FIG. 1

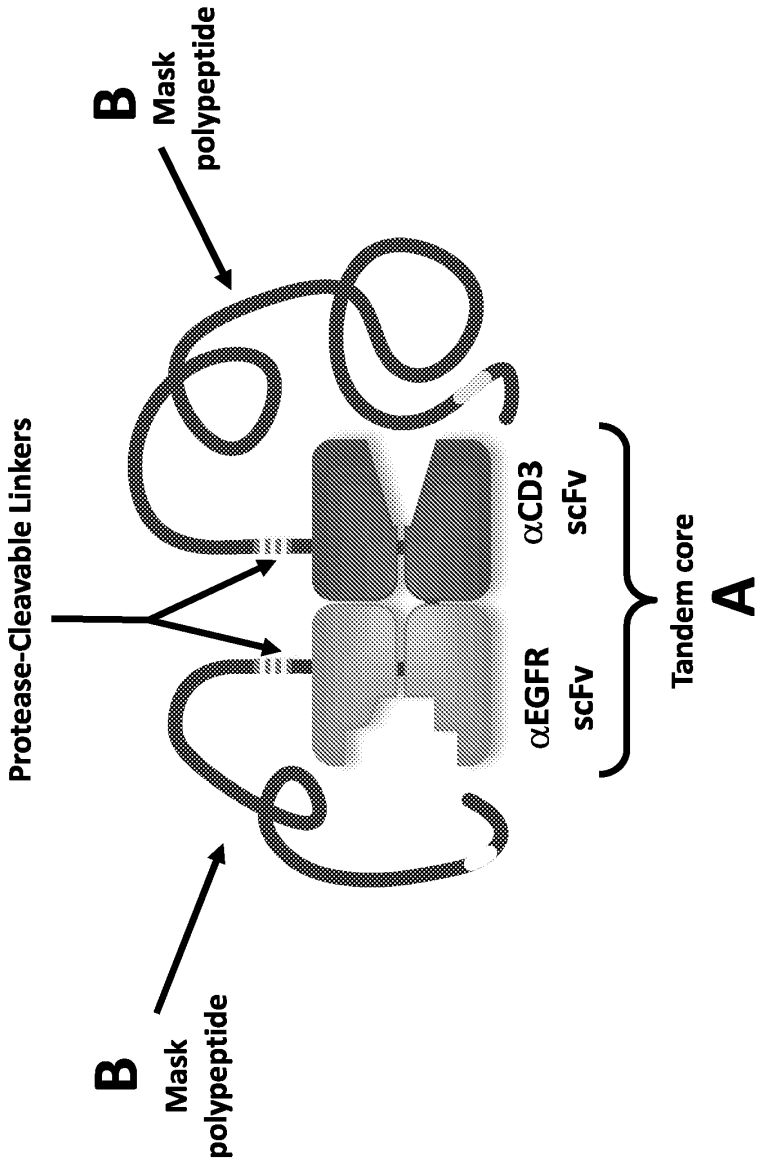

B
Mask polypeptide
C
Protease-Cleavable Linkers
B
Mask polypeptide
αCD3 scFv
αEGFR scFv
Tandem core
A

FIG. 2

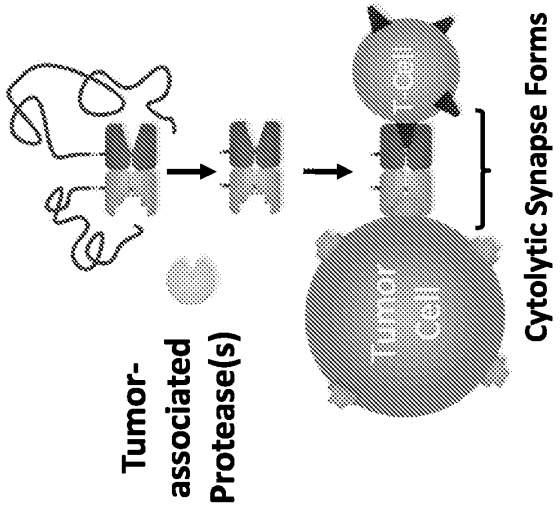

T cell
Tumor cell
Tumor-associated Protease(s)
Cytolytic Synapse Forms

FIG. 3

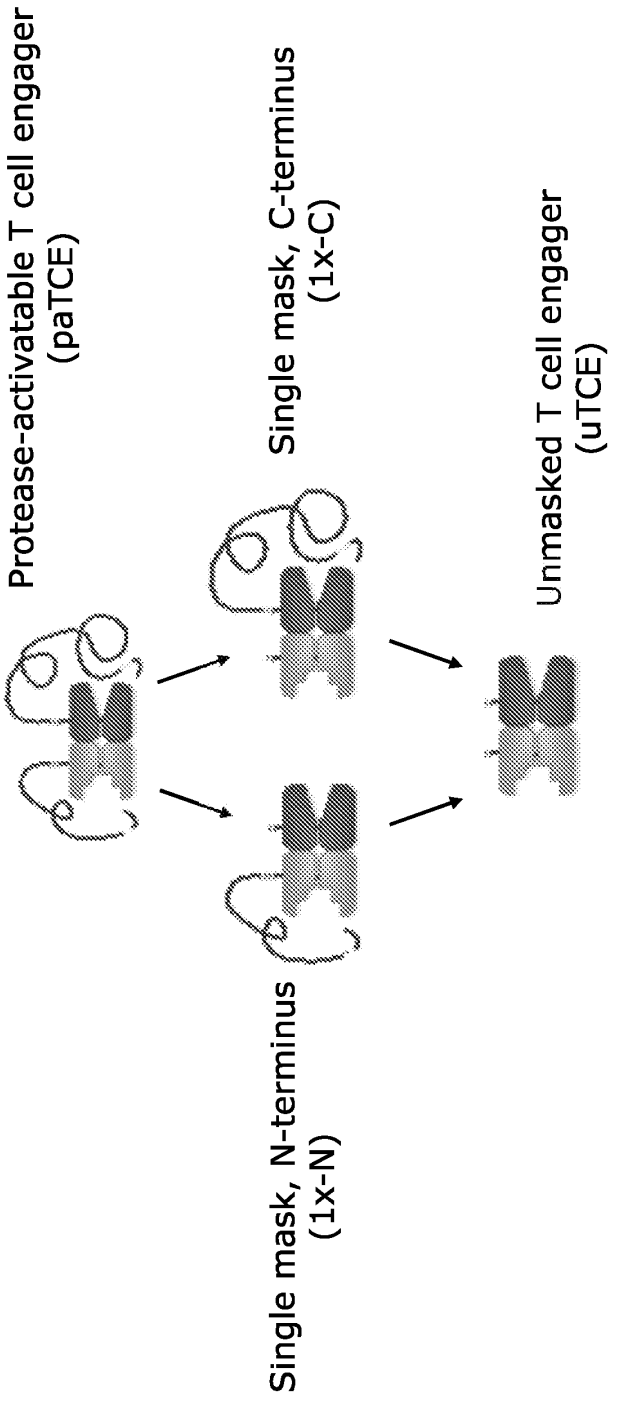

Protease-activatable T cell engager
(paTCE)
Single mask, C-terminus
(1x-C)
Single mask, N-terminus
(1x-N)
Unmasked T cell engager
(uTCE)

FIG. 4

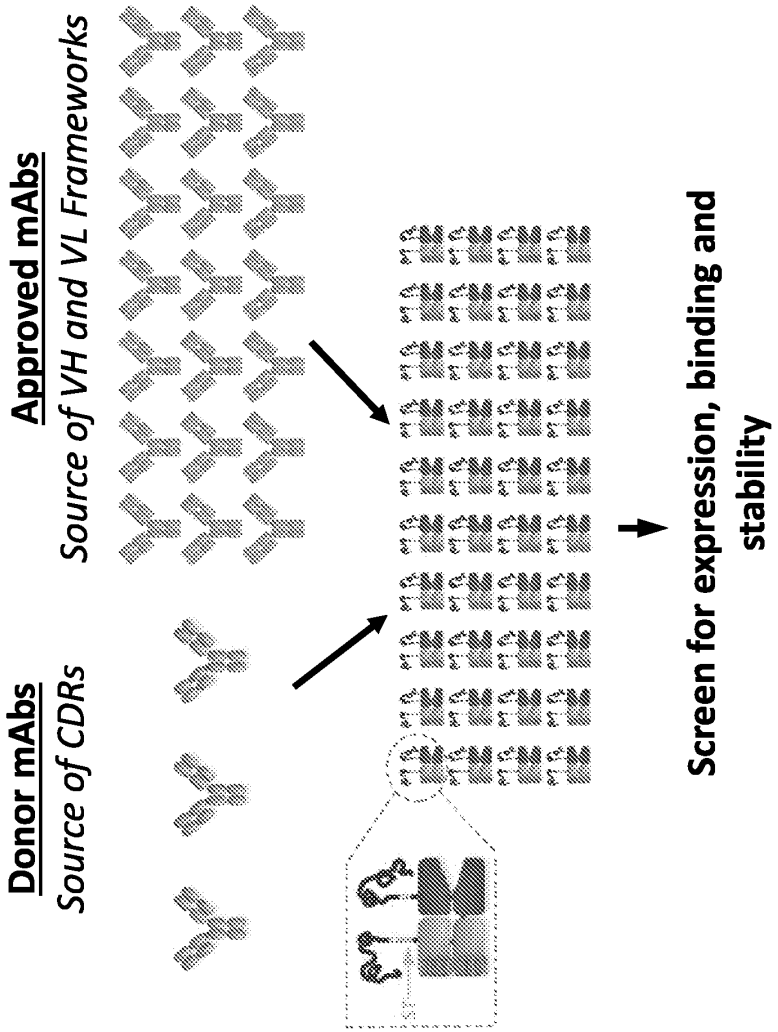

Approved mAbs
Source of VH and VL Frameworks
Donor mAbs
Source of CDRs
Screen for expression, binding and stability

FIG. 5A

Screen for Thermal Stability

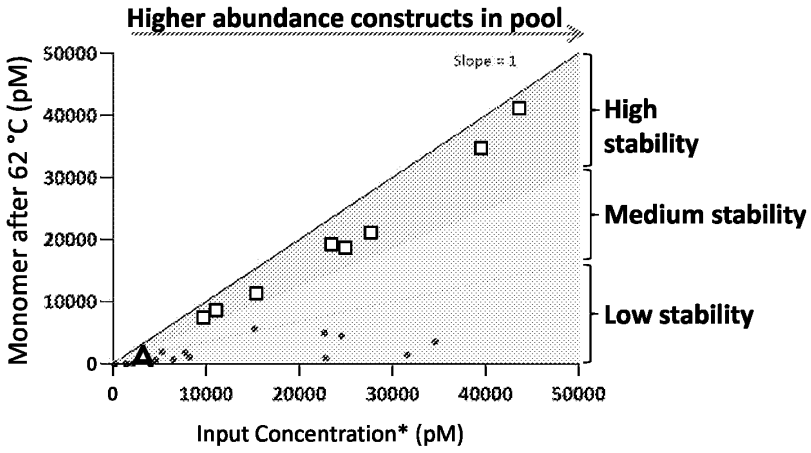
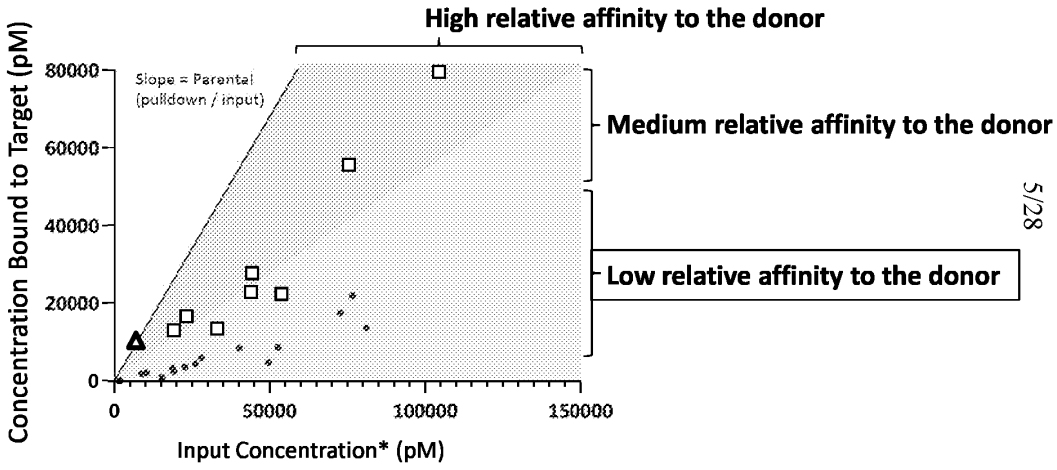


FIG. 5B

Screen for Binding Affinity



△ Donor □ Thermal stable constructs ● Constructs with low Tm

* Same relative abundance of each clone in the pool. Input concentration of pool is assay dependent.

