FIGURE 1A

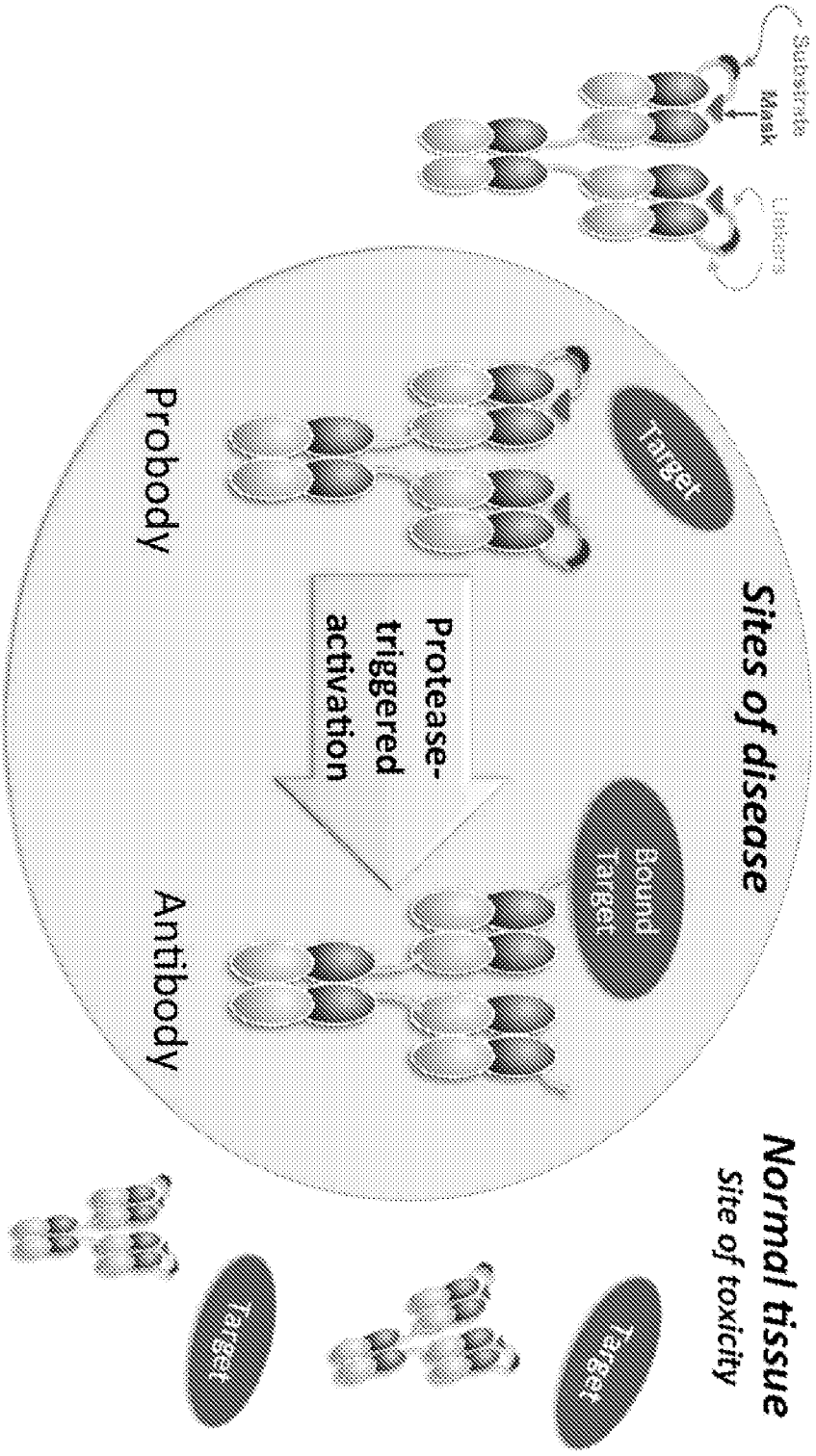

Substrate
Mask
Linker
Probody
Target
Protease-triggered activation
Antibody
Bound Target
Sites of disease
Target
Target
Normal tissue
Site of toxicity

FIGURE 1B

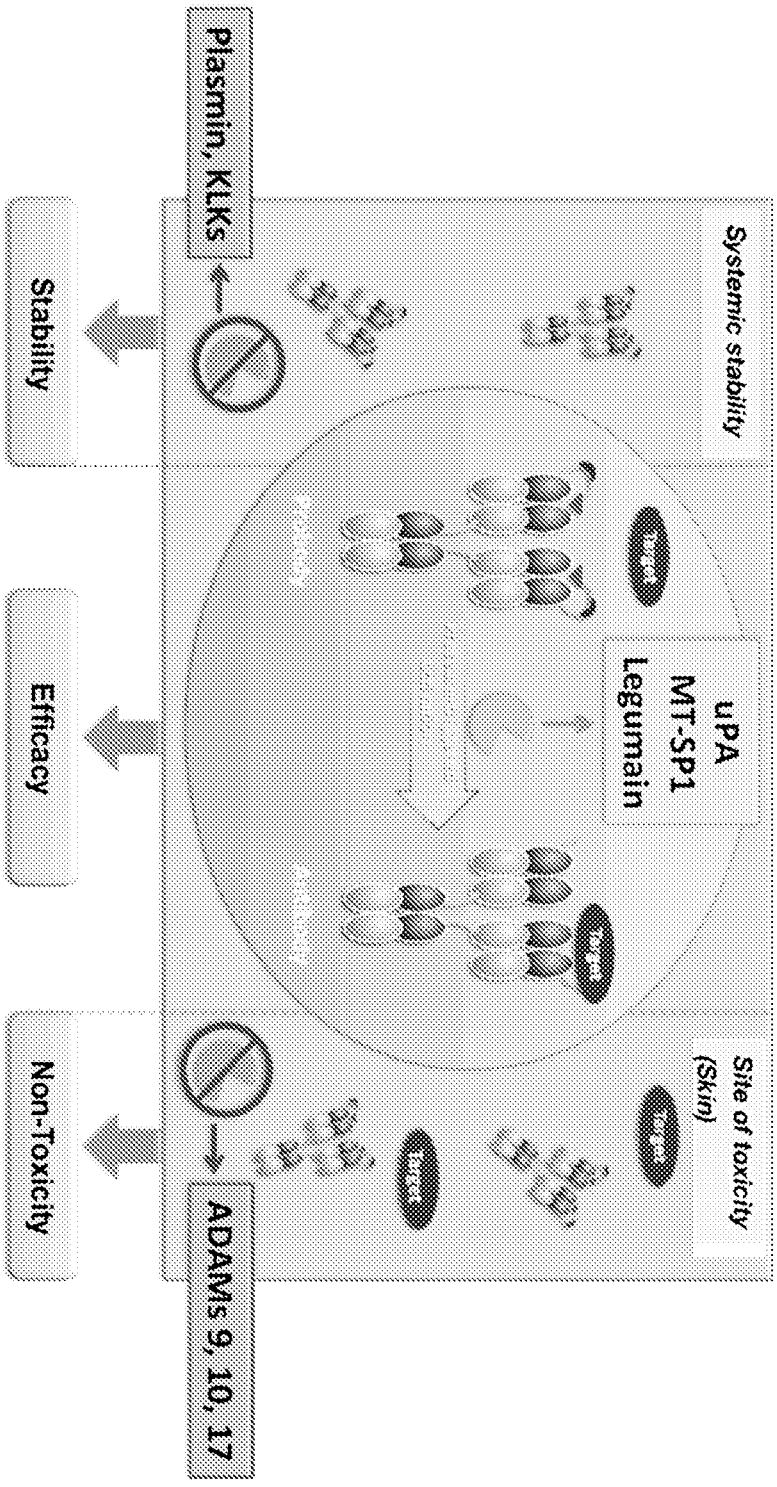

Plasmin, KLKs
Stability
Efficacy
Non-Toxicity
ADAMs 9, 10, 17
Systemic stability
uPA
MT-SP1
Legumain
Site of toxicity
(Skin)

FIGURE 2

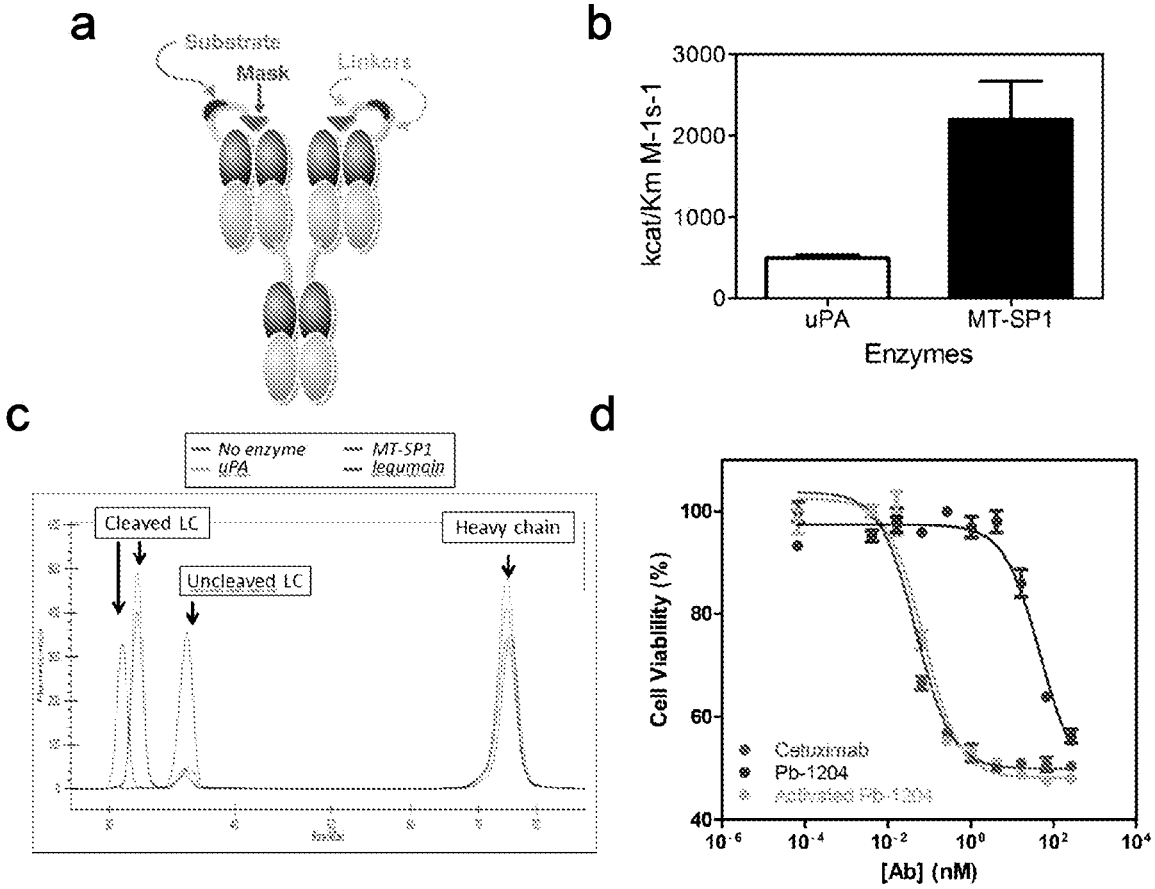

a
Substrate
Mask
Linkers
b
kcat/Km M-1s-1
3000
2000
1000
0
uPA
MT-SP1
Enzymes
c
No enzyme
uPA
MT-SP1
legumain
Cleaved LC
Uncleaved LC
Heavy chain
d
Cell Viability (%)
100
80
60
40
Cetuximab
Pb-1204
Activated Pb-1204
10-6
10-4
10-2
100
102
104
[Ab] (nM)

