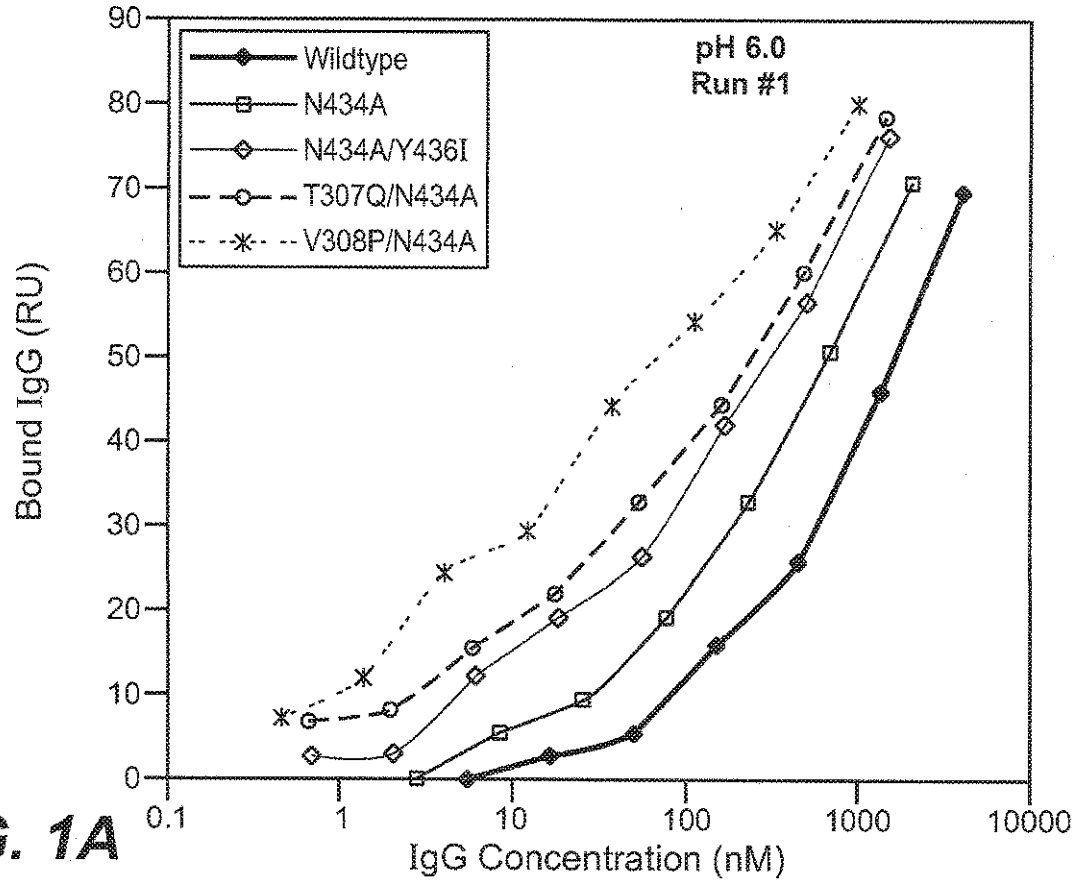

Wildtype
N434A
N434A/Y436I
T307Q/N434A
V308P/N434A
pH 6.0
Run #1
Bound IgG (RU)
IgG Concentration (nM)
0
10
20
30
40
50
60
70
80
90
0.1
1
10
100
1000
10000

FIG. 1A

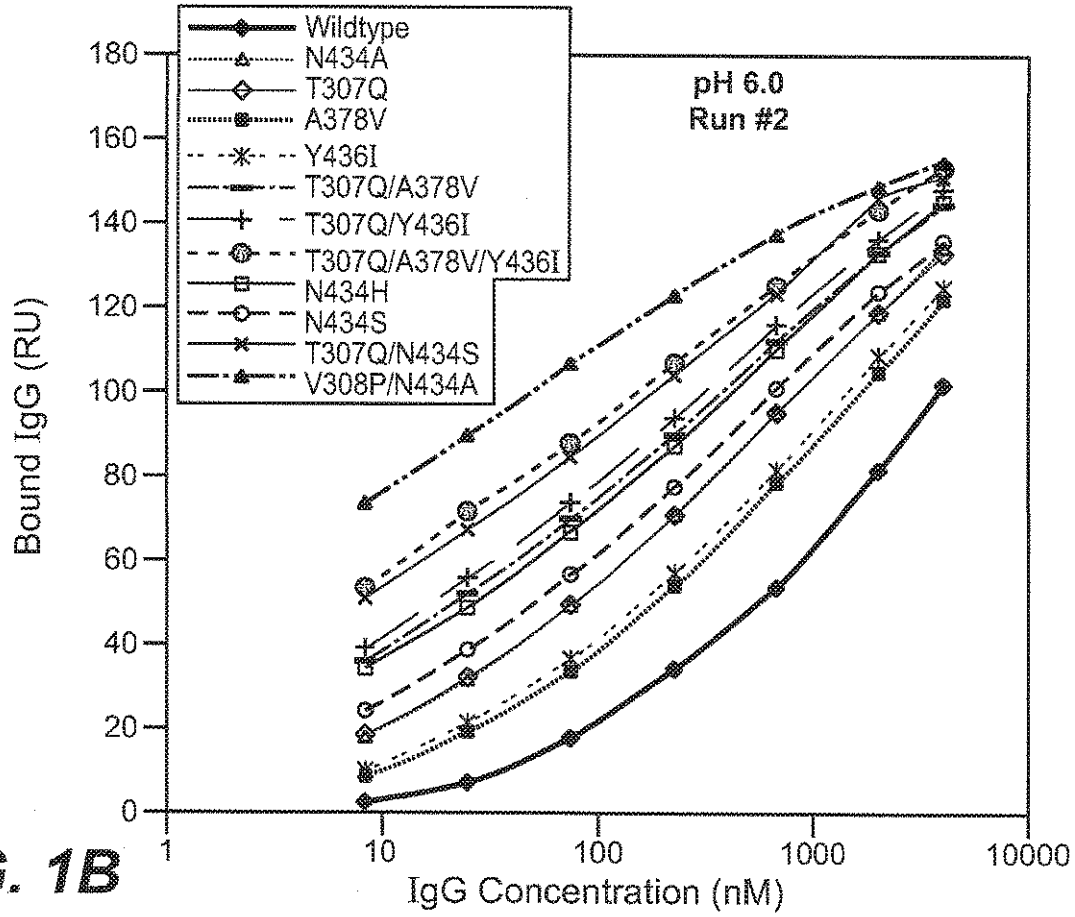

Wildtype
N434A
T307Q
A378V
Y436I
T307Q/A378V
T307Q/Y436I
T307Q/A378V/Y436I
N434H
N434S
T307Q/N434S
V308P/N434A
pH 6.0
Run #2
Bound IgG (RU)
IgG Concentration (nM)
0
20
40
60
80
100
120
140
160
180
1
10
100
1000
10000

FIG. 1B

Variants	KD (nM) Run 1	KD (nM) Run 2
Wildtype	1500	1500
T307Q		250
A378V		500
N434A	550	250
N434H		100
N434S		150
Y436I		450
T307Q/A378V		90
T307Q/N434A	180	
T307Q/N434S		35
T307Q/Y436I		70
T307Q/A378V/Y436I		30
V308P/N434A	40	8
N434A/Y436I	220	

FIG. 2

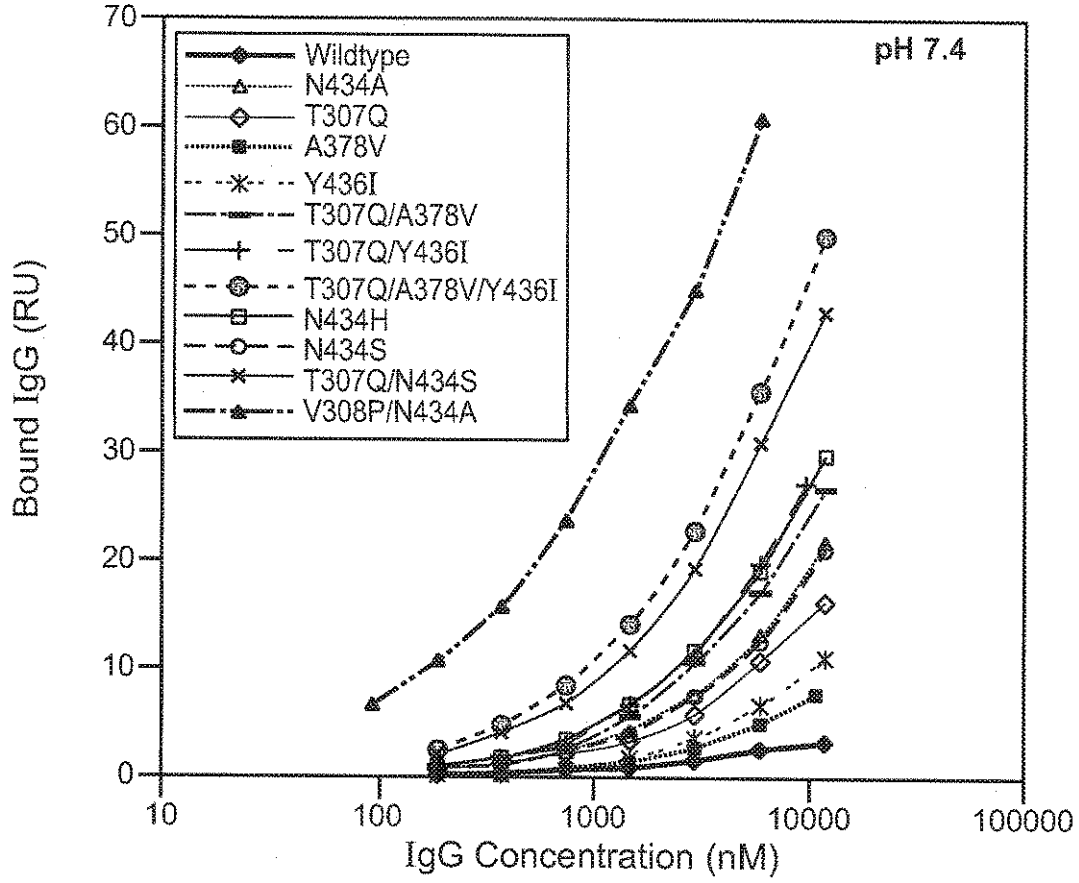


FIG. 3

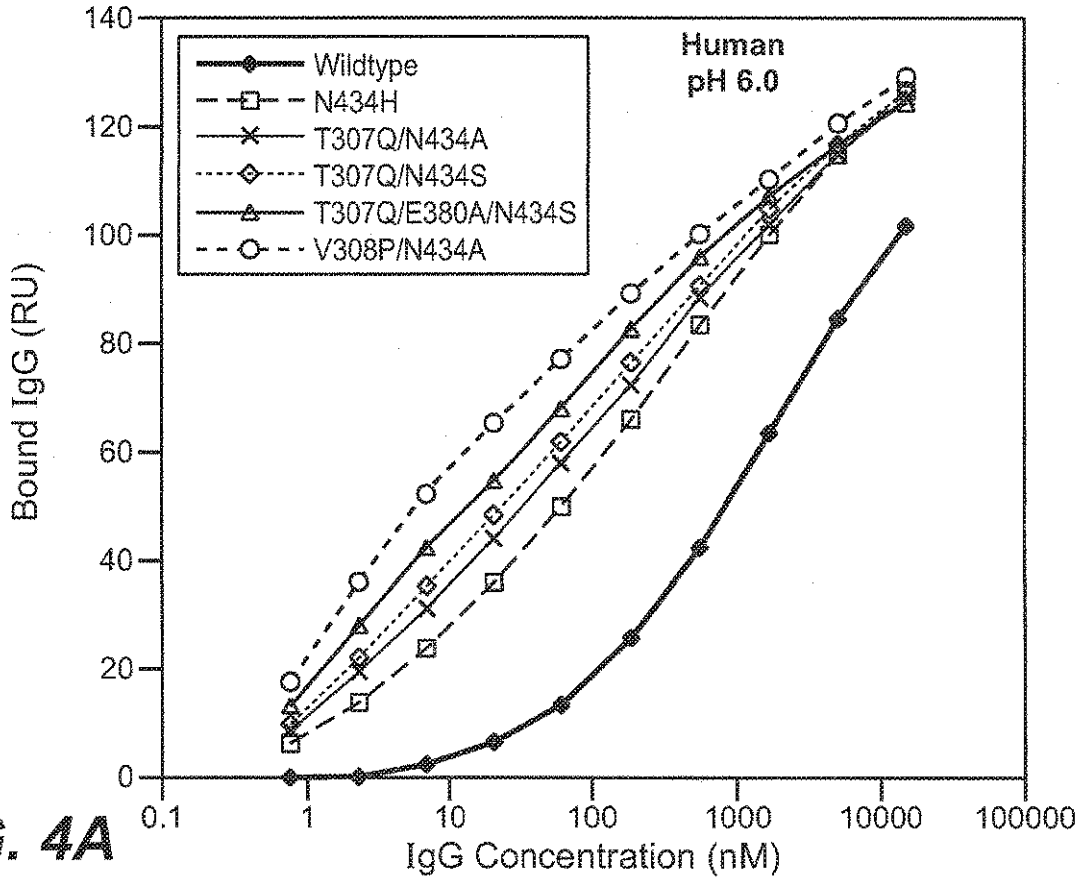

Human
pH 6.0
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A
Bound IgG (RU)
IgG Concentration (nM)
0
20
40
60
80
100
120
140
0.1
1
10
100
1000
10000
100000

FIG. 4A

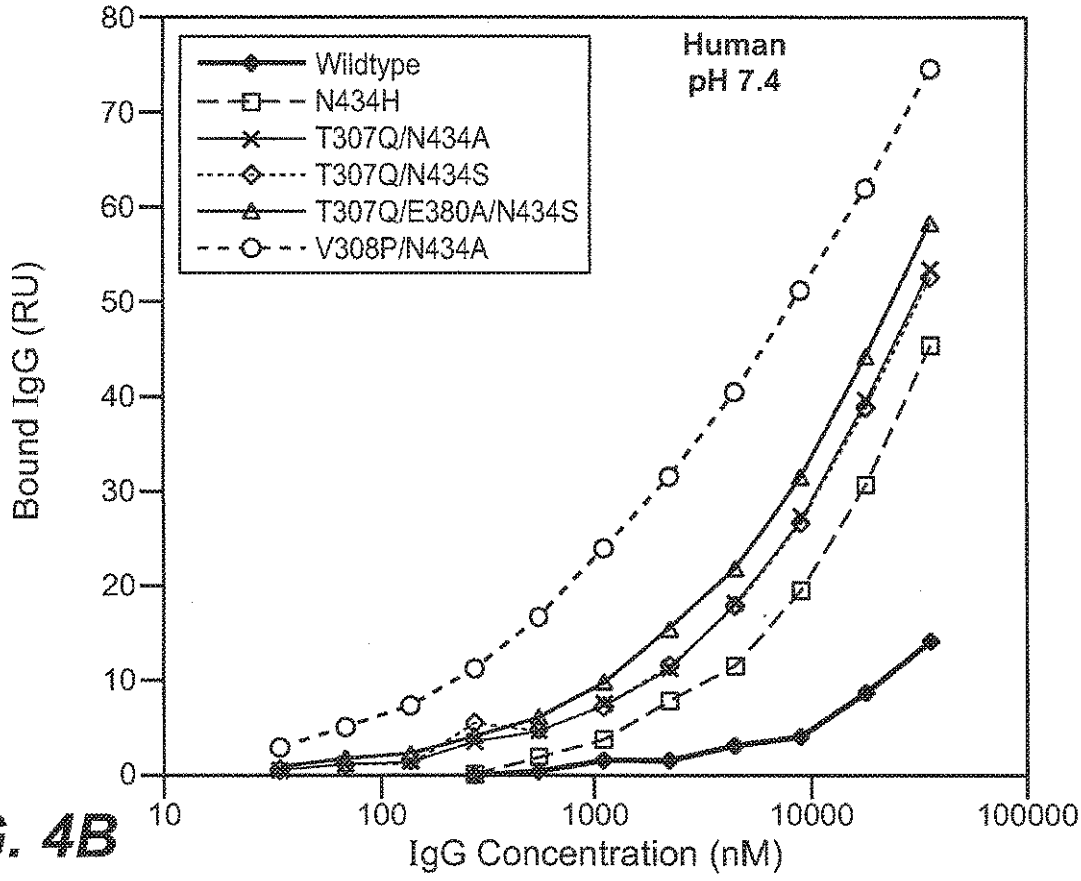

Human
pH 7.4
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A
Bound IgG (RU)
IgG Concentration (nM)
0
10
20
30
40
50
60
70
80
10
100
1000
10000
100000

FIG. 4B

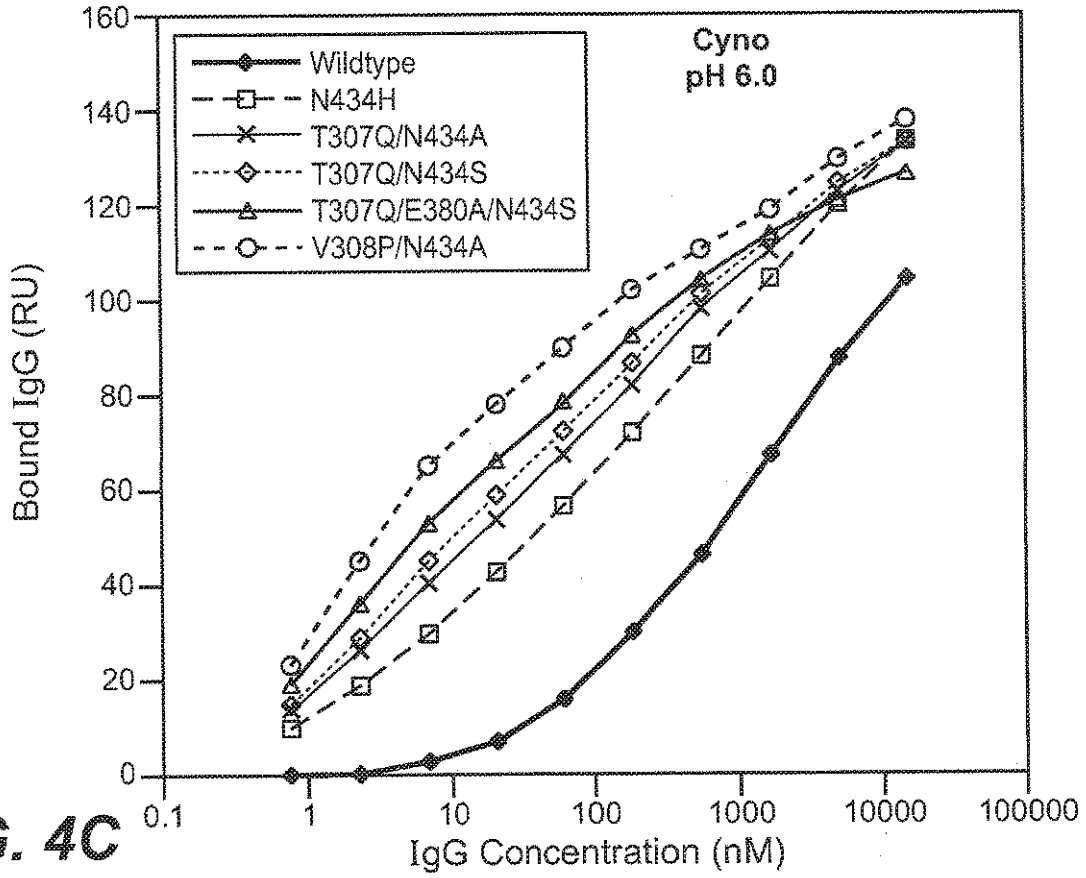

Cyno
pH 6.0
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A
Bound IgG (RU)
IgG Concentration (nM)
0
20
40
60
80
100
120
140
160
0.1
1
10
100
1000
10000
100000