FIG. 5C

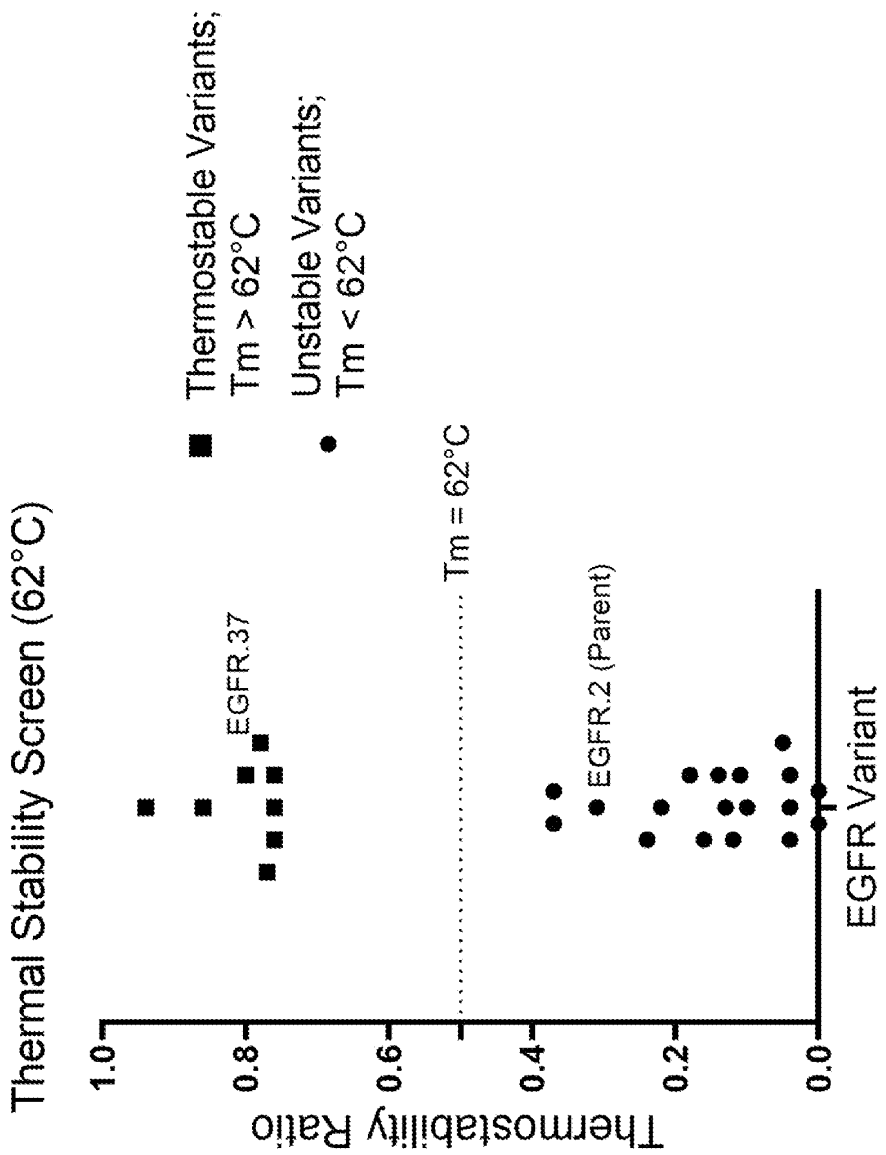

Thermal Stability Screen (62°C)
Thermostable Variants;
Tm > 62°C
Unstable Variants;
Tm < 62°C
Tm = 62°C
EGFR.37
EGFR.2 (Parent)
Thermostability Ratio
1.0
0.8
0.6
0.4
0.2
0.0
EGFR Variant

FIG. 5D

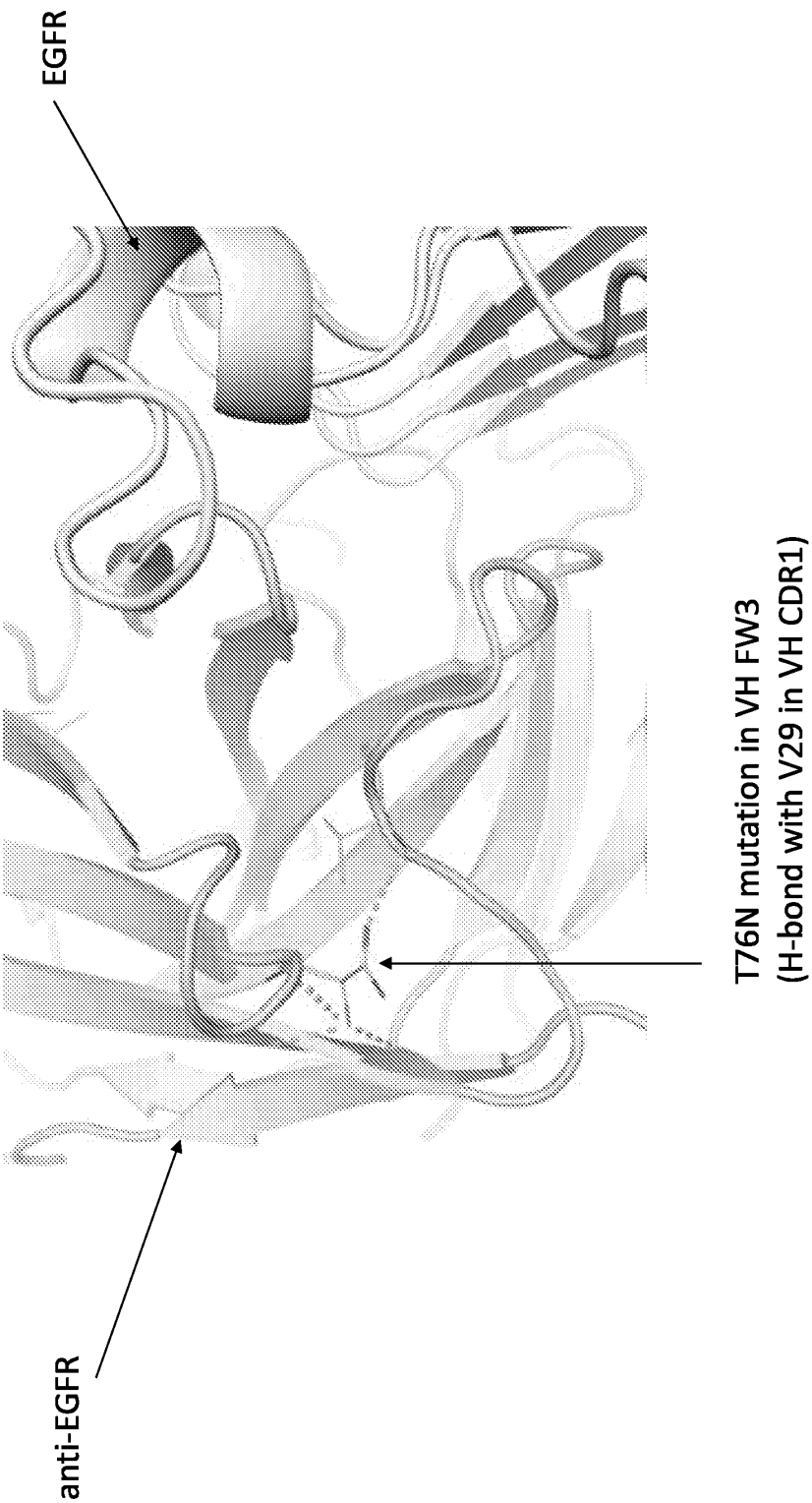

EGFR
T76N mutation in VH FW3
(H-bond with V29 in VH CDR1)
anti-EGFR

FIG. 6

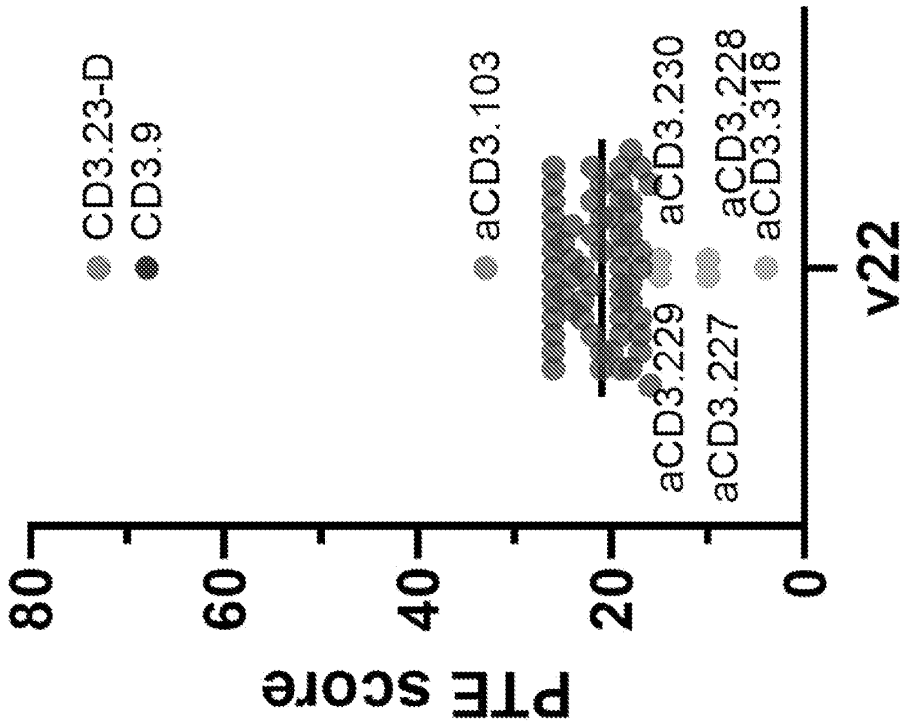

CD3.23-D
CD3.9
aCD3.103
aCD3.229
aCD3.227
aCD3.230
aCD3.228
aCD3.318
PTE score
80
60
40
20
0
v22

