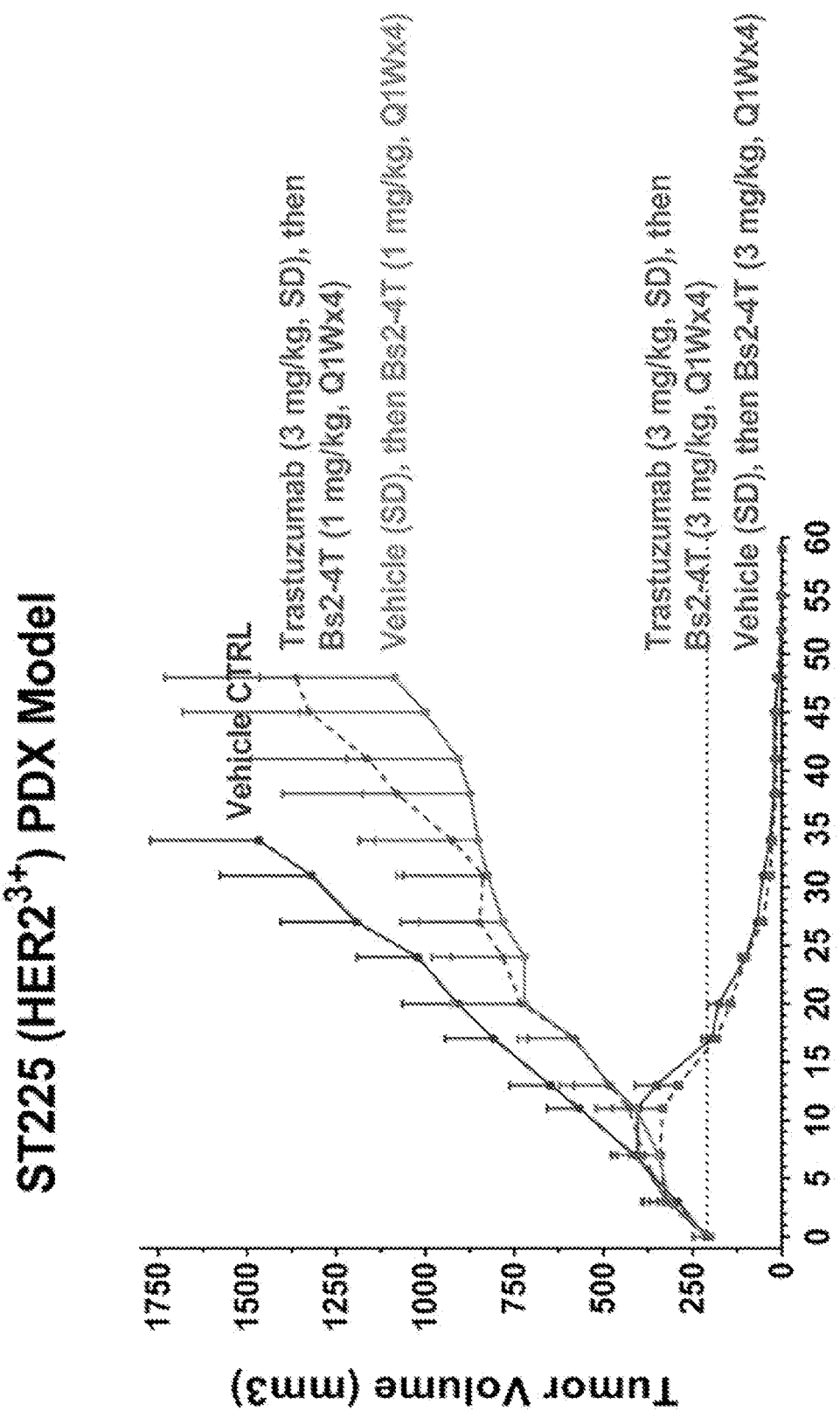

ST225 (HER2$^{3+}$) PDX Model
Tumor Volume (mm3)
Days after Drug Treatment
Vehicle CTRL
Trastuzumab (3 mg/kg, SD), then Bs2-4T (1 mg/kg, Q1Wx4)
Vehicle (SD), then Bs2-4T (1 mg/kg, Q1Wx4)
Trastuzumab (3 mg/kg, SD), then Bs2-4T (3 mg/kg, Q1Wx4)
Vehicle (SD), then Bs2-4T (3 mg/kg, Q1Wx4)
1750
1500
1250
1000
750
500
250
0
0 5 10 15 20 25 30 35 40 45 50 55 60