FIGURE 3

a

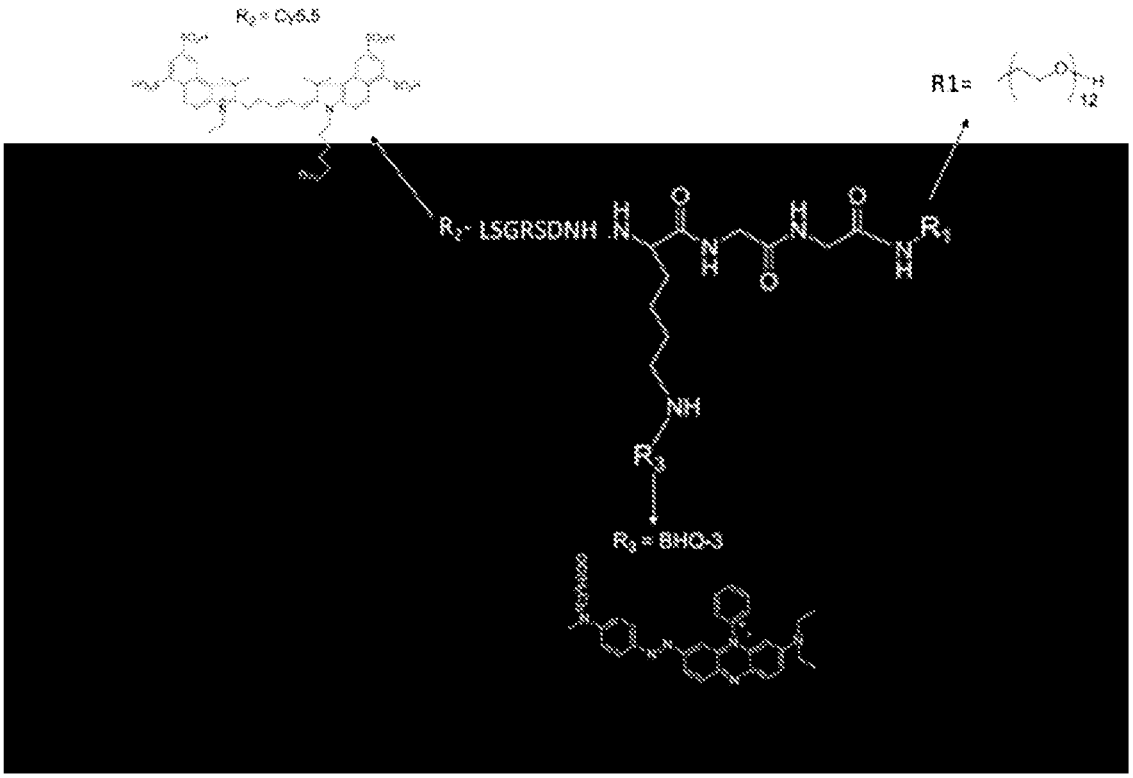


FIGURE 3 CONTINUED

b

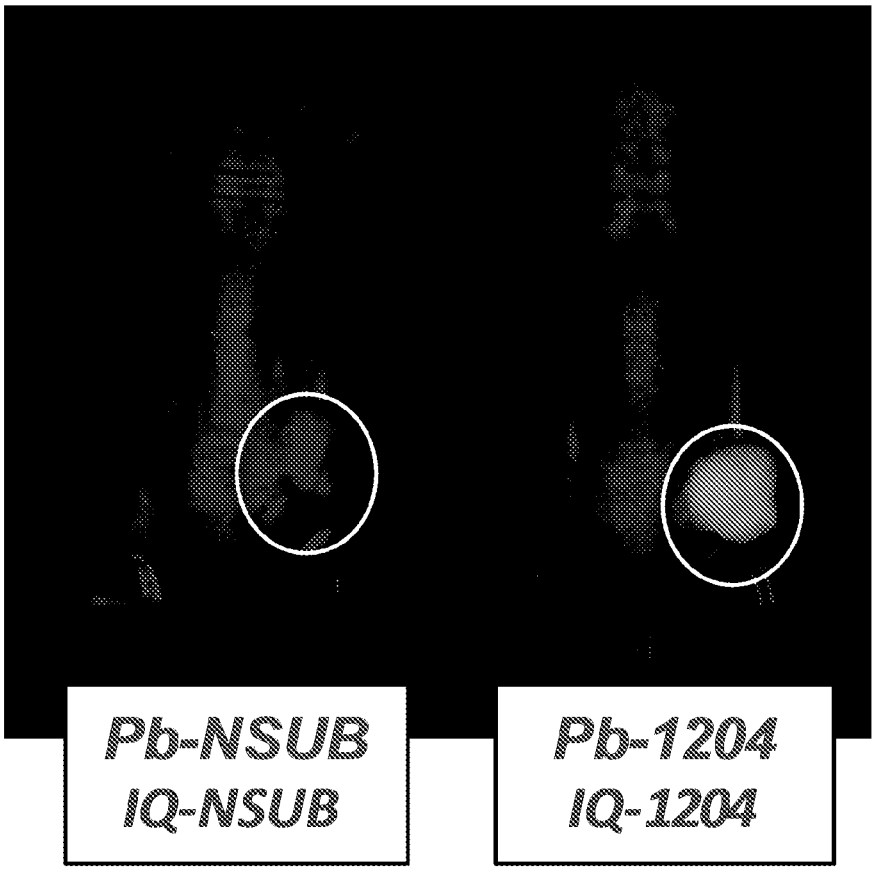


FIGURE 3 CONTINUED

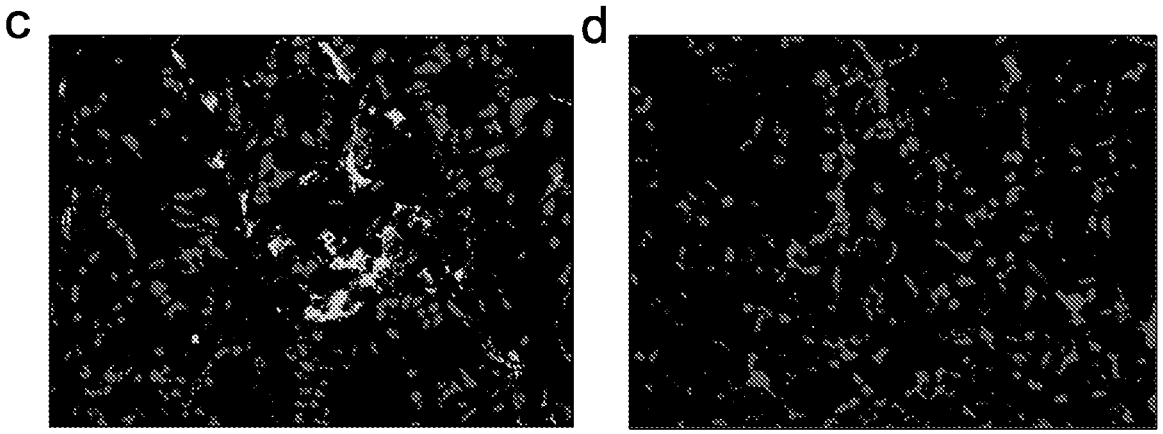


FIGURE 3E

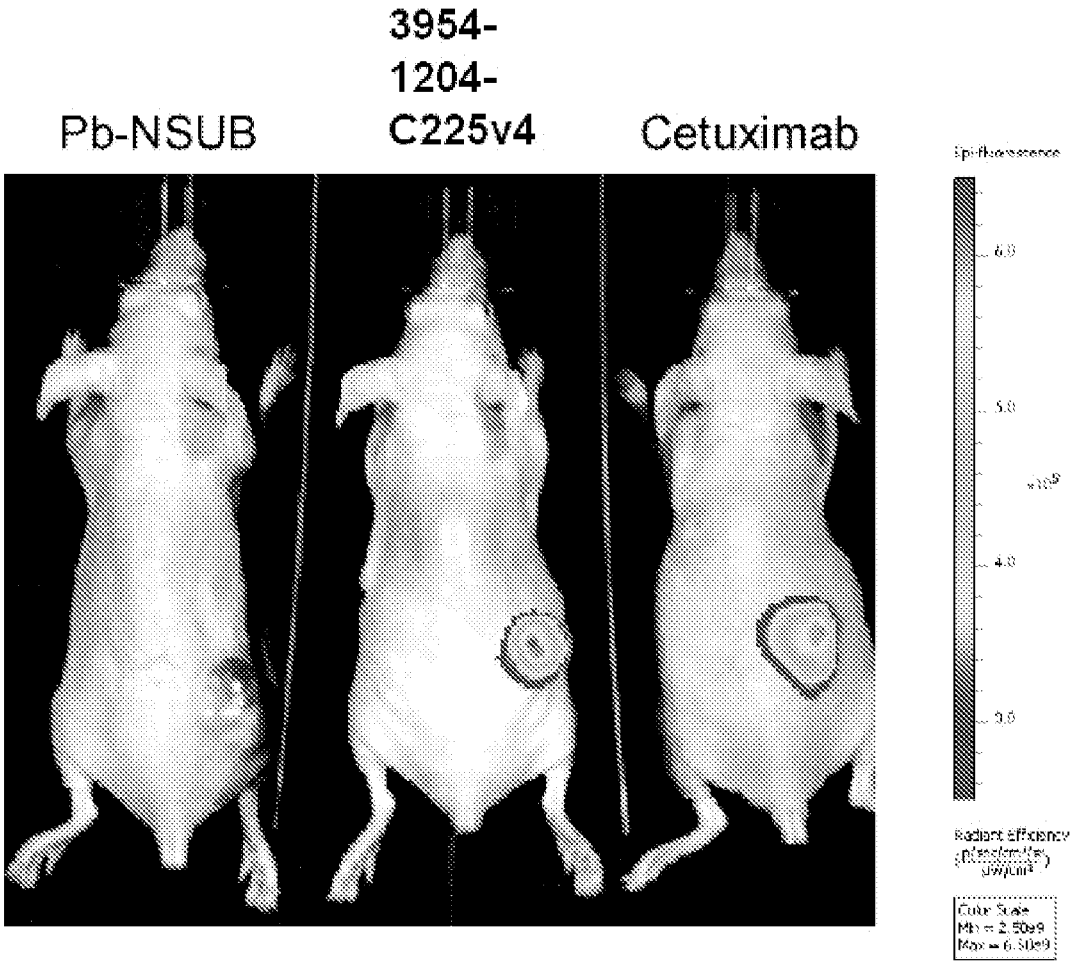


FIGURE 3 CONTINUED

FIGURE 3F

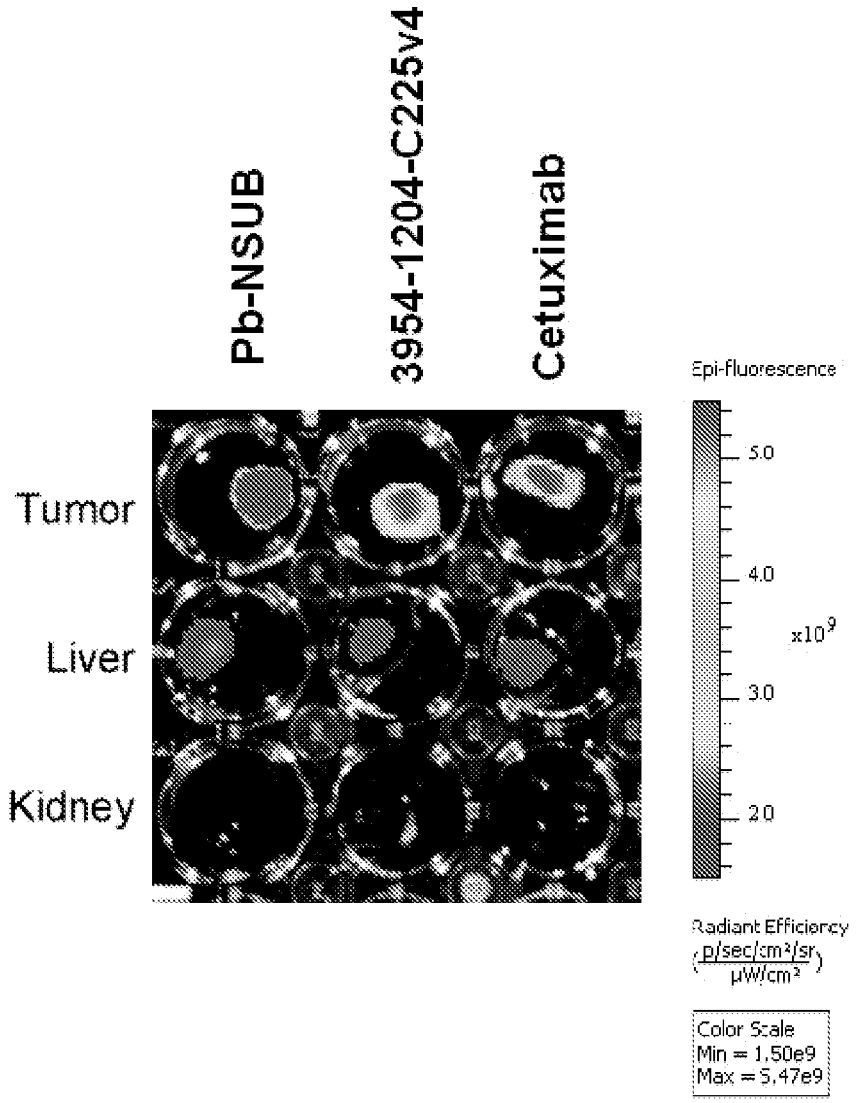


FIGURE 3 CONTINUED

FIGURE 3G

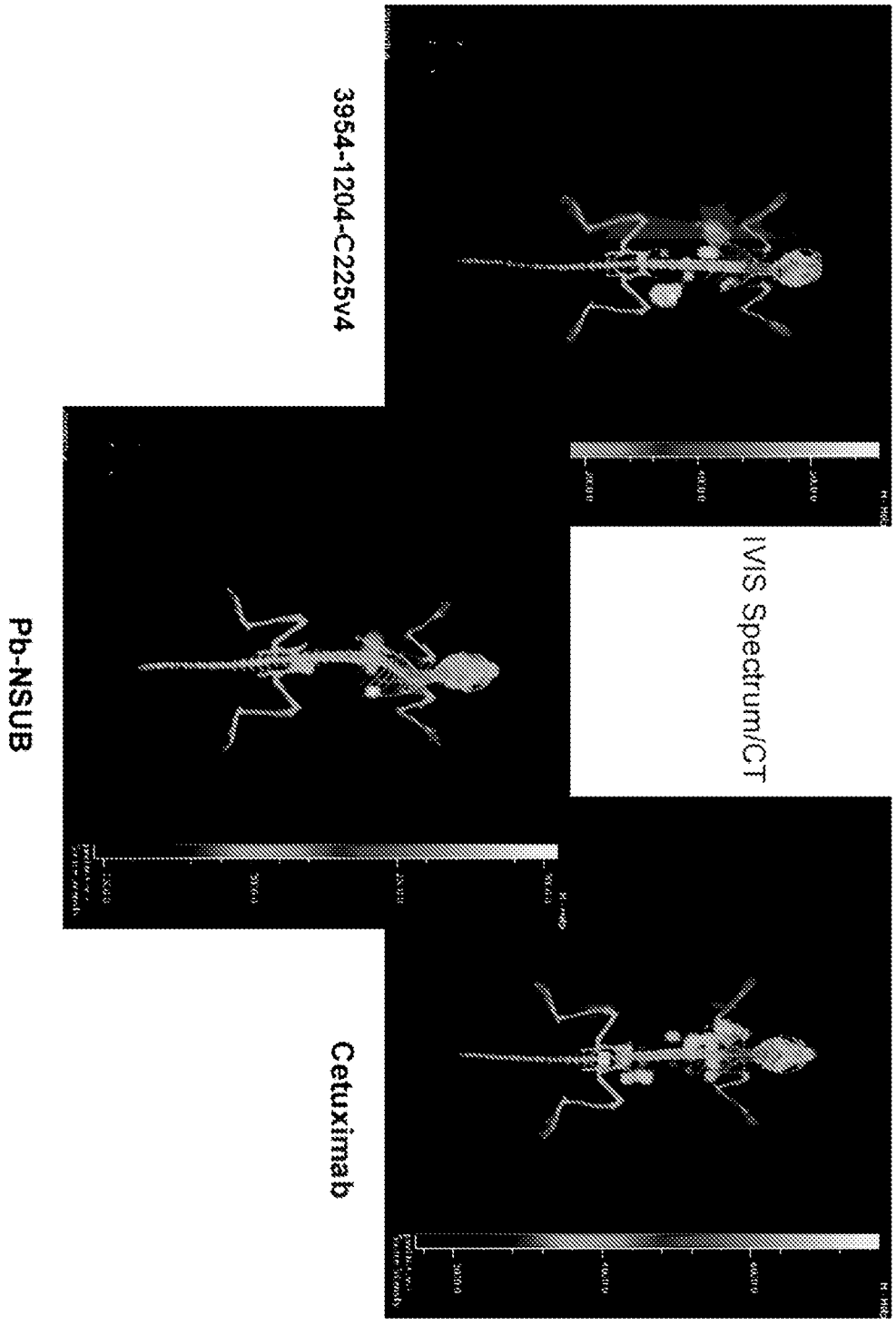


FIGURE 3 CONTINUED

FIGURE 3H

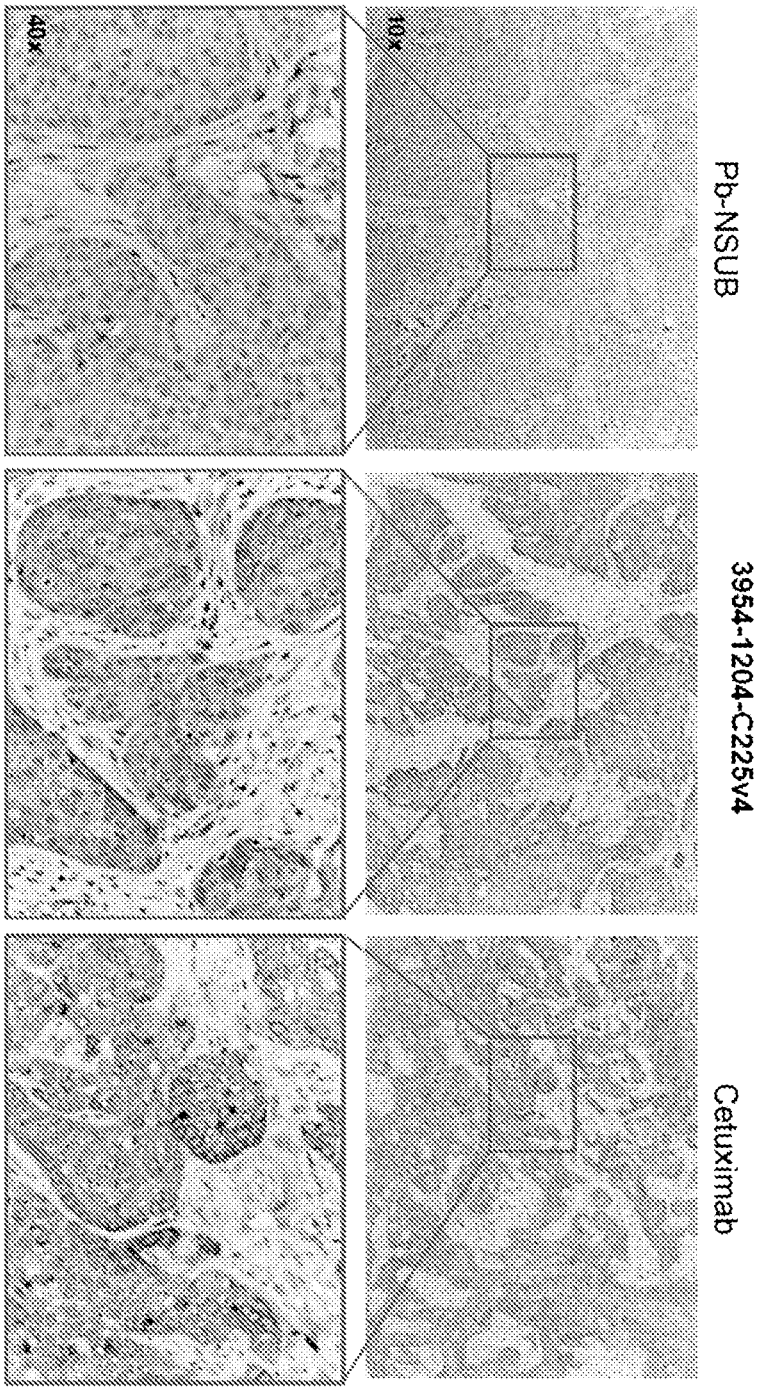


FIGURE 4