FIG. 4C

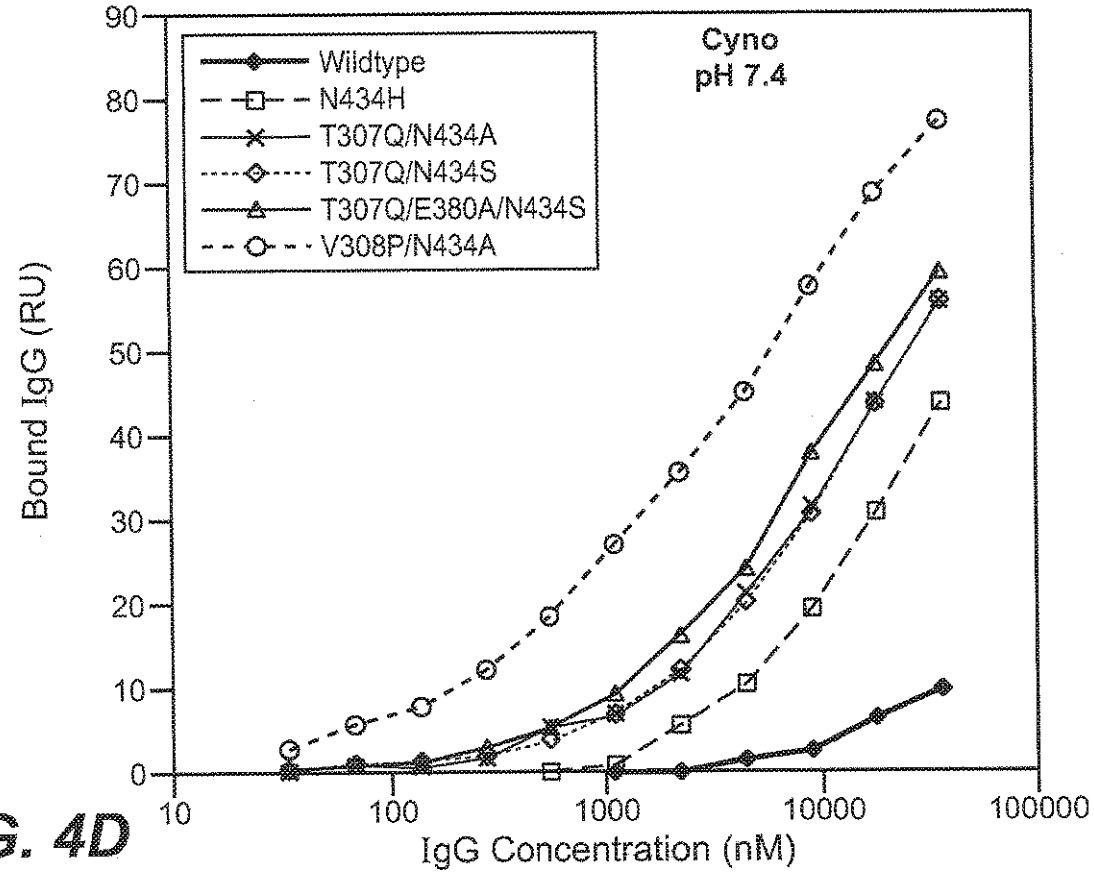

Cyno
pH 7.4
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A
Bound IgG (RU)
IgG Concentration (nM)
0
10
20
30
40
50
60
70
80
90
10
100
1000
10000
100000

FIG. 4D

Human FcRn

Fc Variant	k_{on} ($x10^5$ $M^{-1}s^{-1}$)	k_{off} ($x10^{-2}s^{-1}$)	K_D (nM)[a]	K_D (nM)[b]
Wildtype	1.6 ± 0.4	32 ± 15	1940 ± 520	1870 ± 560
N434H	4.0 ± 0.2	16.6 ± 1.8	420 ± 40	470 ± 30
T307Q/N434A	4.0 ± 0.2	9.6 ± 0.3	240 ± 20	258 ± 4
T307Q/N434S	3.2 ± 0.1	6.0 ± 0.6	185 ± 19	195 ± 13
T307Q/E380A/N434A	3.5 ± 0.2	5.1 ± 0.3	146 ± 18	148 ± 17
V308P/N434A	4.0 ± 0.5	2.9 ± 0.3	72 ± 1	76 ± 3

[a] determined by ratio of k_{off} to k_{on}

[b] determined by fitting the steady state RU against concentration

FIG. 5

Cyno FcRn

Fc Variant	k_{on} ($x10^5$ $M^{-1}s^{-1}$)	k_{off} ($x10^{-2}s^{-1}$)	K_D (nM)[a]	K_D (nM)[b]
Wildtype	2.7 ± 0.9	63 ± 21	2400 ± 100	2400 ± 100
N434H	4.8 ± 0.7	21.8 ± 3.3	450 ± 10	470 ± 10
T307Q/N434A	4.7 ± 0.1	11.2 ± 0.4	240 ± 9	241 ± 5
T307Q/N434S	4.4 ± 0.2	8.5 ± 0.1	195 ± 5	203 ± 6
T307Q/E380A/N434A	4.3 ± 0.1	6.6 ± 0.3	153 ± 4	160 ± 4
V308P/N434A	5.1 ± 0.2	3.4 ± 1.2	66.6 ± 1.8	69.9 ± 2.3

[a] determined by ratio of k_{off} to k_{on}

[b] determined by fitting the steady state RU against concentration

FIG. 6

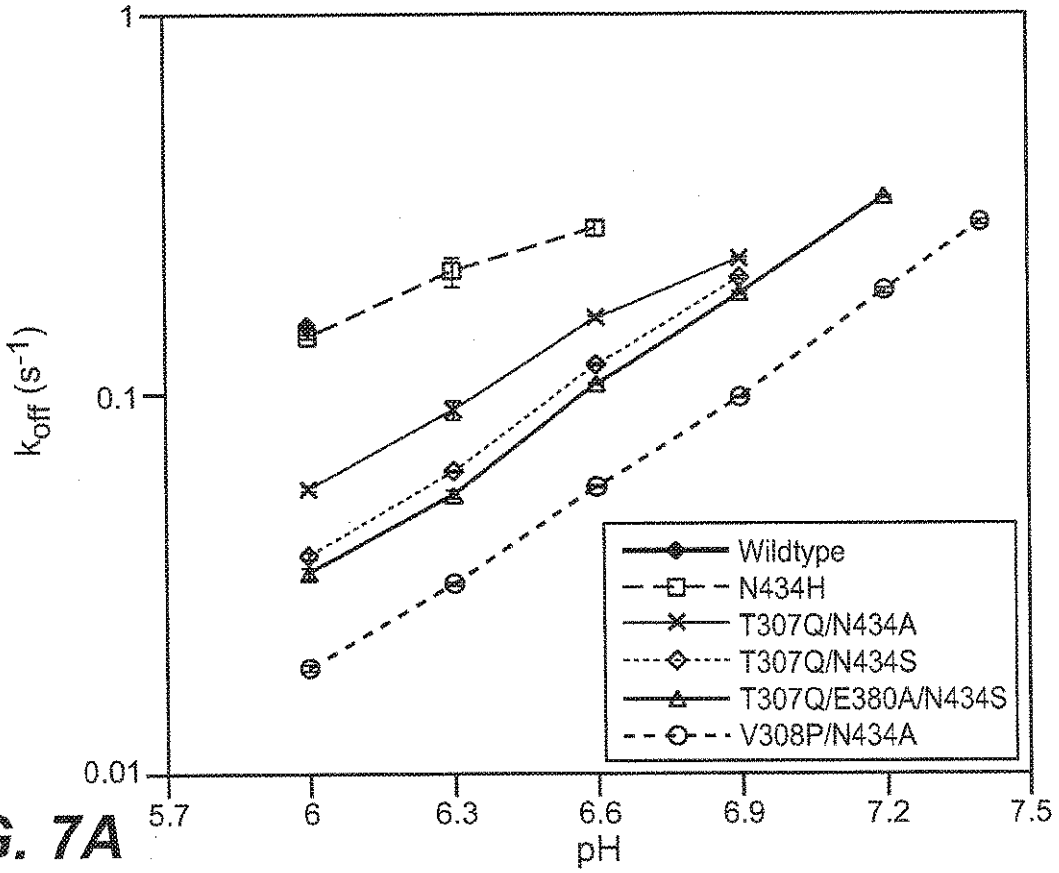

1
0.1
0.01
k_{off} (s^{-1})
5.7
6
6.3
6.6
6.9
7.2
7.5
pH
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A

FIG. 7A

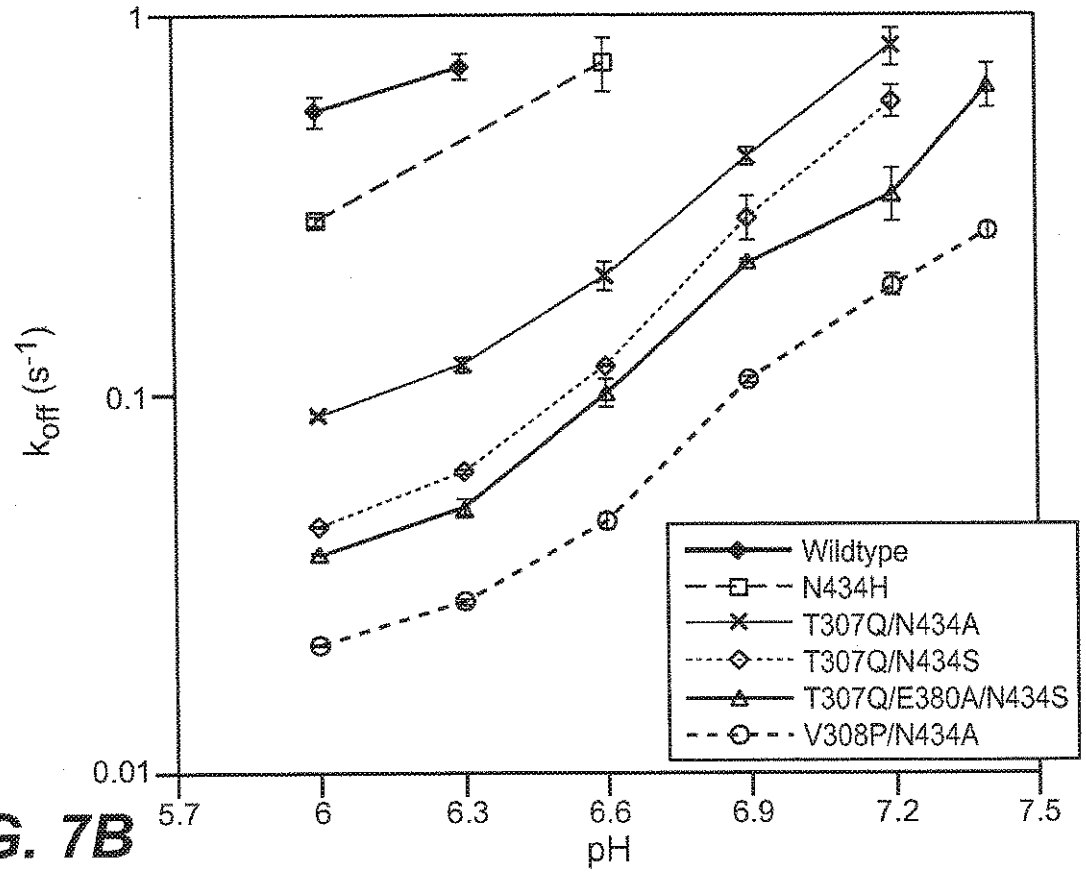

1
0.1
0.01
k_{off} (s^{-1})
5.7
6
6.3
6.6
6.9
7.2
7.5
pH
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A

FIG. 7B

Bevacizumab Fc Variants	Monovalent Affinity Fold ↑ at pH 6.0	Bivalent Affinity Fold ↑ at pH 6.0	Bivalent Affinity Fold ↑ at pH 7.4
Wildtype	1	1	1
N434H	3	9	6
T307Q/N434A	6	15	10
T307Q/N434S	8	21	10
T307Q/E380A/N434A	11	38	17
V308P/N434A	20	83	70

FIG. 8

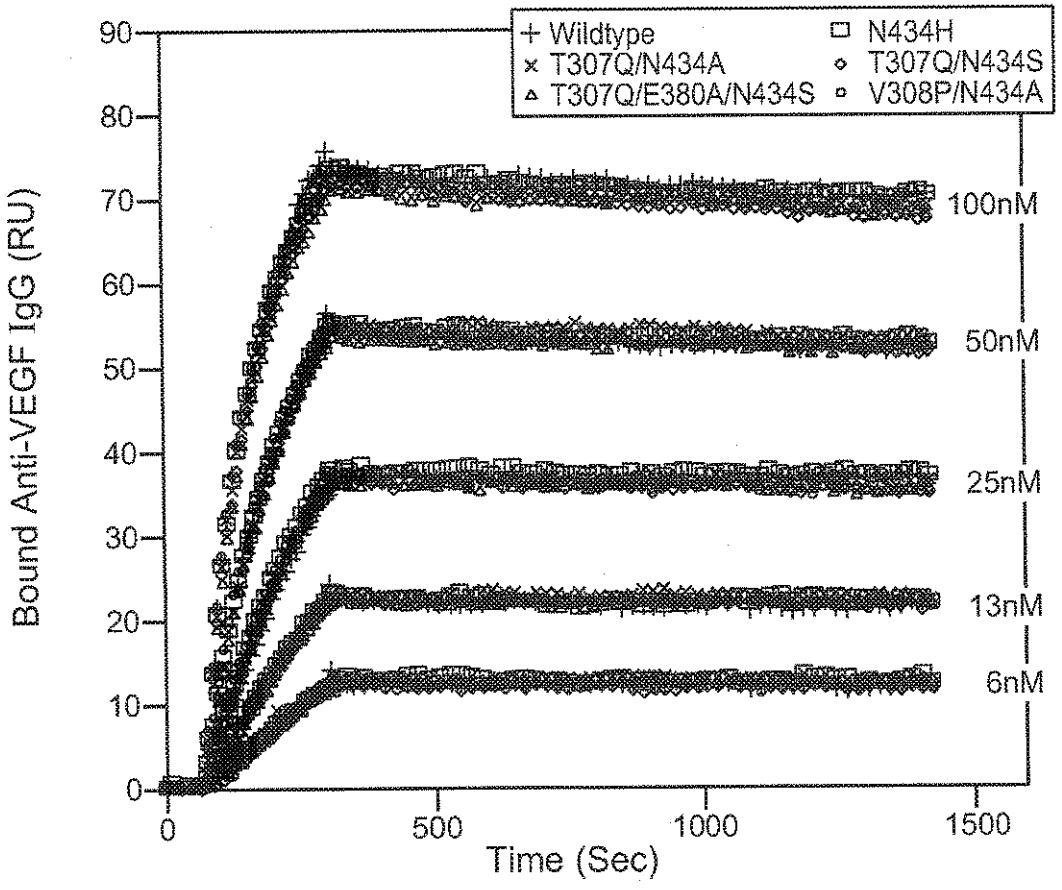


FIG. 9A

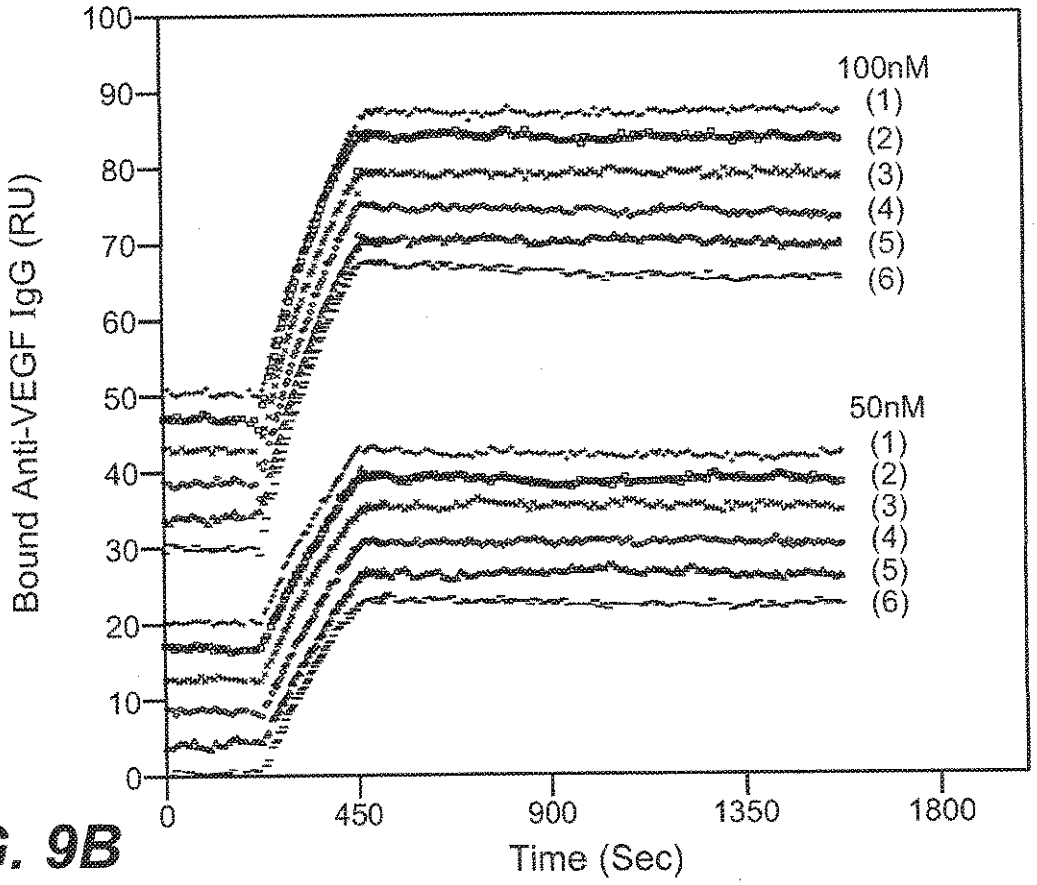

Bound Anti-VEGF IgG (RU)
Time (Sec)
100nM
(1)
(2)
(3)
(4)
(5)
(6)
50nM
(1)
(2)
(3)
(4)
(5)
(6)
0
10
20
30
40
50
60
70
80
90
100
0
450
900
1350
1800

FIG. 9B

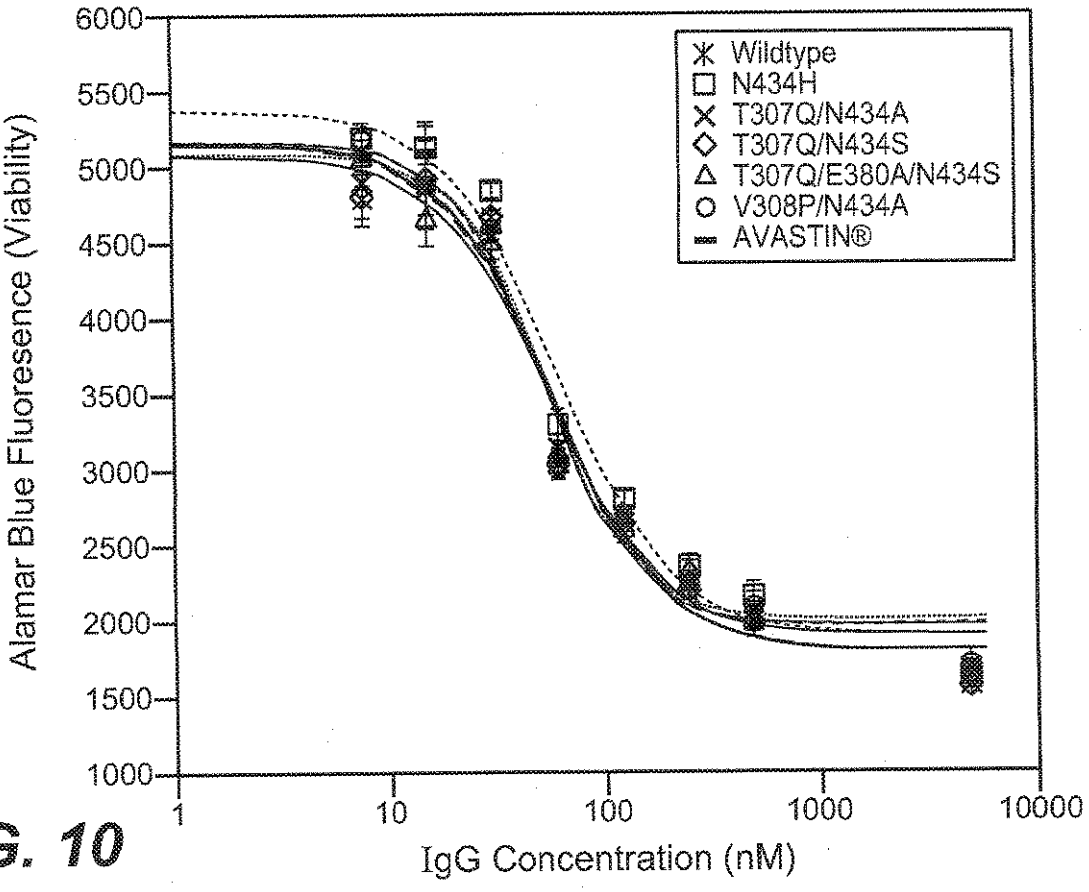

Alamar Blue Fluoresence (Viability)
IgG Concentration (nM)
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A
AVASTIN®
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
1
10
100
1000
10000

