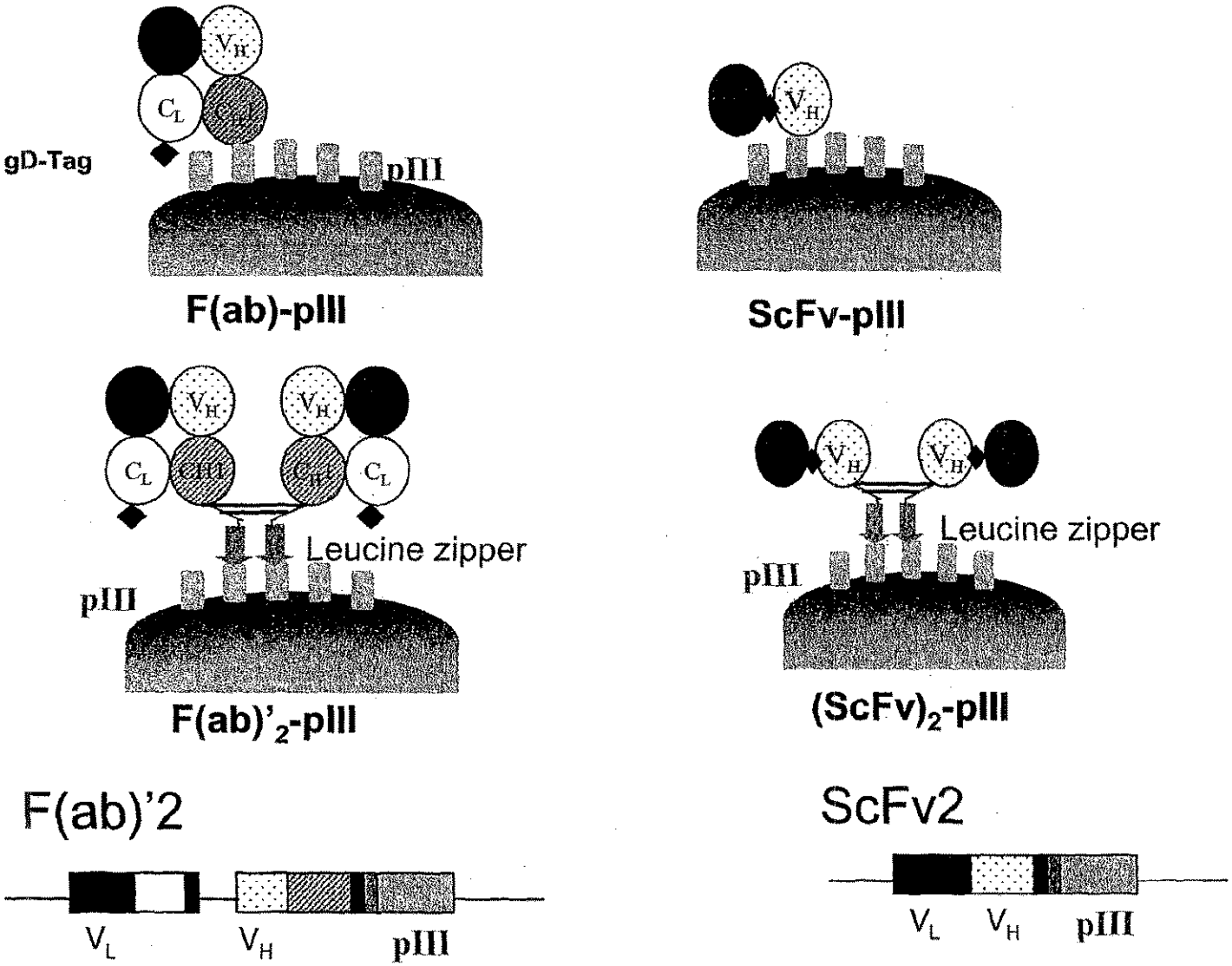

gD-Tag
pIII
F(ab)-pIII
ScFv-pIII
Leucine zipper
pIII
F(ab)'2-pIII
Leucine zipper
pIII
(ScFv)2-pIII
F(ab)'2
ScFv2

Figure 1

Clone	H1				H2							H3								IC50 (nM)	
	30	31	32	33	49	50	52	53	54	56	58	95	96	97	98	99	100		100a	mVEGF	hVEGF
h4D5	K	D	T	Y	A	R	Y	T	N	Y	R	W	G	G	D	G	F		Y	ND	ND
G6	S	D	Y	W	A	G	T	A	G	Y	Y	F	V	F	F	L	P		Y	0.6	1.4
B20	N	A	S	W	G	A	Y	Y	S	Y	N	W	G	H	S	T	S	P	W	22	159
B29	T	G	S	G	G	W	Y	Y	S	N	Y	A	L	W	A	F	V		A	25	>2000
C3	T	G	S	S	A	R	S	A	G	Y	D	Y	F	R	W	Y	M		A	80	696

Figure 2

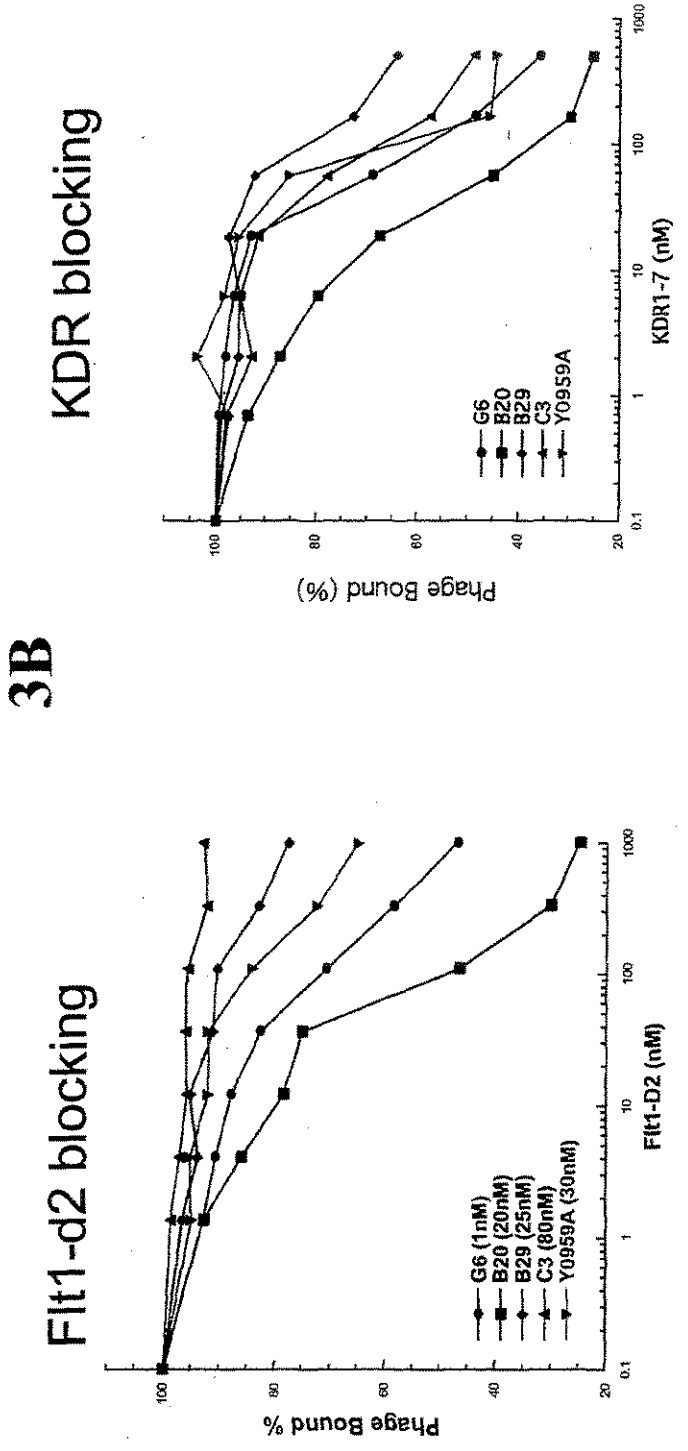

3A
Flt1-d2 blocking
Phage Bound %
Flt1-D2 (nM)
G6 (1nM)
B20 (20nM)
B29 (25nM)
C3 (80nM)
Y0959A (30nM)
3B
KDR blocking
Phage Bound (%)
KDR1-7 (nM)
G6
B20
B29
C3
Y0959A