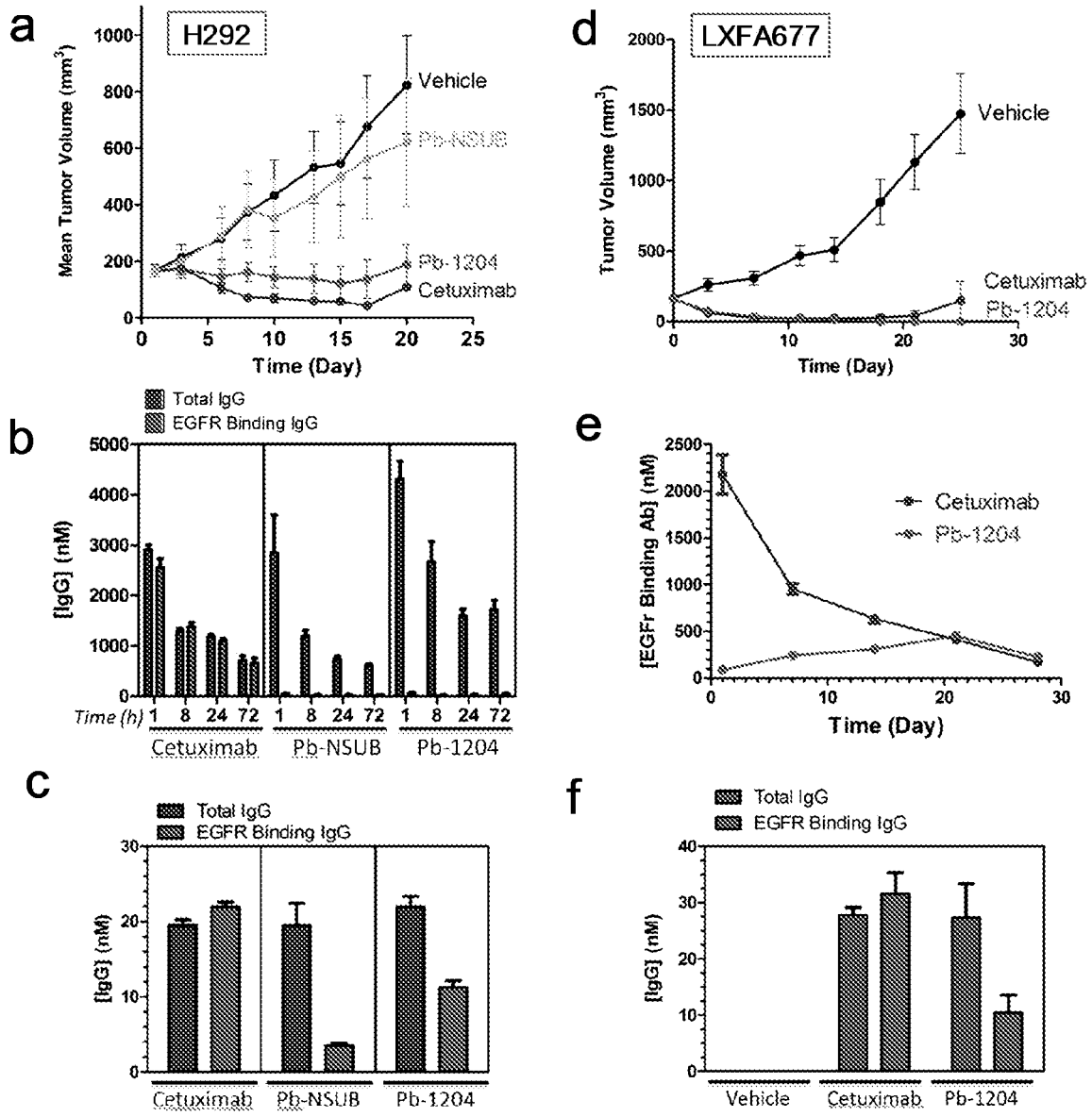

a
H292
Mean Tumor Volume (mm3)
Vehicle
Pb-NSUB
Pb-1204
Cetuximab
Time (Day)
d
LXFA677
Tumor Volume (mm3)
Vehicle
Cetuximab
Pb-1204
Time (Day)
b
Total IgG
EGFR Binding IgG
[IgG] (nM)
Time (h) 1 8 24 72 1 8 24 72 1 8 24 72
Cetuximab
Pb-NSUB
Pb-1204
e
[EGFr Binding Ab] (nM)
Cetuximab
Pb-1204
Time (Day)
c
Total IgG
EGFR Binding IgG
[IgG] (nM)
Cetuximab
Pb-NSUB
Pb-1204
f
Total IgG
EGFR Binding IgG
[IgG] (nM)
Vehicle
Cetuximab
Pb-1204