FIG. 10

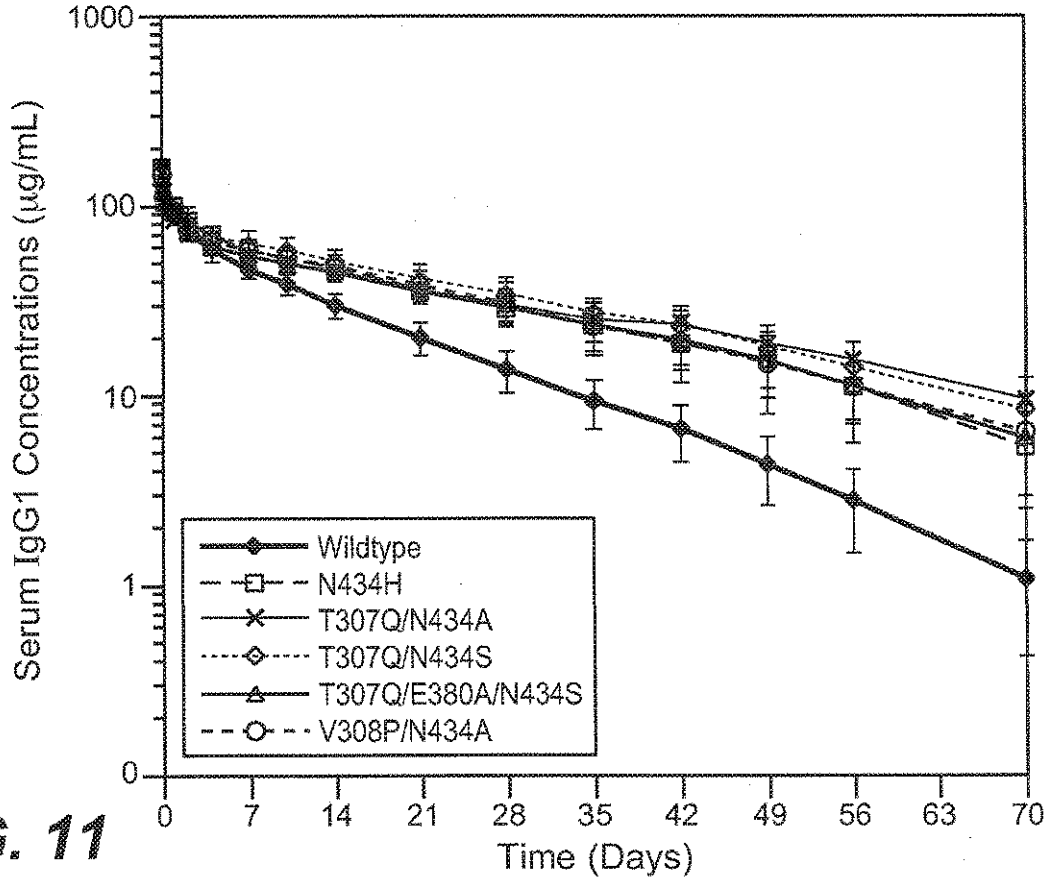

1000
100
10
1
0
Serum IgG1 Concentrations (µg/mL)
0
7
14
21
28
35
42
49
56
63
70
Time (Days)
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A

FIG. 11

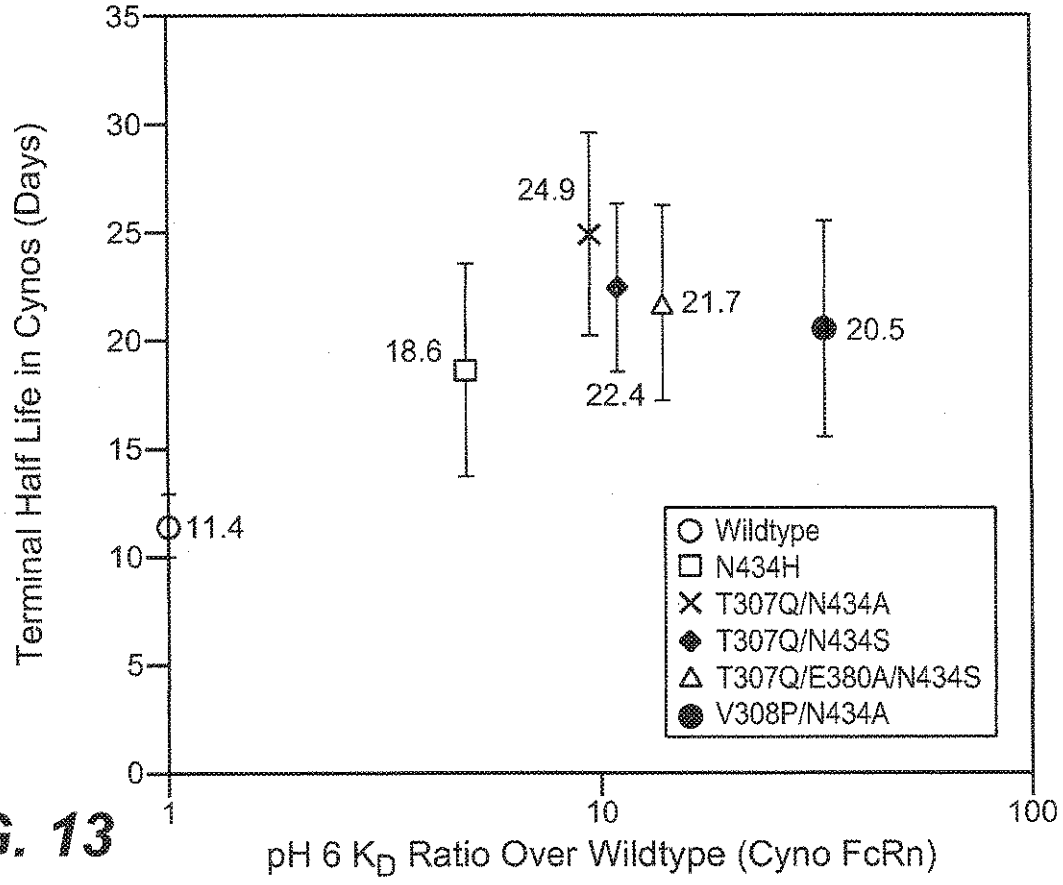

35
30
25
20
15
10
5
0
Terminal Half Life in Cynos (Days)
11.4
18.6
24.9
22.4
21.7
20.5
1
10
100
pH 6 K_D Ratio Over Wildtype (Cyno FcRn)
Wildtype
N434H
T307Q/N434A
T307Q/N434S
T307Q/E380A/N434S
V308P/N434A

FIG. 13

	Wildtype[a]	N434H[a]	T307Q/N434A[a]	T307Q/N434S[a]	T307Q/E380A/N434S[a]	V308P/N434A[b]
AUC_∞ (Day*μg/mL)	1280 ± 210	2190 ± 570*	2480 ± 470*	2680 ± 490*	2250 ± 370*	2360 ± 560*
$t_{1/2,\alpha}$ (Hr)	12.0 ± 6.7	14.0 ± 3.6	10.2 ± 3.2	15.2 ± 5.2	13.8 ± 5.1	11.8 ± 5.5
$t_{1/2,\beta}$ (Day)	11.4 ± 1.5	18.6 ± 4.9*	24.9 ± 4.7*	22.4 ± 3.9*	21.7 ± 4.5*	20.5 ± 5.0*
C_{max} (μg/mL)	136 ± 16	154 ± 15	142 ± 8	163 ± 11	137 ± 11	155 ± 15
CL (μg/day/kg)	4.01 ± 0.67	2.48 ± 0.92*	2.08 ± 0.40*	1.95 ± 0.49*	2.28 ± 0.36*	2.27 ± 0.70*
V_1 (mL/kg)	37.4 ± 4.1	32.8 ± 3.0	35.2 ± 2.0	30.8 ± 2.3	36.7 ± 2.7	32.5 ± 3.1
V_{ss} (mL/kg)	63.1 ± 5.7	59.3 ± 4.4	71.5 ± 6.7	58.9 ± 4.8	67.7 ± 7.4	61.4 ± 6.4

[a] n=12 animals; [b] n=11 animals.

Data is shown as average ± standard deviation

AUC_∞=total drug exposure defined as area under the concentration-time curve extrapolated to infinity;

$t_{1/2,\alpha}$ =half-life of the alpha phase (α half-life)

$t_{1/2,\beta}$=half-life of the beta phase (β half-life)

C_{max}=maximum observed concentration

CL=clearance

V_1=volume of the central compartment

V_{ss}=volume of distribution at steady state

* Statistically significant from wildtype ($p<0.001$)

FIG. 12

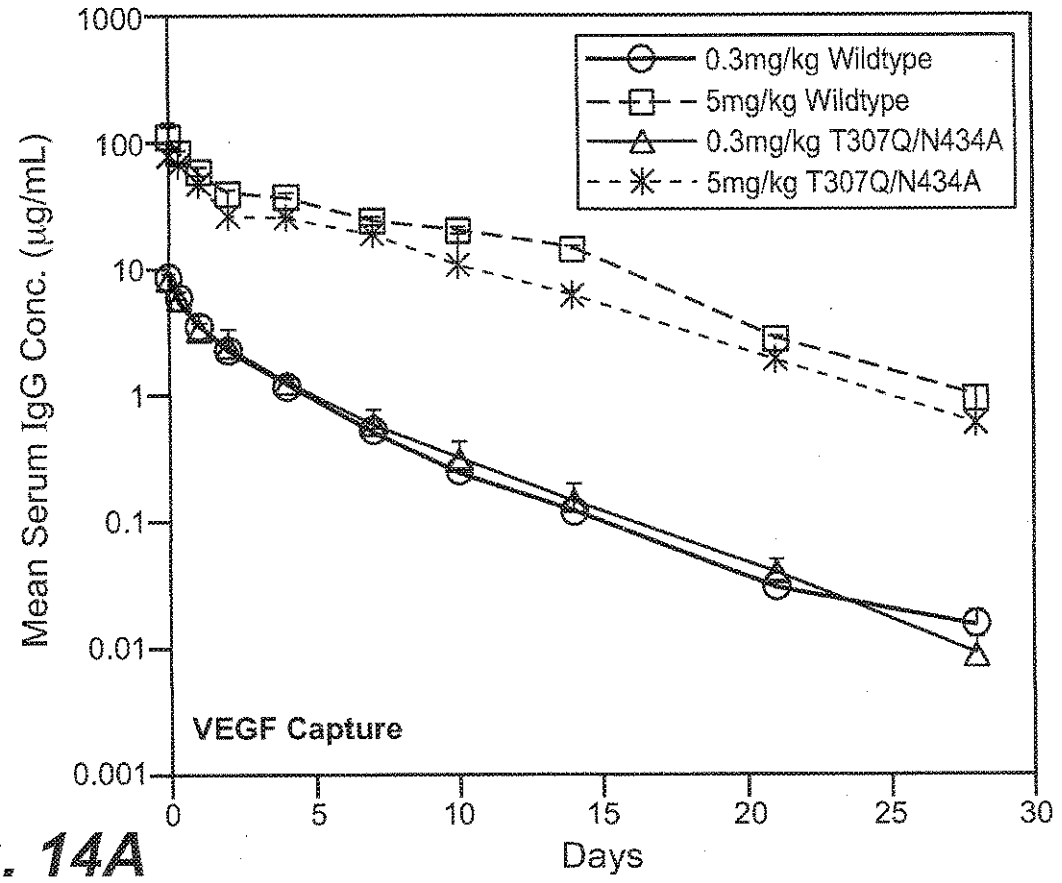


FIG. 14A

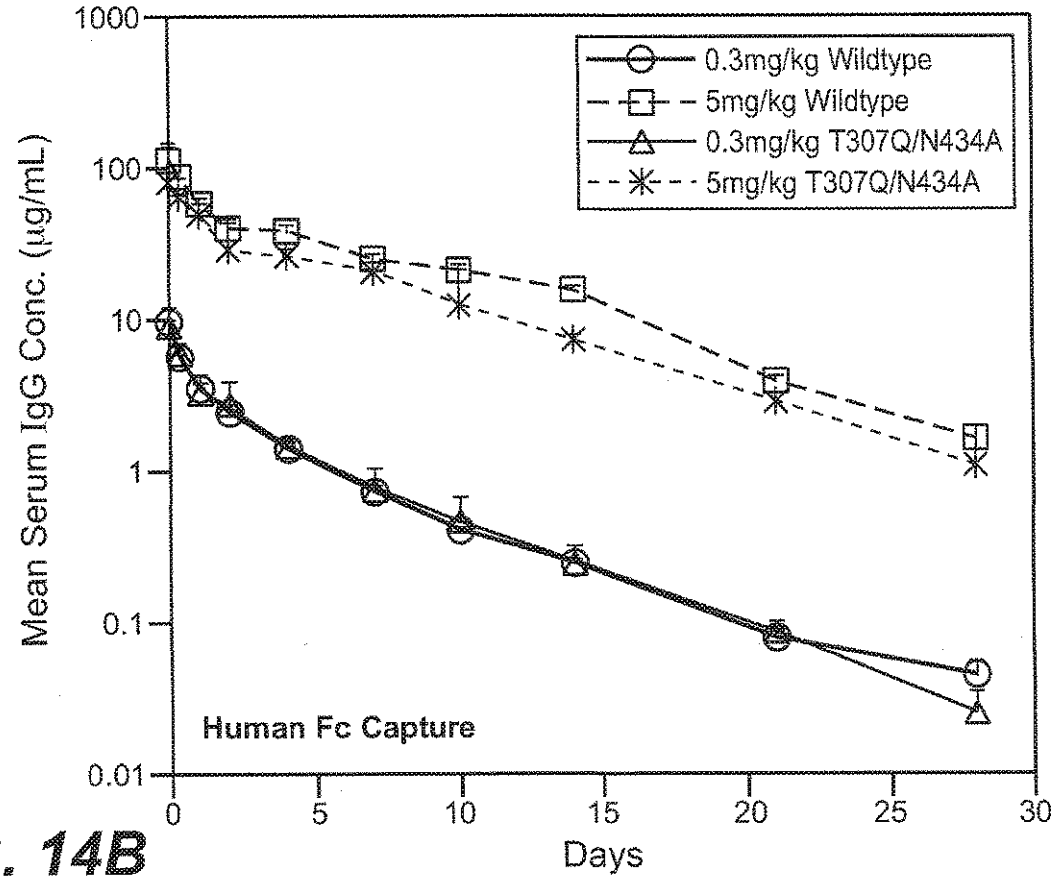


FIG. 14B

From VEGF Capture

Group	Dose (mg/kg)	Cmax (μg/mL)	AUC (Day*μg/mL)	CL (Day*μg/mL)	$t_{1/2,\beta}$ (Day)	V_1 (mL/kg)	V_{ss} (mL/kg)
Wildtype	0.3[a]	8.8	16.4	20.1	2.9	38	71
Wildtype	5[a]	115	519	9.63	6.0	43	79
T307Q/N434A	0.3[a]	8.7	17.7	20.9	2.9	43	78
T307Q/N434A	5[a]	93	352	14.2	5.2	54	99

From Human Fc Capture

Group	Dose (mg/kg)	Cmax (μg/mL)	AUC (Day*μg/mL)	CL (Day*μg/mL)	$t_{1/2,\beta}$ (Day)	V_1 (mL/kg)	V_{ss} (mL/kg)
Wildtype	0.3[a]	9.2	20	17	3.8	36	79
Wildtype	5[a]	113	538	9.3	6.5	44	83
T307Q/N434A	0.3[a]	9.4	21	18	3.5	39	81
T307Q/N434A	5[a]	91	381	13	5.6	55	100

[a] n=8 animals; [b] n=9 animals.