Figure 3

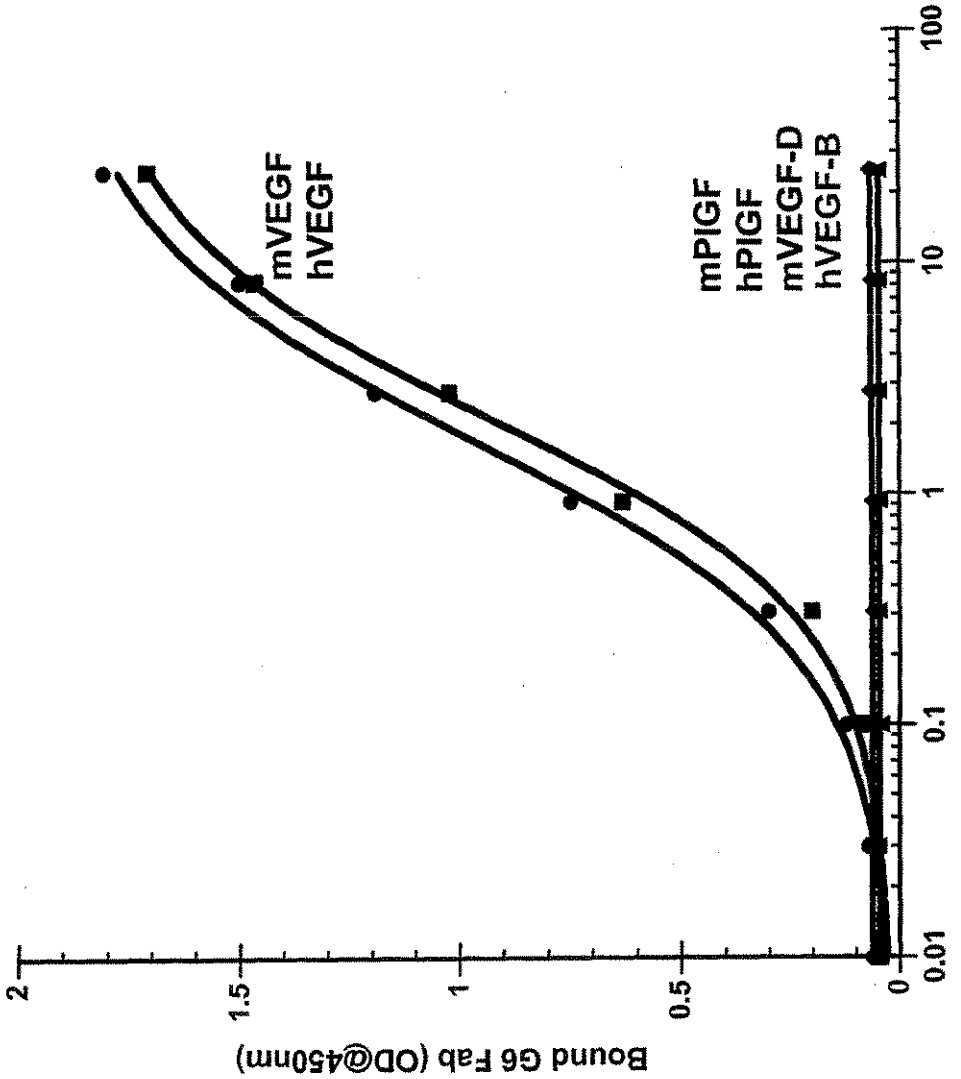

Bound G6 Fab (OD@450nm)
2
1.5
1
0.5
0
0.01
0.1
1
10
100
G6 Fab (nM)
mVEGF
hVEGF
mPlGF
hPlGF
mVEGF-D
hVEGF-B

Figure 4

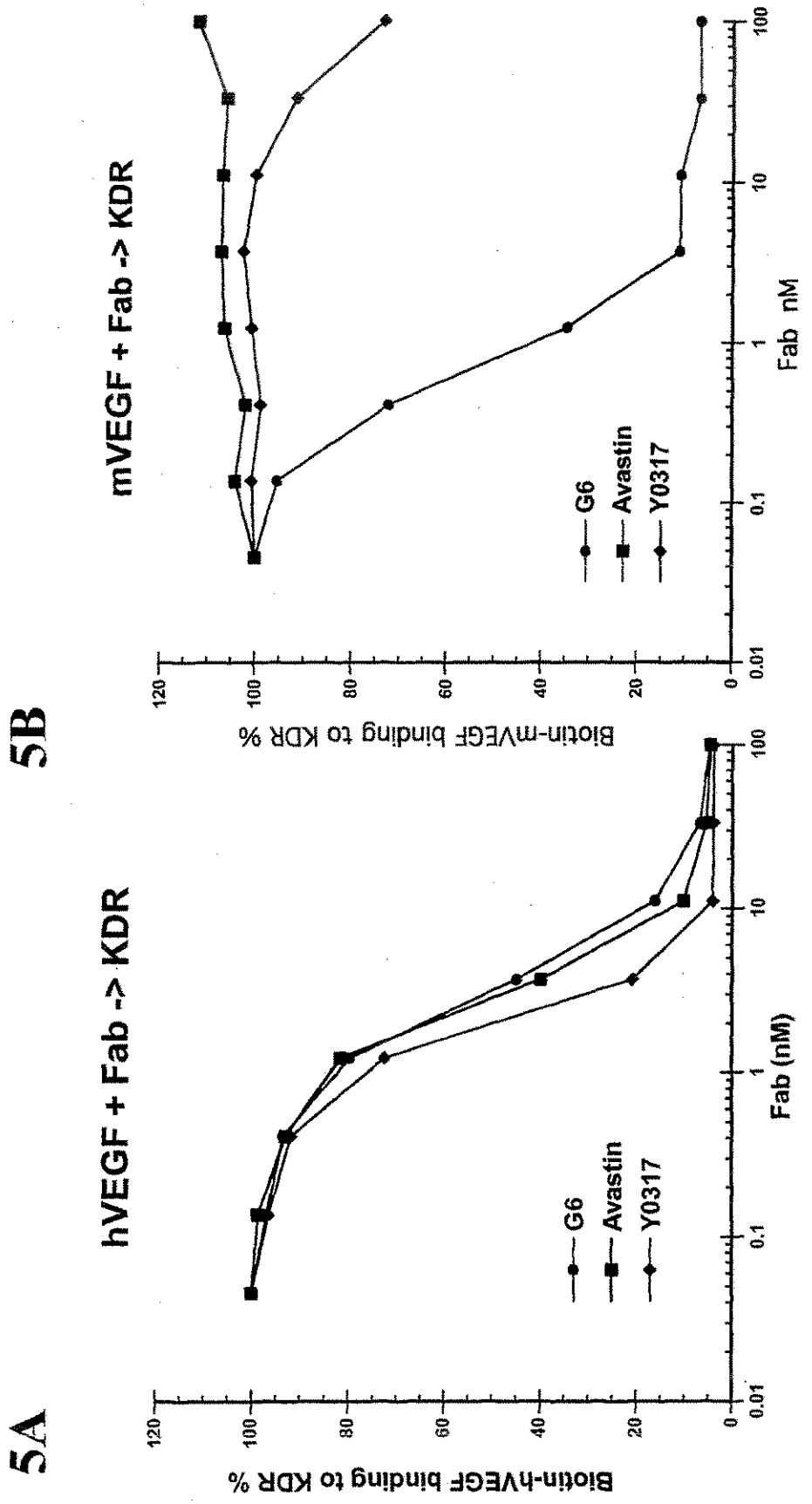

5A
hVEGF + Fab -> KDR
Biotin-hVEGF binding to KDR %
Fab (nM)
G6
Avastin
Y0317
5B
mVEGF + Fab -> KDR
Biotin-mVEGF binding to KDR %
Fab nM
G6
Avastin
Y0317