FIGURE 5

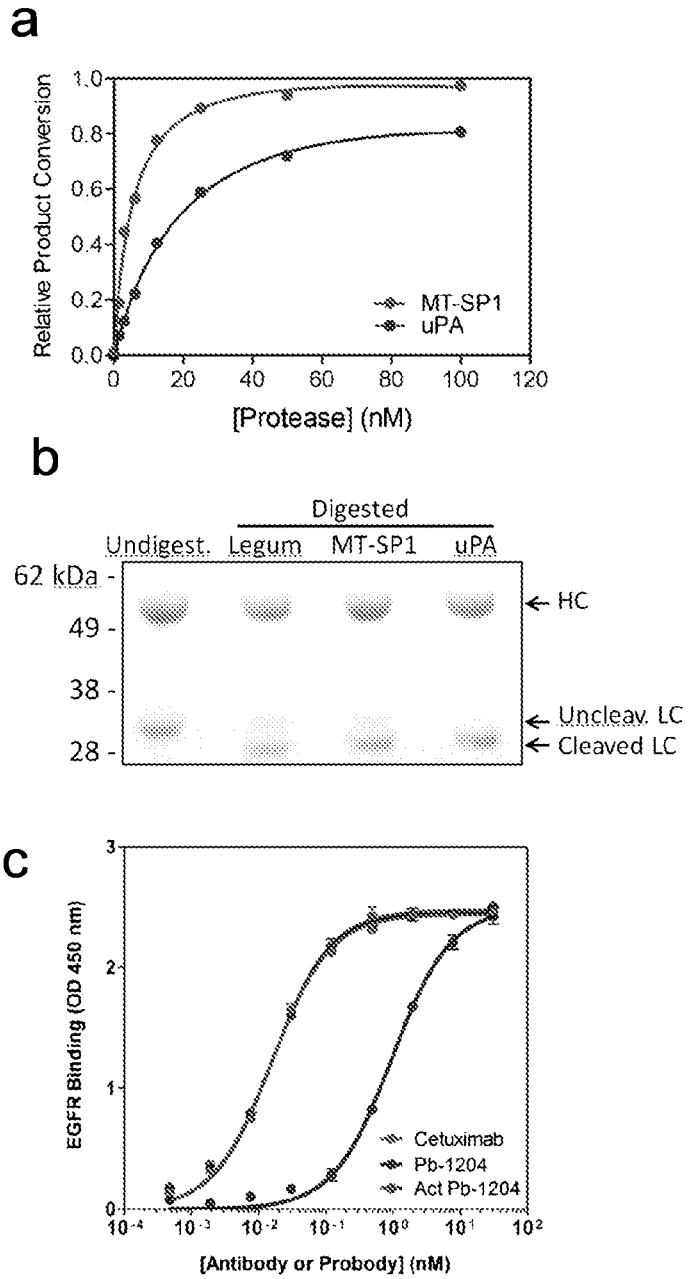

a
Relative Product Conversion
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
[Protease] (nM)
MT-SP1
uPA
b
Digested
Undigest.
Legum
MT-SP1
uPA
62 kDa
49
38
28
HC
Uncleav. LC
Cleaved LC
c
EGFR Binding (OD 450 nm)
3
2
1
0
10-4
10-3
10-2
10-1
100
101
102
[Antibody or Probody] (nM)
Cetuximab
Pb-1204
Act Pb-1204

FIGURE 6

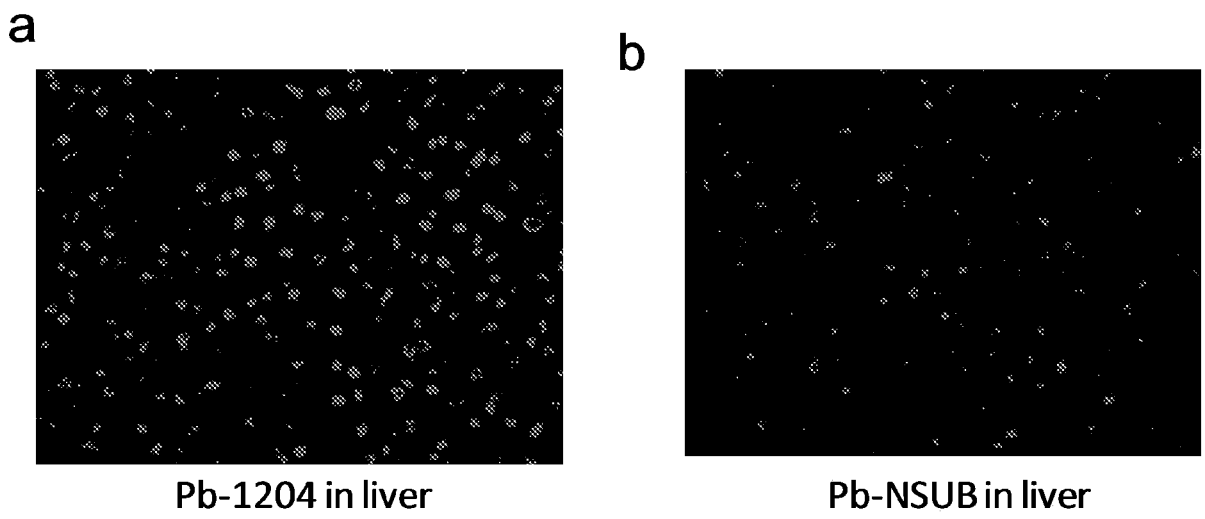


FIGURE 7

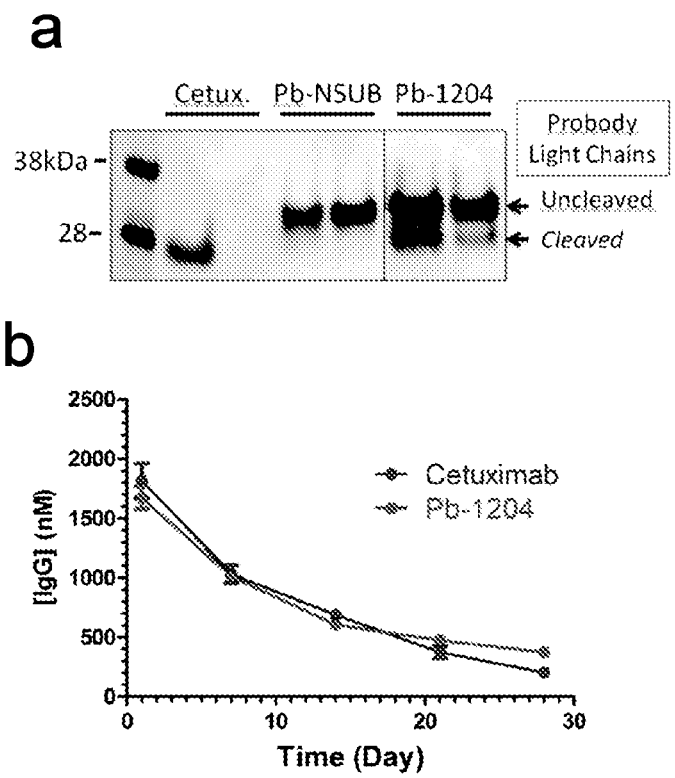
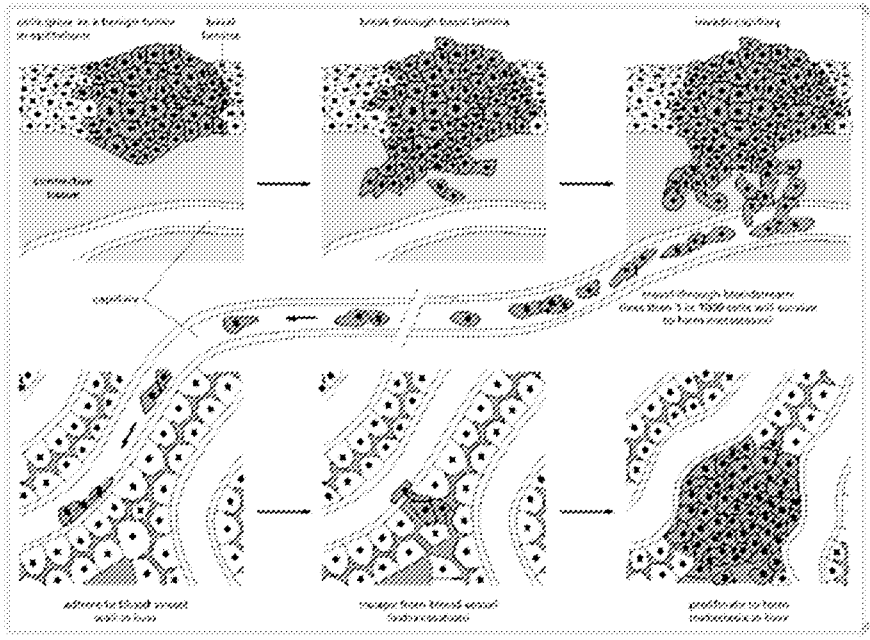
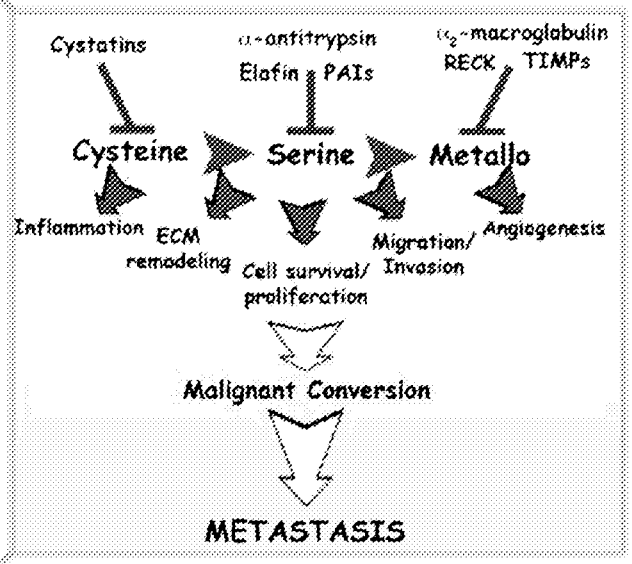


FIGURE 8

A)



B)



Affara et al., (2009) Proteases in Cancer

FIGURE 9

A)

Protease	k_{cat}/K_m $M^{-1}s^{-1}$
Human uPA	2236
Mouse uPA	82
Human MT-SP1	8442
Mouse MT-SP1	22655
Legumain	75000 (pH 4.0)
tPA	43.8
Thrombin	2.3

B)