Data is shown as average ± standard deviation

AUC_∞=total drug exposure defined as area under the concentration-time curve extrapolated to infinity;

$t_{1/2,\beta}$=half-life of the β phase (terminal half-life)

C_{max}=maximum observed concentration

CL=clearance

V_1=volume of the central compartment

V_{ss}=volume of distribution at steady state

FIG. 15

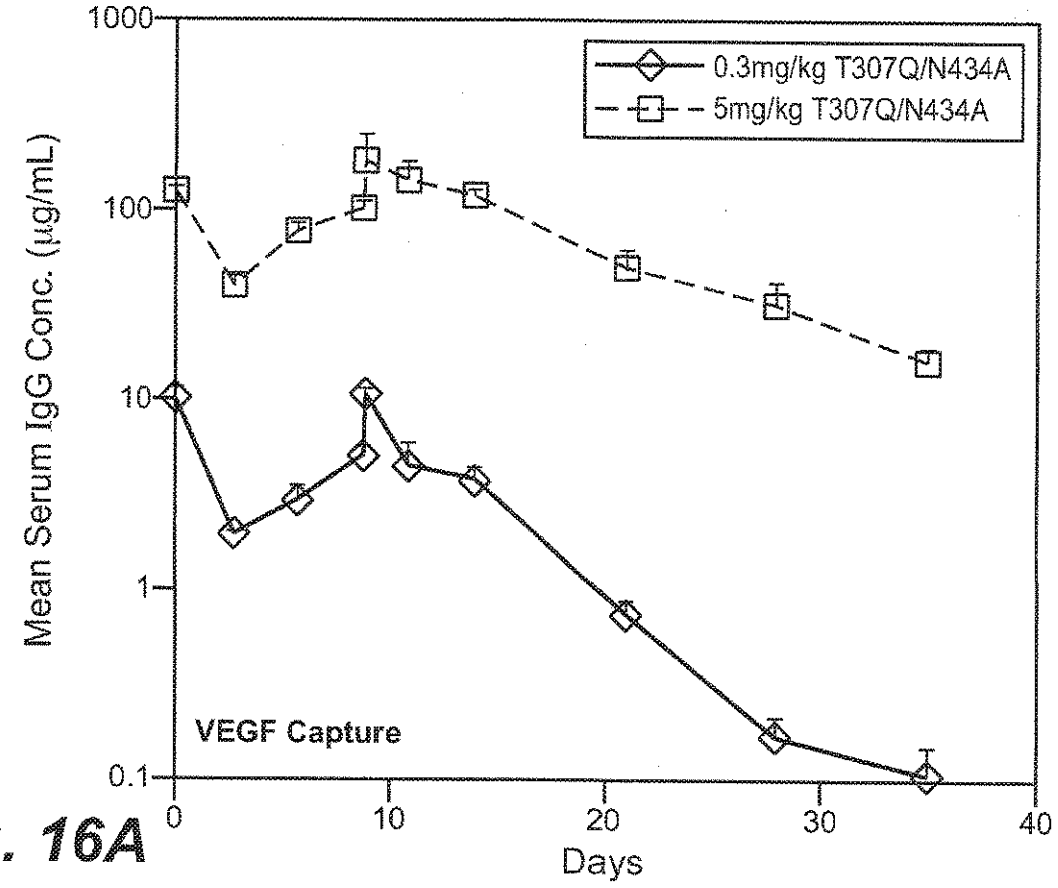


FIG. 16A

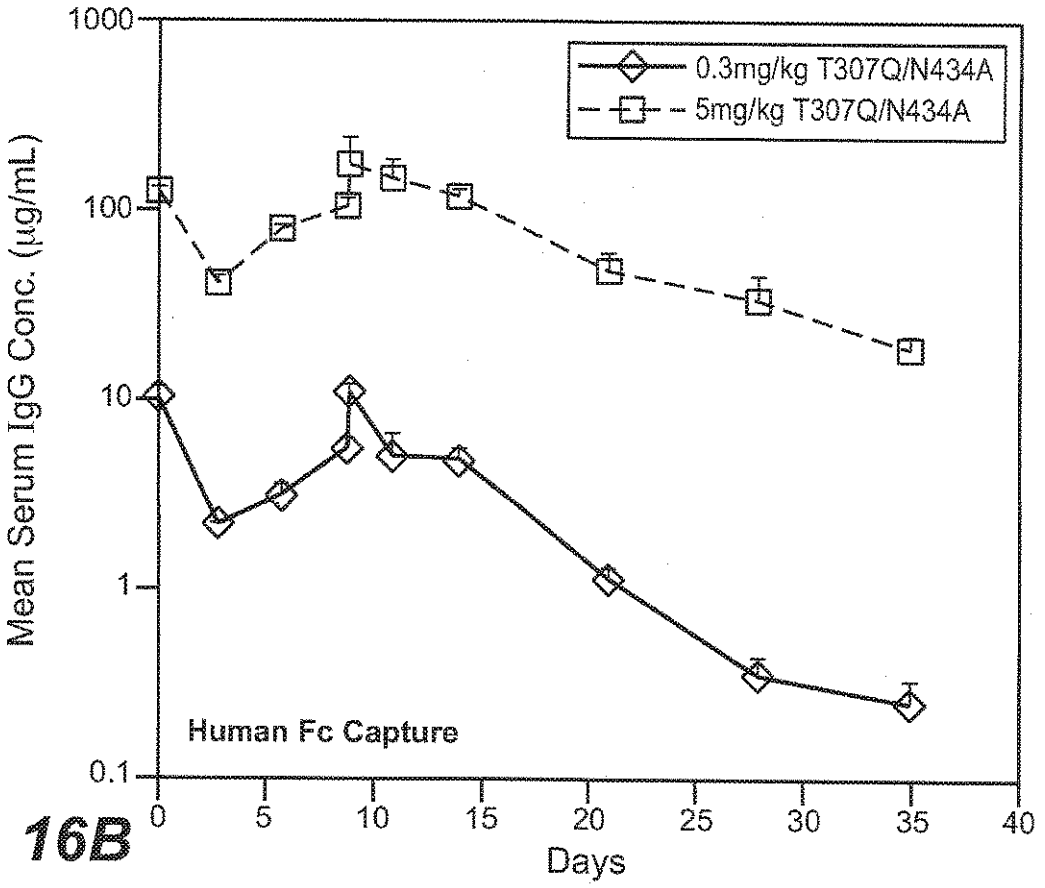


FIG. 16B

From VEGF Capture

Group	Dose (mg/kg)	AUC (Day*µg/mL)	CL (Day*µg/mL)	$t_{1/2,\beta}$ (Day)	V_1 (mL/kg)	V_{ss} (mL/kg)
T307Q/N434A	0.3	25	15	4.3	41	71
T307Q/N434A	5	715	7.0	8.4	44	76

From Human Fc Capture

Group	Dose (mg/kg)	AUC (Day*µg/mL)	CL (Day*µg/mL)	$t_{1/2,\beta}$ (Day)	V_1 (mL/kg)	V_{ss} (mL/kg)
T307Q/N434A	0.3	29.1	13	5.3	40	76
T307Q/N434A	5	757	6.6	9.0	43	77

n=9 animals.

Data is shown as average ± standard deviation

AUC_∞=total drug exposure defined as area under the concentration-time curve extrapolated to infinity;

$t_{1/2,\beta}$=half-life of the β phase (terminal half-life)

CL=clearance

V_1=volume of the central compartment

V_{ss}=volume of distribution at steady state

FIG. 17

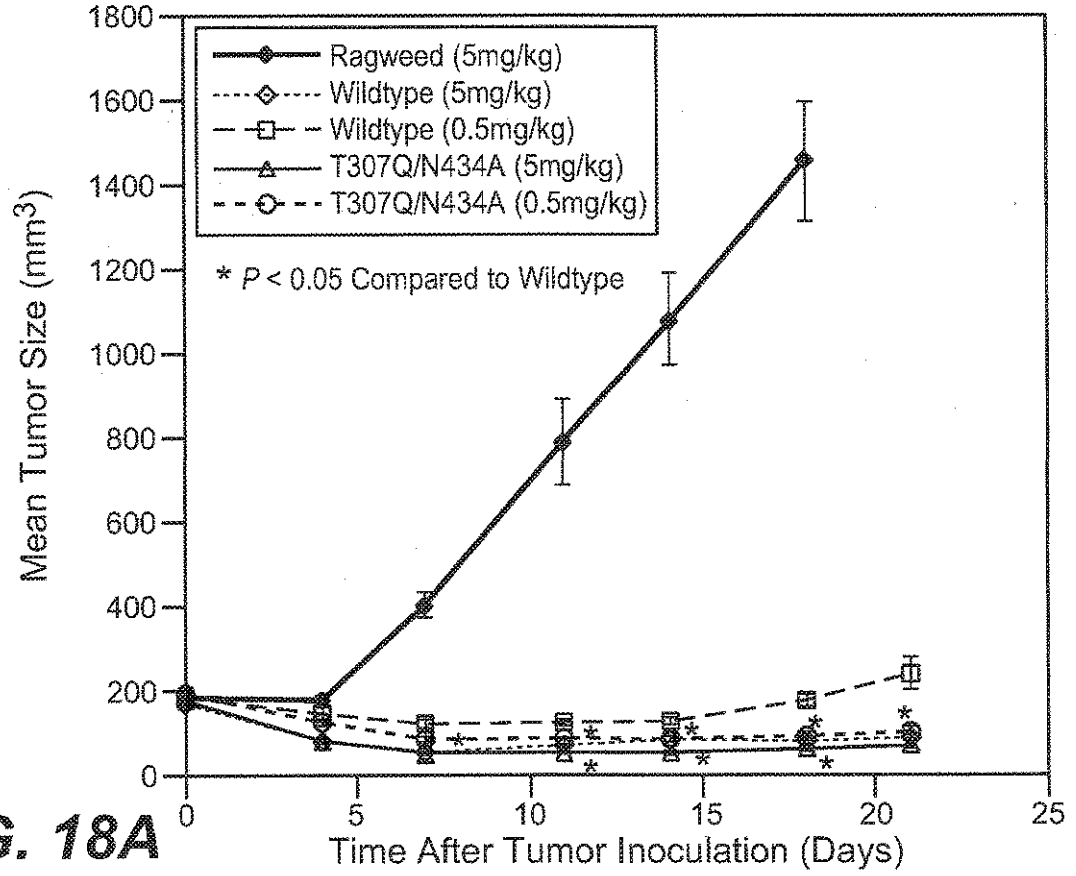


FIG. 18A

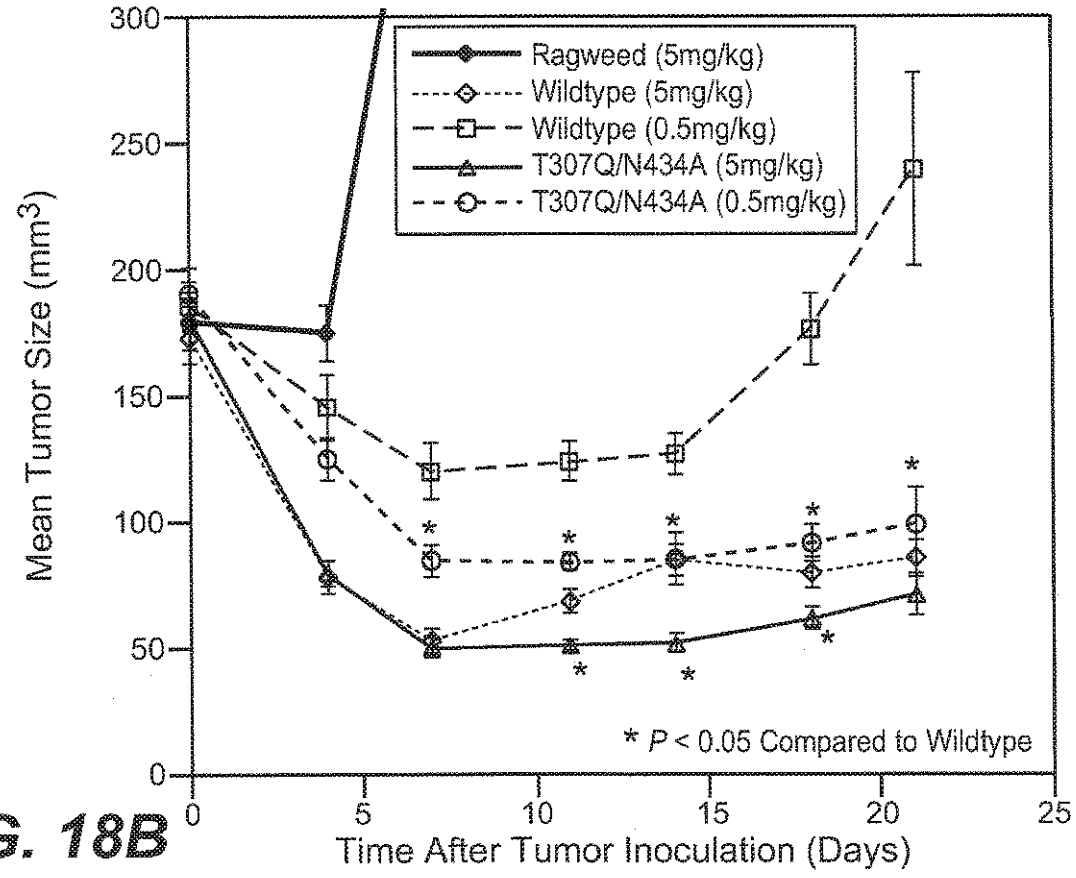


FIG. 18B

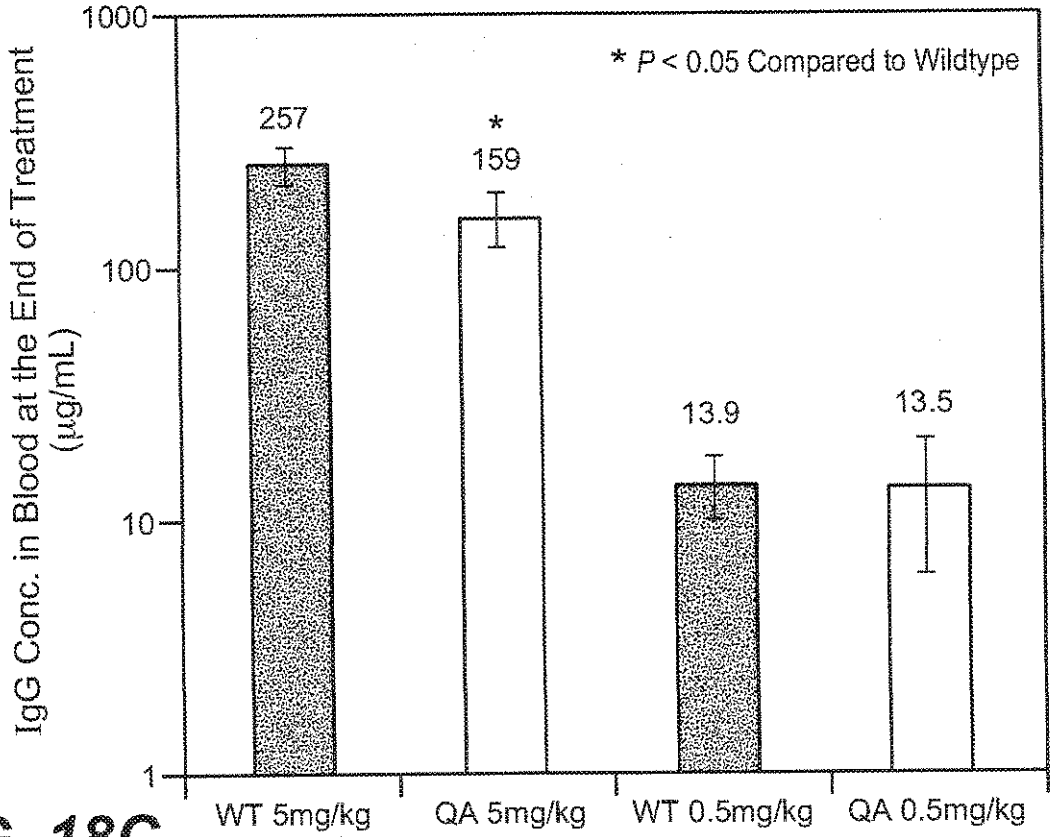


FIG. 18C

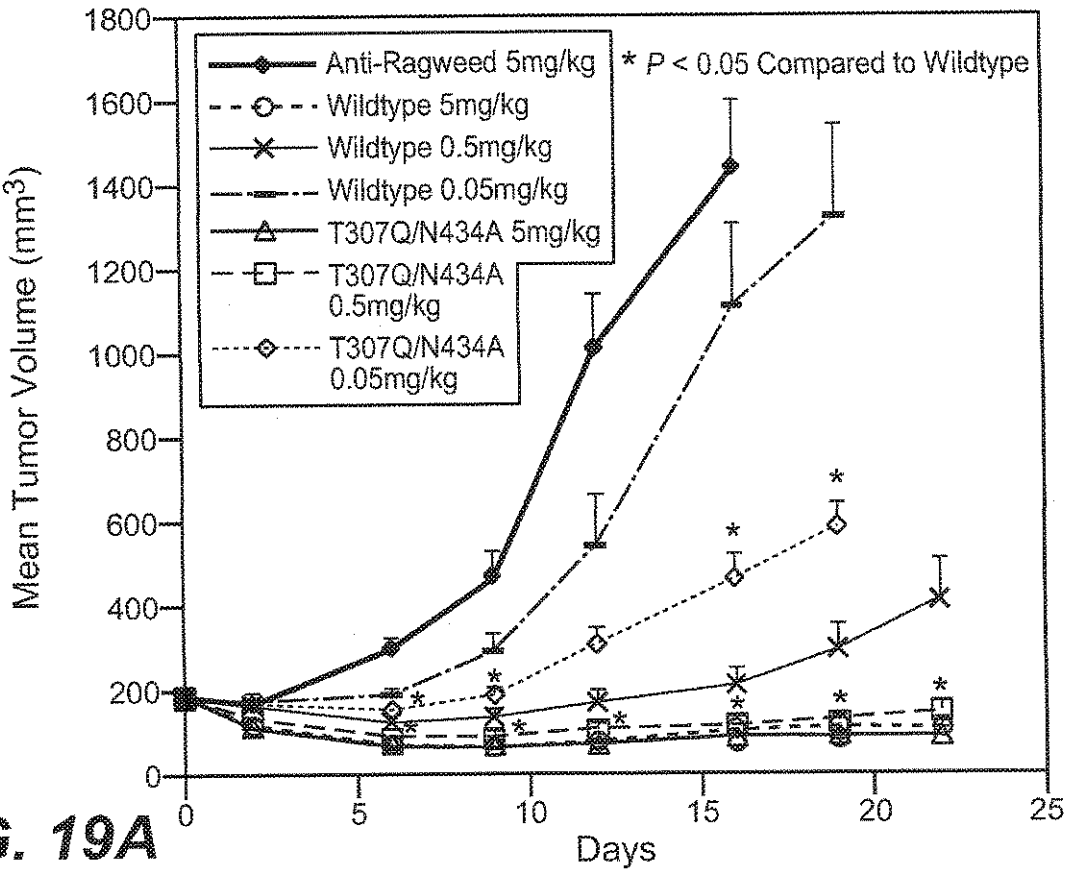


FIG. 19A

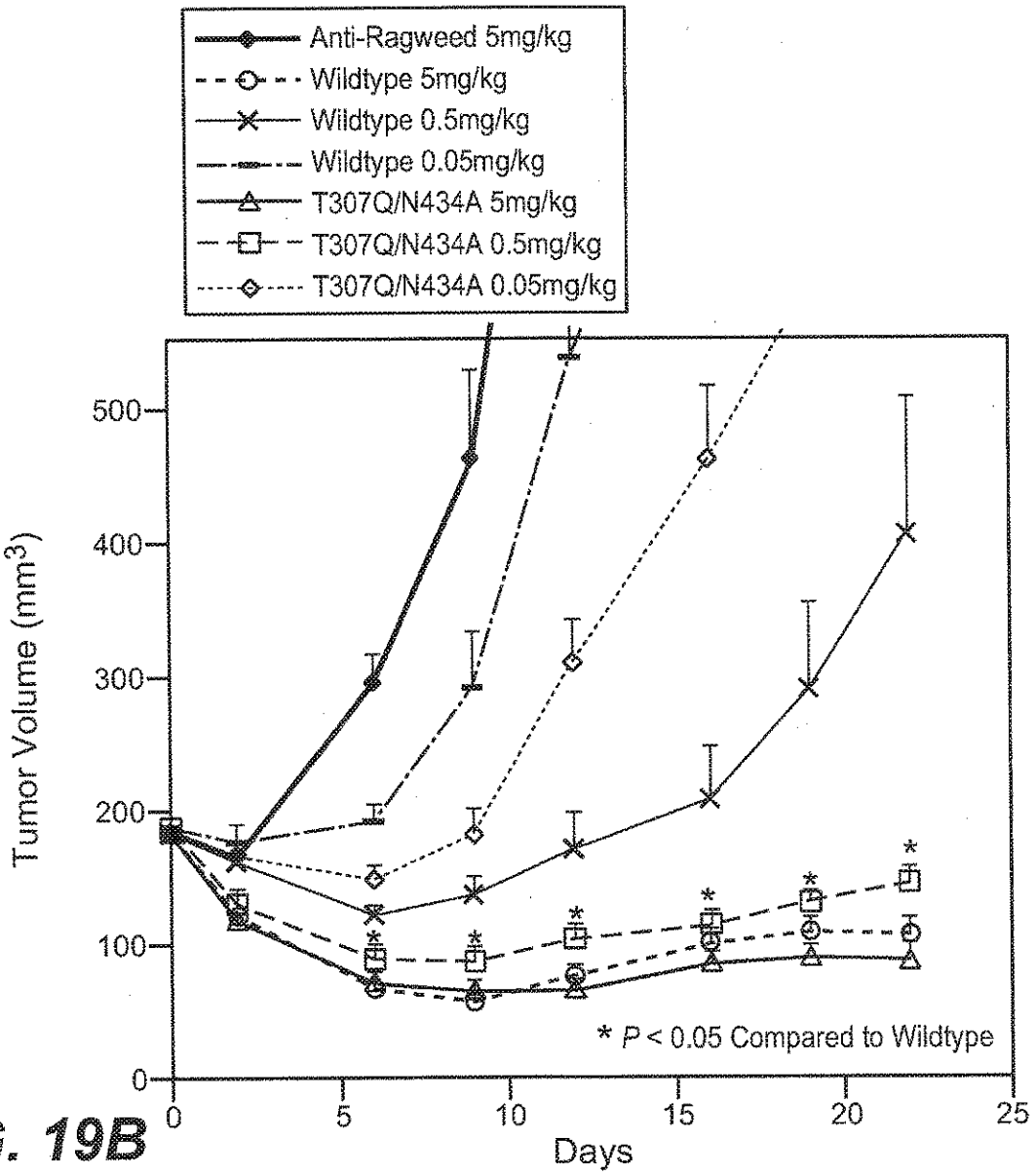

Anti-Ragweed 5mg/kg
Wildtype 5mg/kg
Wildtype 0.5mg/kg
Wildtype 0.05mg/kg
T307Q/N434A 5mg/kg
T307Q/N434A 0.5mg/kg
T307Q/N434A 0.05mg/kg
Tumor Volume (mm3)
0
100
200
300
400
500
0
5
10
15
20
25
Days
* P < 0.05 Compared to Wildtype