Figure 5

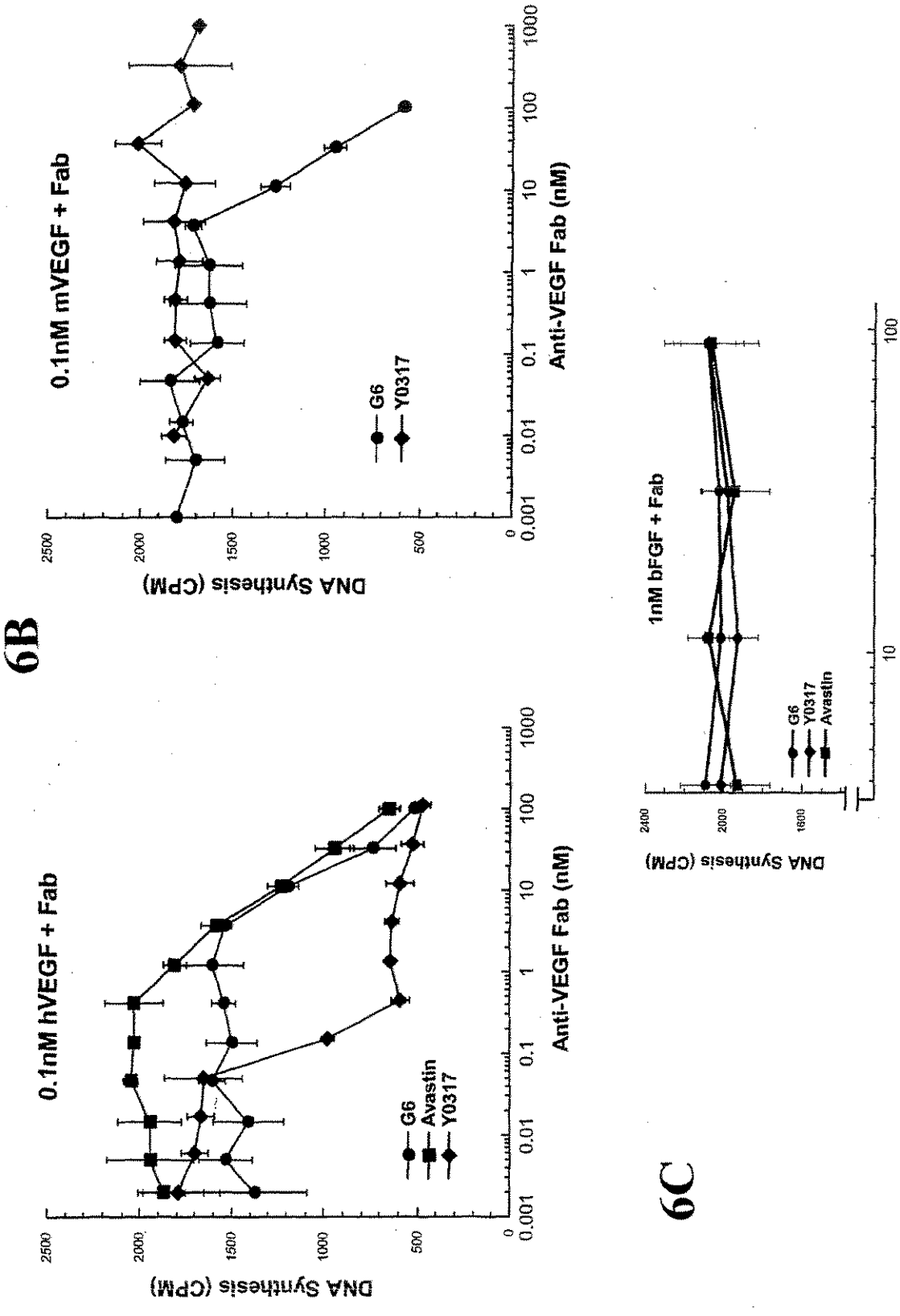
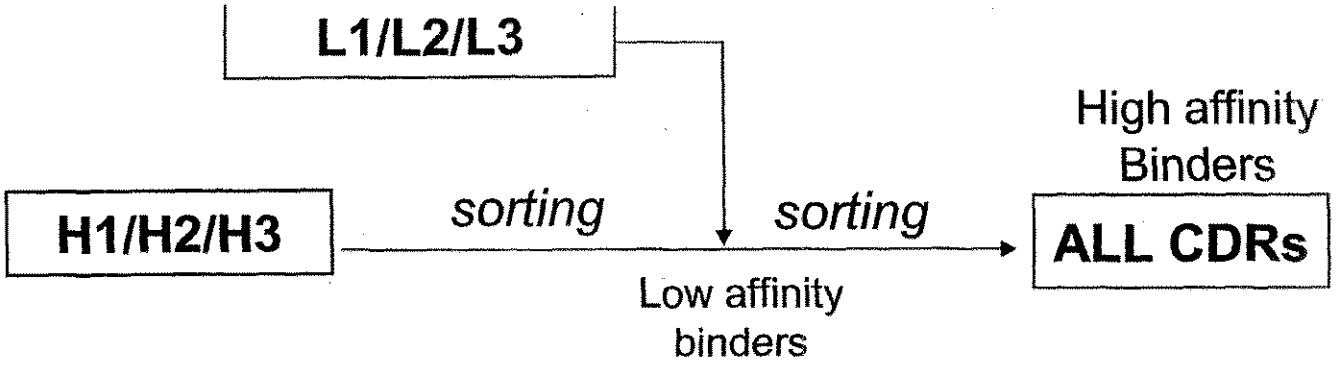

6A
0.1nM hVEGF + Fab
DNA Synthesis (CPM)
Anti-VEGF Fab (nM)
G6
Avastin
Y0317
6B
0.1nM mVEGF + Fab
DNA Synthesis (CPM)
Anti-VEGF Fab (nM)
G6
Y0317
6C
1nM bFGF + Fab
DNA Synthesis (CPM)
Anti-VEGF Fab (nM)
G6
Y0317
Avastin