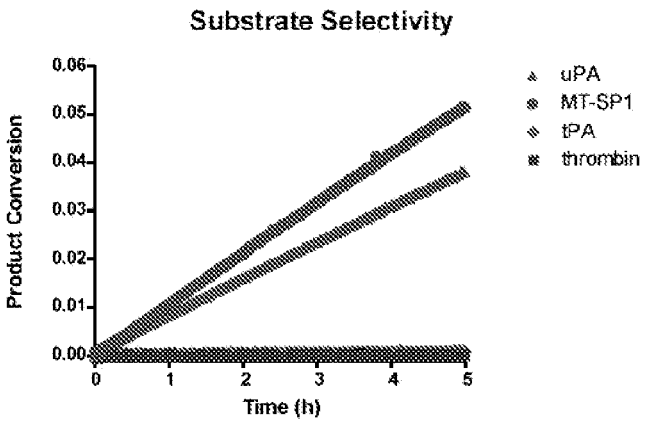
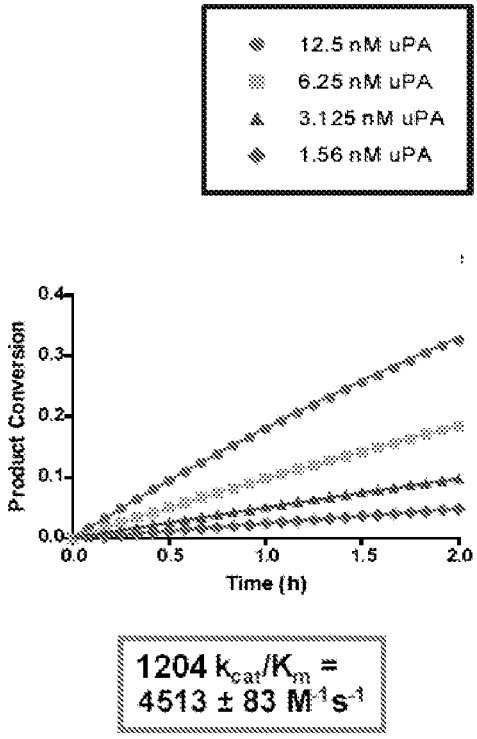
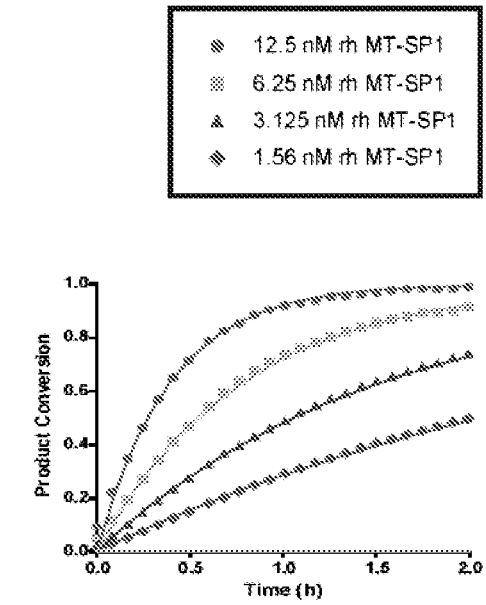


FIGURE 9C



1204 k_{cat}/K_m = 4513 ± 83 $M^{-1}s^{-1}$

FIGURE 9D



1204 k_{cat}/K_m = 57753 ± 1558 $M^{-1}s^{-1}$

FIGURE 10A

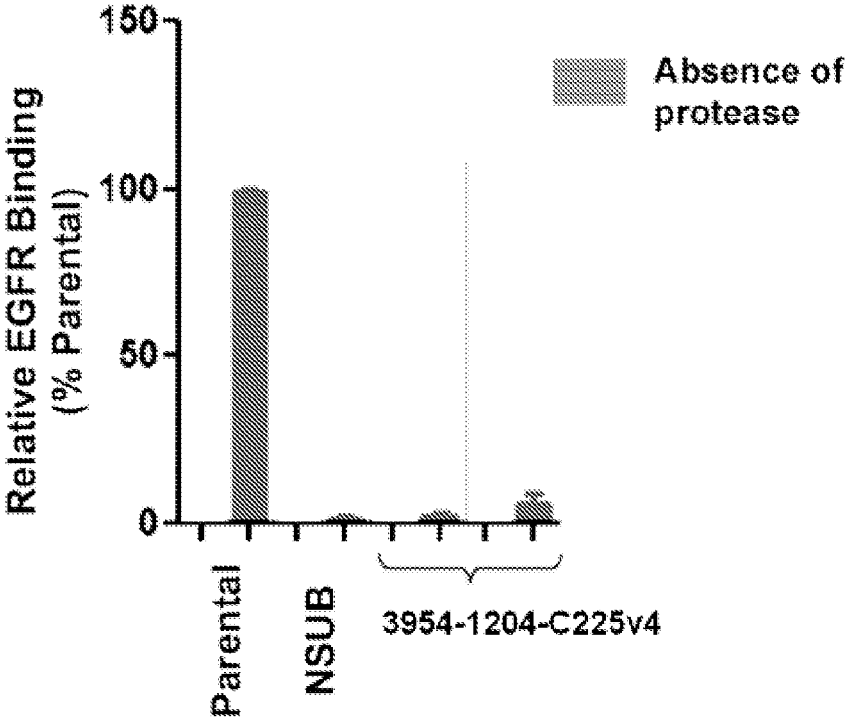


FIGURE 10B

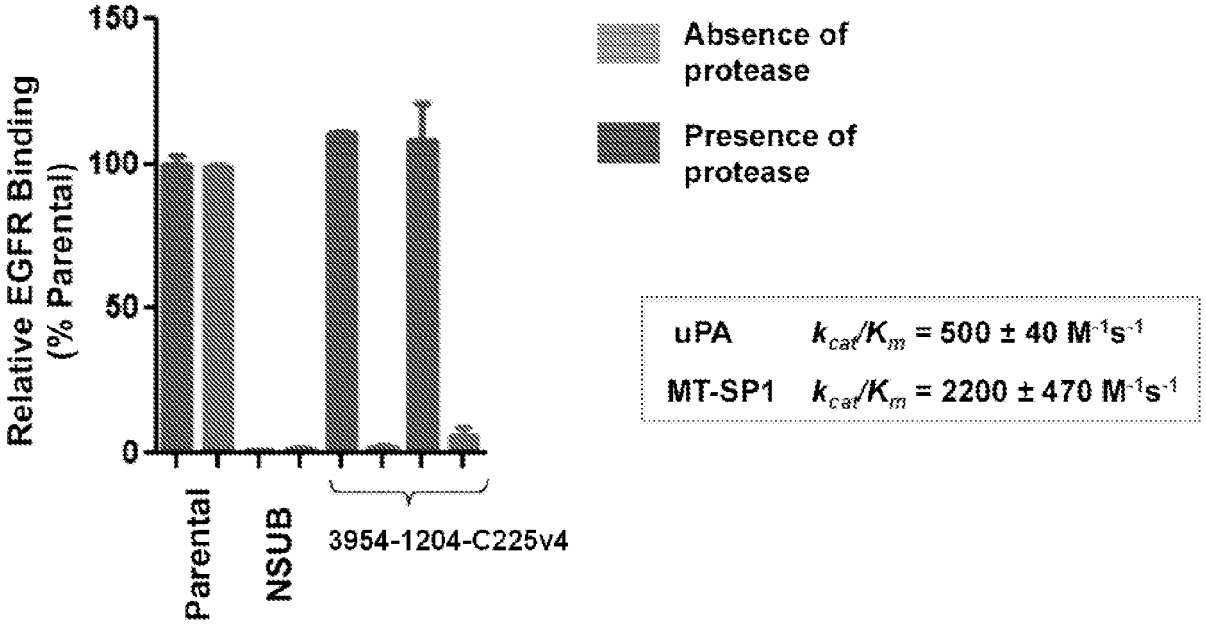


FIGURE 11A

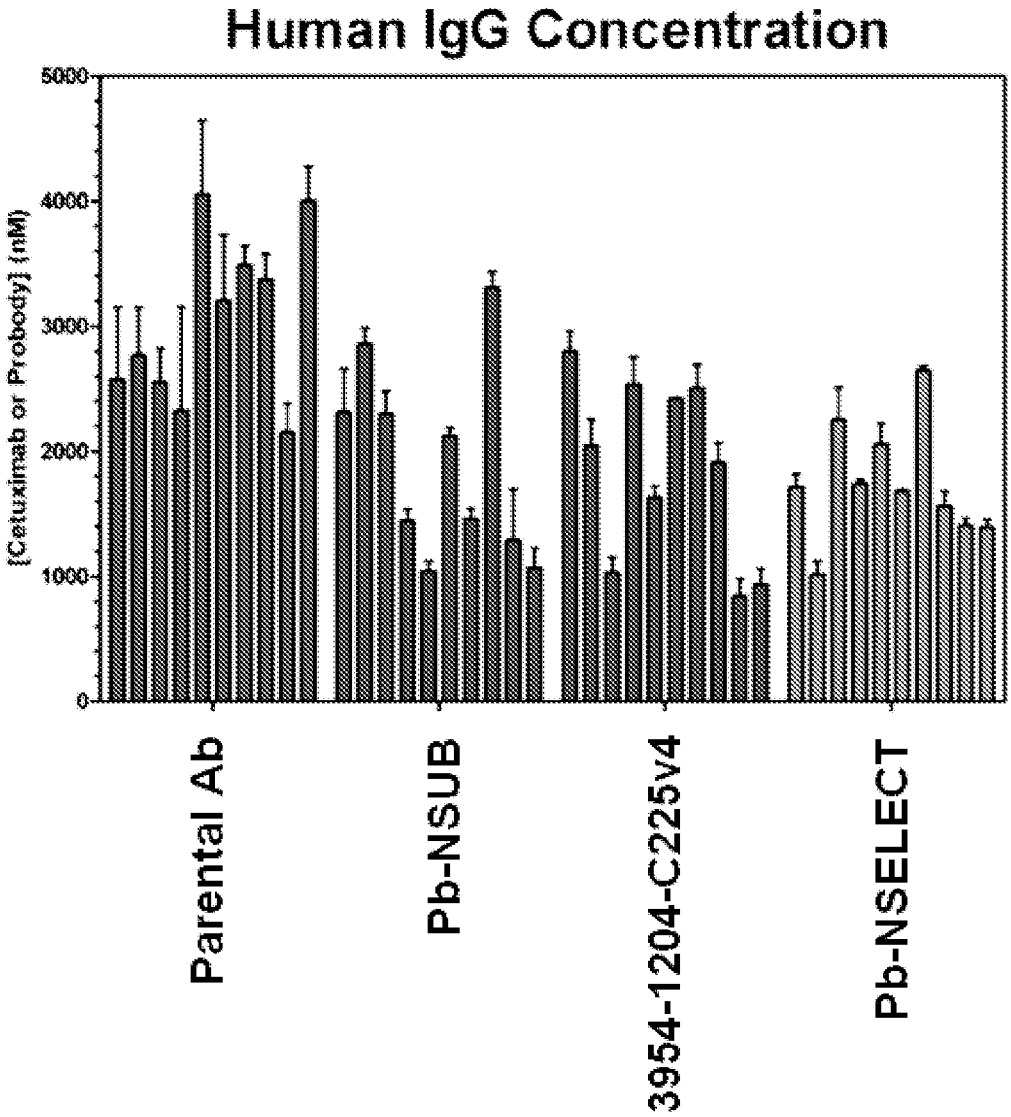

Human IgG Concentration
[Cetuximab or Probody] (nM)
5000
4000
3000
2000
1000
0
Parental Ab
Pb-NSUB
3954-1204-C225v4
Pb-NSELECT