FIG. 27

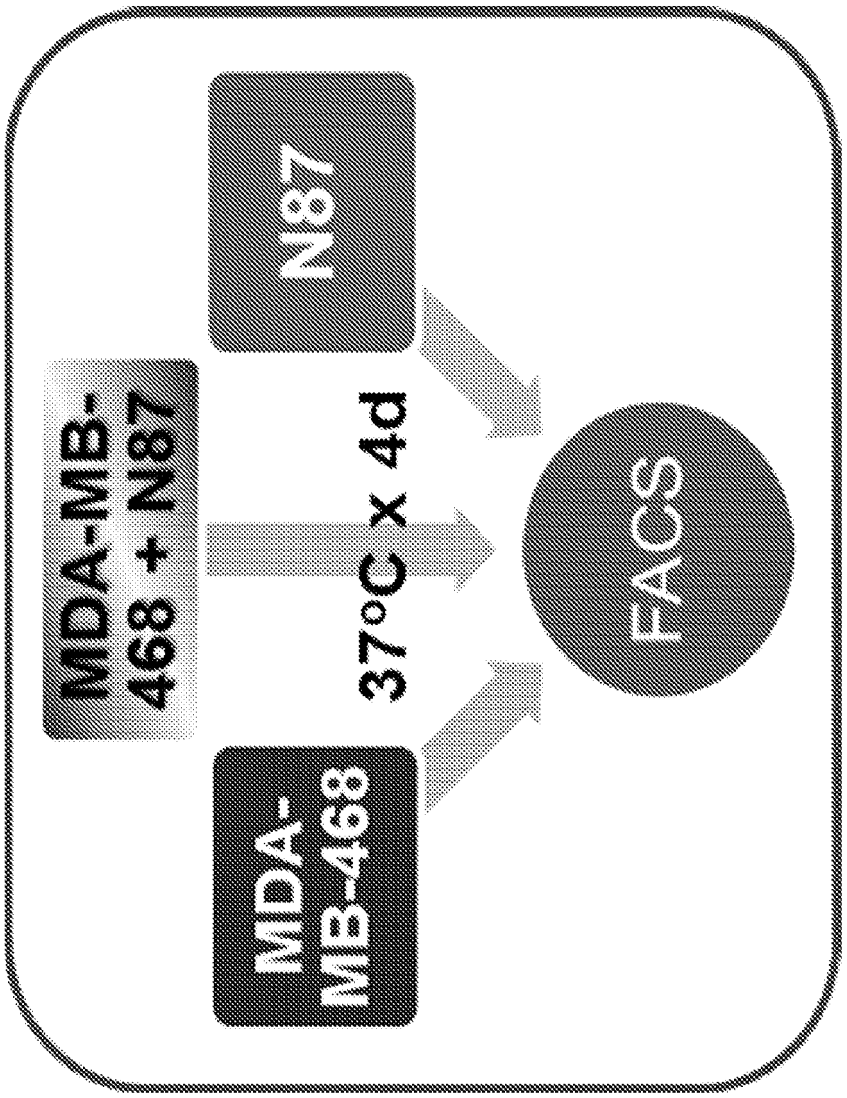



FIG. 28A

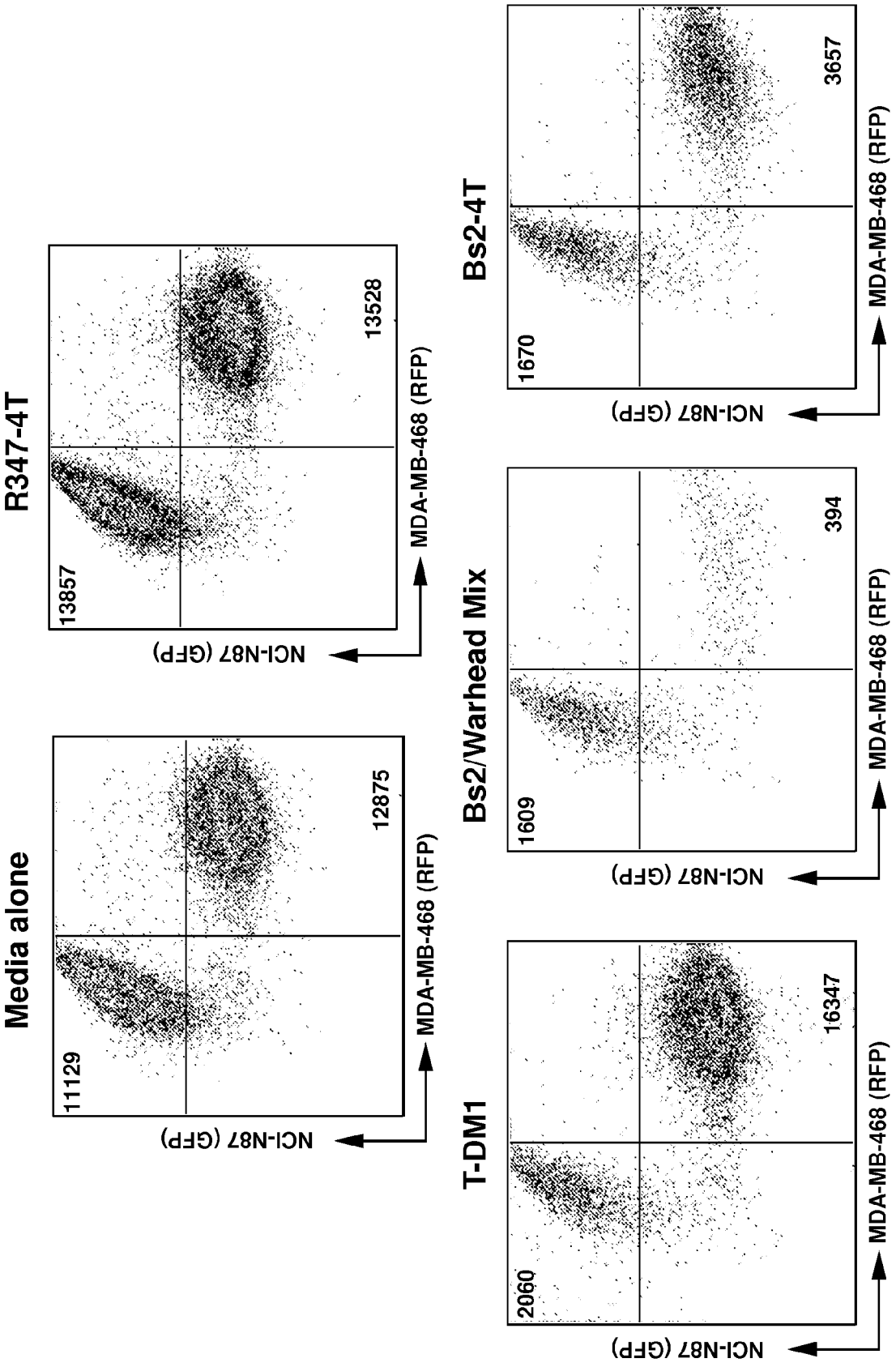
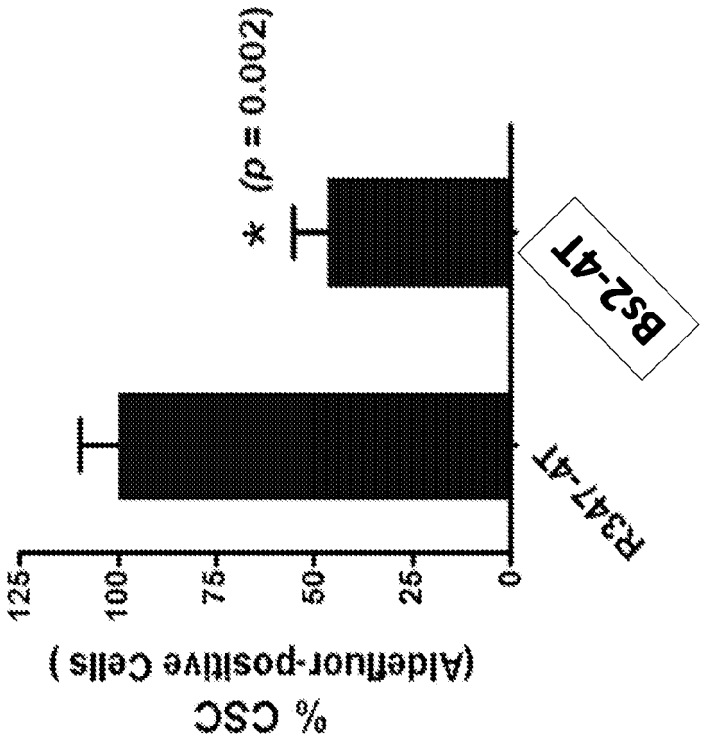