FIG. 7A

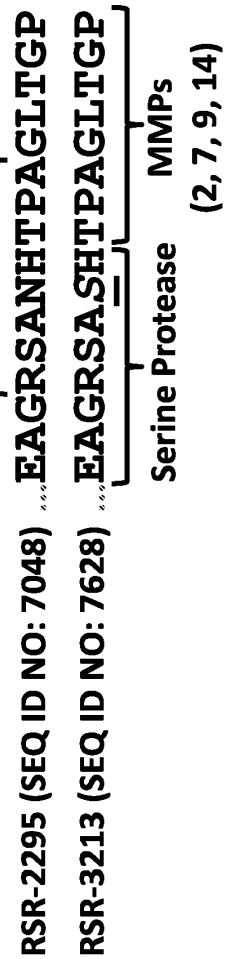

Legumain
RSR-2295 (SEQ ID NO: 7048) ...EAGRSANHTPAGLTGP
RSR-3213 (SEQ ID NO: 7628) ...EAGRSASHTPAGLTGP
Serine Protease
MMPs
(2, 7, 9, 14)

FIG. 7B

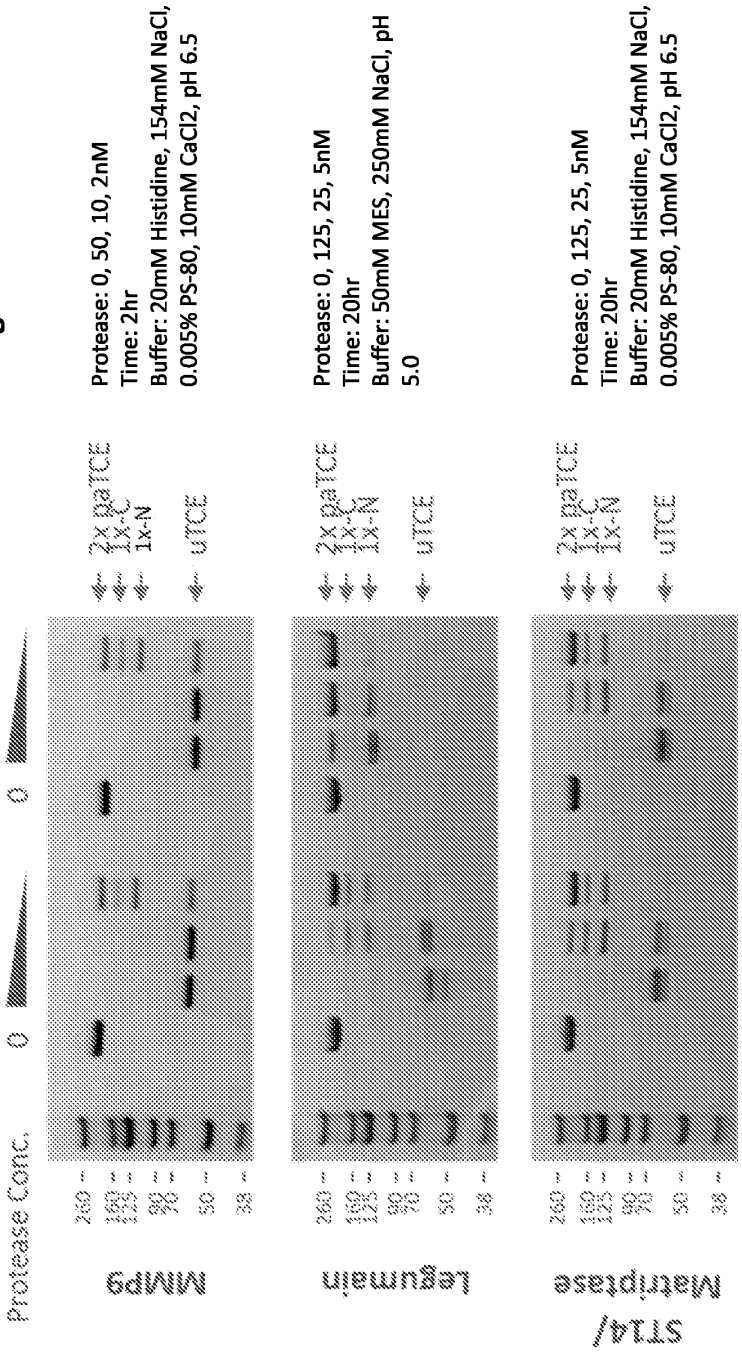

Digest conditions
Protease: 0, 50, 10, 2nM
Time: 2hr
Buffer: 20mM Histidine, 154mM NaCl, 0.005% PS-80, 10mM CaCl2, pH 6.5
Protease: 0, 125, 25, 5nM
Time: 20hr
Buffer: 50mM MES, 250mM NaCl, pH 5.0
RSR-2295
RSR-3213
Protease Conc.
0
0
2x paTCE
1x-C
1x-N
uTCE
MMP9
Legumain
ST14/ Matriptase
260
160
125
90
70
50
38

FIG. 8A

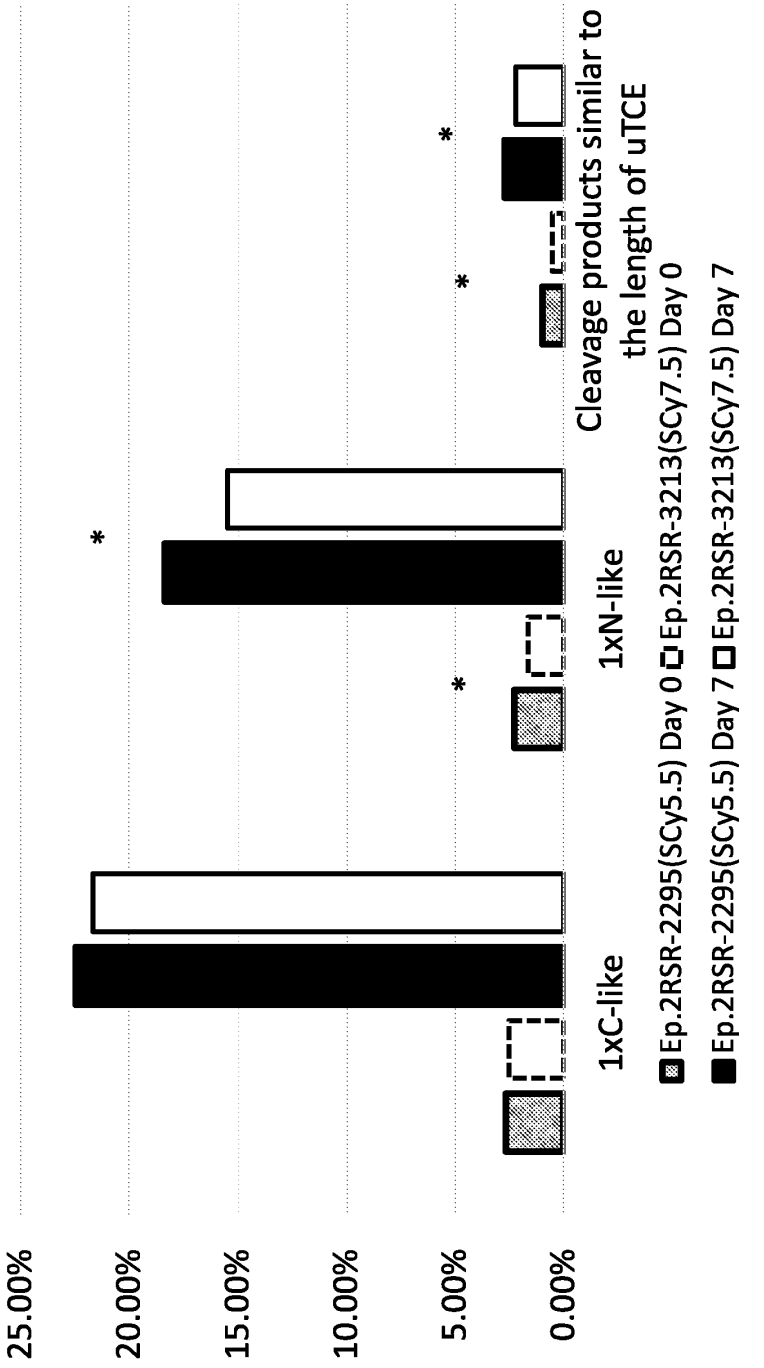

Comparing Plasma Stability of Release Sites
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
*
*
*
*
1xC-like
1xN-like
Cleavage products similar to the length of uTCE
Ep.2RSR-2295(SCy5.5) Day 0
Ep.2RSR-3213(SCy7.5) Day 0
Ep.2RSR-2295(SCy5.5) Day 7
Ep.2RSR-3213(SCy7.5) Day 7
*p < 0.05