FIGURE 11B

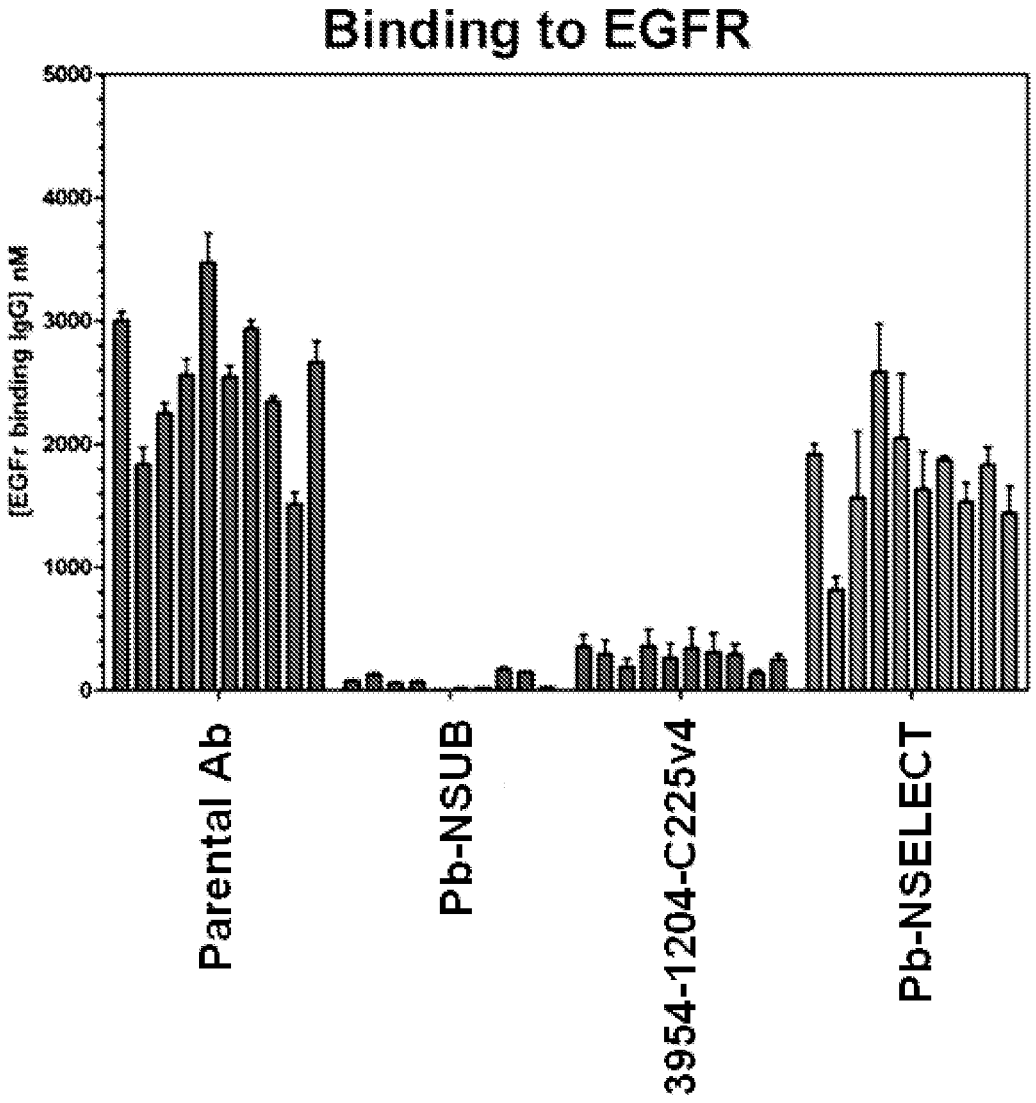

Binding to EGFR
{EGFr binding IgG} nM
5000
4000
3000
2000
1000
0
Parental Ab
Pb-NSUB
3954-1204-C225v4
Pb-NSELECT

FIGURE 12

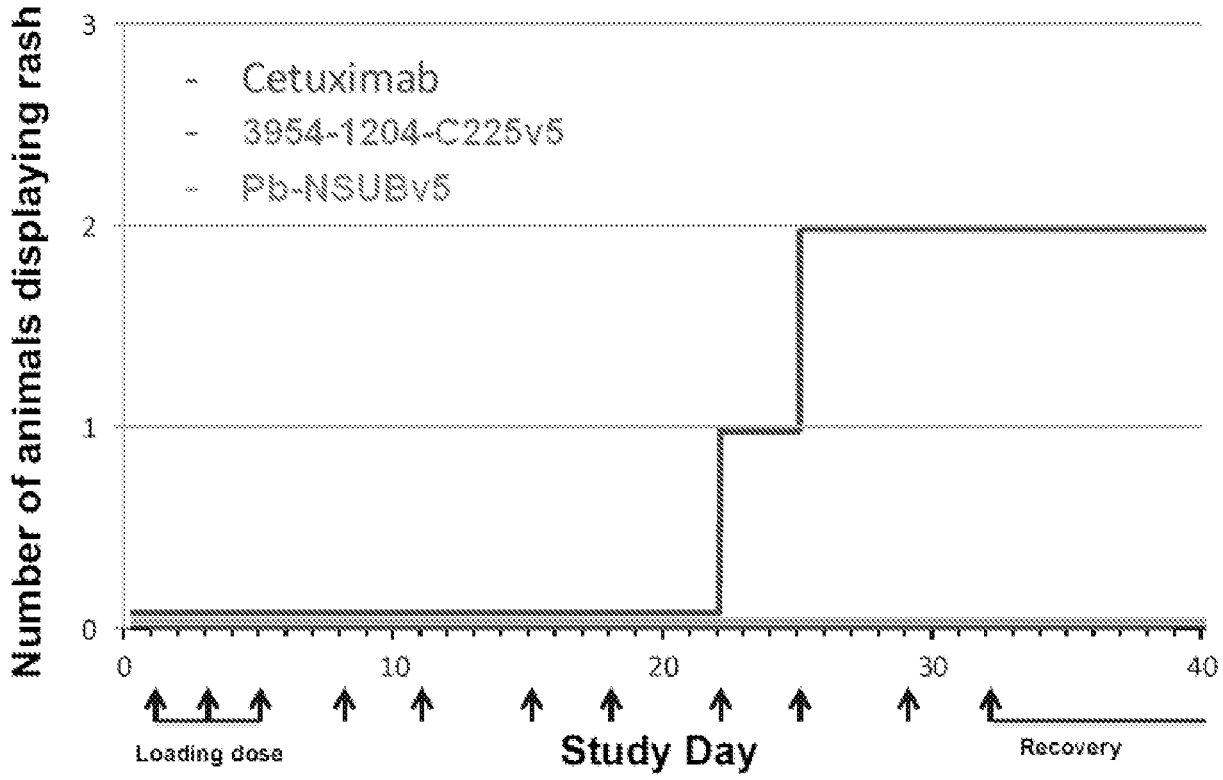

Number of animals displaying rash
3
2
1
0
Cetuximab
3954-1204-C225v5
Pb-NSUBv5
0
10
20
30
40
Loading dose
Study Day
Recovery

FIGURE 13

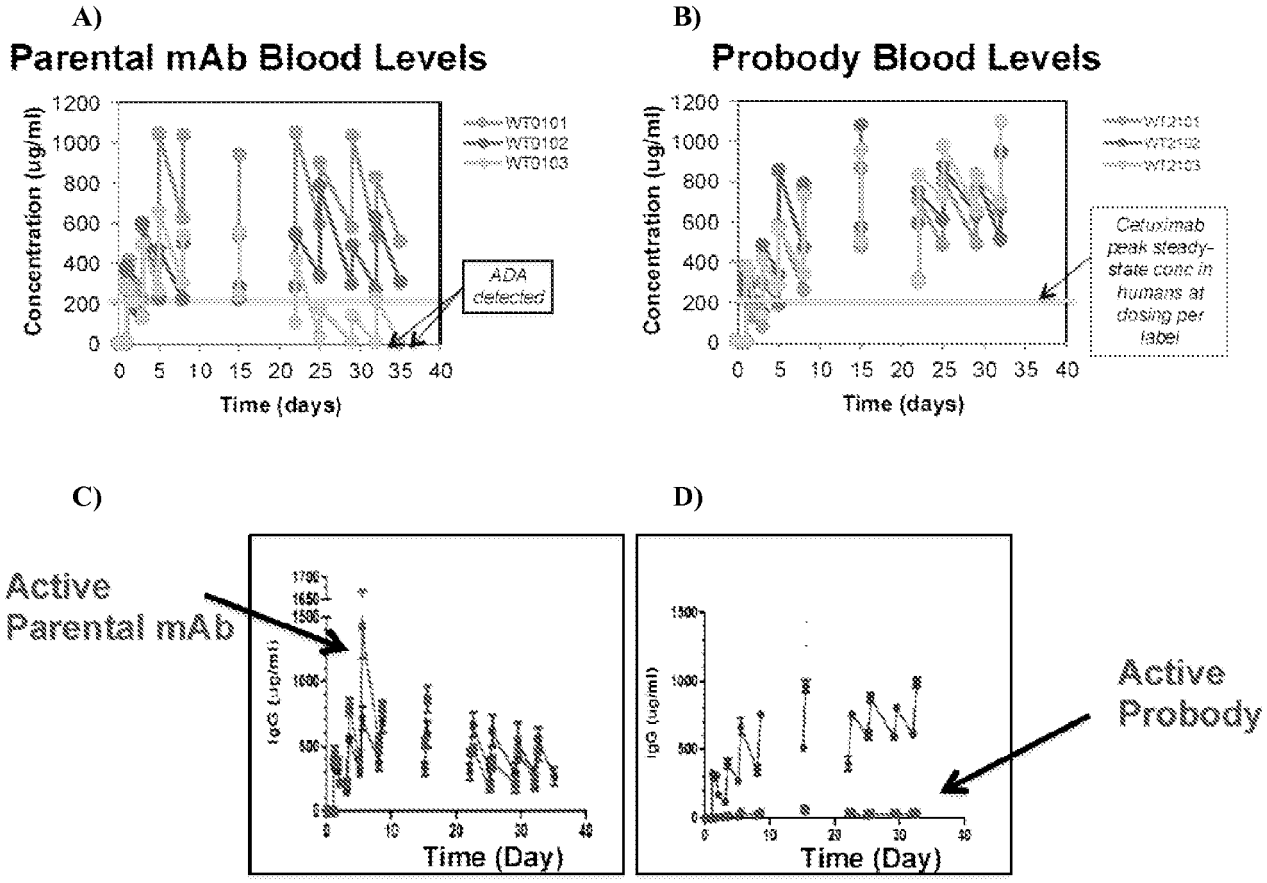

A)
Parental mAb Blood Levels
Concentration (ug/ml)
Time (days)
WT0101
WT0102
WT0103
ADA detected
B)
Probody Blood Levels
Concentration (ug/ml)
Time (days)
Cetuximab peak steady-state conc in humans at dosing per label
C)
Active Parental mAb
IgG (ug/ml)
Time (Day)
D)
IgG (ug/ml)
Time (Day)
Active Probody

FIGURE 14

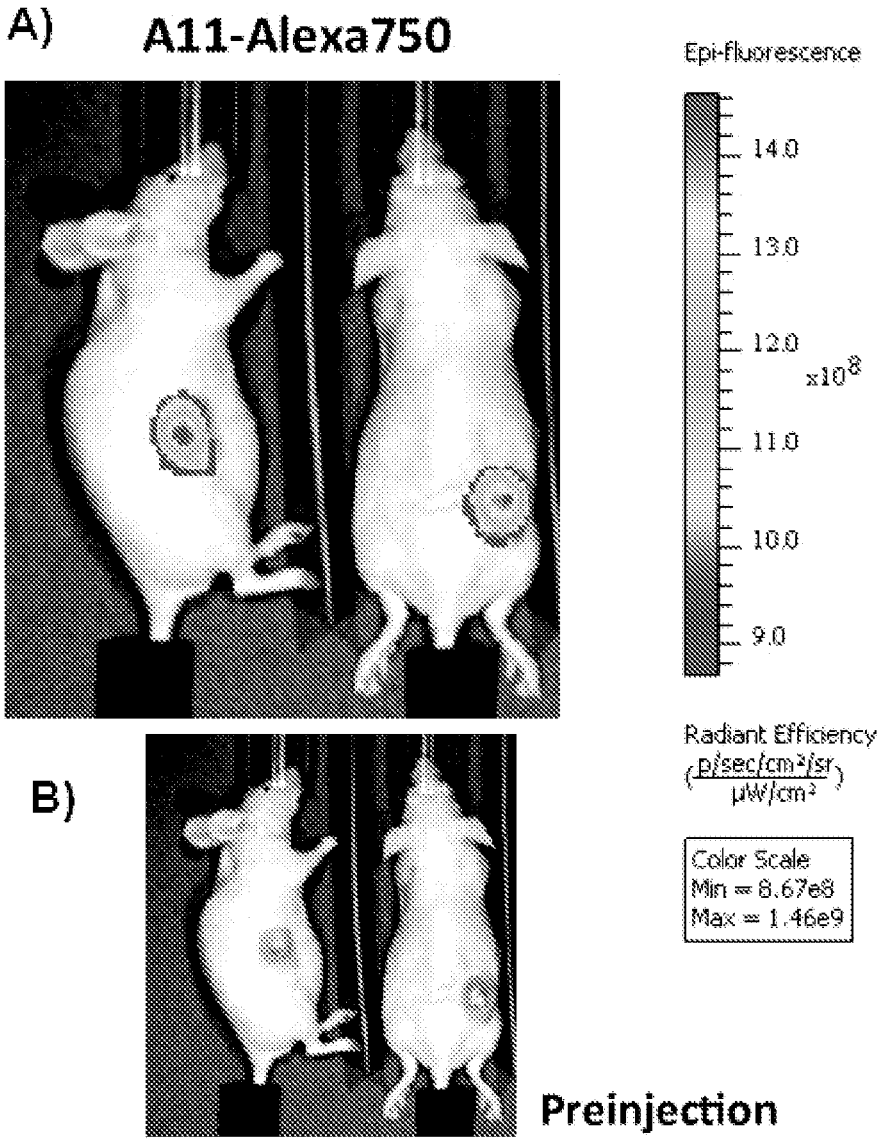

A)
A11-Alexa750
Epi-fluorescence
14.0
13.0
12.0
x10^8
11.0
10.0
9.0
Radiant Efficiency
(p/sec/cm²/sr / µW/cm²)
Color Scale
Min = 8.67e8
Max = 1.46e9
B)
Preinjection