Media alone
11129
12875
NCI-N87 (GFP)
MDA-MB-468 (RFP)
R347-4T
13857
13528
NCI-N87 (GFP)
MDA-MB-468 (RFP)
T-DM1
2060
16347
NCI-N87 (GFP)
MDA-MB-468 (RFP)
Bs2/Warhead Mix
1609
394
NCI-N87 (GFP)
MDA-MB-468 (RFP)
Bs2-4T
1670
3657
NCI-N87 (GFP)
MDA-MB-468 (RFP)

FIG. 28B


* (p = 0.002)
B52-4T
R347-4T
125
100
75
50
25
0
% CSC
(Aldefluor-positive cells)

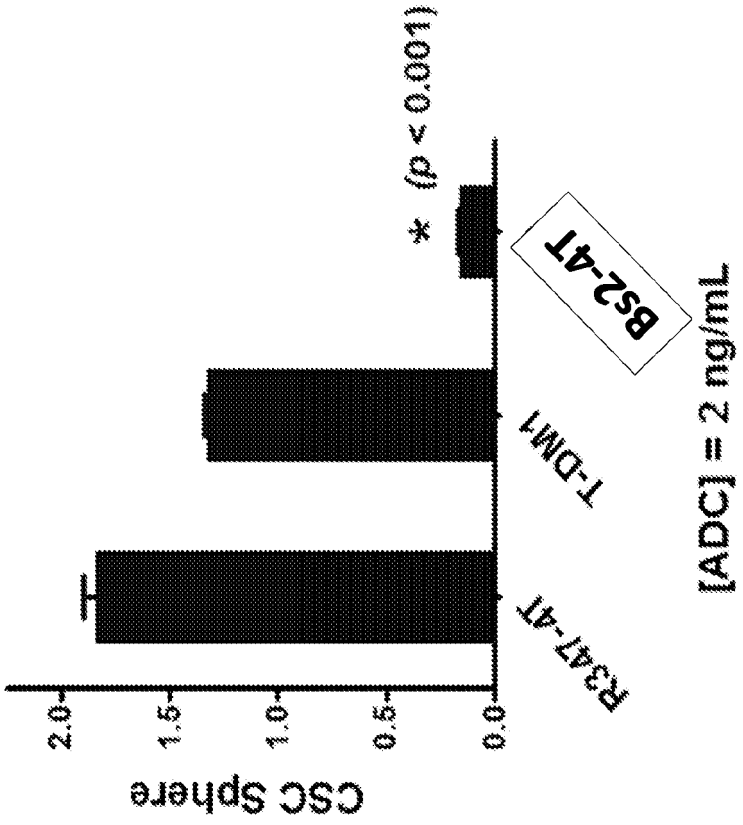

* (p < 0.001)
B52-4T
T-DM1
R347-4T
2.0
1.5
1.0
0.5
0.0
CSC Sphere
[ADC] = 2 ng/mL