FIG. 19B

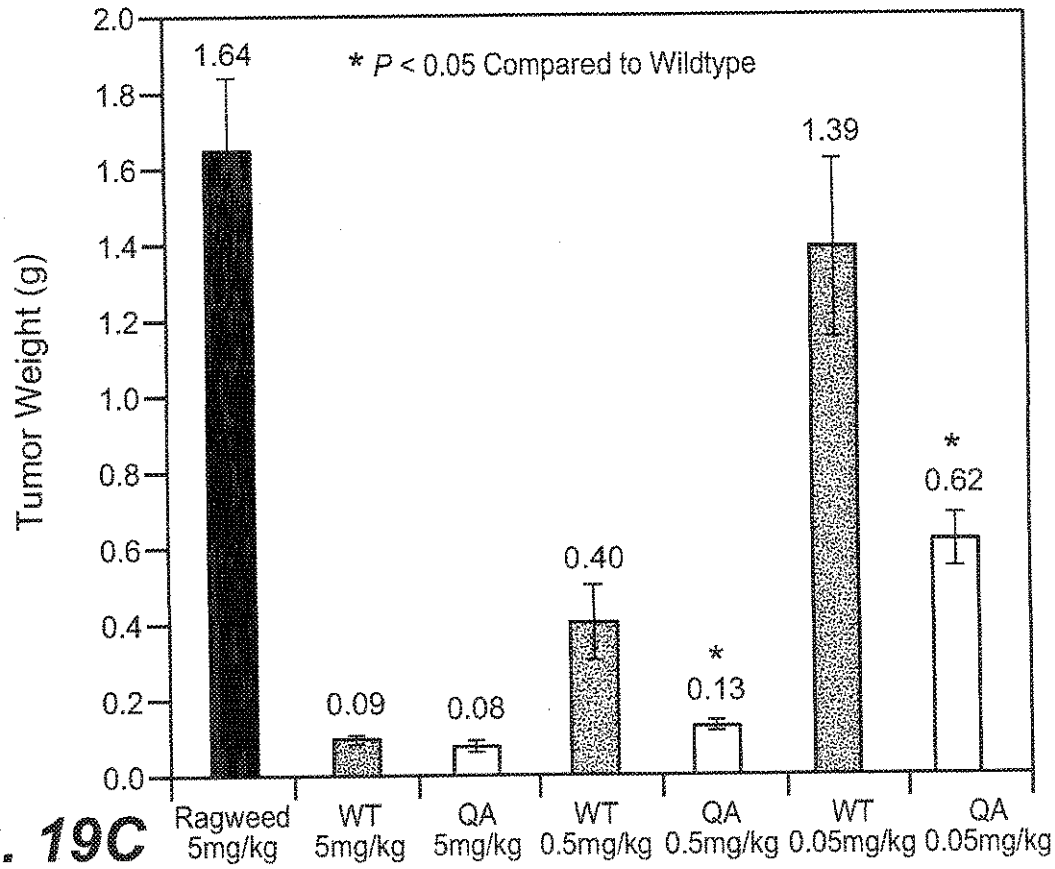

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Tumor Weight (g)
* P < 0.05 Compared to Wildtype
1.64
0.09
0.08
0.40
*
0.13
1.39
*
0.62
Ragweed 5mg/kg
WT 5mg/kg
QA 5mg/kg
WT 0.5mg/kg
QA 0.5mg/kg
WT 0.05mg/kg
QA 0.05mg/kg