Figure 6

7A

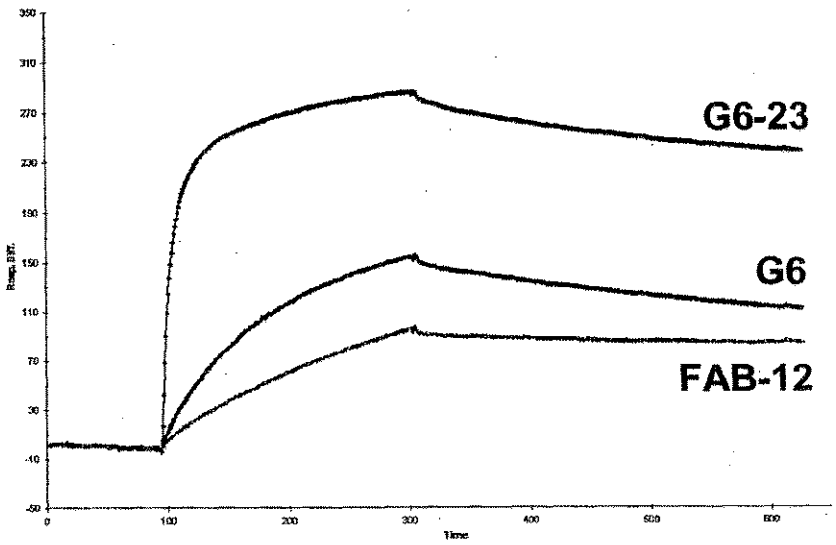


7B

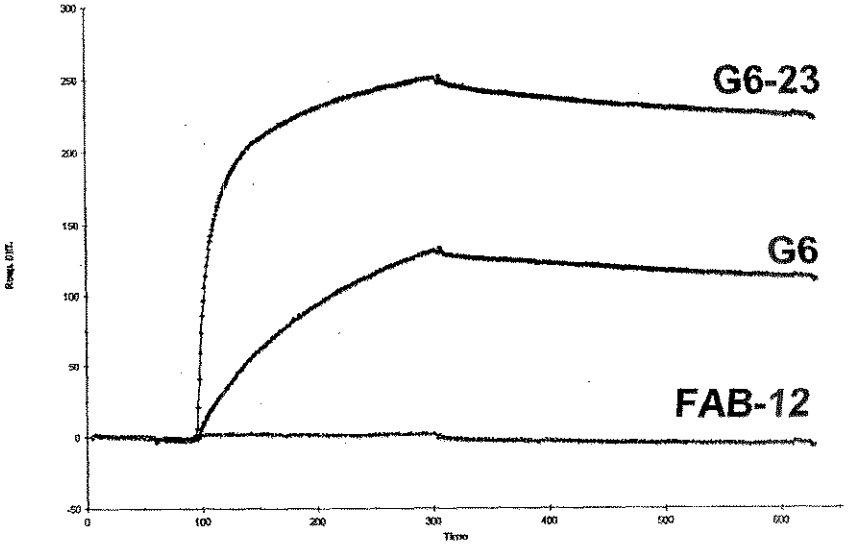
Clone	CDR-L1						CDR-L2			CDR-L3					IC50 (nM)		Kd (nM)	
	28	29	30	31	32	33	50	53	55	91	92	93	94	96	mVEGF	hVEGF	mVEGF	hVEGF
G6	D	V	S	T	A	V	S	F	Y	S	Y	T	T	P	0.60	1	1.1	1.3
G6-6a	N	V	A	T	F	L	S	N	A	S	Y	G	S	L	0.02	0.04	n/d	n/d
G6-23	D	V	S	T	A	V	S	F	Y	G	Y	A	N	W	0.01	0.02	<0.09	<0.2
G6-31	D	V	S	T	A	V	S	F	Y	G	Y	G	N	F	0.03	0.04	<0.06	<0.09
G6-2a	D	V	S	T	A	V	S	F	Y	G	Y	G	S	L	0.05	0.09		
G6-2b	G	G	T	T	S	L	S	N	A	G	Y	G	S	F	0.07	0.16		
G6-6b	I	V	T	N	S	L	L	S	A	G	A	T	D	R	0.22	0.66		
G6-48	D	V	S	T	A	V	S	F	Y	G	T	S	S	P	0.06	0.07		
G6-1a	D	V	S	T	A	V	S	F	Y	G	T	G	N	P	0.02	0.04		
G6-8	D	V	S	T	A	V	S	F	Y	G	A	G	S	L	0.06	0.11	0.12	0.45
G6-3	D	V	S	T	A	V	A	F	Y	G	S	G	H	F	0.60	1.38		
G6-34	D	V	S	T	A	V	S	F	Y	G	T	G	S	L	0.07	0.09		
G6-4a	D	V	S	T	A	V	S	F	Y	G	S	G	N	F	0.06	0.13		
G6-1a	D	V	S	T	A	V	S	F	Y	G	T	G	N	L	0.07	0.12		
G6-4b	D	V	S	T	A	V	S	F	Y	G	S	G	S	F	0.04	0.10		
G6-5	D	V	S	T	A	V	S	F	Y	G	A	G	S	F	0.04	0.08	n/d	n/d
G6-15	D	V	S	T	A	V	S	F	Y	G	V	N	S	L	0.06	0.10		

Figure 7

8A



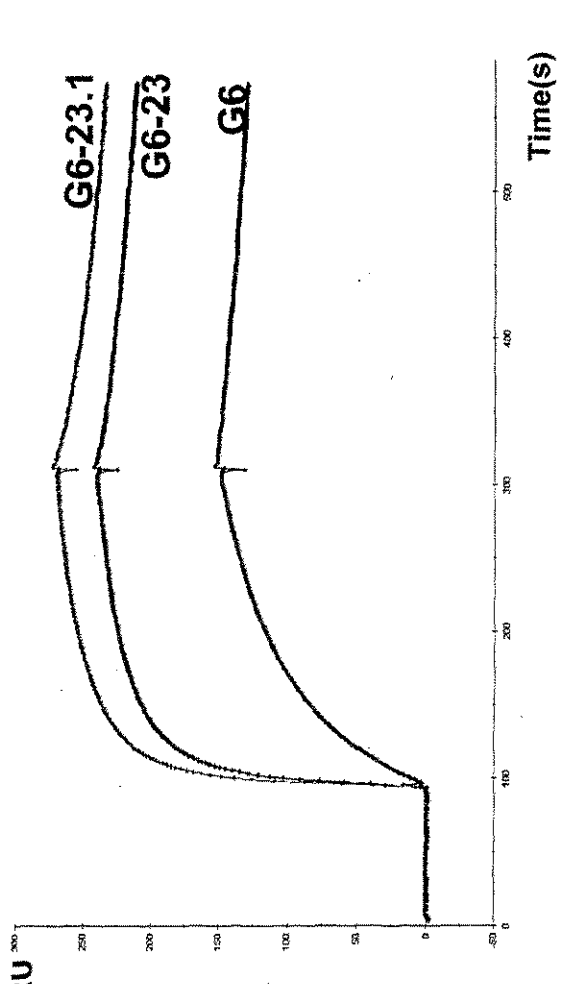
8B



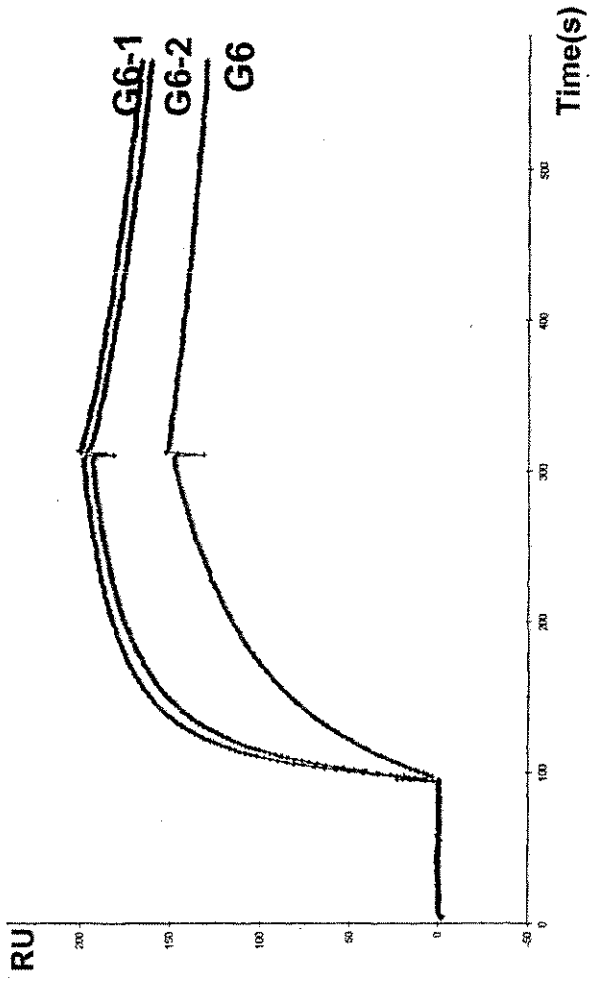
8C

	$k_{on}/10^4$		$k_{off}/10^{-4}$		Kd (nM)	
	hVEGF	mVEGF	hVEGF	mVEGF	hVEGF	mVEGF
G6	15.2	19.3	2.5	1.3	1.6	0.7
G6-23	126	114	2.6	1.3	<0.2	<0.1
Avastin	4.1	NB	1.2	NB	2.9	NB