FIG. 29

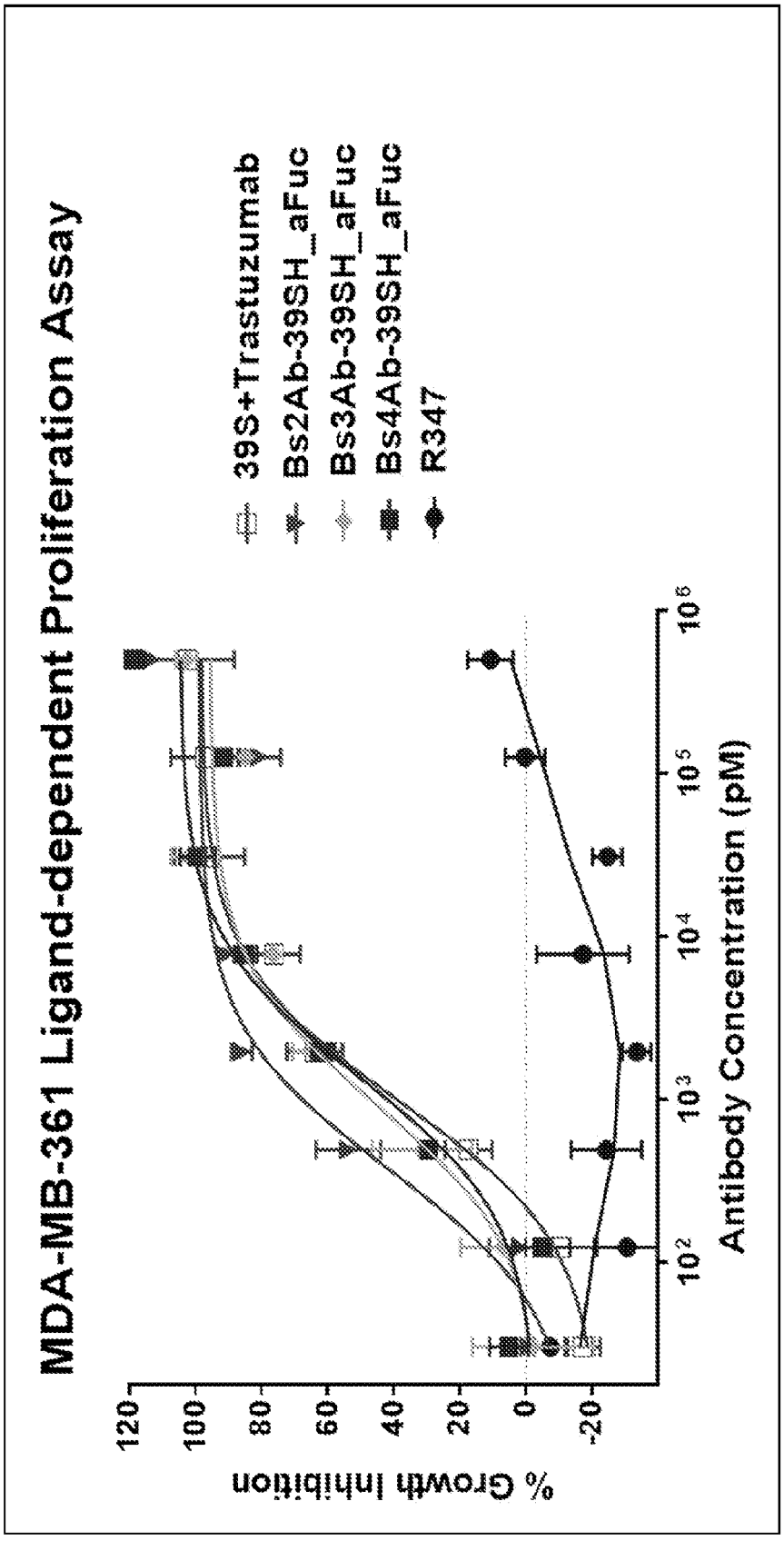

MDA-MB-361 Ligand-dependent Proliferation Assay
39S+Trastuzumab
Bs2Ab-39SH_aFuc
Bs3Ab-39SH_aFuc
Bs4Ab-39SH_aFuc
R347
% Growth Inhibition
120
100
80
60
40
20
0
-20
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Antibody Concentration (pM)