FIG. 8B

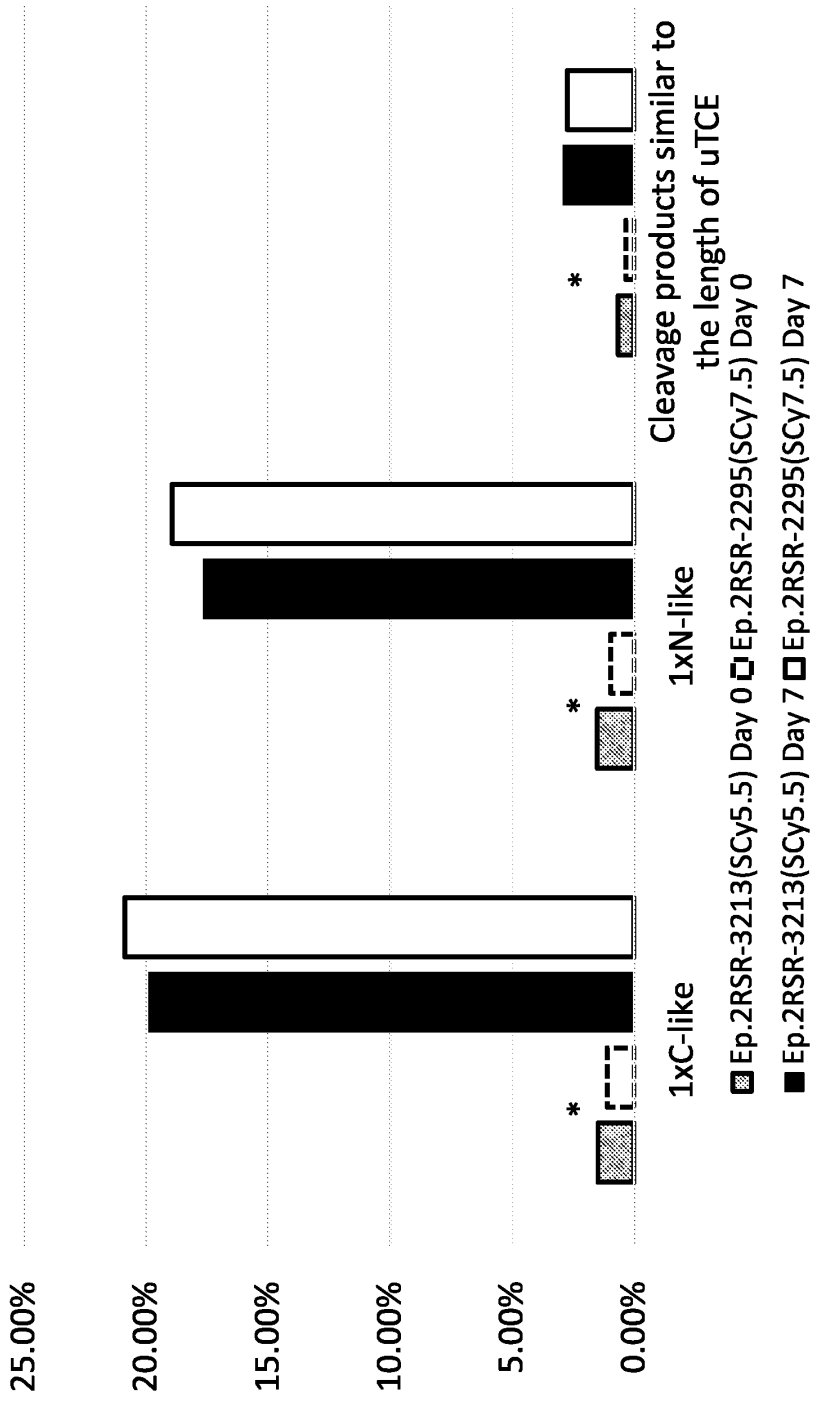

Comparing Plasmas Stability of Release Sites
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
*
*
*
1xC-like
1xN-like
Cleavage products similar to the length of uTCE
Ep.2RSR-3213(SCy5.5) Day 0
Ep.2RSR-2295(SCy7.5) Day 0
Ep.2RSR-3213(SCy5.5) Day 7
Ep.2RSR-2295(SCy7.5) Day 7
*p < 0.05

FIG. 8C

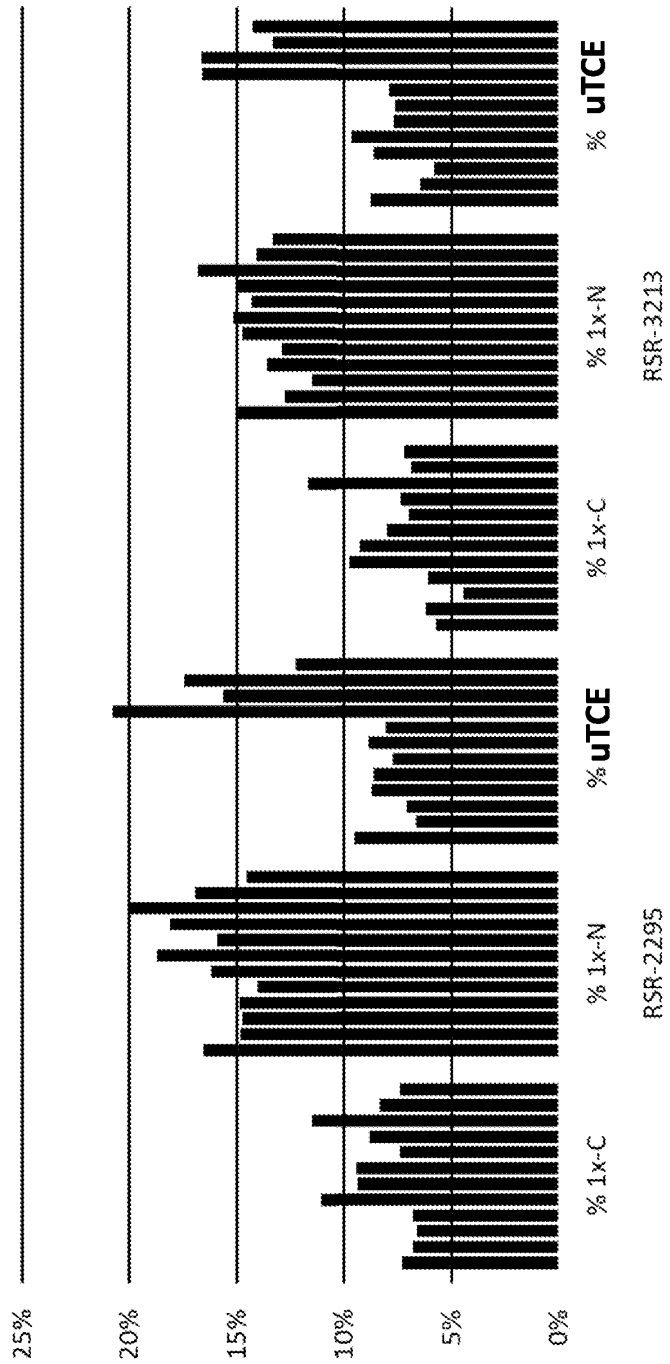


FIG. 8D

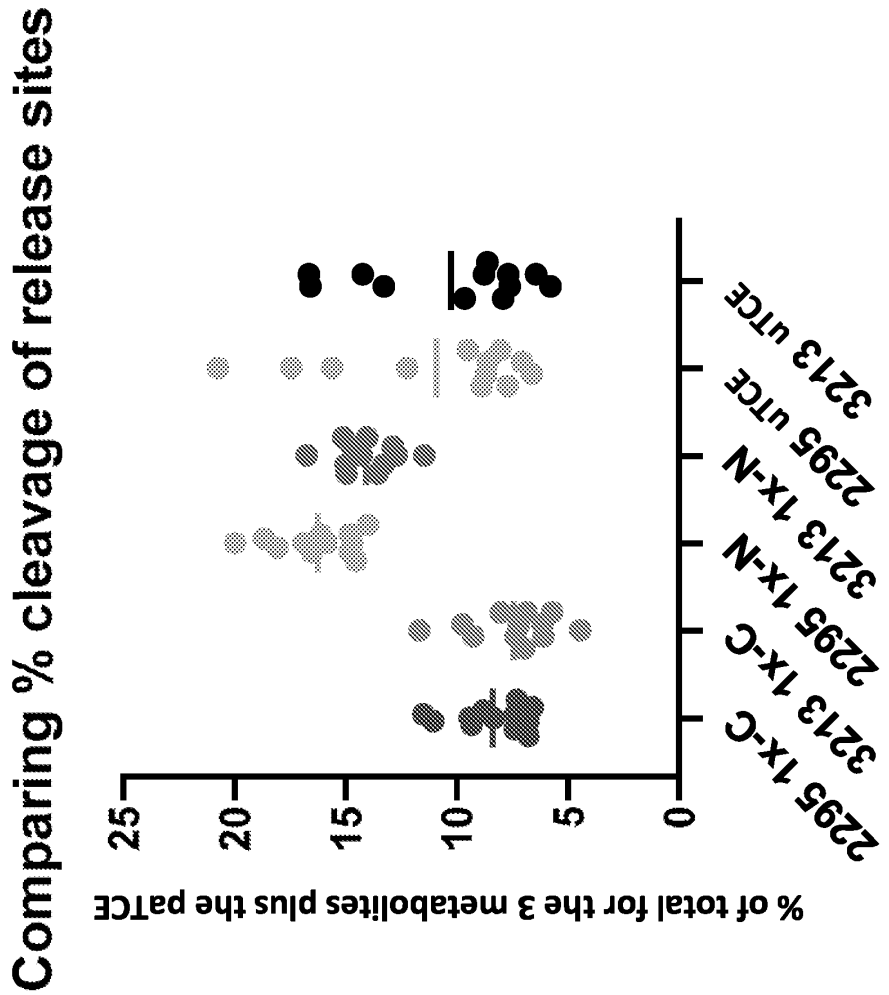

Comparing % cleavage of release sites
% of total for the 3 metabolites plus the paTCE
25
20
15
10
5
0
2295 1x-C
3213 1x-C
2295 1x-N
3213 1x-N
2295 uTCE
3213 uTCE

FIG. 9

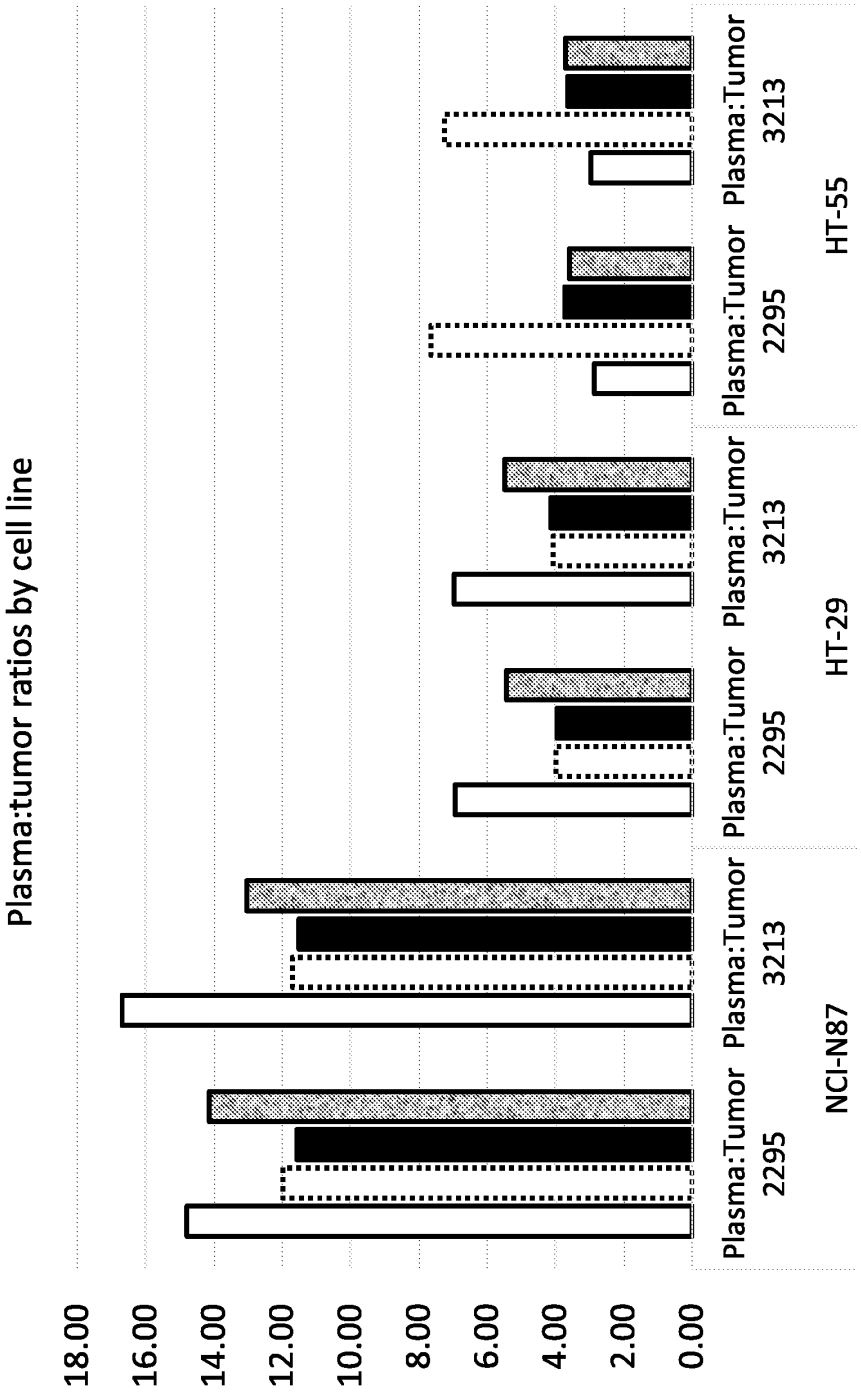

Plasma:tumor ratios by cell line
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Plasma:Tumor 2295
Plasma:Tumor 3213
NCI-N87
Plasma:Tumor 2295
Plasma:Tumor 3213
HT-29
Plasma:Tumor 2295
Plasma:Tumor 3213
HT-55
Mouse 1
Mouse 2
Mouse 3
Mouse 4