Figure 8



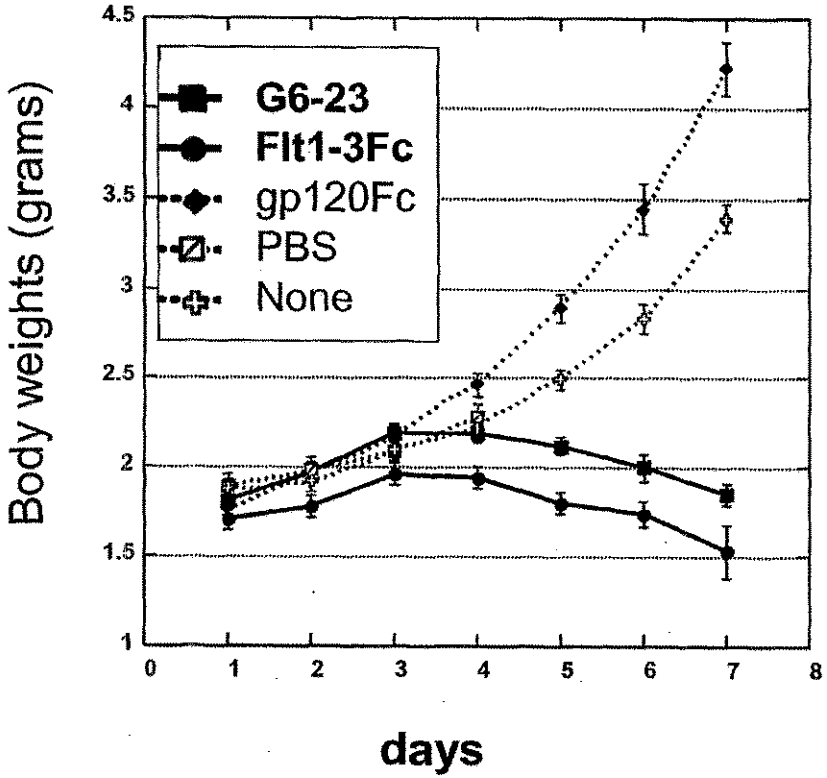
9A



9B

Figure 9

10A



10B

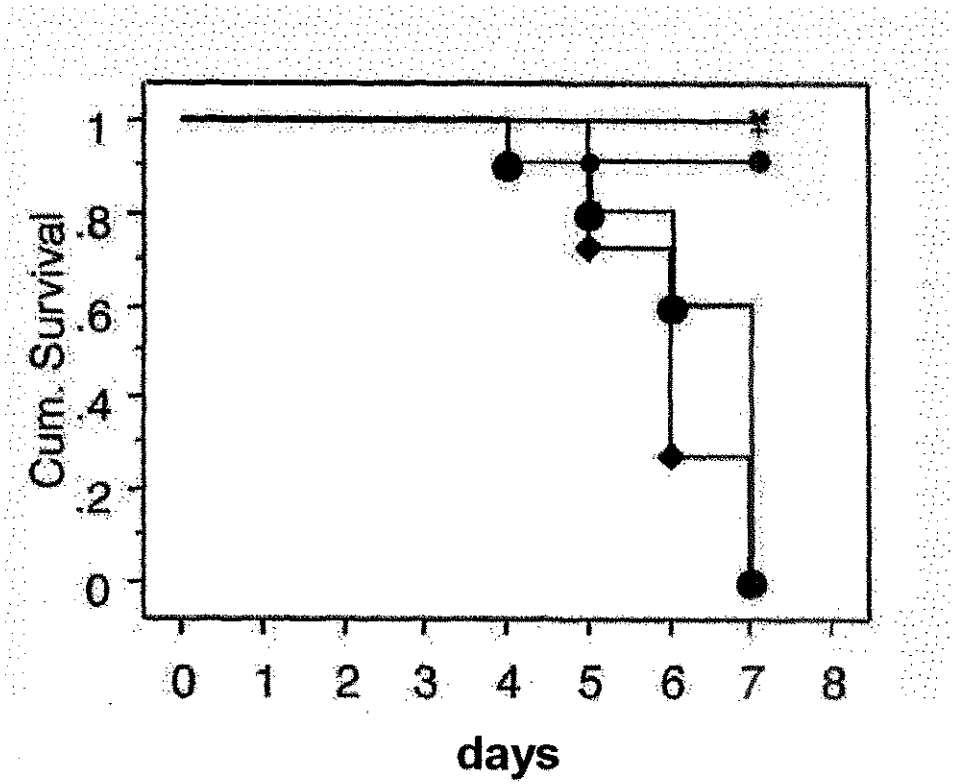
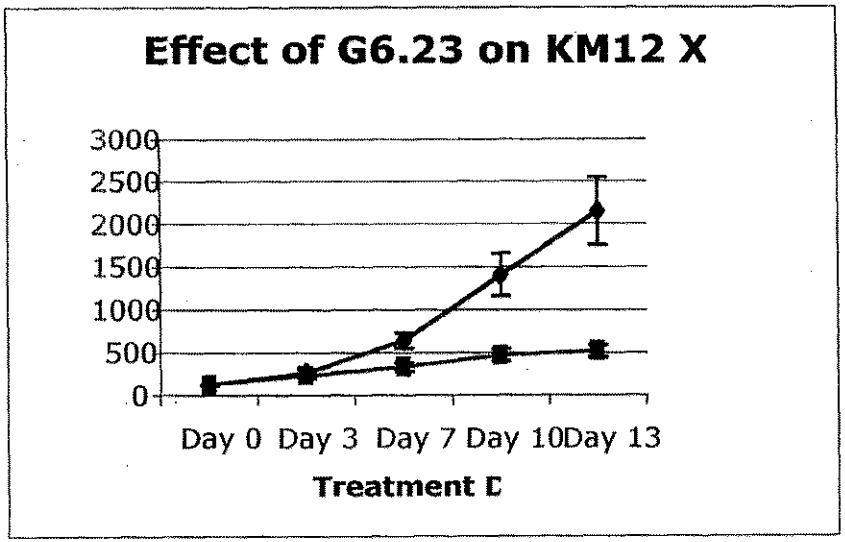