FIG. 19C

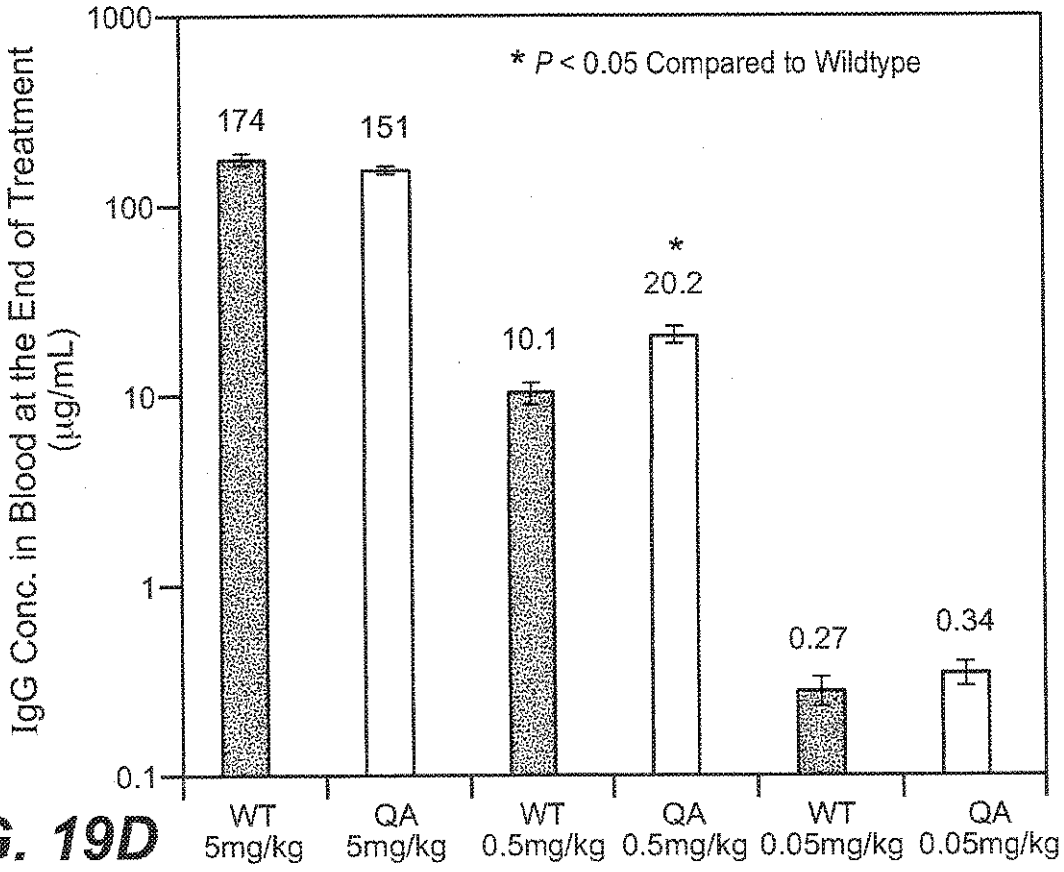


FIG. 19D

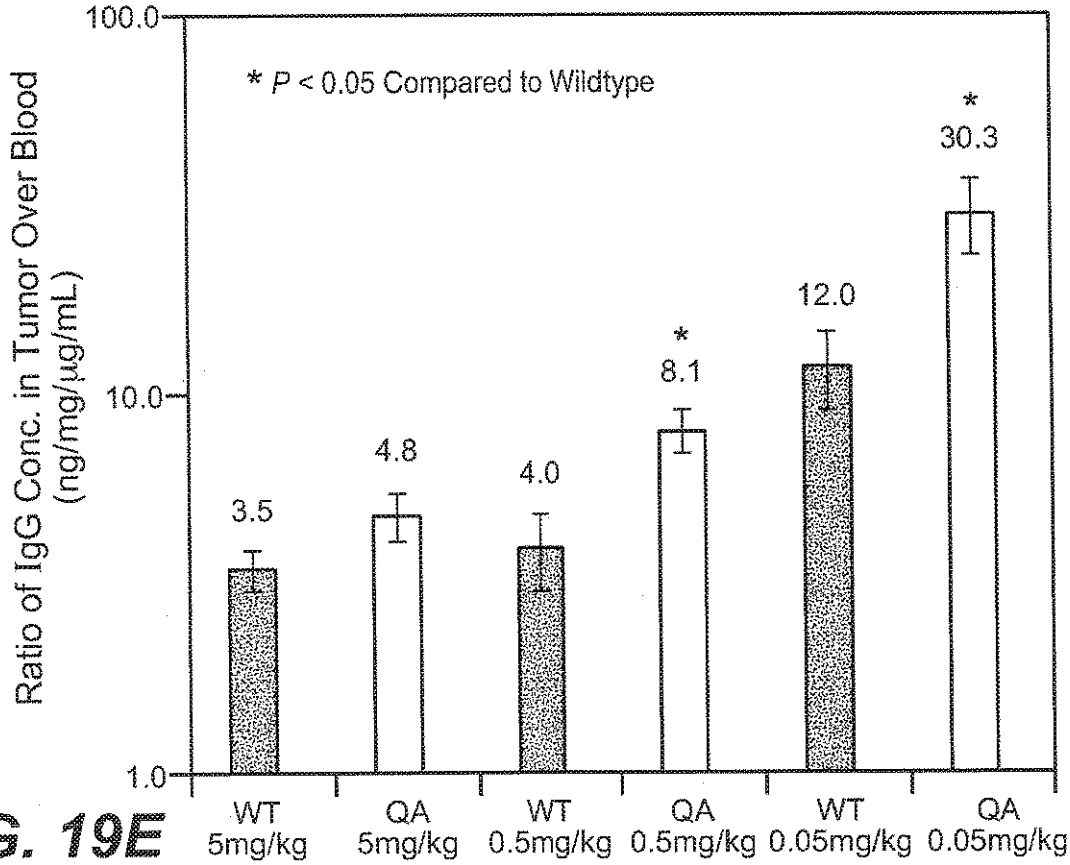


FIG. 19E

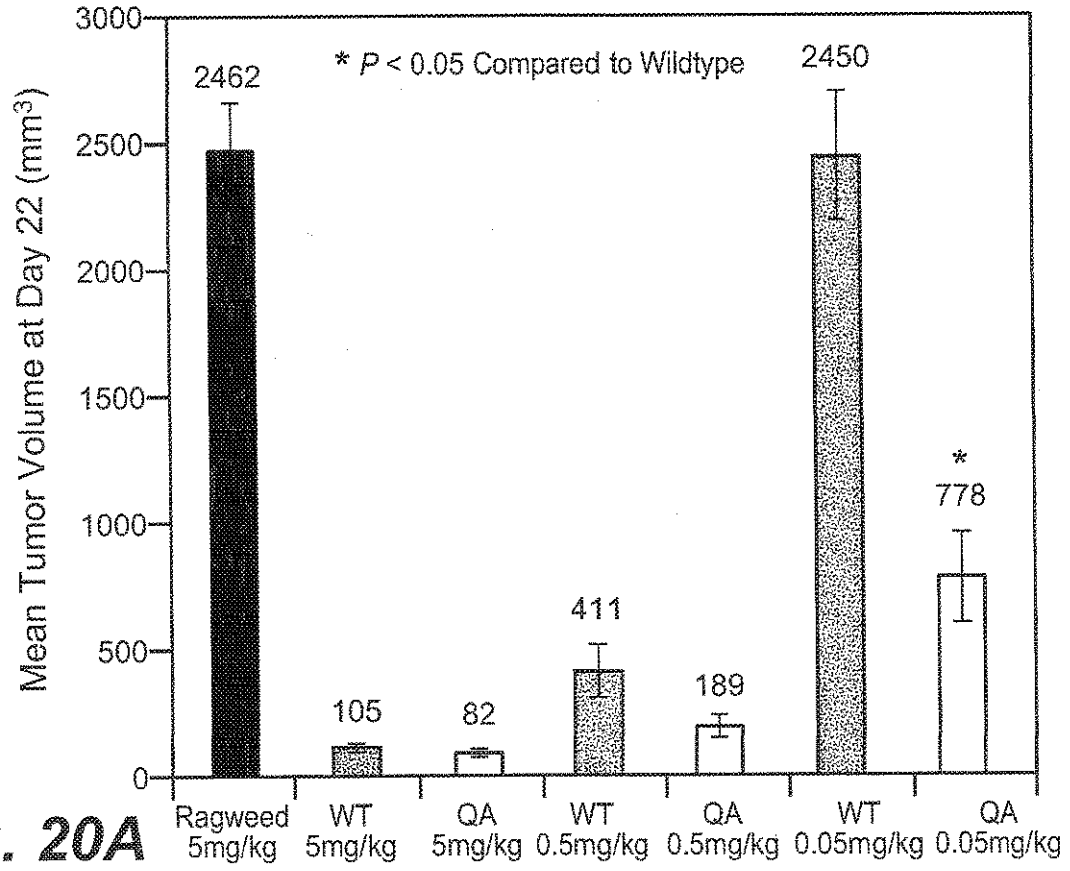


FIG. 20A

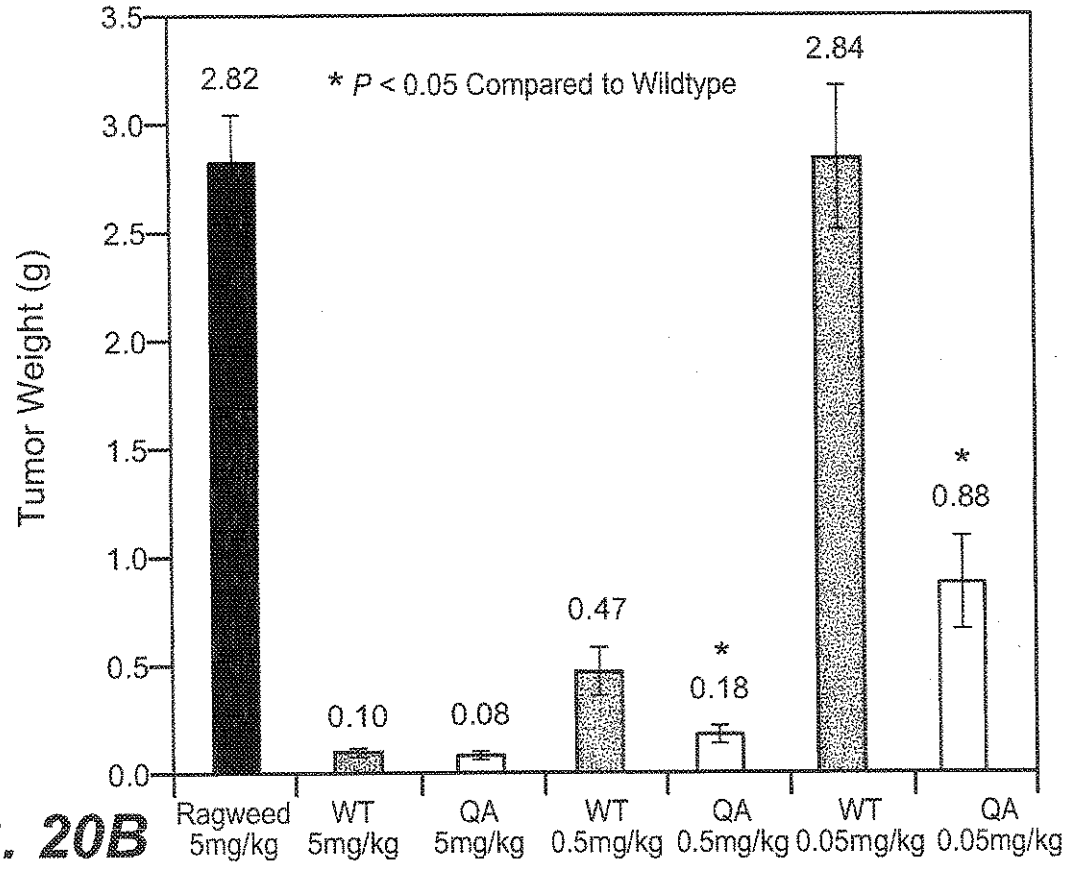


FIG. 20B

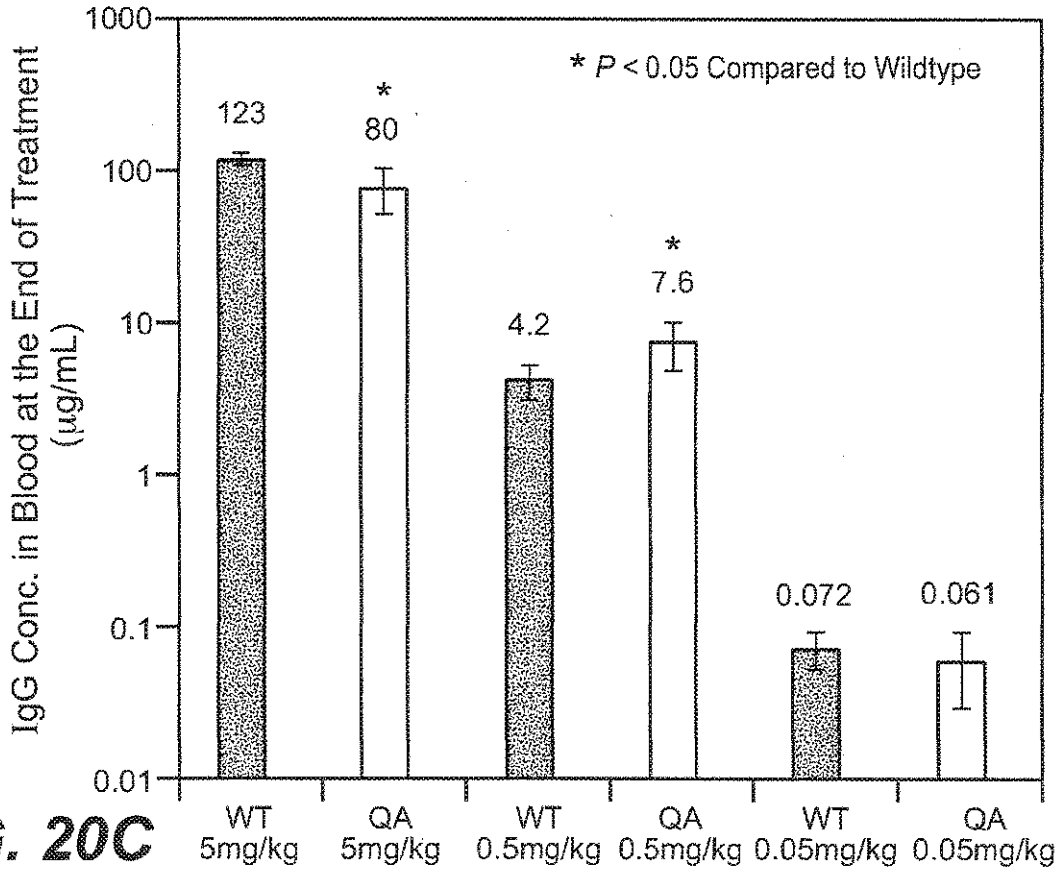


FIG. 20C

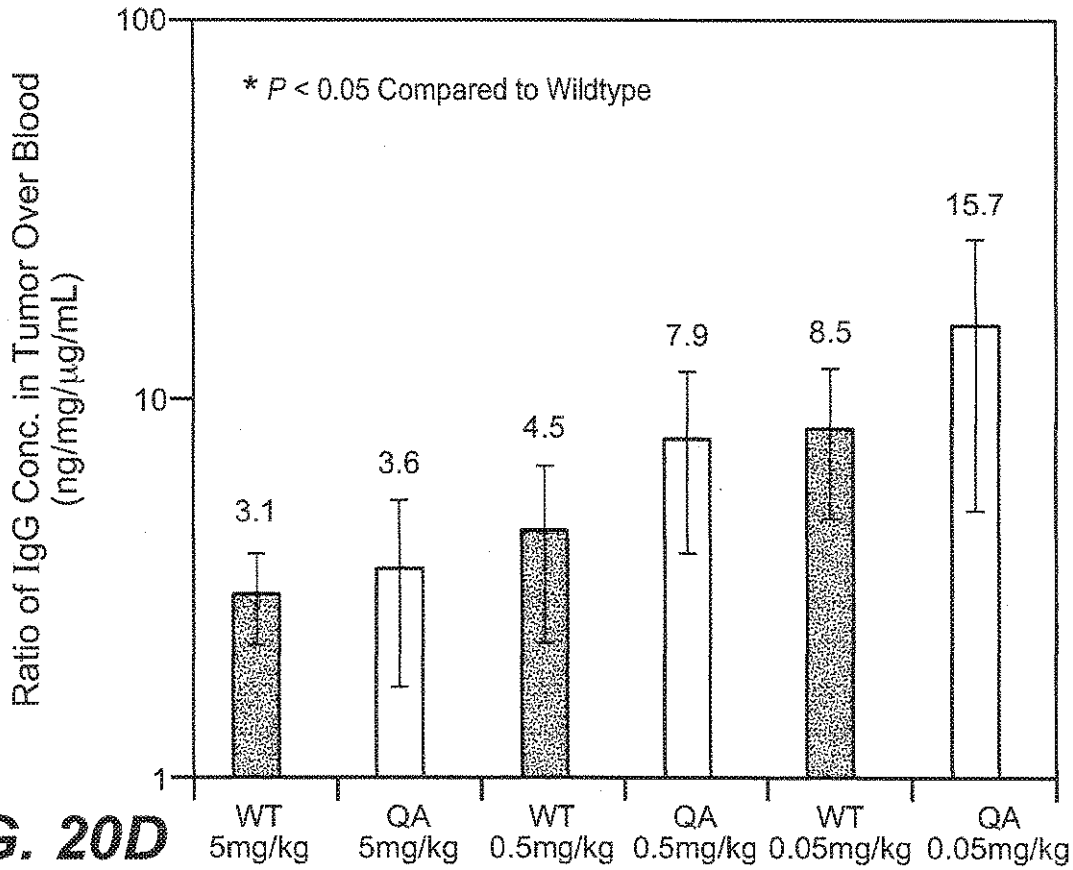


FIG. 20D

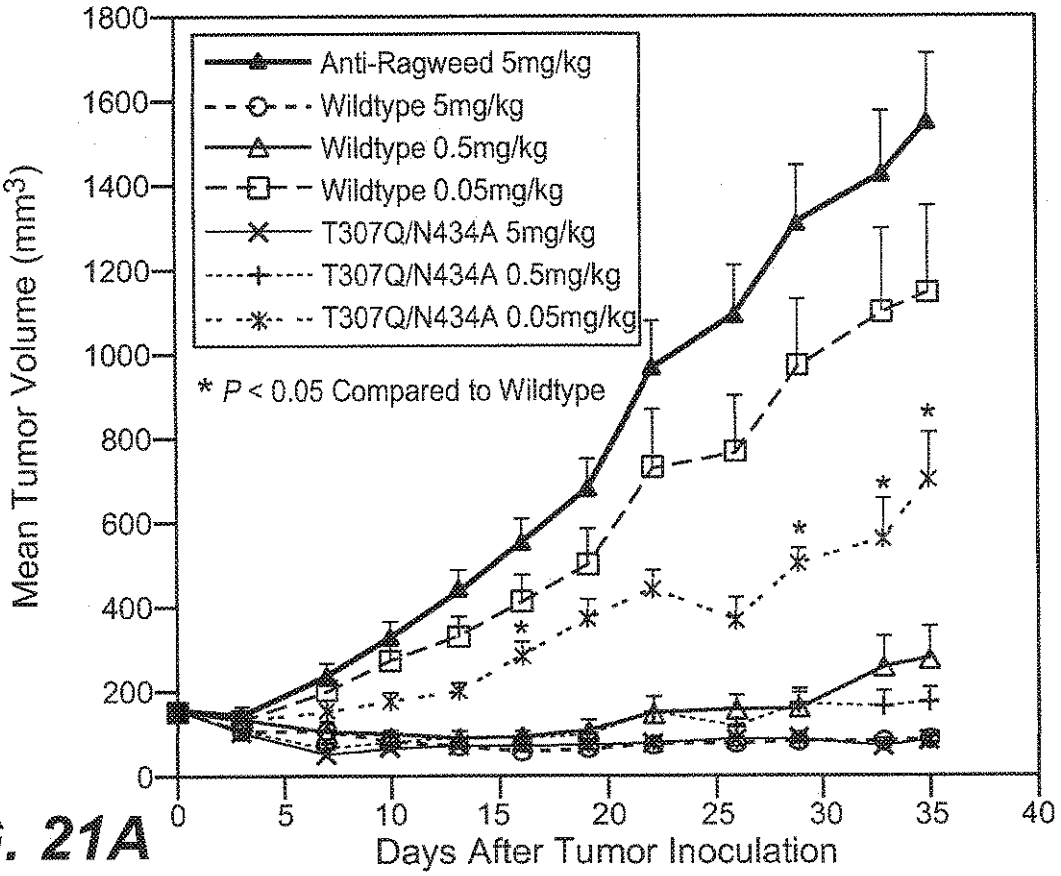


FIG. 21A

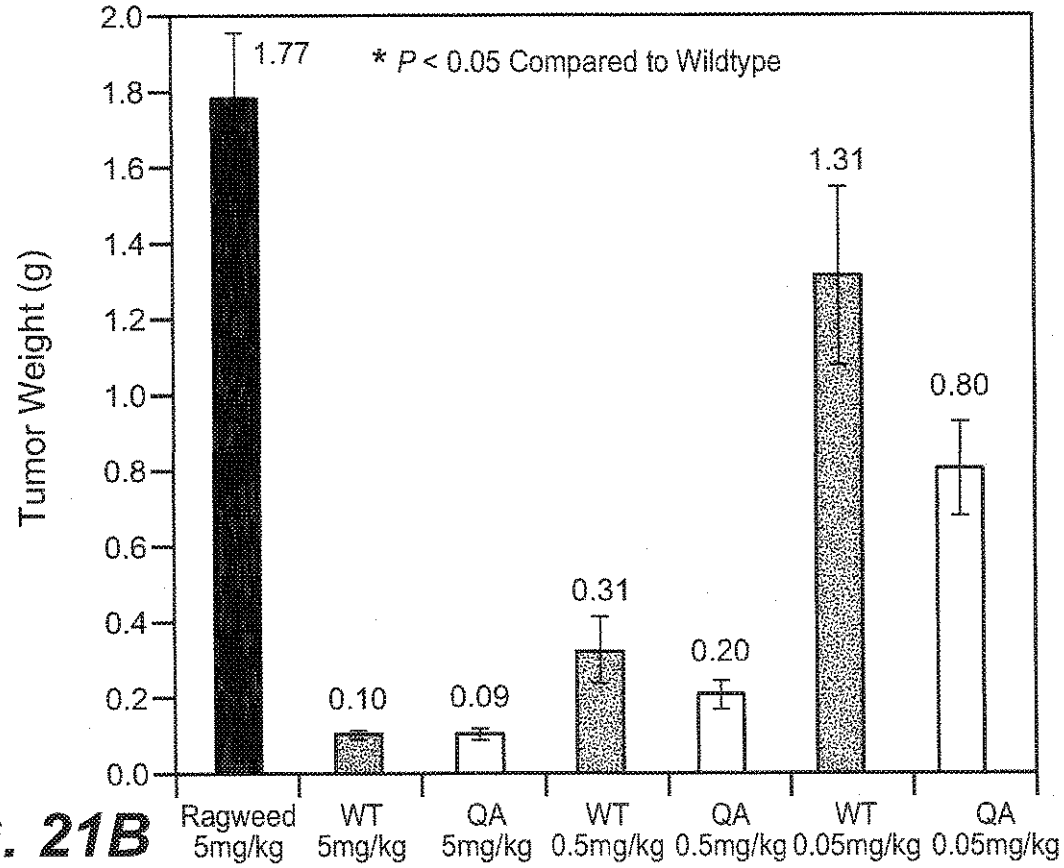


FIG. 21B

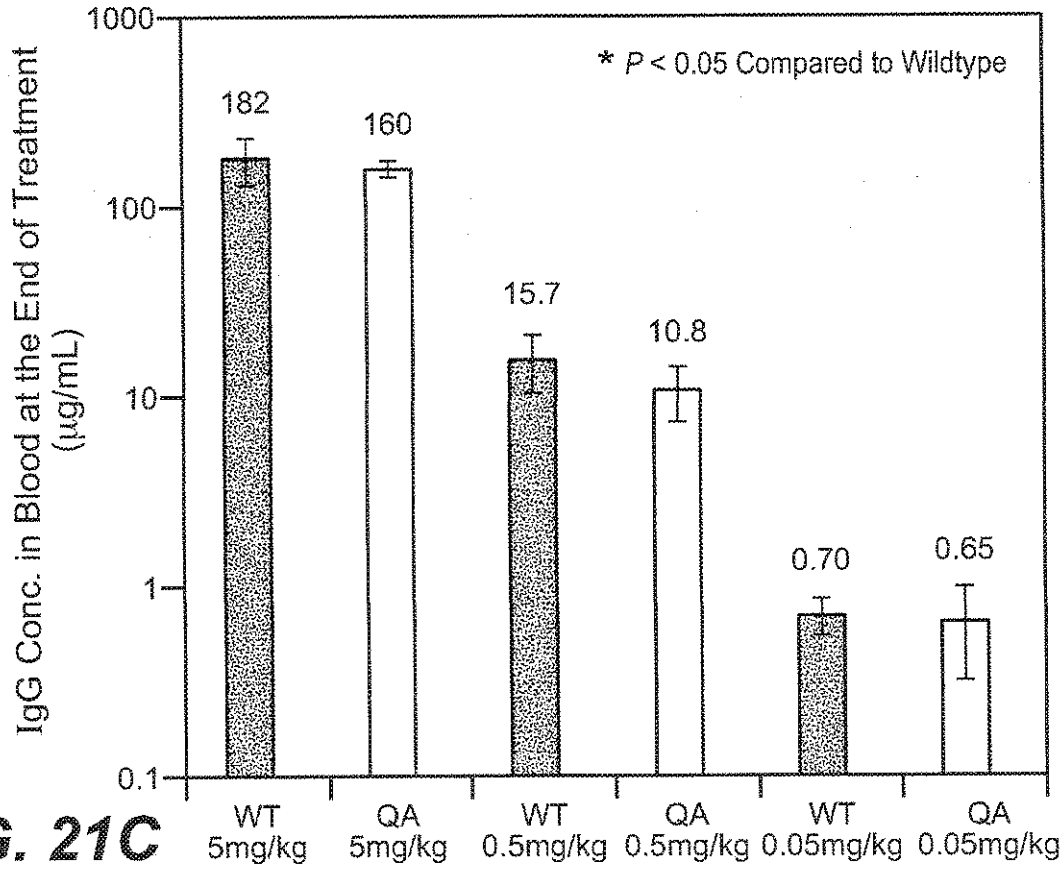


FIG. 21C

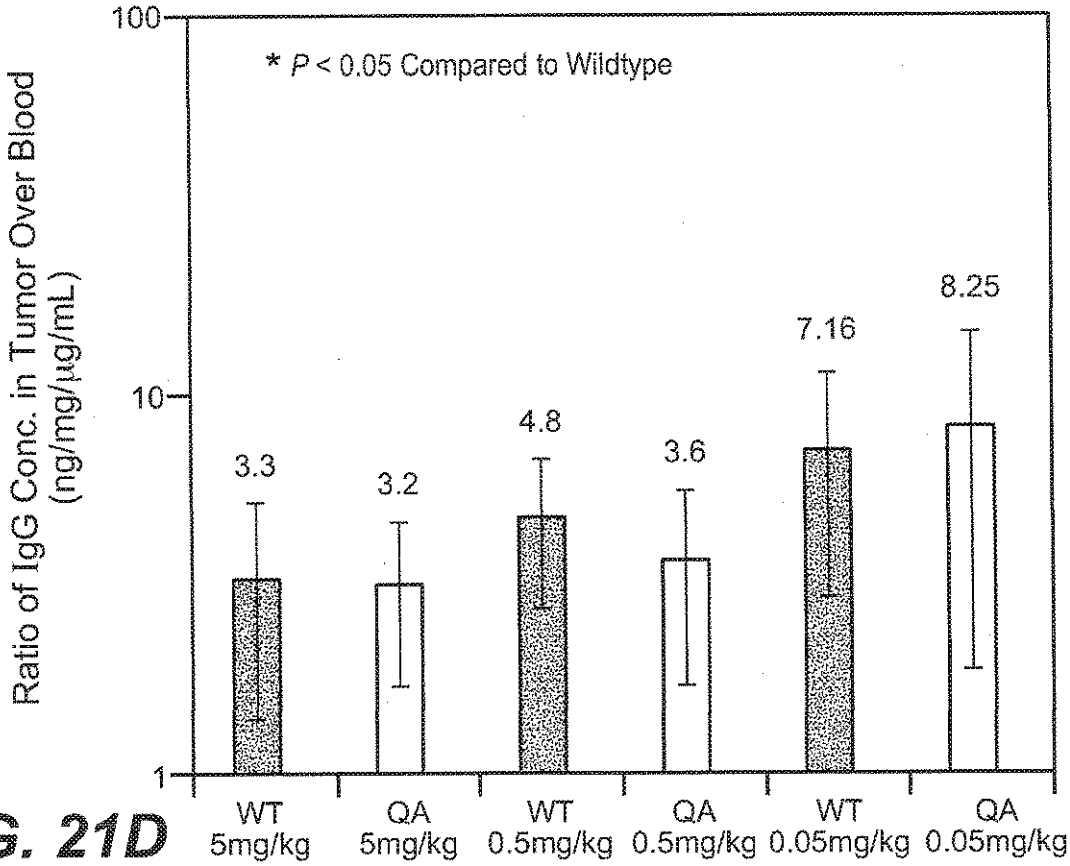


FIG. 21D

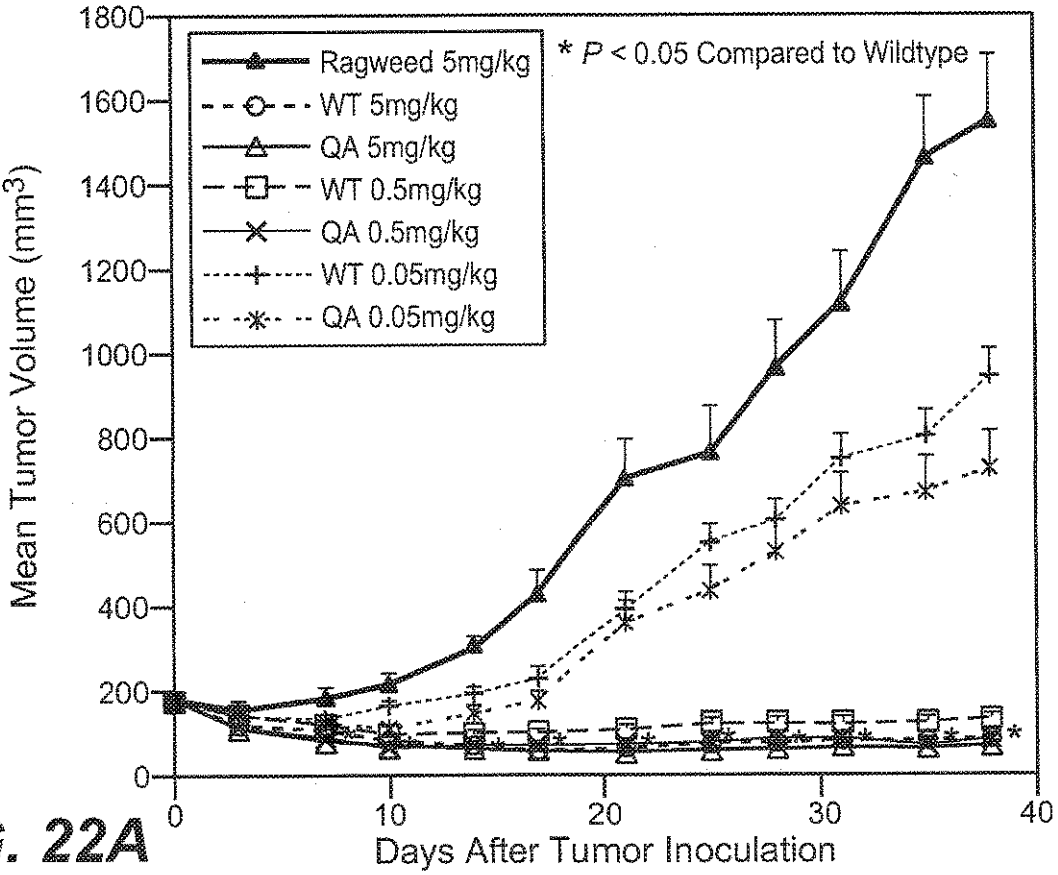


FIG. 22A

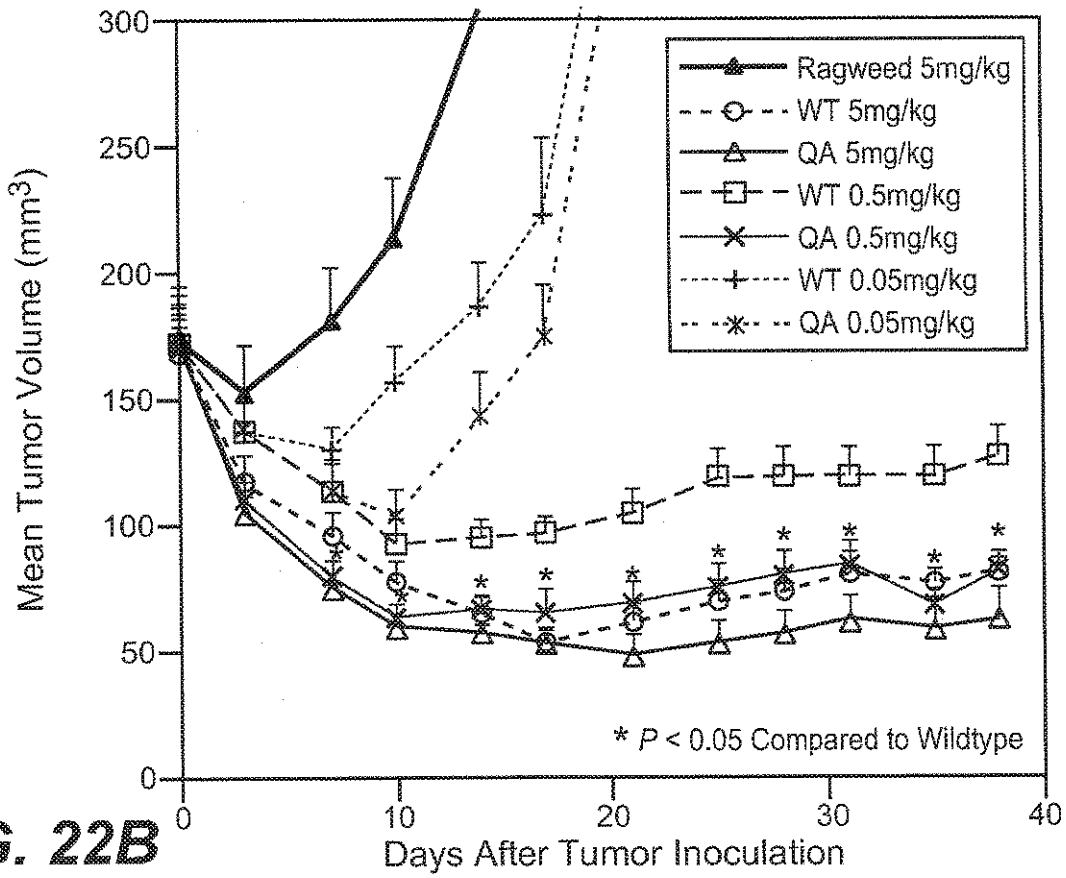


FIG. 22B

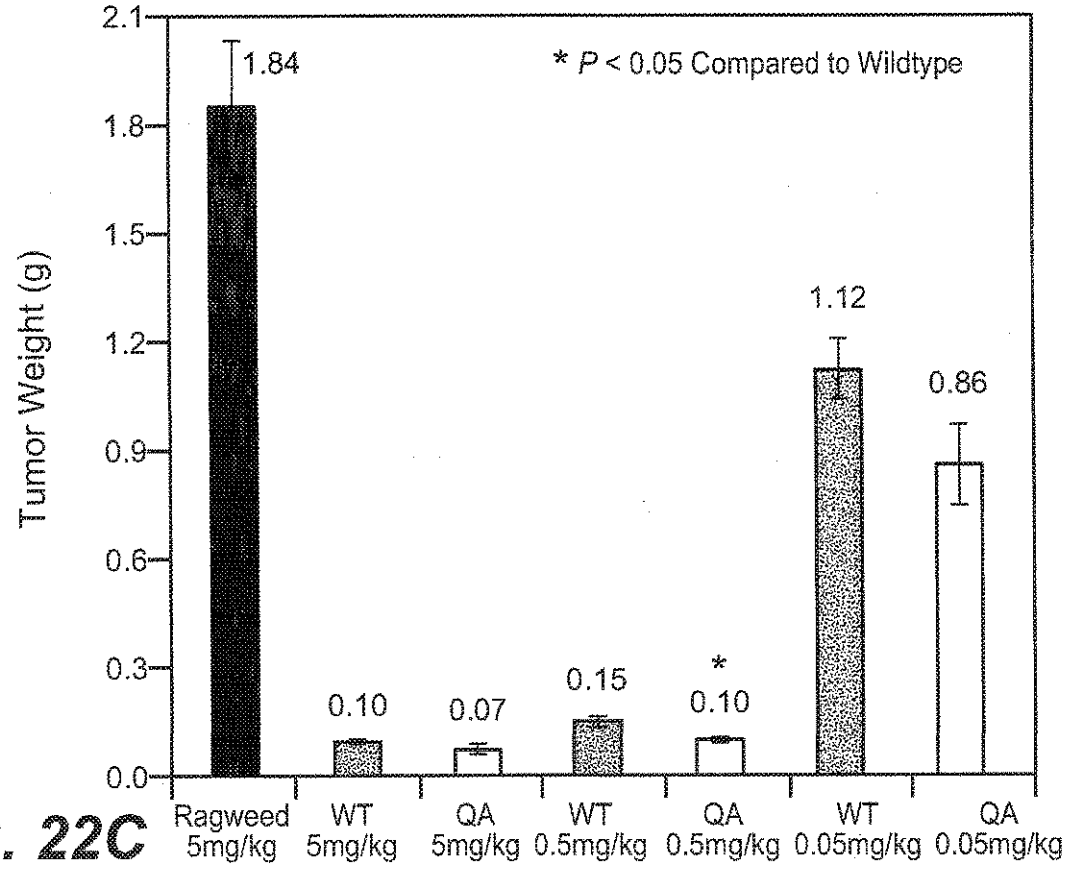