FIG. 10A

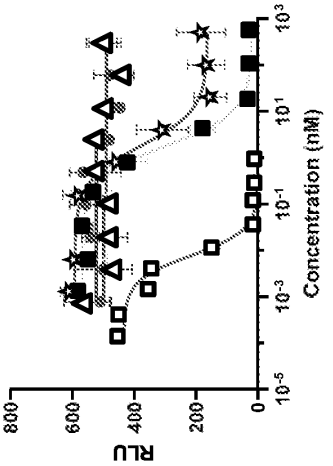


FIG. 10B

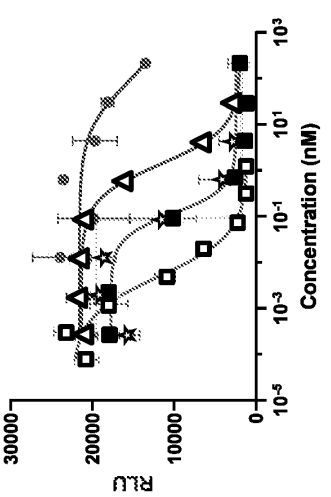


FIG. 10C

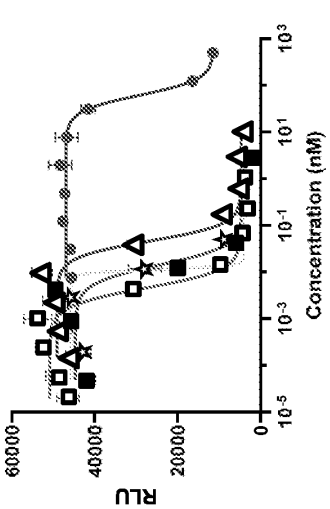




FIG. 11A

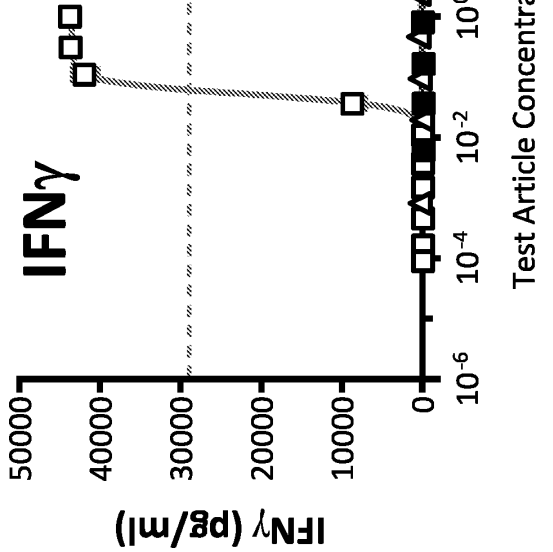
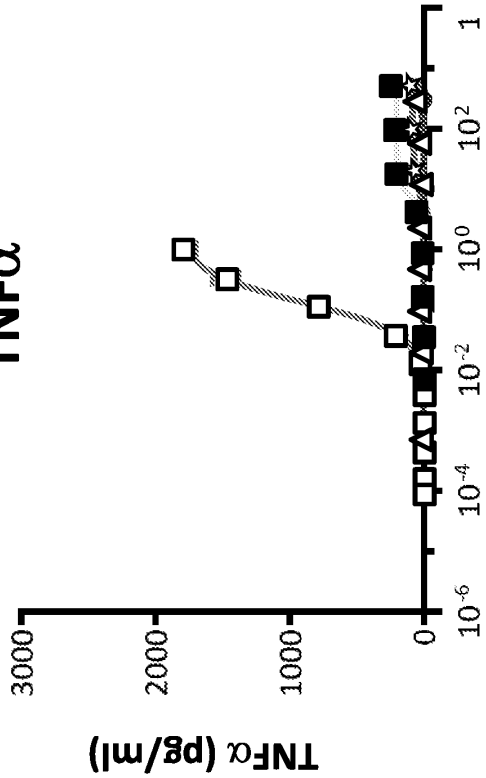


FIG. 11B



HT-29 Cells (22K EGFR/cell)

- AMX-525(uTCE)
- AMX-525
- AMX-525(NoCivSite)
- AMX-525(1x-N)
- AMX-525(1x-C)

FIG. 11C

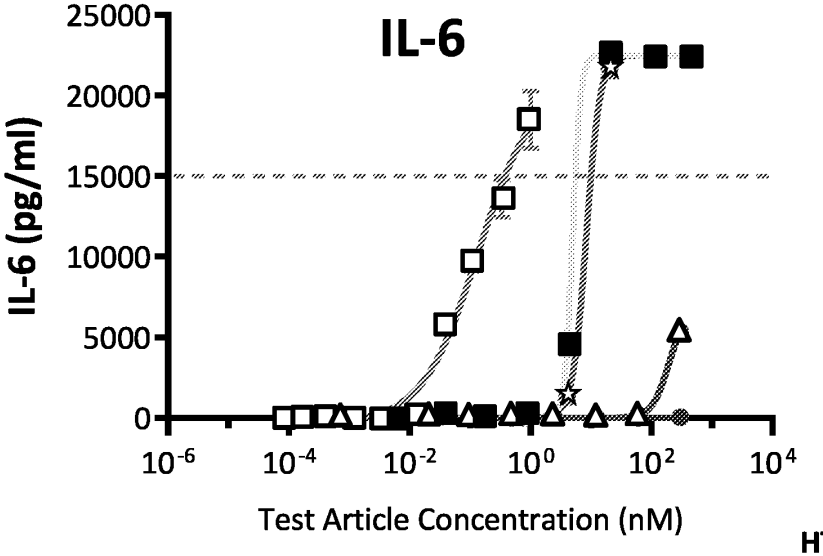
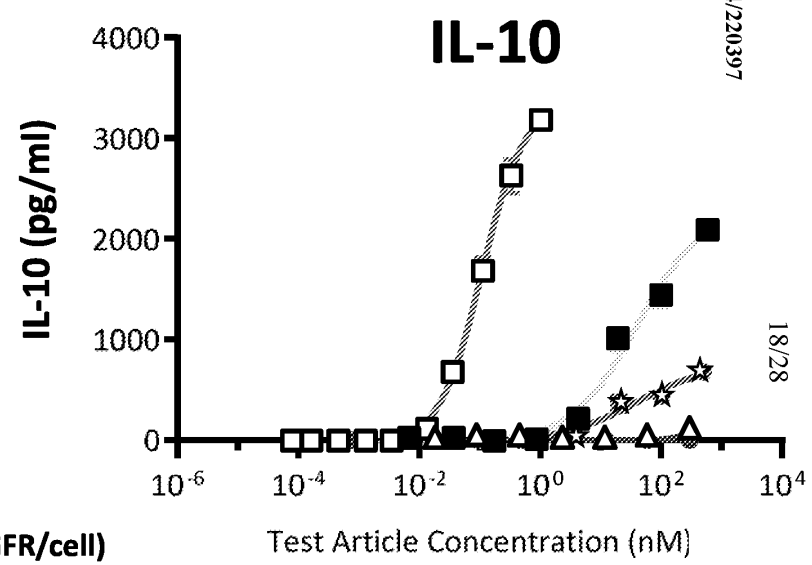


FIG. 11D



HT-29 Cells (22K EGFR/cell)

AMX-525(uTCE)

AMX-525

AMX-525(NoClvSite)

AMX-525(1x-N)

AMX-525(1x-C)

FIG. 12

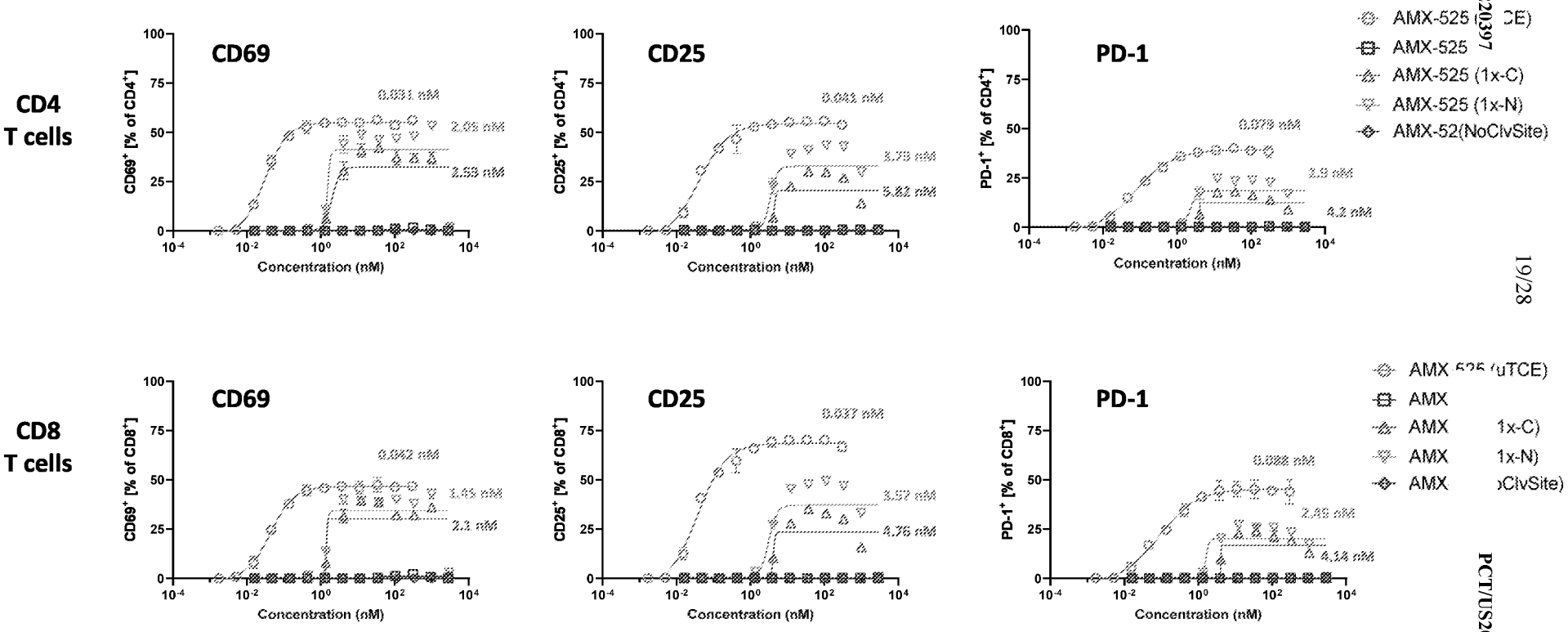

CD4
T cells
CD8
T cells
CD69
CD25
PD-1
CD69+ [% of CD4+]
CD25+ [% of CD4+]
PD-1+ [% of CD4+]
CD69+ [% of CD8+]
CD25+ [% of CD8+]
PD-1+ [% of CD8+]
Concentration (nM)
0.031 nM
2.05 nM
2.53 nM
0.041 nM
3.79 nM
5.82 nM
0.079 nM
2.9 nM
4.2 nM
0.042 nM
1.45 nM
2.1 nM
0.037 nM
3.57 nM
4.76 nM
0.088 nM
2.45 nM
4.14 nM
AMX-525
AMX-525 (1x-C)
AMX-525 (1x-N)
AMX-52(NoClvSite)