FIG. 30A

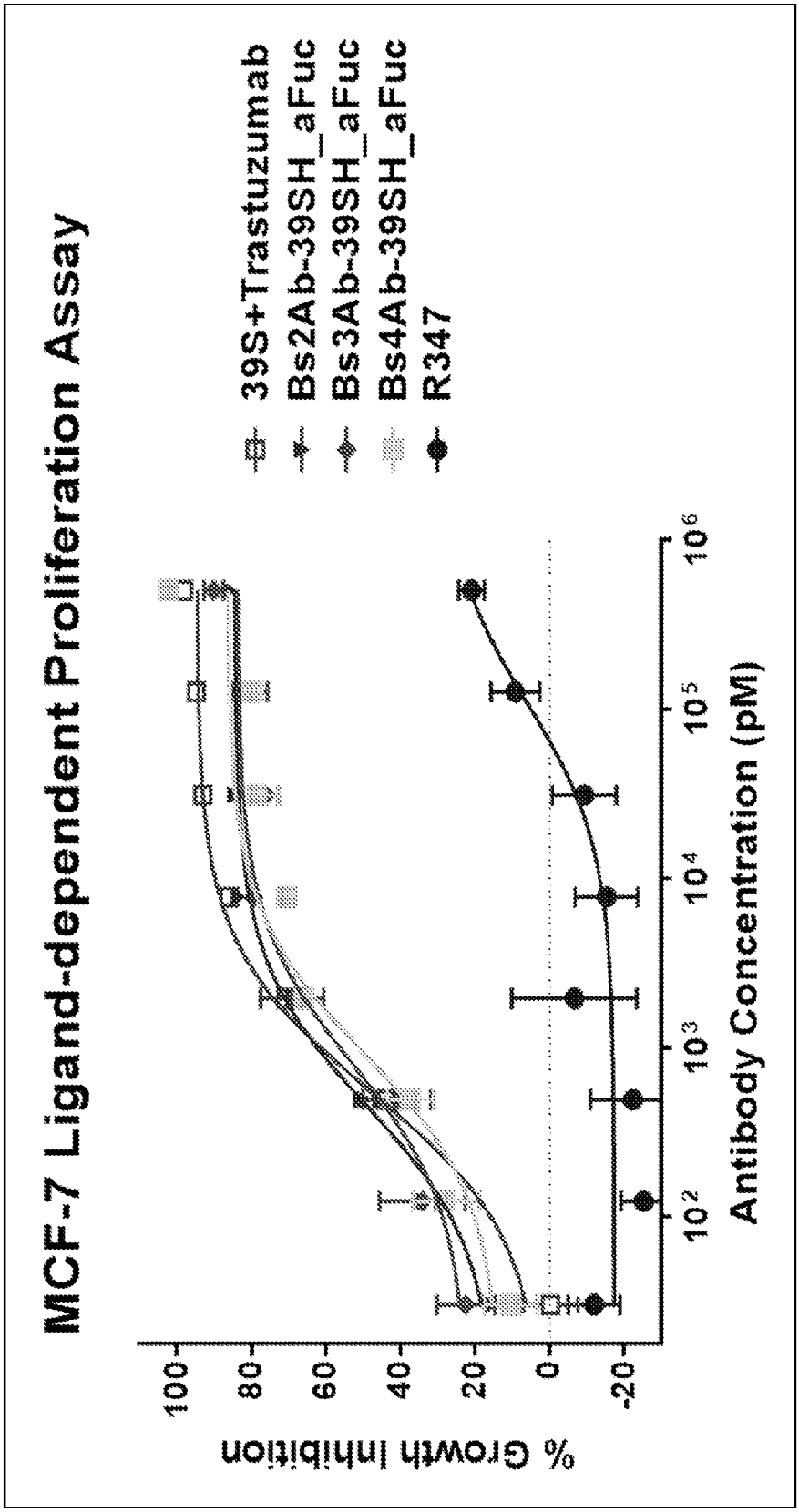

MCF-7 Ligand-dependent Proliferation Assay
39S+Trastuzumab
Bs2Ab-39SH_aFuc
Bs3Ab-39SH_aFuc
Bs4Ab-39SH_aFuc
R347
% Growth Inhibition
100
80
60
40
20
0
-20
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Antibody Concentration (pM)