Figure 10

11A



11B

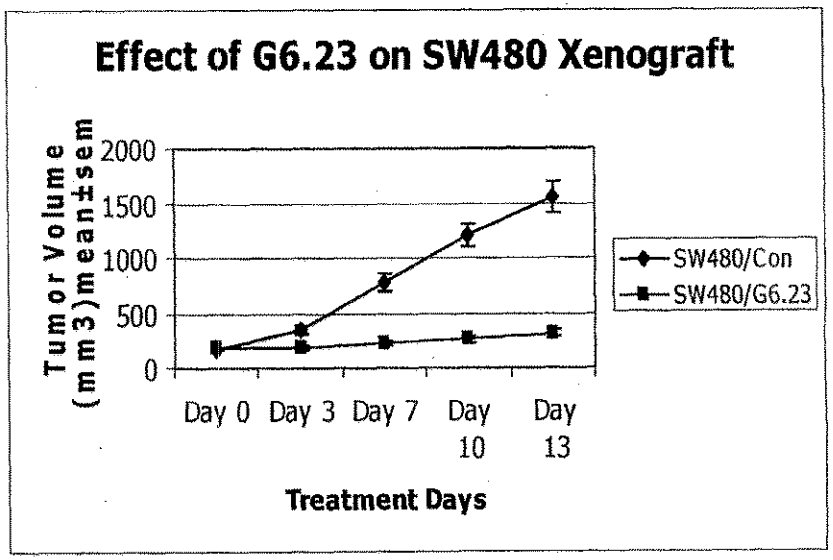
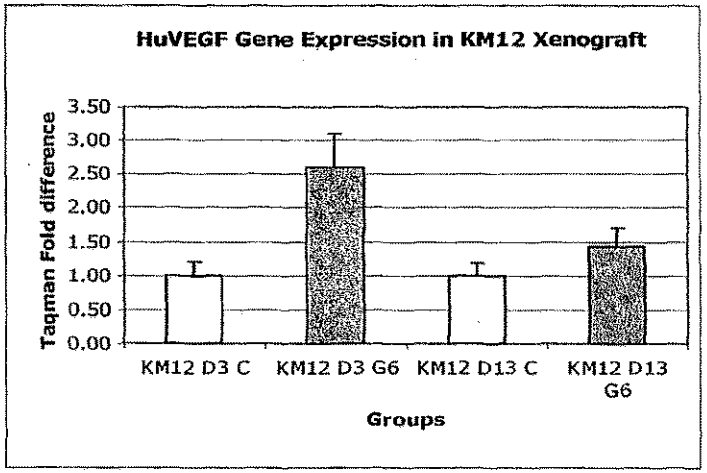


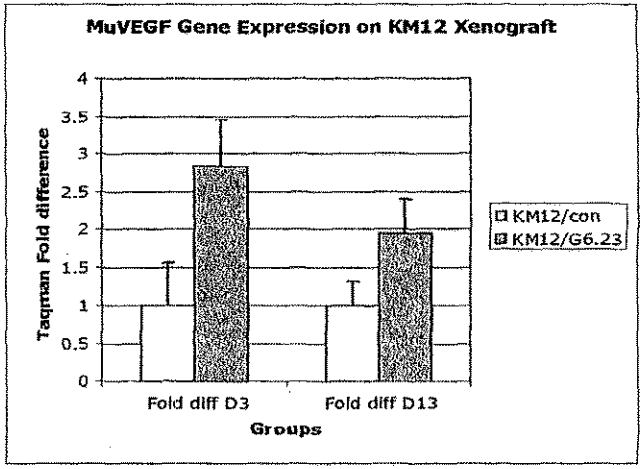
Figure 11

12A



hVEGF

12B



mVEGF

Figure 12

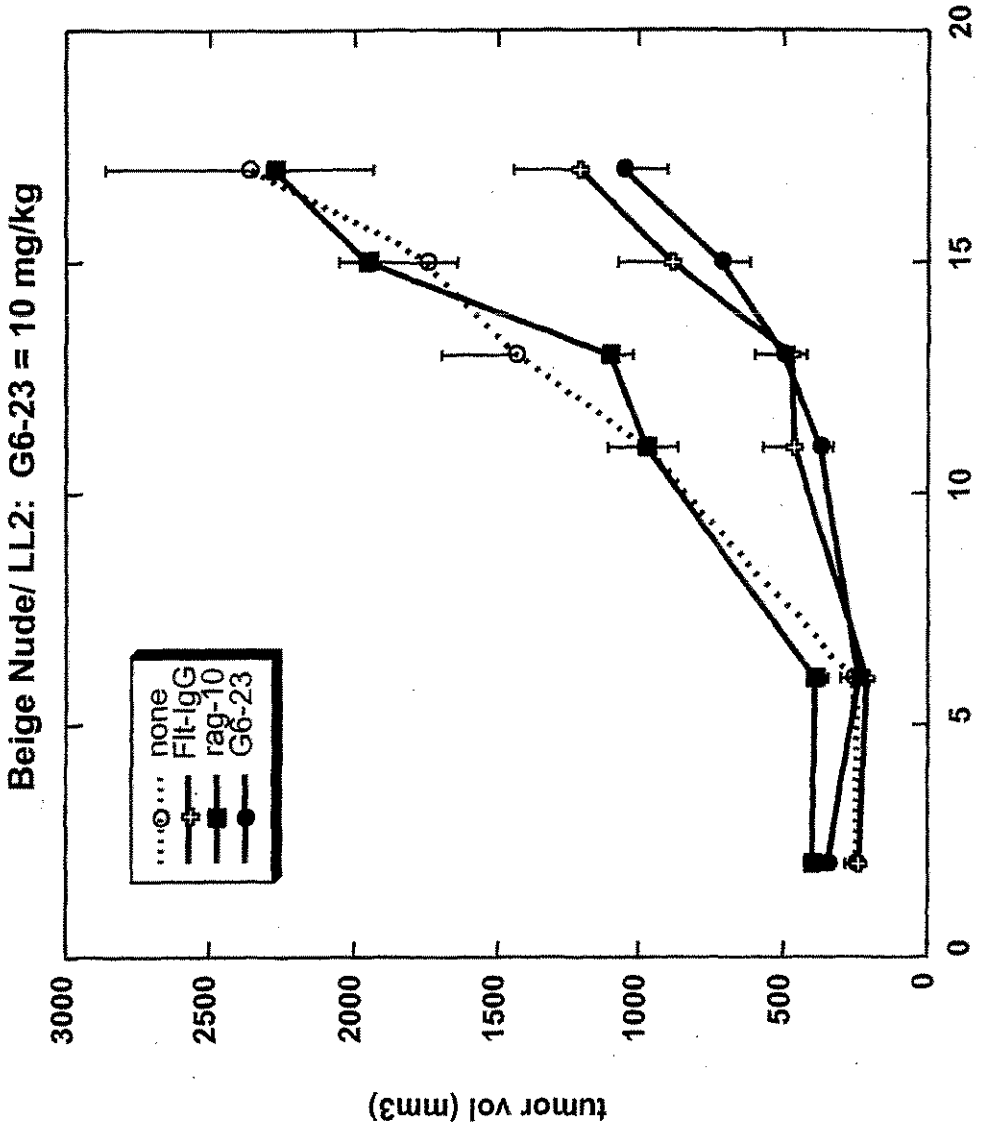

Beige Nude/ LL2: G6-23 = 10 mg/kg
none
Fit-IgG
rag-10
G6-23
tumor vol (mm3)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
day

Figure 13

G6 & G6-23 HC Shotgun Scan Codon								
Wild-type		Alanine-scan				Homolog-scan		
		Codon	m1	m2	m3	Codon	m4	m5
CDR-H1	*S30	KCC	A			KCC	A	
	D31	GMT	A			GAM	E	
	Y32	KMT	A	D	S	TWC	F	
	W33	KSG	A	G	S	YKG	L	R
CDR-H2	*G50	GST	A			GST	A	
	I51					RTT	V	
	T52	RCT	A			ASC	S	
	P52a					SCA	A	
	A53	GST	G			KCT	S	
	*G54	GST	A			GST	A	
	G55					GST	A	
	Y56	KMT	A	D	S	TWC	F	
	T57					ASC	S	
	Y58	KMT	A	D	S	TWC	F	
CDR-H3	F95	KYT	A	S	V	TWC	Y	
	V96	GYT	A			RTT	I	
	F97	KYT	A	S	V	TWC	Y	
	F98	KYT	A	S	V	TWC	Y	
	L99	SYT	A	P	V	MTC	I	
	*P100	SCA	A			SCA	A	
	Y100a	KMT	A	D	S	TWC	F	

Figure 14(A)