FIG. 13

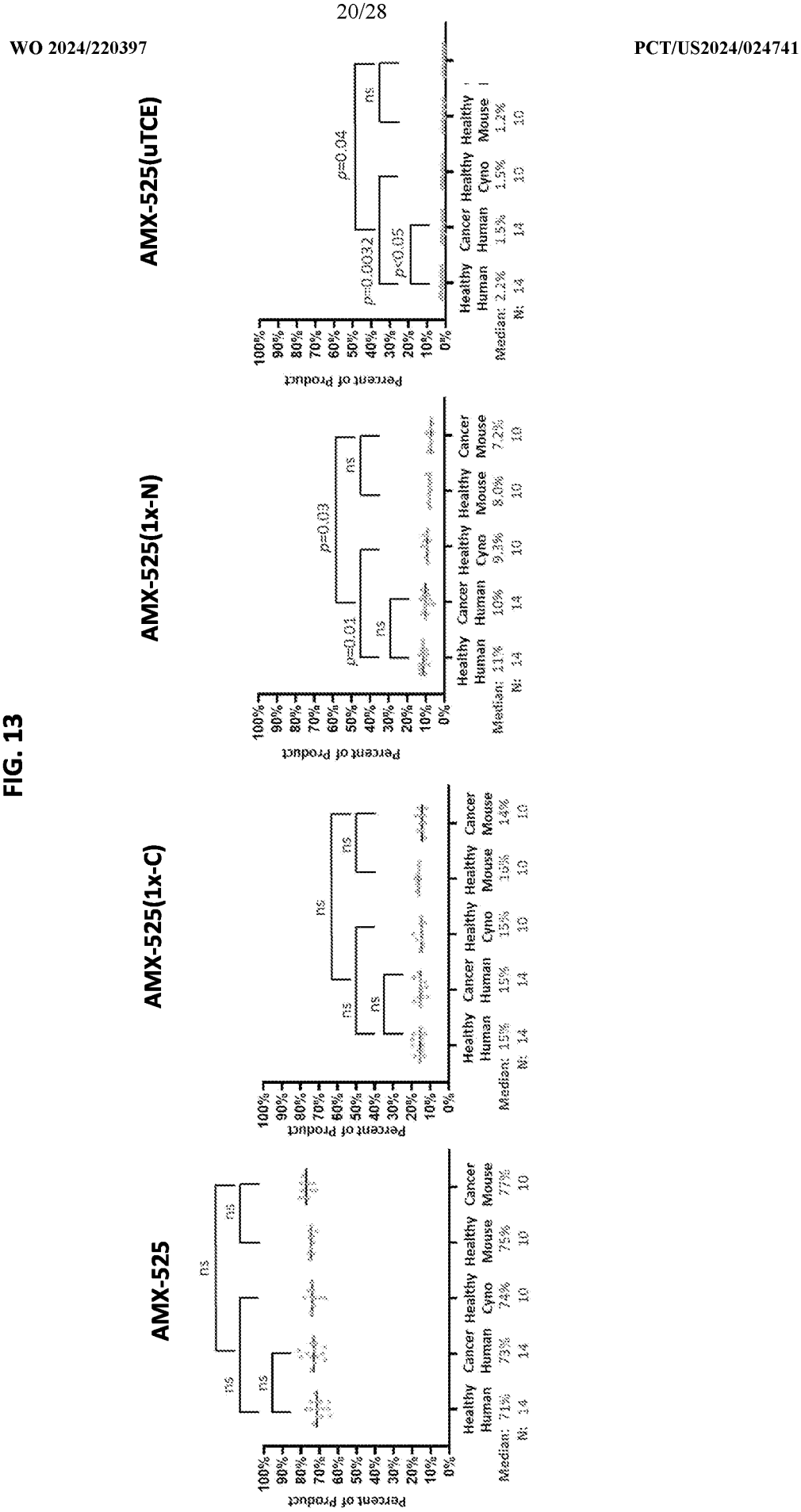

AMX-525
AMX-525(1x-C)
AMX-525(1x-N)
AMX-525(uTCE)
Percent of Product
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
ns
p=0.01
p=0.03
p=0.04
p=0.0032
p<0.05
Healthy Human
Cancer Human
Healthy Cyno
Healthy Mouse
Cancer Mouse
Median: 71% 73% 74% 75% 77%
N: 14 14 10 10 10
Median: 15% 15% 15% 15% 14%
N: 14 14 10 10 10
Median: 11% 10% 9.3% 8.0% 7.2%
N: 14 14 10 10 10
Median: 2.2% 1.5% 1.5% 1.2%
N: 14 14 10 10

FIG. 14

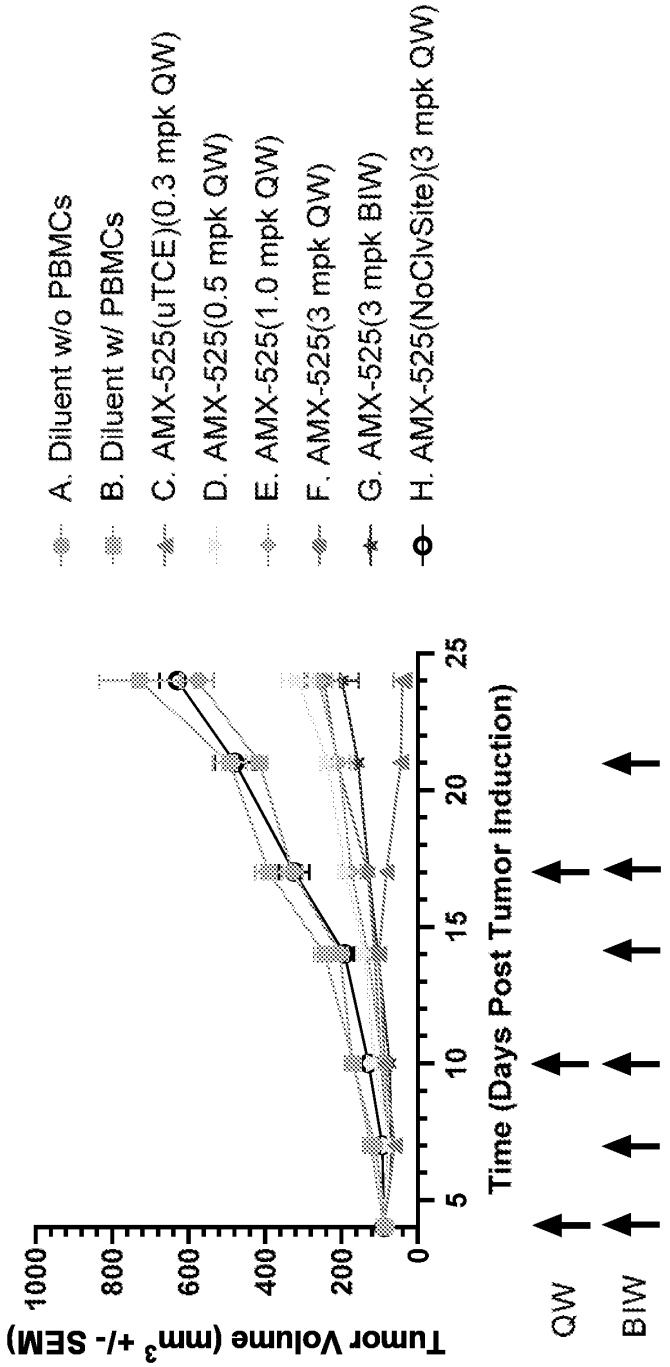

HT-29 (CRC; 22K EGFR/cell)
A. Diluent w/o PBMCs
B. Diluent w/ PBMCs
C. AMX-525(uTCE)(0.3 mpk QW)
D. AMX-525(0.5 mpk QW)
E. AMX-525(1.0 mpk QW)
F. AMX-525(3 mpk QW)
G. AMX-525(3 mpk BIW)
H. AMX-525(NoClvSite)(3 mpk QW)
Tumor Volume (mm3 +/- SEM)
1000
800
600
400
200
0
5
10
15
20
25
Time (Days Post Tumor Induction)
QW
BIW

FIG. 15

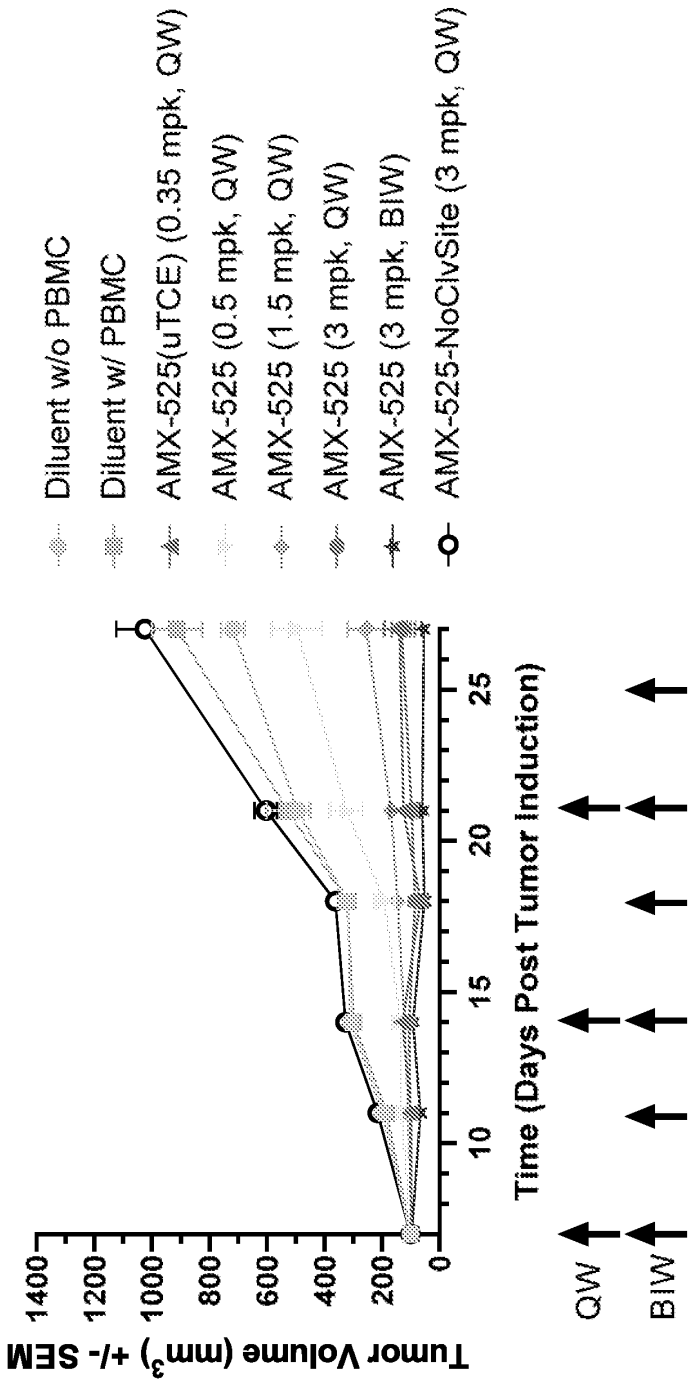

LoVo (CRC; 28K EGFR/cell)
Tumor Volume (mm3) +/- SEM
1400
1200
1000
800
600
400
200
0
10
15
20
25
Time (Days Post Tumor Induction)
QW
BIW
Diluent w/o PBMC
Diluent w/ PBMC
AMX-525(uTCE) (0.35 mpk, QW)
AMX-525 (0.5 mpk, QW)
AMX-525 (1.5 mpk, QW)
AMX-525 (3 mpk, QW)
AMX-525 (3 mpk, BIW)
AMX-525-NoClvSite (3 mpk, QW)