FIGURE 14 CONTINUED

C)



FIGURE 15

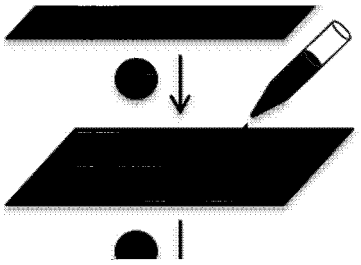


FIGURE 16

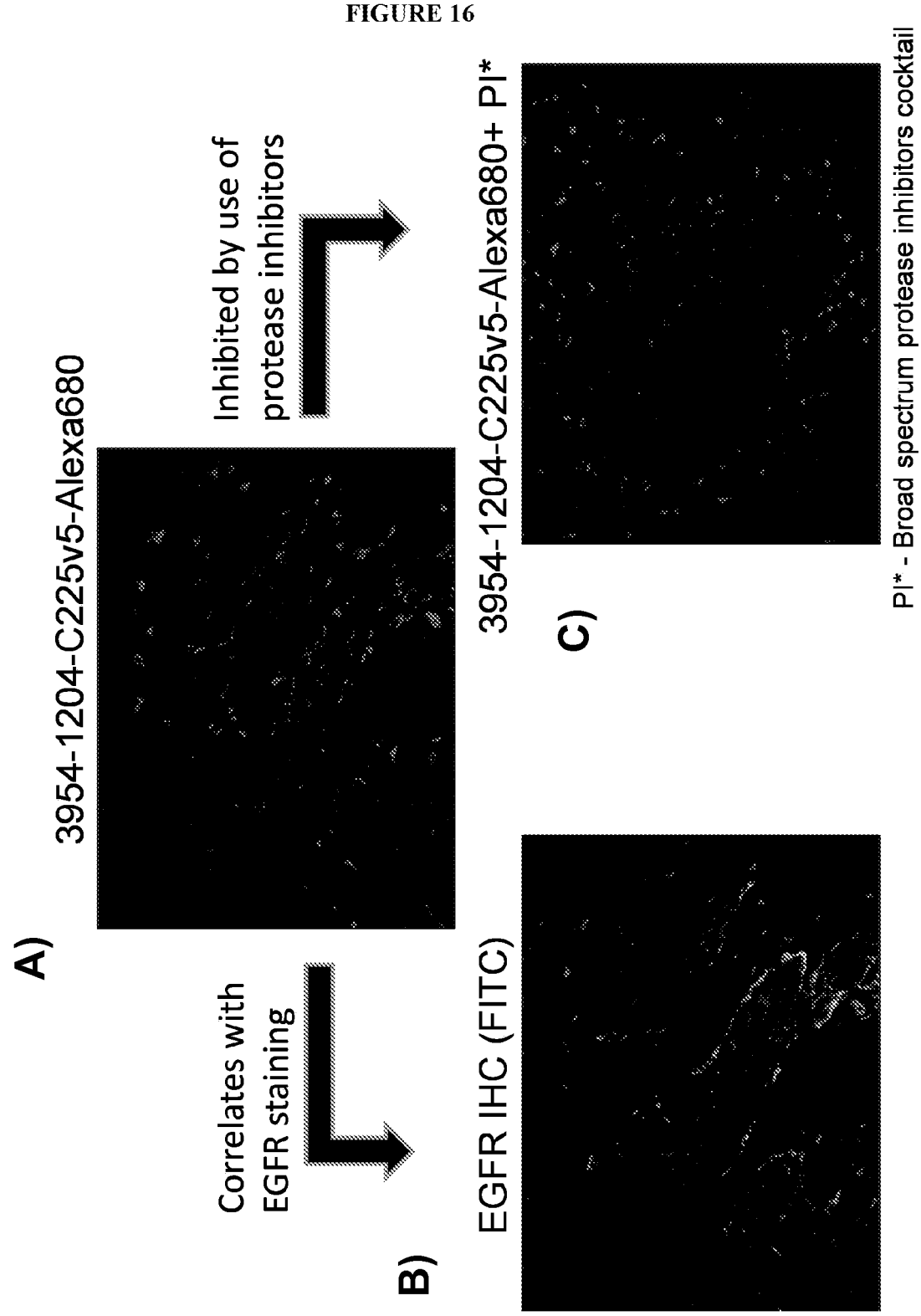

A)
3954-1204-C225v5-Alexa680
Inhibited by use of protease inhibitors
C)
3954-1204-C225v5-Alexa680+ PI*
Correlates with EGFR staining
B)
EGFR IHC (FITC)
PI* - Broad spectrum protease inhibitors cocktail

FIGURE 17

Lung Tumor

Patient #	EGFR	MT-SP1	Cetux	3954-1204-C225v5
5608	-	+++		
5610	-	-		
5613	+	-	++	+
5615	-	-		
5621	+++	++	+++	++
5625	+++	++	+++	++
5627	-	-		
5646	+/-	+	+	+
5648	-	-		
5654	+/-	++		

Colon Cancer

Patient #	EGFR	MT-SP1	Cetux	3954-1204-C225v5
5577	+++	+++		
5579	+++	-	++	++
5638	++	+	++	+
5642	+++	+++		
5650	++	+	+/++	+
5652	+++	+++	+++	+++
5656	+++	++		
5658	-	+++		
5660	++	+++		

* - scoring is based on comparison with Cetuximab staining

FIGURE 18

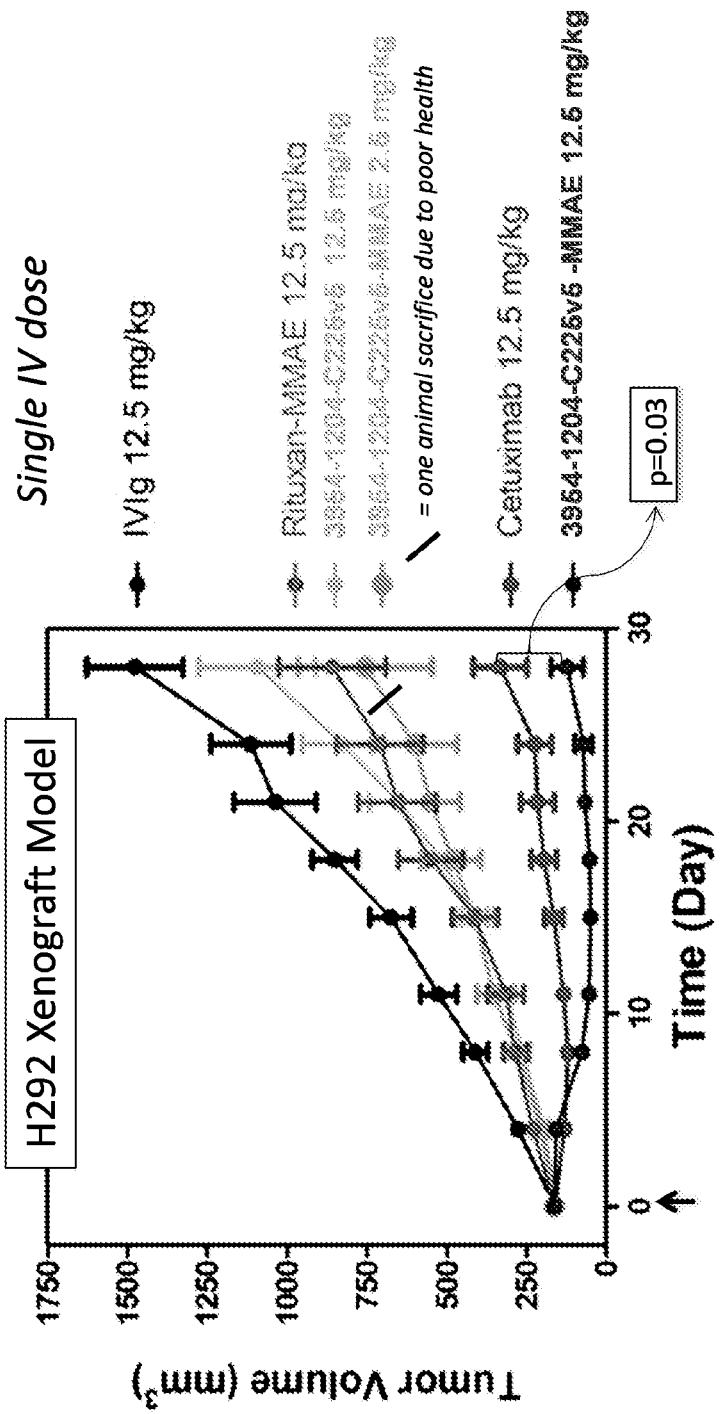

Single IV dose
IVig 12.5 mg/kg
Rituxan-MMAE 12.5 mg/kg
3954-1204-C226v6 12.5 mg/kg
3954-1204-C226v6-MMAE 2.5 mg/kg
= one animal sacrifice due to poor health
Cetuximab 12.5 mg/kg
3954-1204-C226v5 -MMAE 12.5 mg/kg
p=0.03
H292 Xenograft Model
Tumor Volume (mm³)
Time (Day)
0
250
500
750
1000
1250
1500
1750
0
10
20
30