FIG. 22C

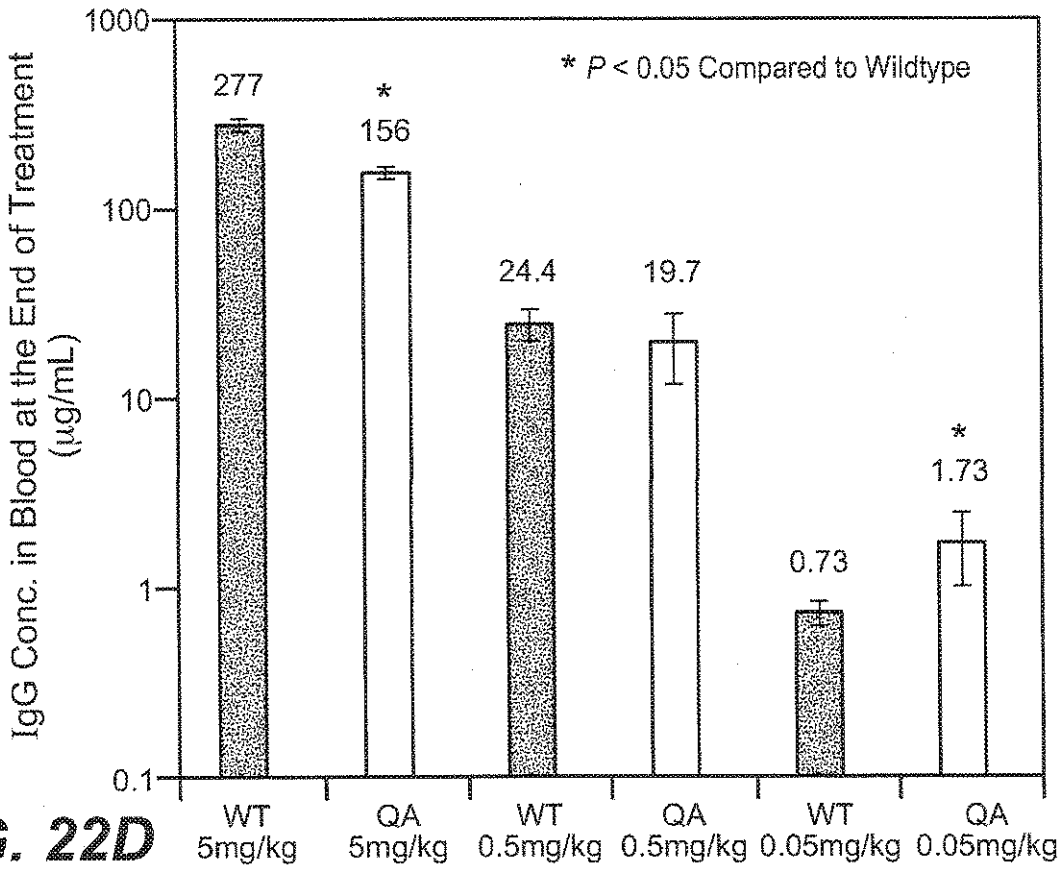


FIG. 22D

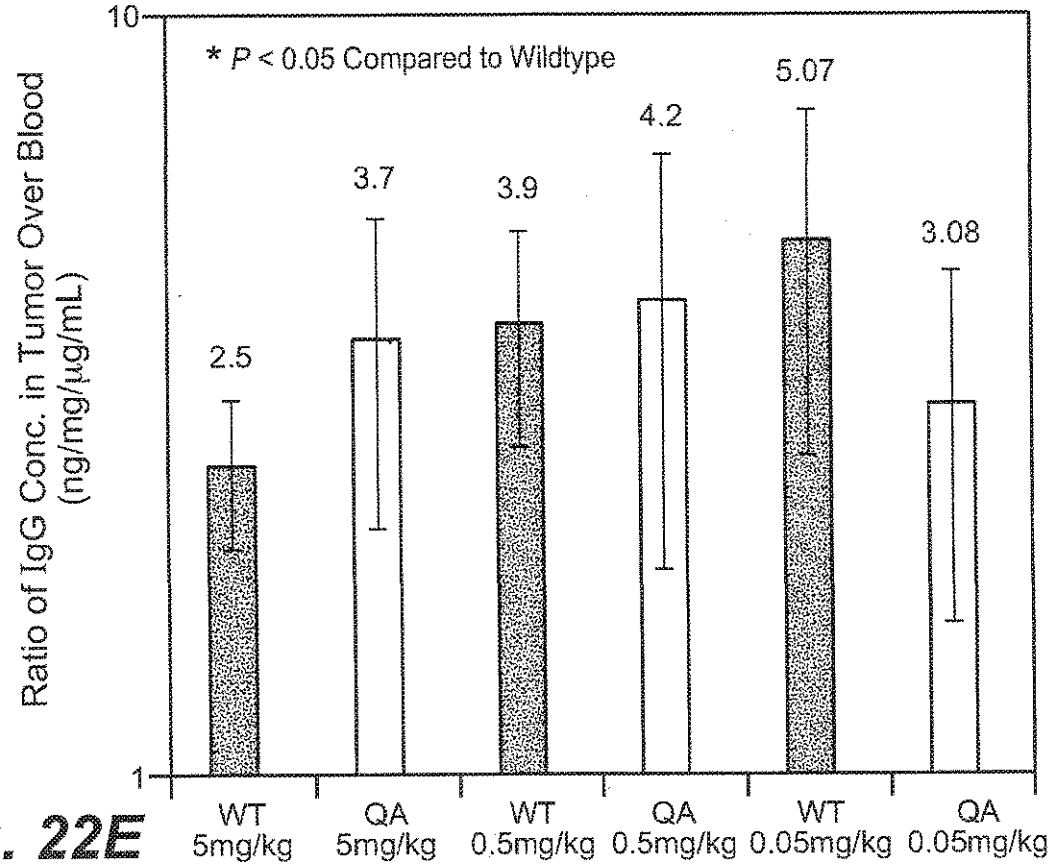


FIG. 22E

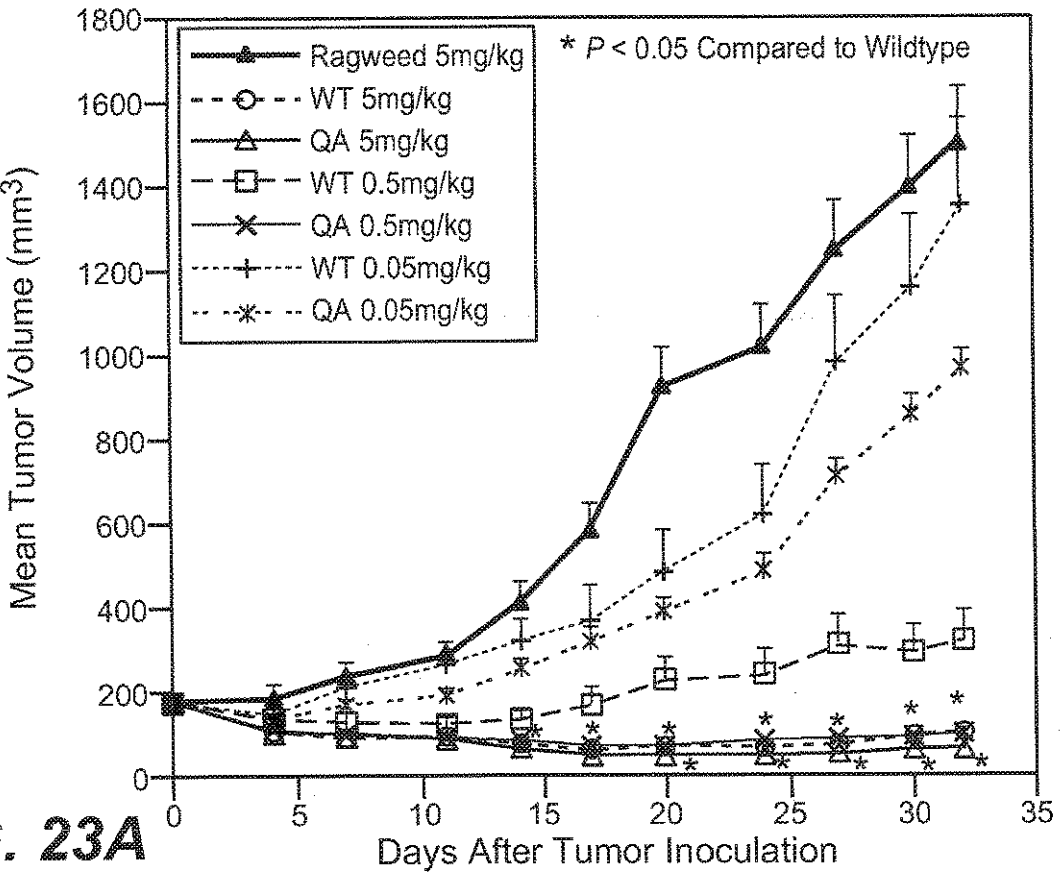


FIG. 23A

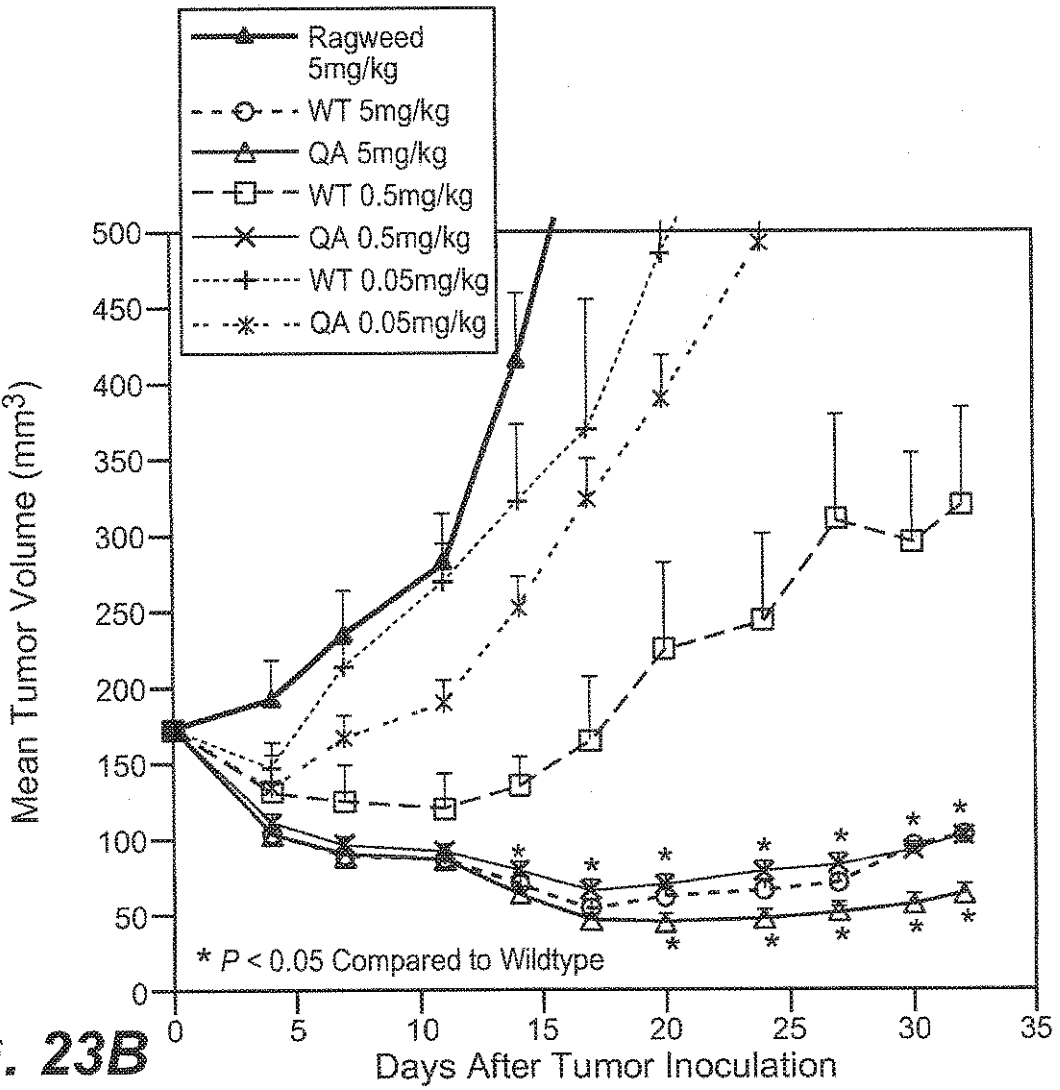

Ragweed 5mg/kg
WT 5mg/kg
QA 5mg/kg
WT 0.5mg/kg
QA 0.5mg/kg
WT 0.05mg/kg
QA 0.05mg/kg
Mean Tumor Volume (mm3)
Days After Tumor Inoculation
* P < 0.05 Compared to Wildtype
0
50
100
150
200
250
300
350
400
450
500
0
5
10
15
20
25
30
35

FIG. 23B

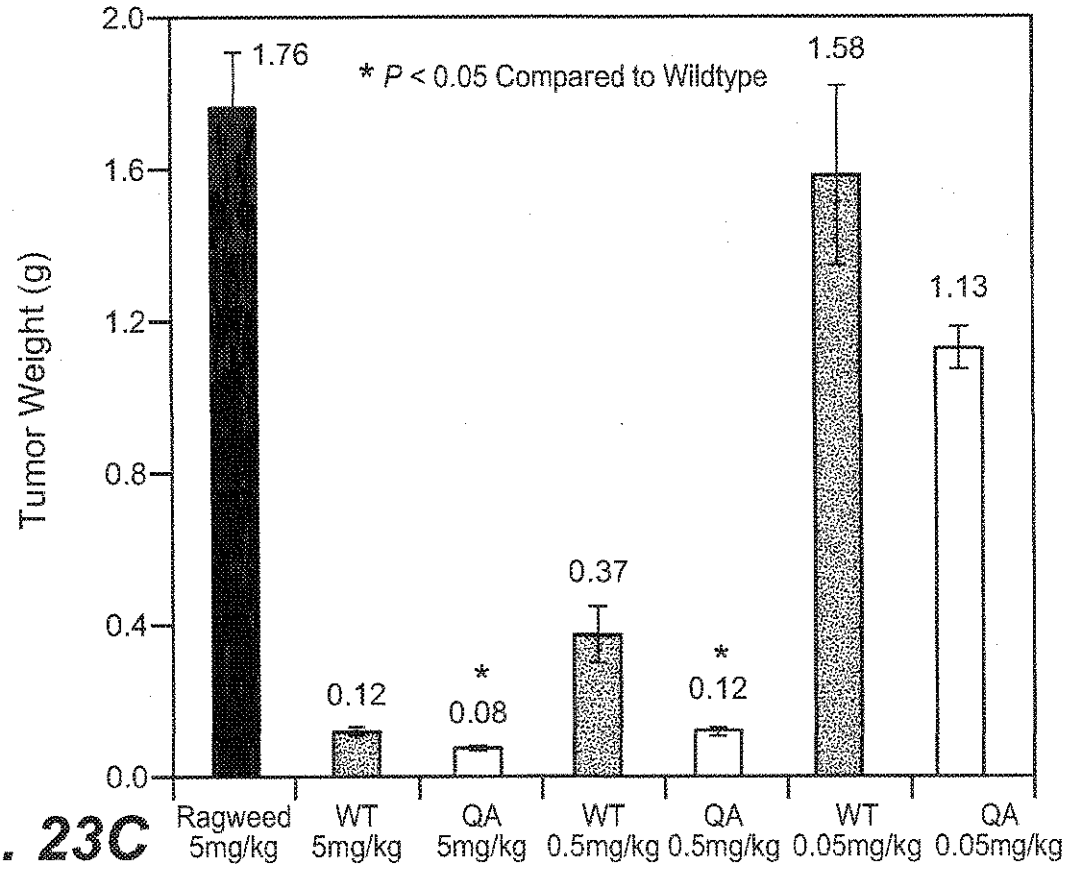

2.0
1.6
1.2
0.8
0.4
0.0
Tumor Weight (g)
* P < 0.05 Compared to Wildtype
1.76
0.12
*
0.08
0.37
*
0.12
1.58
1.13
Ragweed 5mg/kg
WT 5mg/kg
QA 5mg/kg
WT 0.5mg/kg
QA 0.5mg/kg
WT 0.05mg/kg
QA 0.05mg/kg