FIG. 30B

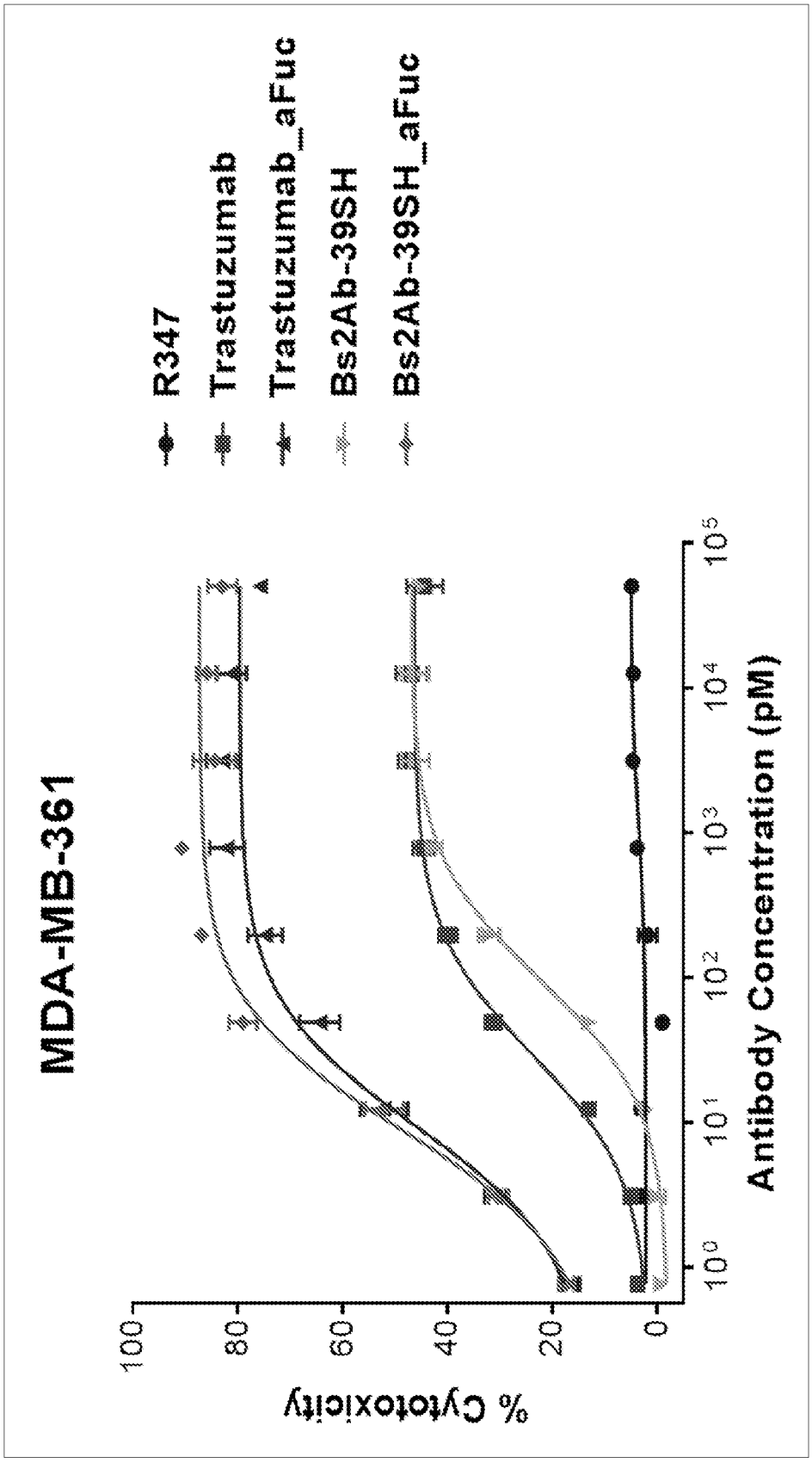

MDA-MB-361
R347
Trastuzumab
Trastuzumab_aFuc
Bs2Ab-39SH
Bs2Ab-39SH_aFuc
% Cytotoxicity
Antibody Concentration (pM)
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
10^5