G6 & G6-23 LC Shotgun Scan Codons								
Wild-type		Alanine-scan				Homolog-scan		
		Codon	m1	m2	m3	Codon	m4	m5
CDR-L1	*Q27	SMA	A	E	P	SAA	E	
	D28	GMT	A			GAM	E	
	V29	GYT	A			RTT	I	
	*S30	KCC	A			KCC	A	
	T31	RCT	A			ASC	S	
	A32	GST	G			KCT	S	
CDR-L2	Y49	KMT	A	D	S	TWC	F	
	*S50	KCC	A			KCC	A	
	F53	KYT	A	S	V	TWC	Y	
	Y55	KMT	A	D	S	TWC	F	
CDR-L3	*Q89K	SMA/**RMA**	A/**A**	E/**E**	P/**T**	VAR	E/**E**	K/**Q**
	Q90					VAR	E	K
	*S91G	KCC/**GST**	A/**A**			KCC	A/**A**	
	Y92	KMT	A	D	S	TWC	F	
	T93A	RCT/**GST**	A/**G**			ASC	S/**S**	
	*T94N	RCT	A/**A**	**D**	**T**	ASC	S/**D**	
	*P96W	SCA/**KSG**	A/**A**	**G**	**S**	SCA	A/**L**	

Figure 14(B)

Library	Mutated CDR residues	Mutagenic oligos	Diversity	
			Theoretical	Actual
Alanine-scan				
hcA-G6 hcA-G6-23	CDR-H1: S30, D31, Y32, W33 CDR-H2: G50, T52, A53, G54, Y56, Y58 CDR-H3: F95, V96, F97, F98, L99, P100, Y100a	H1-A H2-A H3-A	6.7E+07	2.1E+09
lcA-G6	CDR-L1: Q27, D28, V29, S30, T31, A32 CDR-L2: Y49, S50, F53, Y55 CDR-L3: Q89, S91, Y92, T93, T94, P96	L1-A L2-A L3-A1	4.2E+06	4.8E+09
lcA-G6-23	CDR-L1: Q27, D28, V29, S30, T31, A32 CDR-L2: Y49, S50, F53, Y55 CDR-L3: K89, G91, Y92, A93, N94, W96	L1-A L2-A L3-A2	8.3E+06	4.5E+09
Homolog-scan				
hcH-G6 hcH-G6-23	CDR-H1: S30, D31, Y32, W33 CDR-H2: G50, I51, T52, P52a, A53, G54, G55, Y56, T57, Y58 CDR-H3: F95, V96, F97, F98, L99, P100, Y100a	H1-H H2-H H3-H	3.1E+06	2.3E+09
lcH-G6	CDR-L1: Q27, D28, V29, S30, T31, A32 CDR-L2: Y49, S50, F53, Y55 CDR-L3: Q89, Q90, S91, Y92, T93, T94, P96	L1-H L2-H L3-H1	2.9E+05	4.5E+09
lcH-G6-23	CDR-L1: Q27, D28, V29, S30, T31, A32 CDR-L2: Y49, S50, F53, Y55 CDR-L3: K89, Q90, G91, Y92, A93, N94, P96	L1-H L2-H L3-H2	2.9E+05	5.3E+09

Figure 15

G6 Fab heavy chain shotgun scan																	
		Target selection (VEGF)					Display selection (Anti-gD tag)					$F_{wt/mut}$				
		Alanine-scan			Homolog-scan		Alanine-scan			Homolog-scan		Alanine-scan			Homolog-scan	
	Residue	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	m1	m2	m3	m4	m5
CDR-H1	S30	1.55			2.47		1.63			3		0.9			0.9	
	D31	3.46			2.47		0.96			2.08		3.6			1.2	
	Y32	52	>104	104.0	0.61		1.70	1.62	1.03	0.88		**30.6**	**>64.2**	**100.9**	0.7	
	W33	103	103	51.5	54.5	>109	1.14	1.09	0.66	0.34	1.1	**90.6**	**94.8**	**78.3**	**159.1**	**>95.9**
CDR-H2	G50	53			>111		1.45			1		**36.1**			**>93.9**	
	I51				0.12					0.62					0.2	
	T52	52.50			54.5		1.20			0.50		**43.6**			**109.0**	
	P52a				2					1.55					1.3	
	A53	3.65			1.27		1			0.64		3.8			2.0	
	G54	11			54.5		1.04			1.50		**10.5**			**36.3**	
	G55				36					2.16					**16.7**	
	Y56	3.06	6	1.79	0.91		0.57	0.74	0.45	1		5.4	7.8	4.0	0.8	
	T57				0.56					0.76					0.7	
	Y58	0.05	0.02	0.02	1.02		0.93	1.13	0.84	1.31		0.05	0.02	0.03	0.8	
CDR-H3	F95	>104	>104	35	3.11		5	1.69	69	1.55		**>21.2**	**>61.6**	0.5	2.0	
	V96	26			>111		2.18			1.86		**11.8**			**>59.8**	
	F97	105	105.00	>71	>111		3	1.6	1.8	2.24		**37.5**	**67.5**	**>40.6**	**>49.5**	
	F98	106.00	>106	>106	17.5		2.79	1.34	1.50	2.64		**38.1**	**>79**	**>71**	6.6	
	L99	52	>104	104.00	>111		0.75	4.0	0.52	1.67		**69.3**	**>26**	**199.3**	**>66.6**	
	P100	8			>111		0.89			3.62		8.8			**>30.7**	
	Y100a	89	89.00	5.56	1.71		1.19	1.92	0.51	0.48		**74.8**	**46.3**	**10.9**	3.5	

Figure 16(A)

G6-23 Fab heavy chain shotgun scan																	
		Target selection (VEGF)					Display selection (Anti-gD tag)					$F_{wt/mut}$				
		Alanine-scan			Homolog-scan		Alanine-scan			Homolog-scan		Alanine-scan			Homolog-scan	
	Residue	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	m1	m2	m3	m4	m5
CDR-H1	S30	2.54			2.29		1.55			4		1.6			0.6	
	D31	2.44			2.75		0.67			2.59		3.7			1.1	
	Y32	16	>113	28.3	0.78		1.29	1.64	0.82	0.82		**12.6**	**>69.1**	**34.5**	0.9	
	W33	>123	>123	123	1.5	>81	1.21	1.43	1.38	0.64	1.5	**>101.5**	**>86.1**	**89.2**	2.3	**>53.3**
CDR-H2	G50	>124			>135		0.94			2		**>132**			**>54.3**	
	I51				0.61					0.82					0.7	
	T52	40.33			4.6		1.00			0.56		**44.3**			8.2	
	P52a				44					1.98					**22.3**	
	A53	5.53			0.47		1			0.56		5.4			0.8	
	G54	7			32.8		0.69			2.13		**10.6**			**15.4**	
	G55				7					2.21					3.1	
	Y56	2.55	4	1.31	0.78		1.27	1.73	0.95	1		2.0	2.1	1.4	1.1	
	T57				1.01					0.77					1.3	
	Y58	0.89	1.79	0.44	1.70		1.45	0.91	0.59	1.22		0.84	2.34	0.93	1.4	
CDR-H3	F95	>118	>118	20	3.82		4	2.50	3	1.98		**>30.9**	**>47.2**	6.7	1.9	
	V96	9			14.00		1.95			1.98		4.4			7.1	
	F97	>123	>123	123.00	66.50		4	1.3	1.9	1.39		**>33.8**	**>91.7**	**65.1**	**47.8**	
	F98	>123	123.00	>123	6.5		3.47	2.11	2.27	2.05		**>35.4**	**58.4**	**>54.2**	3.2	
	L99	4	44.00	9.78	15.88		1.40	2.1	0.66	1.65		2.5	**21.4**	**14.8**	9.6	
	P100	2			6.94		0.73			2.21		2.5			3.1	
	Y100a	27	>109	9.91	2.14		1.36	2.24	0.81	1.14		**20.1**	**>48.8**	**12.3**	1.9	

Figure 16(B)