FIG. 23C

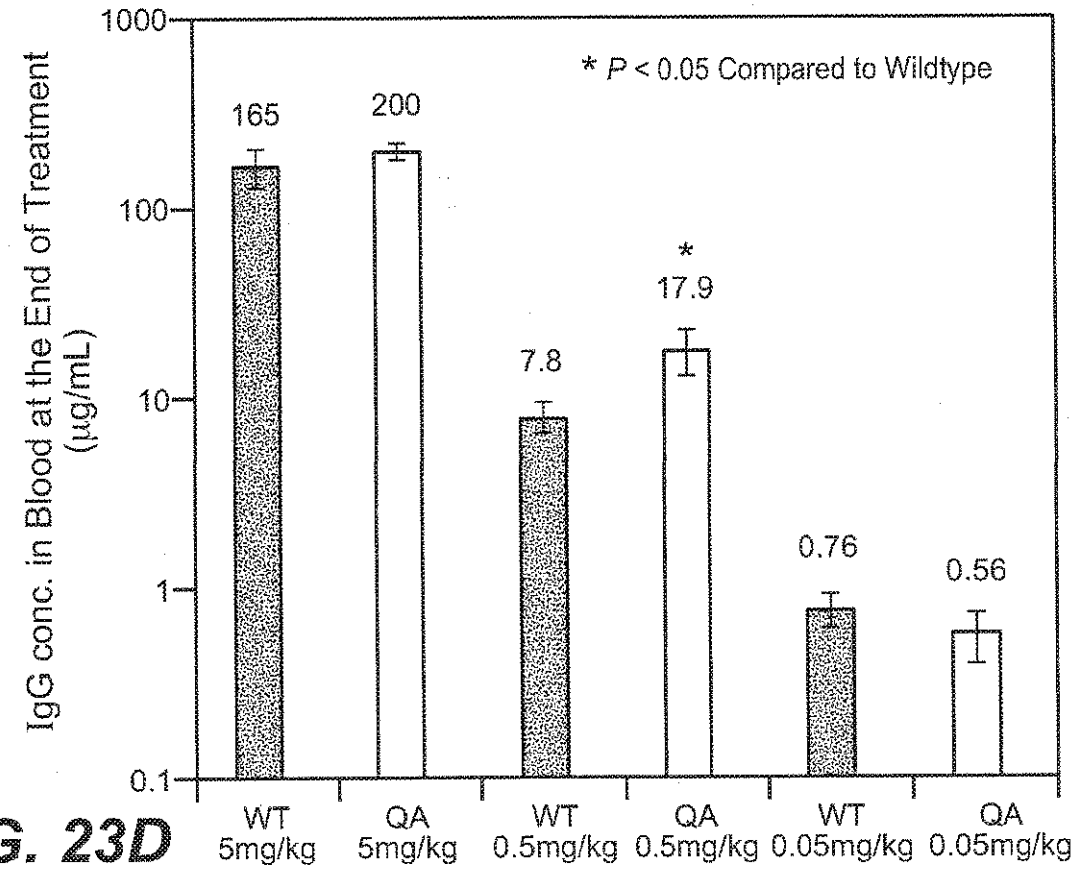


FIG. 23D

h4D5 Variant	K_D (nM)
Wildtype	1700 ± 200
N434A	570 ± 30
N434H	370 ± 30
N434Y	78 ± 12
N434W	44 ± 5
M252Y/N434A	220 ± 20
T307Q/N434A	235 ± 20
V308P/N434A	76 ± 10
T250Q/M428L	234 ± 22
T307A/E380A/N434A	280 ± 10
A378V/N434A	390 ± 40
M428L/N434A	300 ± 40
N434A/Y436I	340 ± 80
V308P/N434Y	10.2 ± 1.8
M252Y/V308P/N434Y	3.7 ± 1.0

FIG. 24

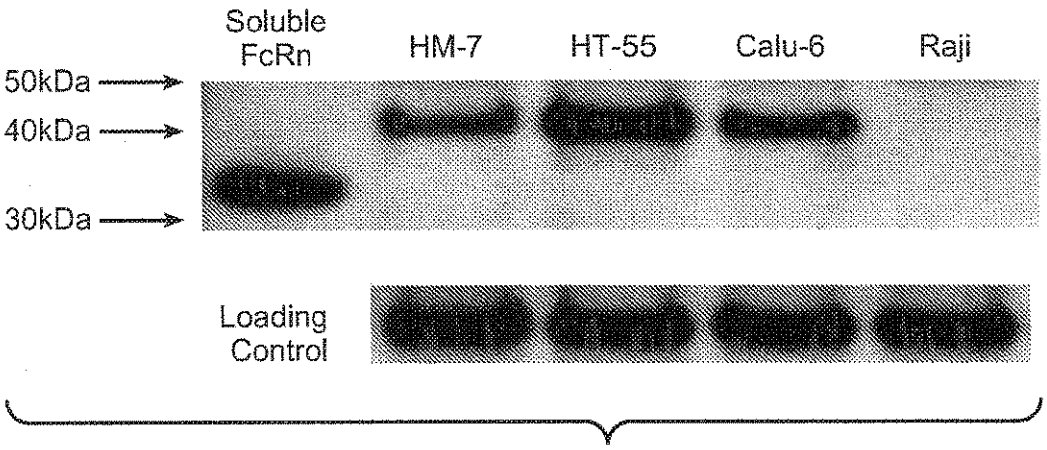

Soluble FcRn
HM-7
HT-55
Calu-6
Raji
50kDa
40kDa
30kDa
Loading Control

FIG. 25

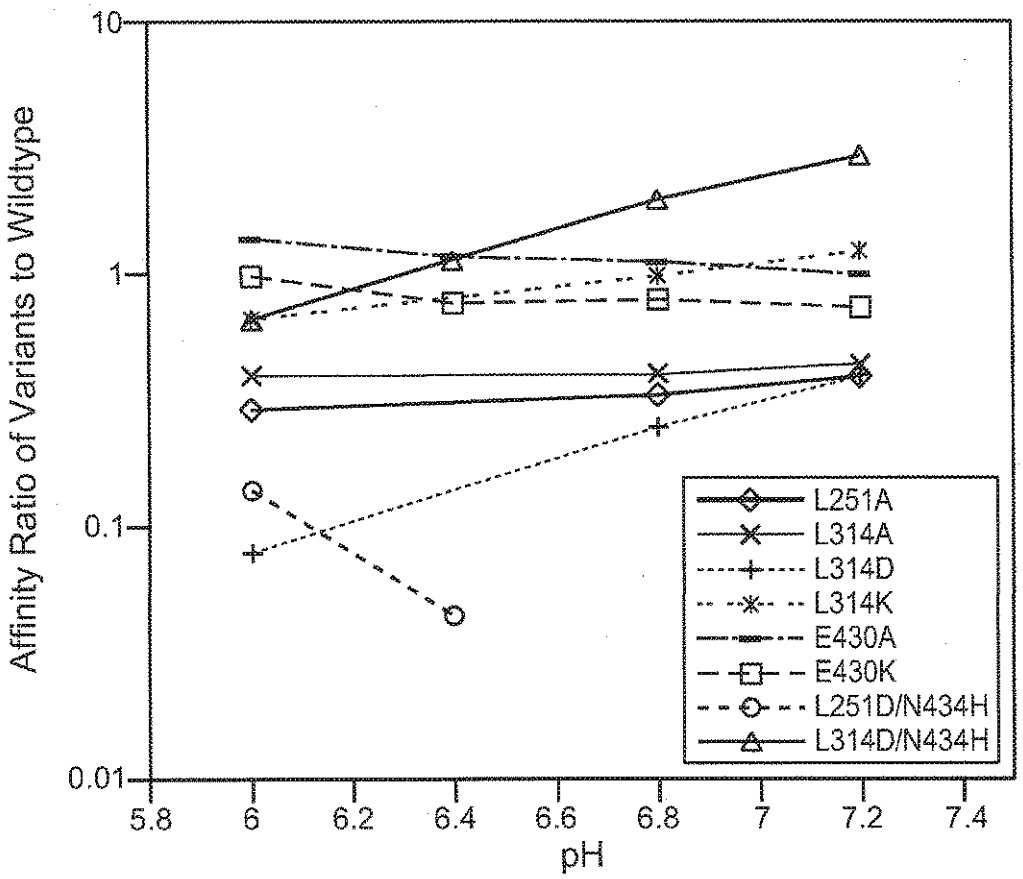

Affinity Ratio of Variants to Wildtype
10
1
0.1
0.01
5.8
6
6.2
6.4
6.6
6.8
7
7.2
7.4
pH
L251A
L314A
L314D
L314K
E430A
E430K
L251D/N434H
L314D/N434H

FIG. 26

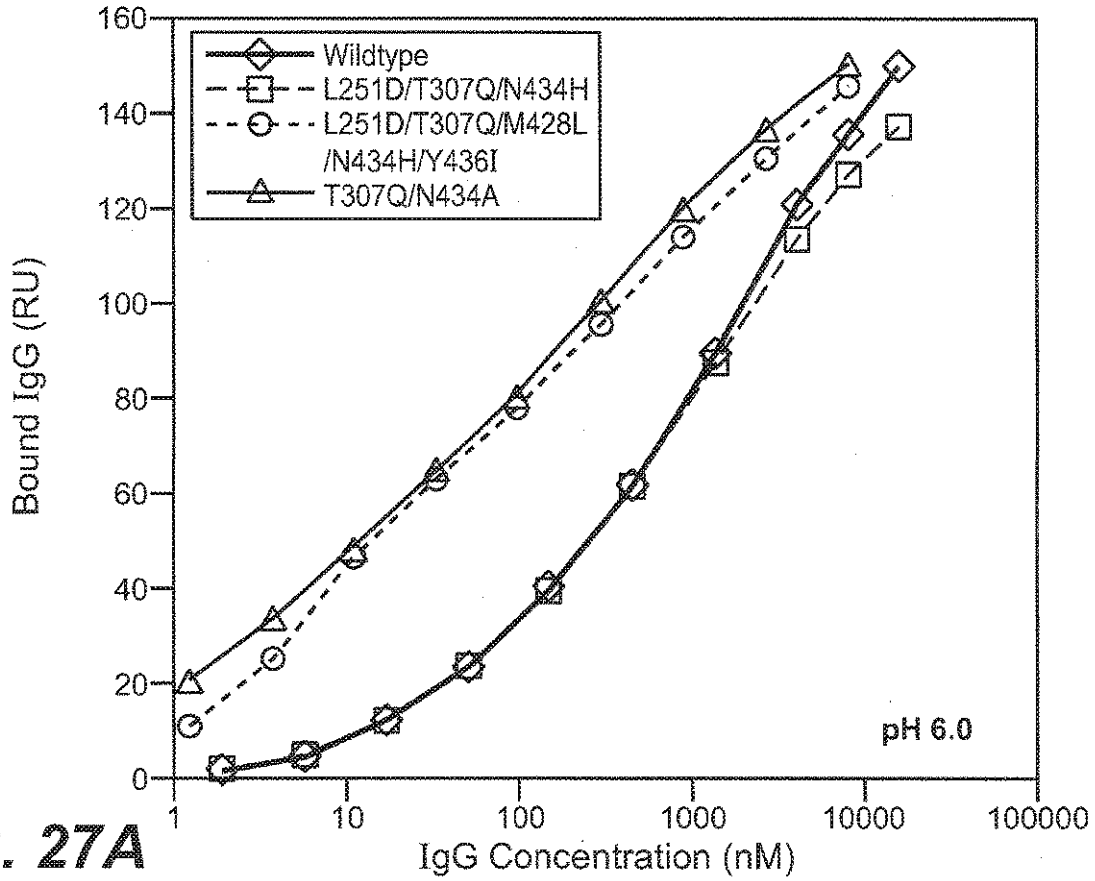


FIG. 27A

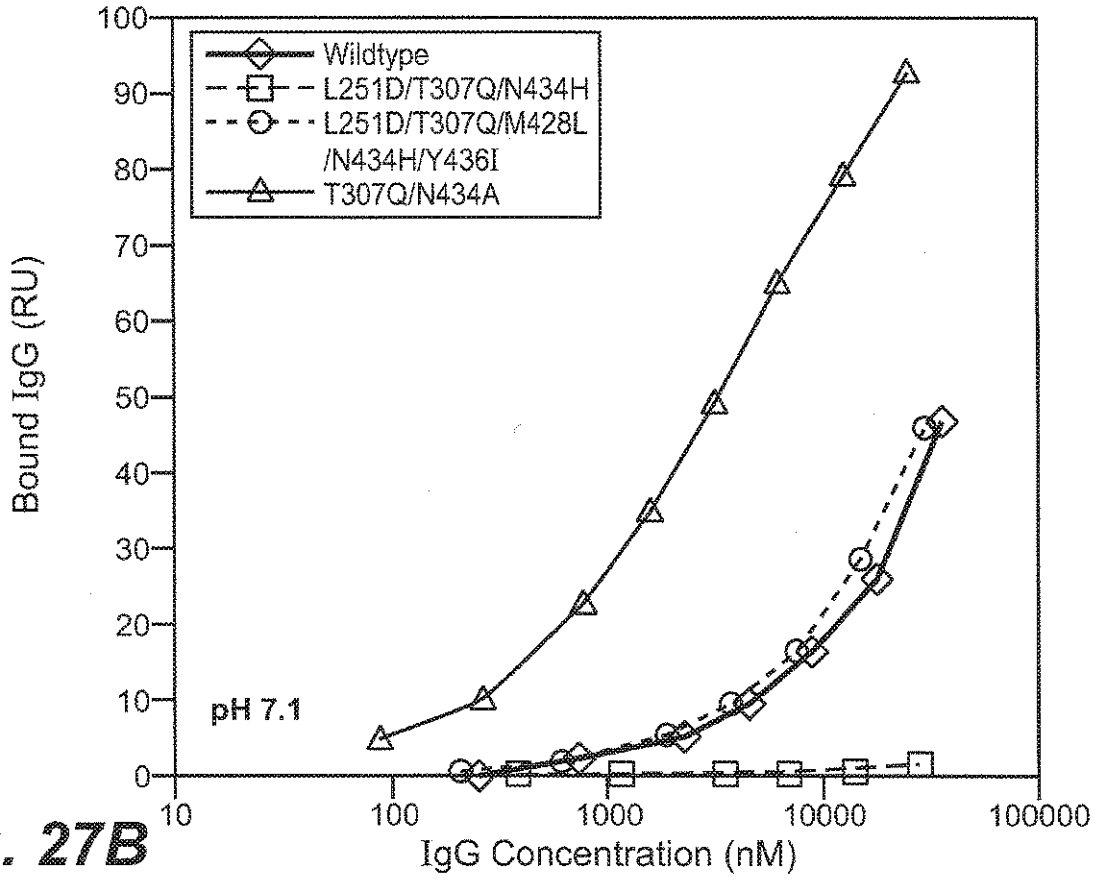


FIG. 27B

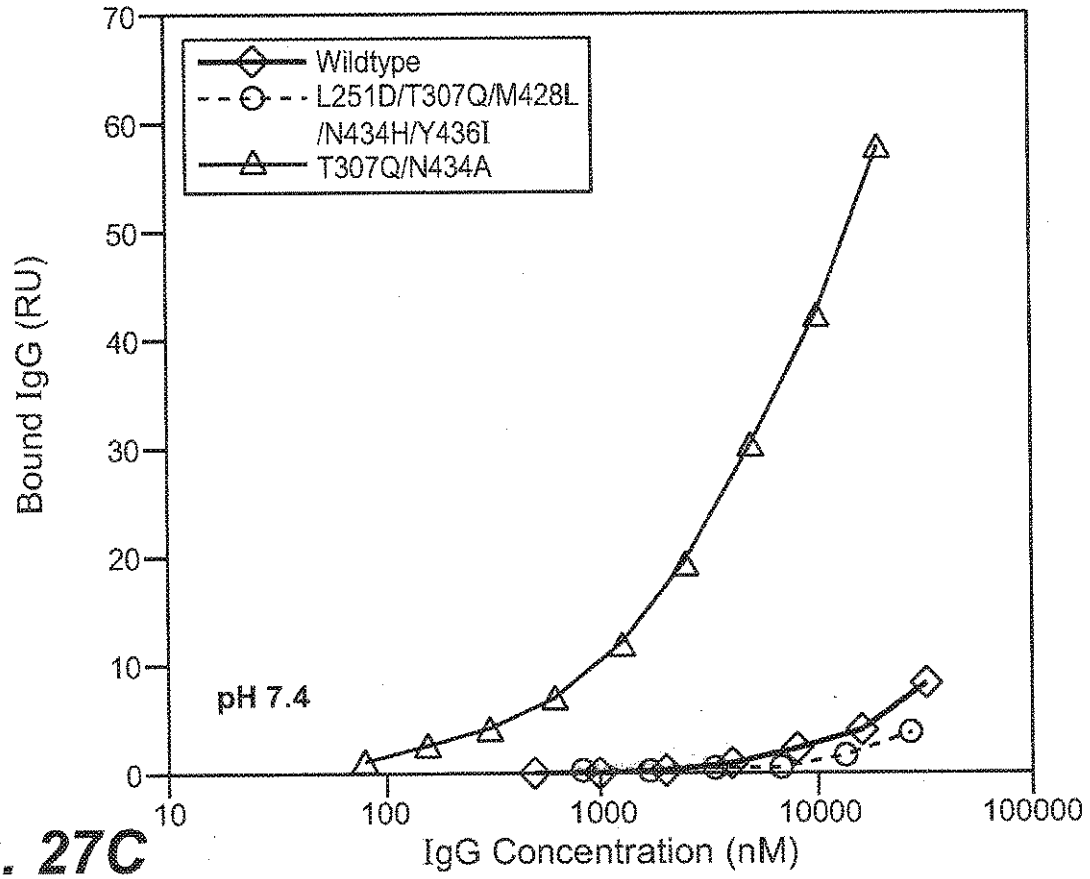


FIG. 27C

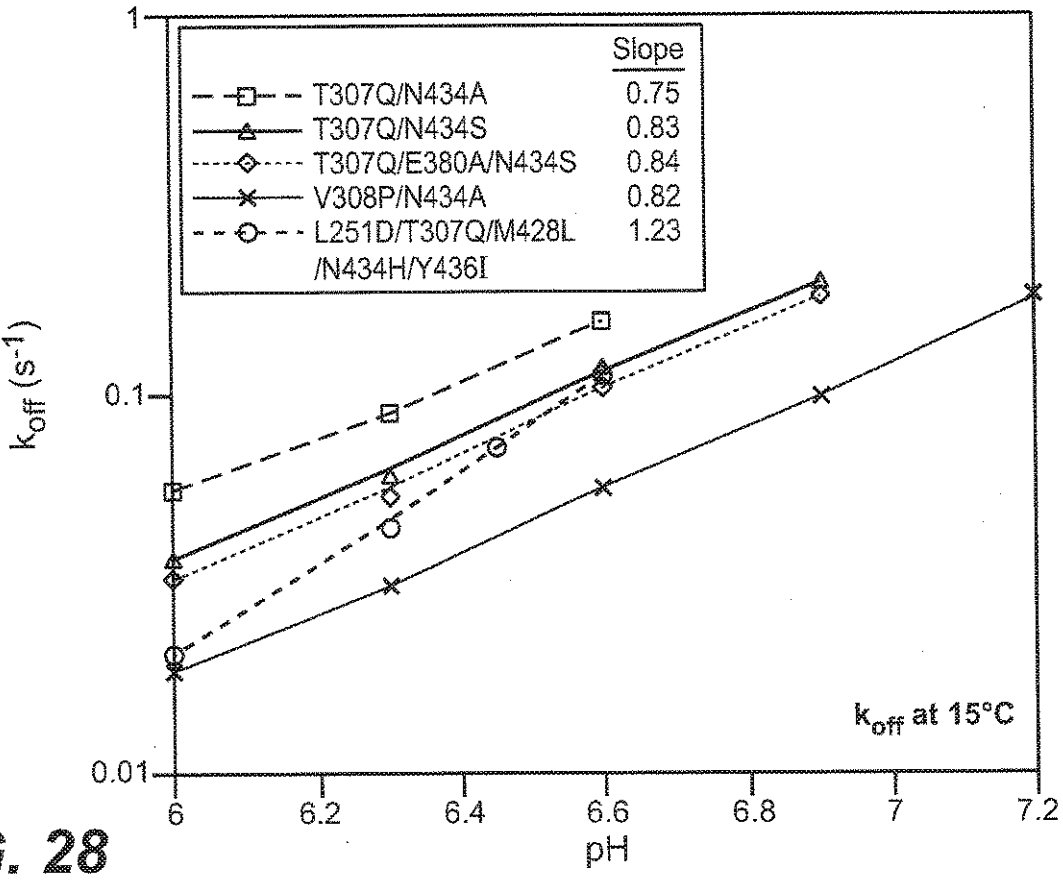


FIG. 28