FIG. 31A

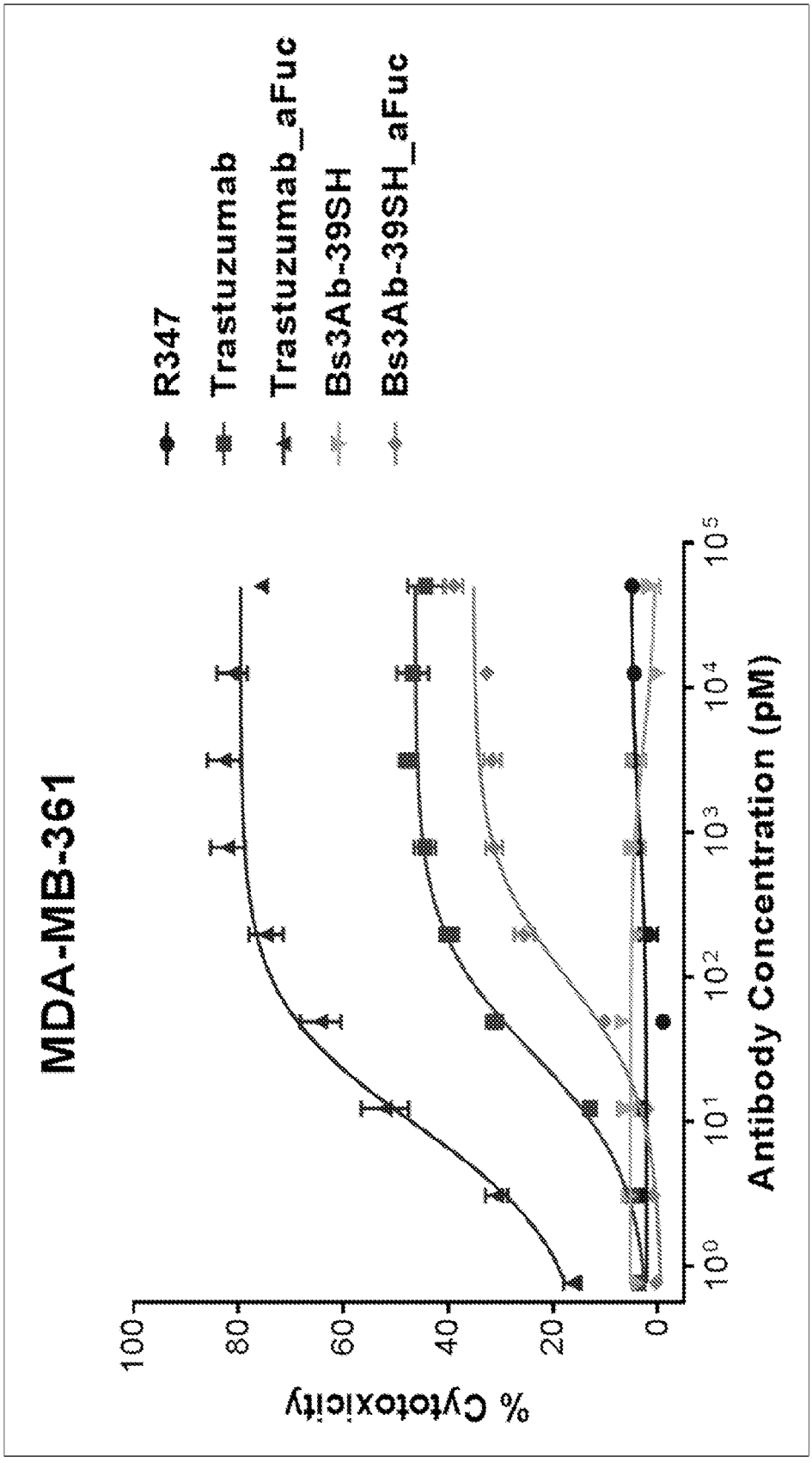

MDA-MB-361
R347
Trastuzumab
Trastuzumab_aFuc
Bs3Ab-39SH
Bs3Ab-39SH_aFuc
% Cytotoxicity
100
80
60
40
20
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
Antibody Concentration (pM)

FIG. 31B

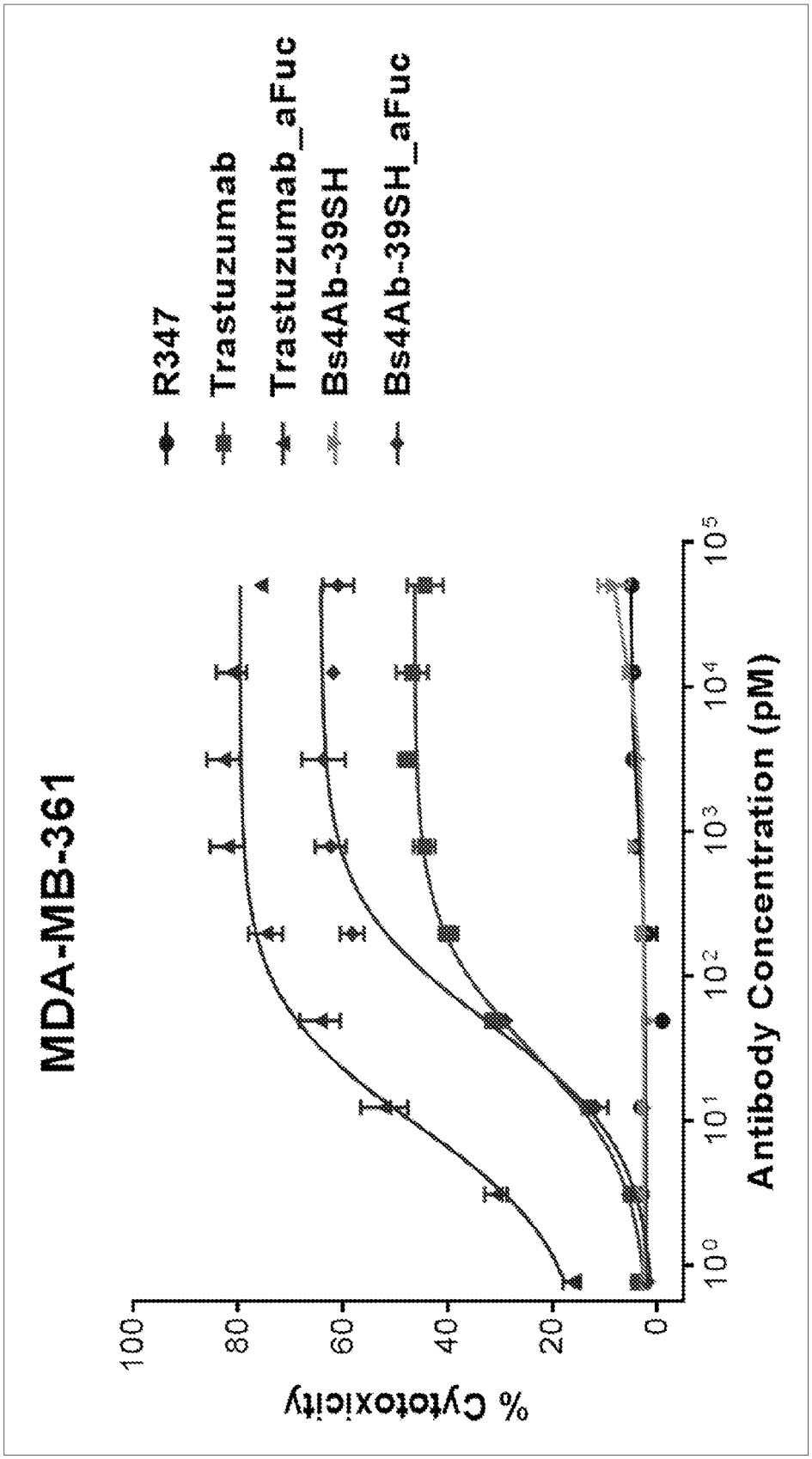

MDA-MB-361
R347
Trastuzumab
Trastuzumab_aFuc
Bs4Ab-39SH
Bs4Ab-39SH_aFuc
% Cytotoxicity
Antibody Concentration (pM)
100
80
60
40
20
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$