FIG. 16

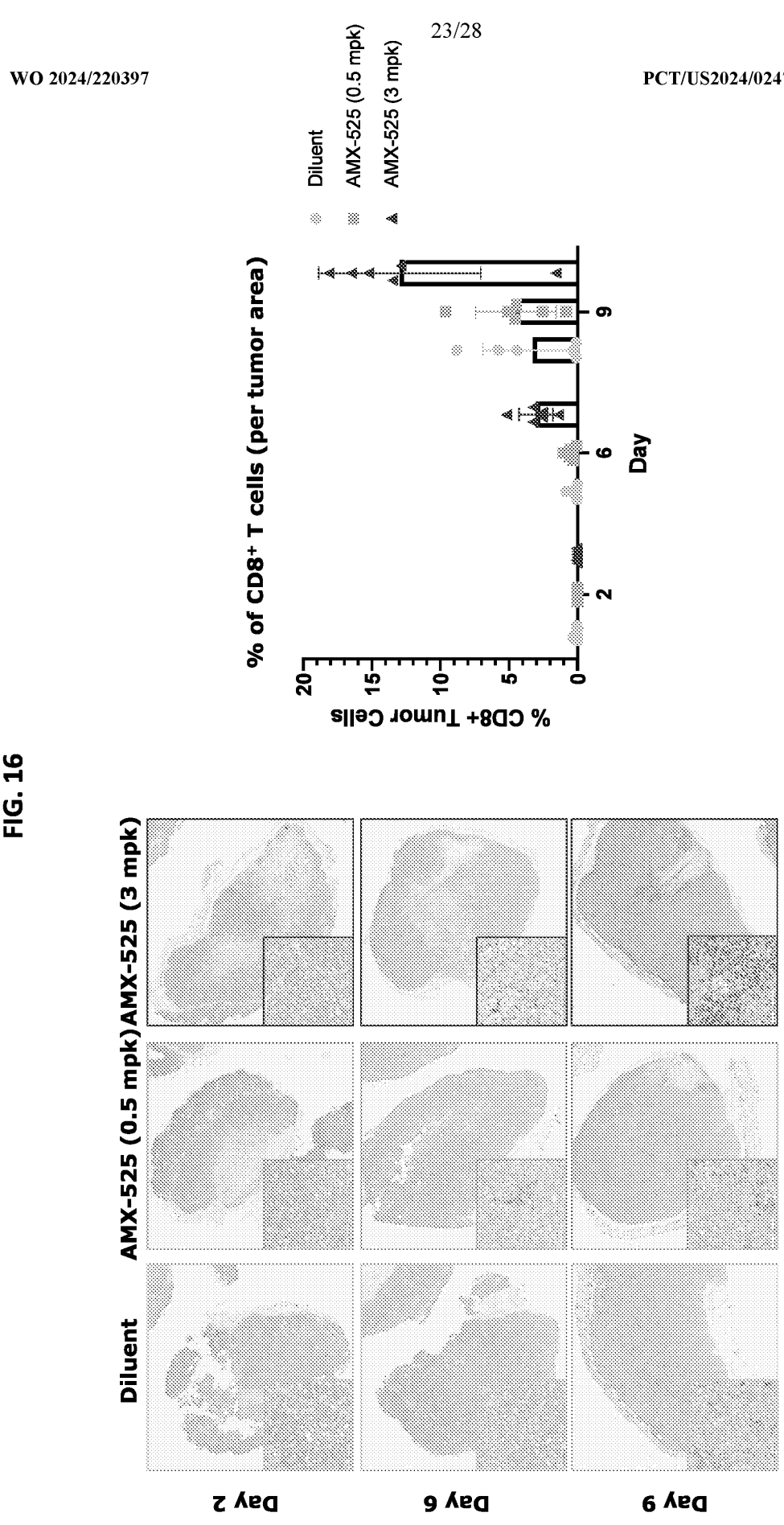

Diluent
AMX-525 (0.5 mpk)
AMX-525 (3 mpk)
Day 2
Day 6
Day 9
% of CD8+ T cells (per tumor area)
Diluent
AMX-525 (0.5 mpk)
AMX-525 (3 mpk)
% CD8+ Tumor Cells
0
5
10
15
20
2
6
9
Day

FIG. 17

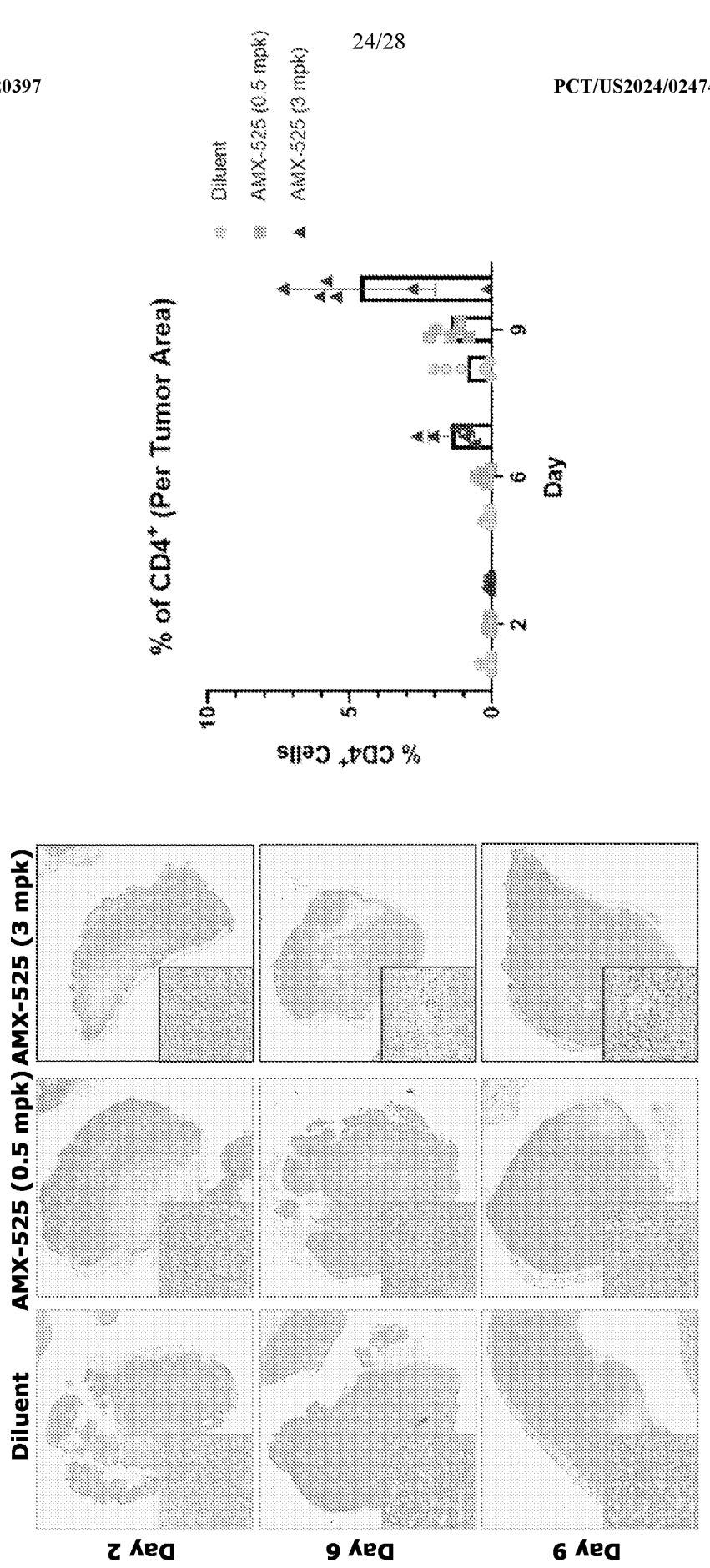

Diluent
AMX-525 (0.5 mpk)
AMX-525 (3 mpk)
Day 2
Day 6
Day 9
% CD4+ Cells
% of CD4+ (Per Tumor Area)
Day
Diluent
AMX-525 (0.5 mpk)
AMX-525 (3 mpk)