FIGURE 19

Molecule	Half-life (days)
Cetuximab	3.2 ± 0.4
3954-1204-C225v5	6.6 ± 0.5

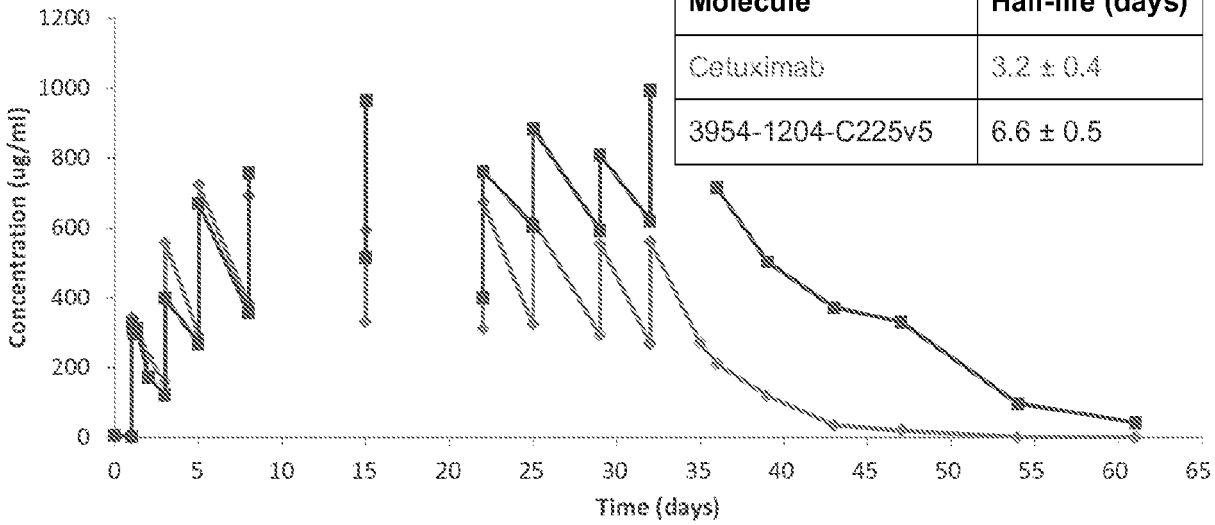


FIGURE 20A

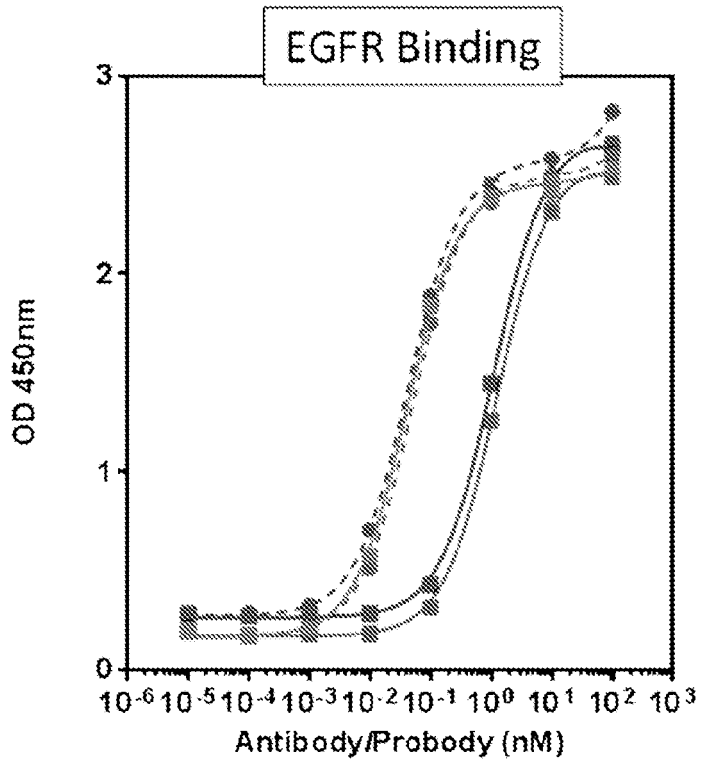


FIGURE 20B

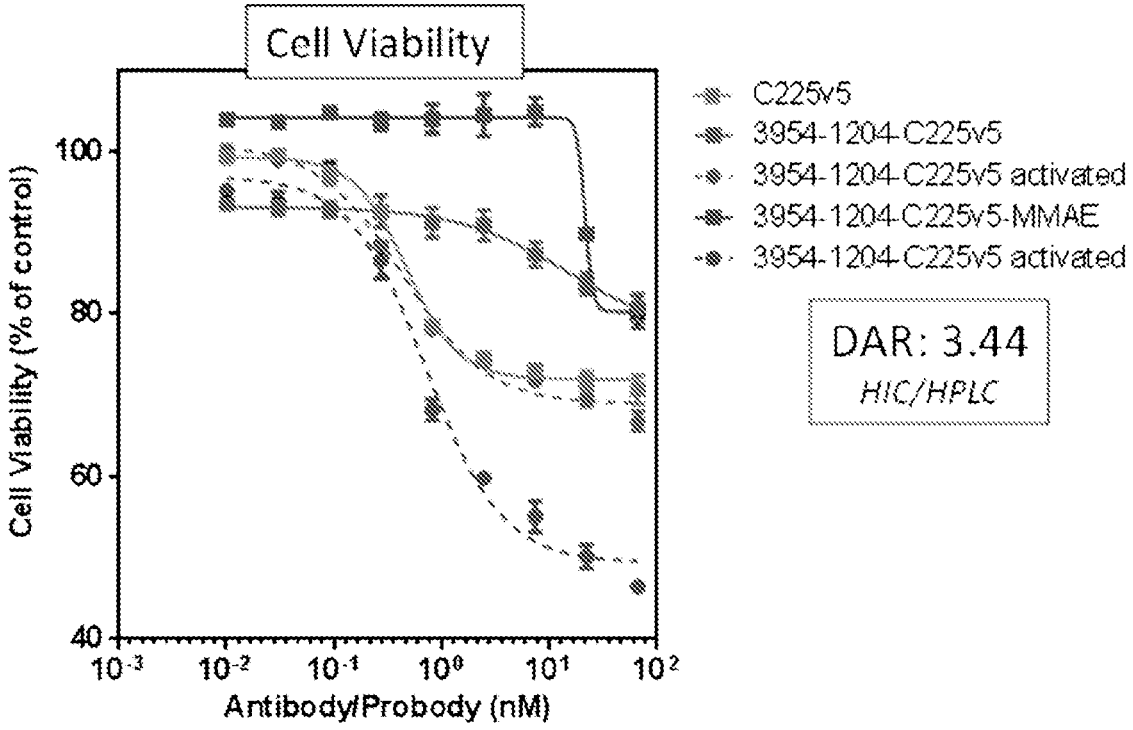


FIGURE 21

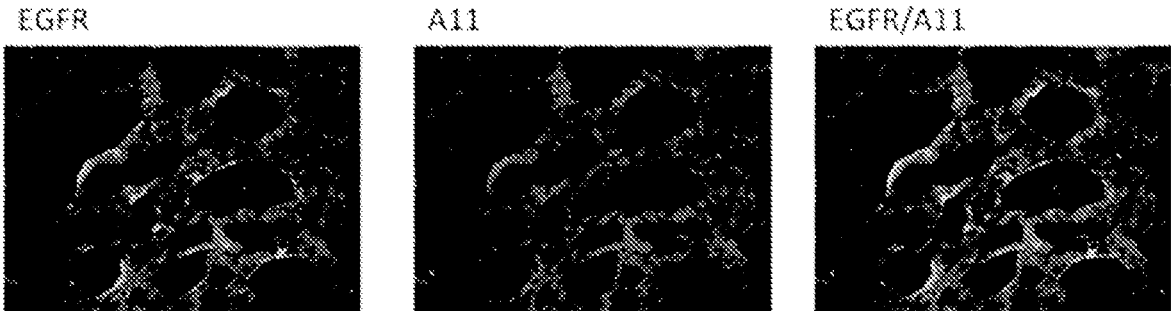


FIGURE 22

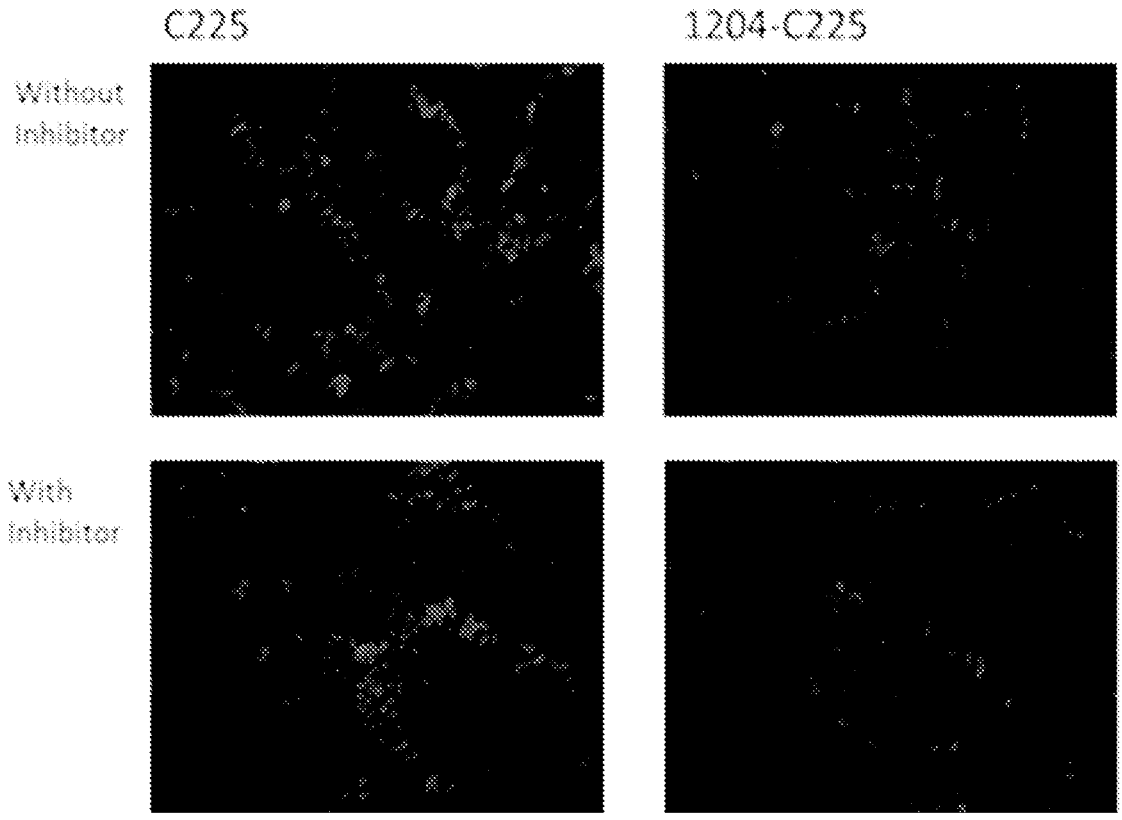
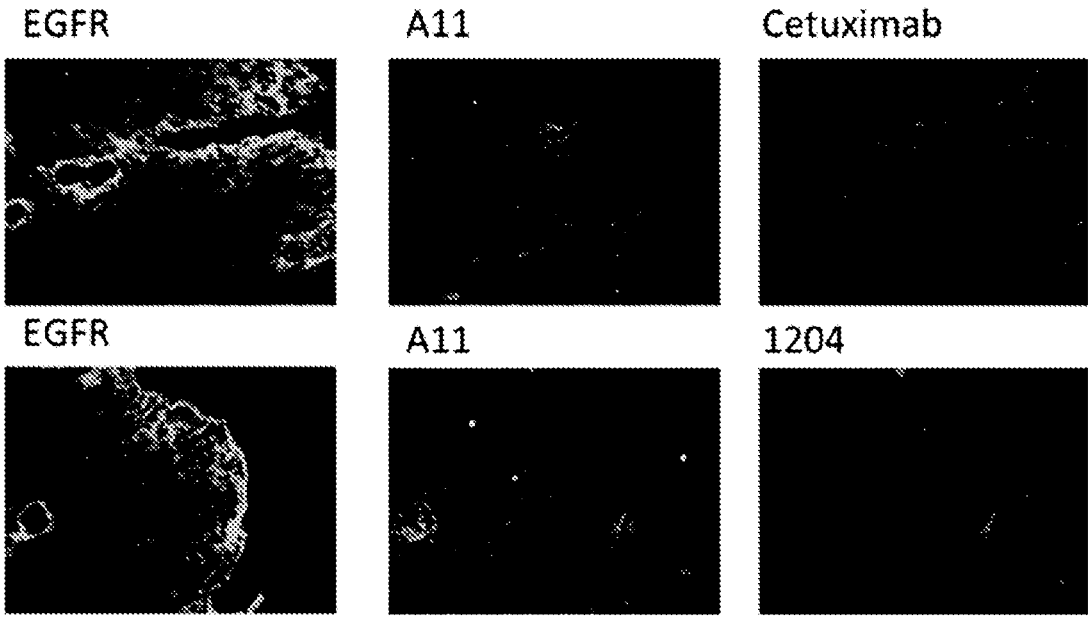


FIGURE 23



Patient #	EGFR	MT-SP1	Cetuximab	3954-1204-C225	Cancer Type
5594	+++	++	+++	~90%	Esophageal