FIG. 31C

Tubulysin 1508 Payload (*compound* T-32)

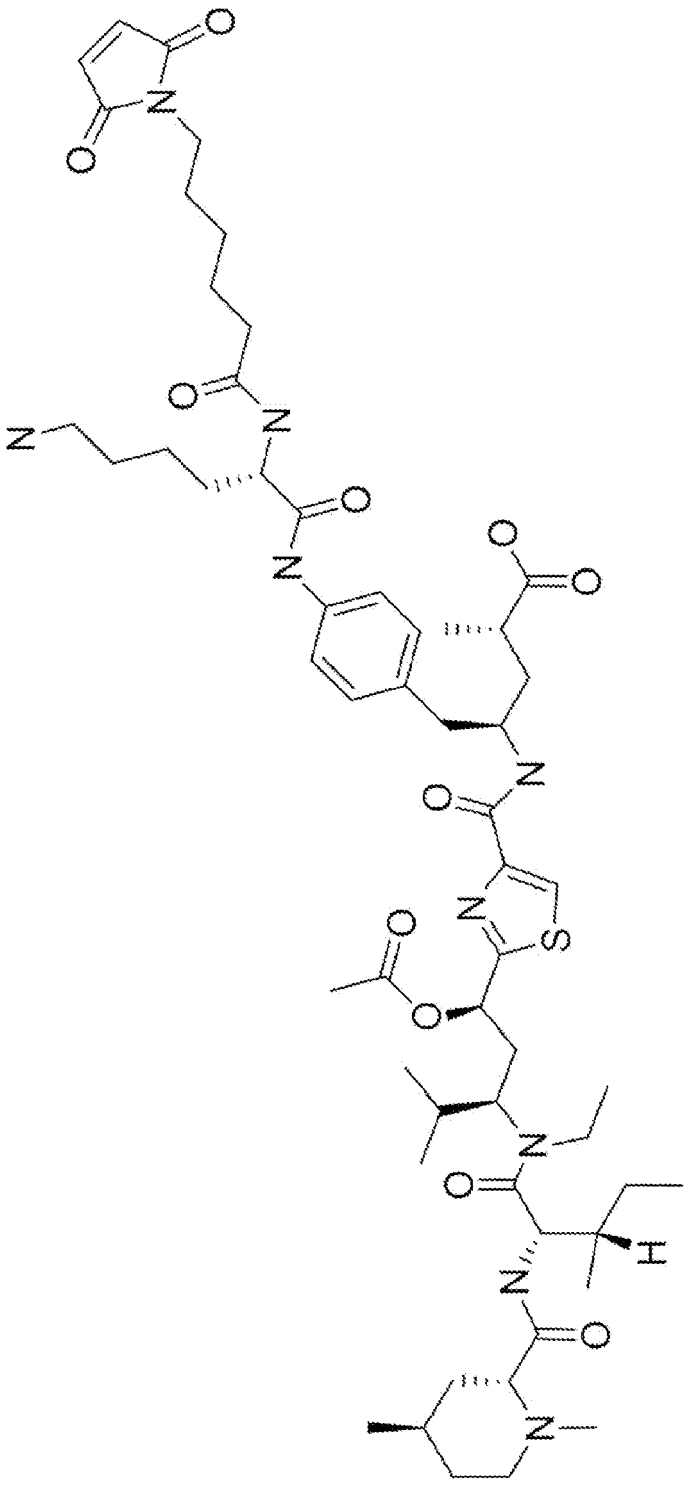


FIG. 32

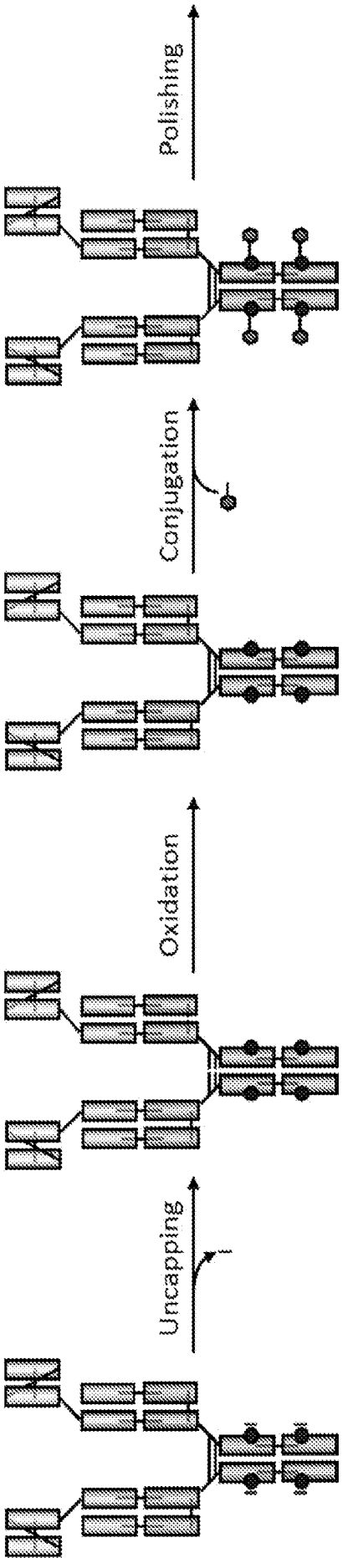



FIG. 33