FIG. 18

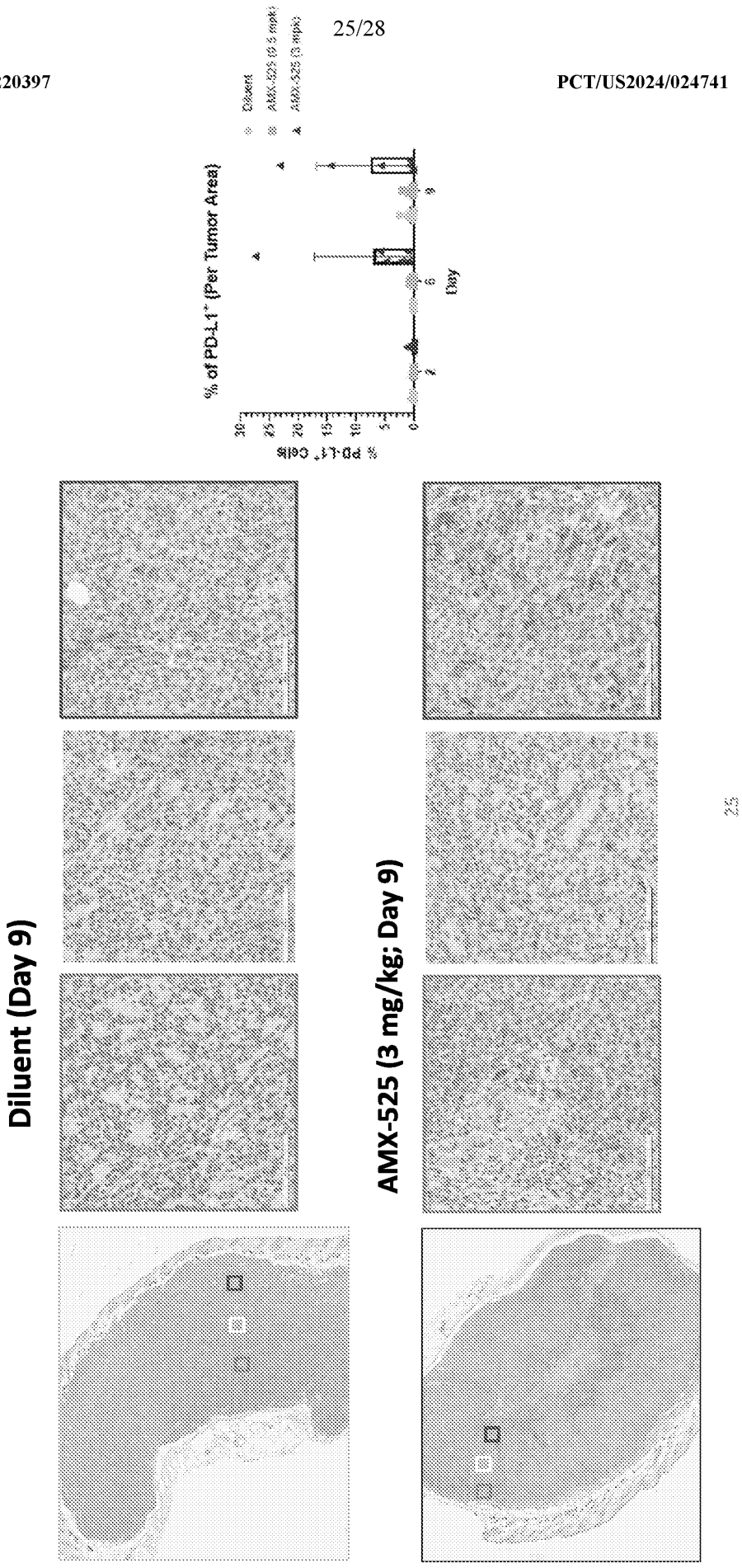

Diluent (Day 9)
AMX-525 (3 mg/kg; Day 9)
% of PD-L1+ (Per Tumor Area)
Day
Diluent
AMX-525 (3 mg/kg)
% PD-L1+ cells

FIG. 19

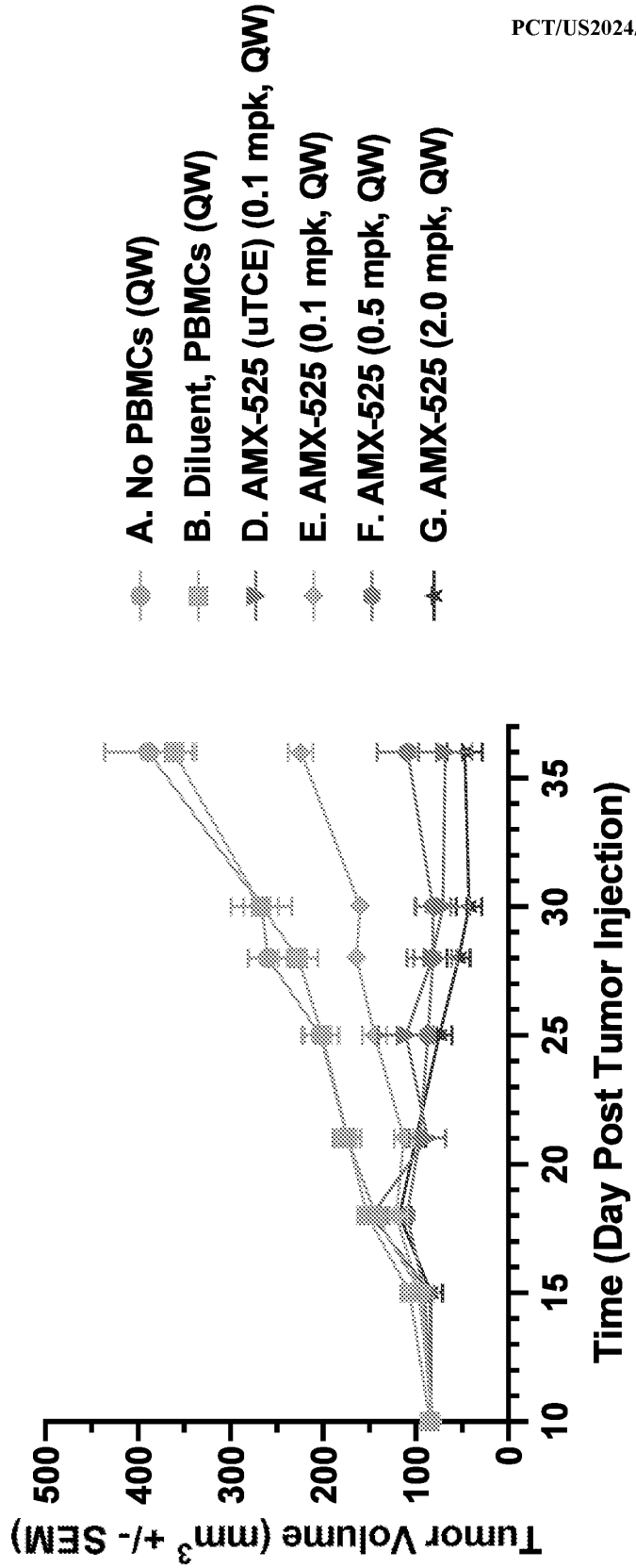

MDA-MB-231 (Breast; 75K EGFR/cell)
A. No PBMCs (QW)
B. Diluent, PBMCs (QW)
D. AMX-525 (uTCE) (0.1 mpk, QW)
E. AMX-525 (0.1 mpk, QW)
F. AMX-525 (0.5 mpk, QW)
G. AMX-525 (2.0 mpk, QW)
Tumor Volume (mm3 +/- SEM)
500
400
300
200
100
0
10
15
20
25
30
35
Time (Day Post Tumor Injection)

FIG. 20

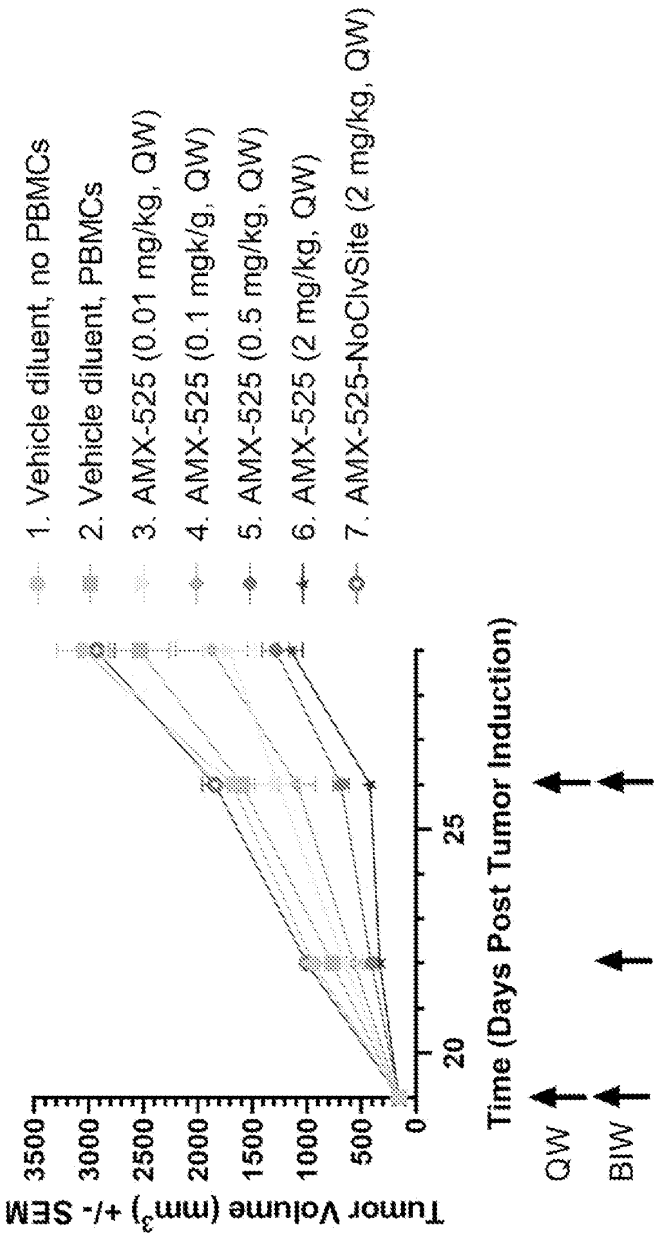
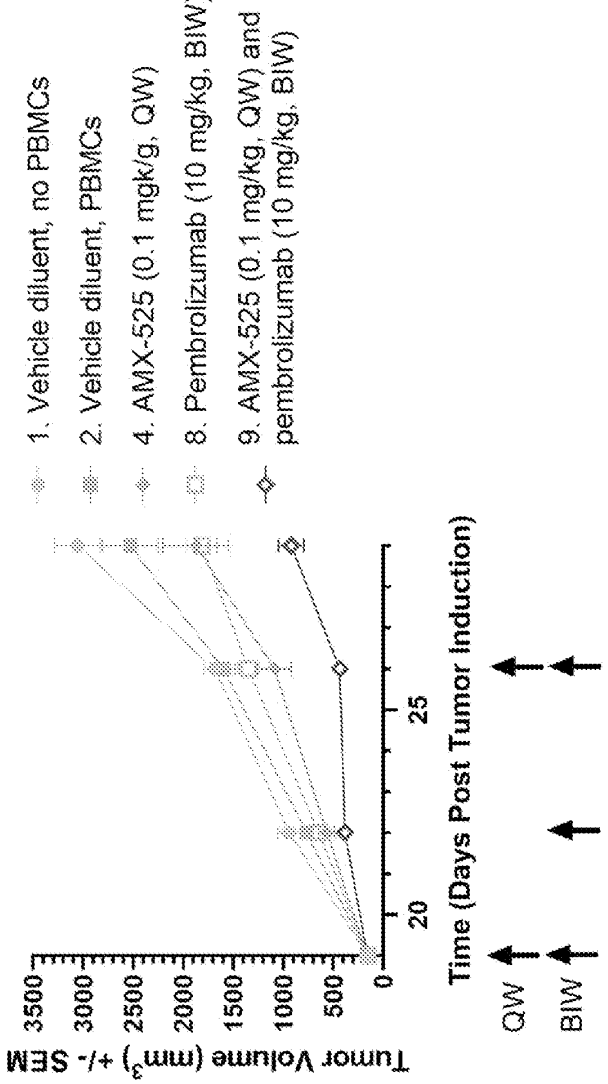

1. Vehicle diluent, no PBMCs
2. Vehicle diluent, PBMCs
3. AMX-525 (0.01 mg/kg, QW)
4. AMX-525 (0.1 mgk/g, QW)
5. AMX-525 (0.5 mg/kg, QW)
6. AMX-525 (2 mg/kg, QW)
7. AMX-525-NoClvSite (2 mg/kg, QW)
Tumor Volume (mm3) +/- SEM
3500
3000
2500
2000
1500
1000
500
0
20
25
Time (Days Post Tumor Induction)
QW
BIW

FIG. 21


1. Vehicle diluent, no PBMCs
2. Vehicle diluent, PBMCs
4. AMX-525 (0.1 mgk/g, QW)
8. Pembrolizumab (10 mg/kg, BIW)
9. AMX-525 (0.1 mg/kg, QW) and pembrolizumab (10 mg/kg, BIW)
Tumor Volume (mm3) +/- SEM
3500
3000
2500
2000
1500
1000
500
0
20
25
Time (Days Post Tumor Induction)
QW
BIW