G6 Fab light chain shotgun scan																	
		Target selection (VEGF)					Display selection (Anti-gD tag)					$F_{wt/mut}$				
		Alanine-scan			Homolog-		Alanine-scan			Homolog-		Alanine-scan			Homolog-	
	Residue	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	m1	m2	m3	m4	m5
CDR-L1	Q27	1.24	1.9	0.8	1.58		0.88	1.75	0.93	2.00		1.4	1.1	0.9	0.8	
	D28	0.61			0.97		0.49			1.52		1.2			0.6	
	V29	2			1.76		2.42			1.22		0.8			1.4	
	S30	2			1.2		1.59			1.06		1.0			1.2	
	T31	1			0.9		0.66			0.88		1.0			1.1	
	A32	2			1.83		1.72			1.85		1.0			1.0	
CDR-L2	Y49	9.78	88.00	6.77	1.83		0.92	2.40	0.52	0.98		**10.6**	**36.7**	**13.0**	1.9	
	S50	1.467			0.90		1.16			1.41		1.3			0.6	
	F53	4.92	2.03	5.36	1.47		2.53	2.15	1.65	1.13		1.9	0.9	3.2	1.3	
	Y55	0.97	11	0.87	3.30		0.88	3.50	0.74	1.41		1.1	3.2	1.2	2.3	
CDR-L3	Q89	0.57	2	16.50	1.50	2.71	0.96	4.78	4.78	2.63	5.46	0.6	0.4	3.5	0.6	0.5
	Q90				0.98	3.92				1.47	1.47				0.7	2.7
	S91	0.98			0.41		1.41			0.91		0.7			0.5	
	Y92	1.17	17.50	0.80	0.40		1.00	3.00	1.14	0.59		1.2	5.8	0.7	0.7	
	T93	0.48			1.42		0.45			0.71		1.06			2.0	
	T94	1			0.73		0.89			0.68		0.56			1.1	
	P96	5			3.64		2.66			4.55		1.7			0.8	

Figure 17(A)

G6-23 Fab light chain shotgun scan																	
		Target selection (VEGF)					Display selection (Anti-gD tag)					$F_{wt/mut}$				
		Alanine-scan			Homolog-		Alanine-scan			Homolog-		Alanine-scan			Homolog-	
	Residue	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	Wt/m1	Wt/m2	Wt/m3	Wt/m4	Wt/m5	m1	m2	m3	m4	m5
CDR-L1	Q27	1.75	2.69	1.03	1.00		1.24	1.63	0.76	1.76		1.41	1.66	1.35	0.57	
	D28	0.62			1.18		0.64			1.37		0.96			0.86	
	V29	1.76			1.65		2.23			0.82		0.79			2.01	
	S30	2.29			1.18		2.46			1.00		0.93			1.18	
	T31	0.65			1.09		0.90			1.04		0.72			1.04	
	A32	2.00			1.72		2.23			1.49		0.90			1.16	
CDR-L2	Y49	13.17	19.75	6.08	3.26		2.11	9.50	1.03	2.00		6.24	2.08	5.92	1.63	
	S50	1.49			1.58		1.62			1.37		0.92			1.15	
	F53	3.53	2.94	3.31	0.92		4.67	1.68	2.00	1.08		0.76	1.75	1.66	0.85	
	Y55	0.94	7.75	0.91	2.63		0.76	8.67	0.76	1.83		1.23	0.89	1.19	1.43	
CDR-L3	K89	0.05	0.31	2.00	0.82	0.21	0.07	0.45	0.71	0.39	0.13	0.71	0.68	2.80	2.10	1.63
	Q90				1.80	3.86				2.48	8.38				0.73	0.46
	G91	0.55			0.92		0.43			0.82		1.28			1.12	
	Y92	1.95	20.50	1.08	0.36		1.19	3.57	0.57	0.48		1.64	5.74	1.90	0.76	
	A93	2.19			0.78		1.49			0.55		1.47			1.43	
	N94	0.11	0.44	0.47	1.28		0.09	0.42	0.23	0.79		1.27	1.05	2.05	1.62	
	W96	0.31	1.10	0.24	0.21		2.29	12.00	2.00	0.57		0.13	0.09	0.12	0.37	

Figure 17(B)

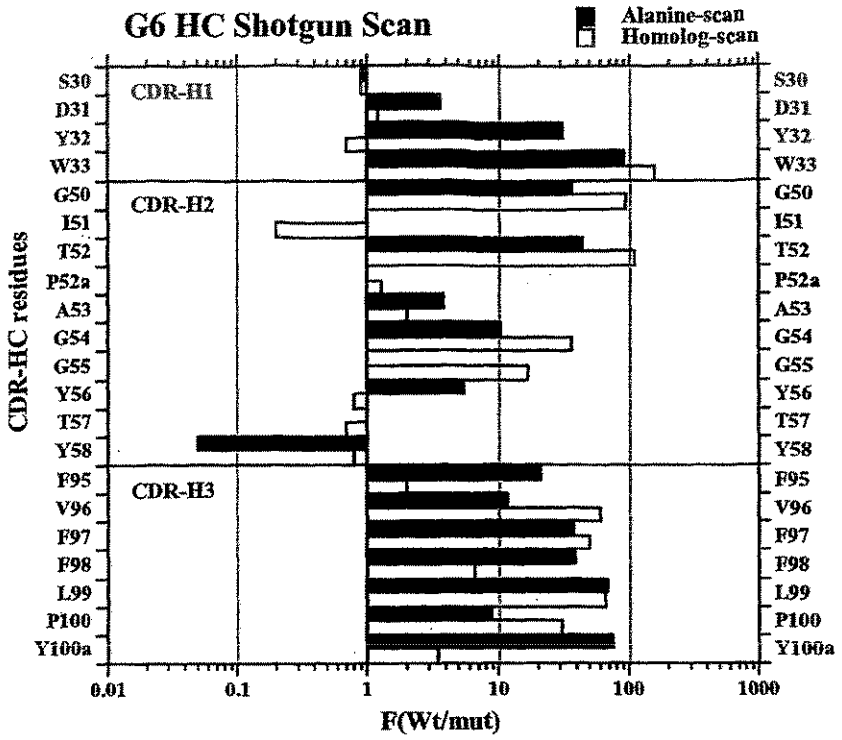
Origin	CDR	Mutants	Conventional scanning		Shotgun scanning	
			$IC_{50,mut}/IC_{50,wt}$	ΔG_{mut-wt} (kcal/mo	$F_{wt/mut}$	ΔG_{mut-wt} (kcal/mo
FabG6	H1	W33A	>400	>3.55	90.6	2.67
	H2	G50A	>400	>3.55	36.1	2.12
		I51V	0.08	-1.5	0.2	-1.0
		T52A	113.6±24.7	2.8±1.9	43.6	2.2
		P52aA	1.9	0.38	1.3	0.2
		G54A	20.8±4.3	1.8±0.86	10.5	1.4
		Y58A	0.14	-1.2	0.05	-1.8
	H3	V96A	>400	>3.55	11.8	1.46
		V96I	25.8±5.1	1.92±0.96	>59.8	>2.42
		F97A	>400	>3.55	37.5	2.15
		F97Y	>400	>3.55	>49.5	>2.31
		L99A	>400	>3.55	69.3	2.51
		P100A	>400	>3.55	8.8	1.29
FabG6-23	H1	W33A	>5000	>5.04	>101.5	>2.74
	H2	G50A	>5000	>5.04	>132	>2.89
		I51V	0.96	-0.02	0.7	-0.21
		T52A	243±28	3.25±1.97	44.3	2.24
		P52aA	711±113	3.9±2.8	22.3	1.84
		G54A	44±3	2.24±0.65	10.5	1.39
		Y58A	0.7	-0.21	0.6	-0.30
	H3	V96A	95±9	2.7±1.3	4.4	0.88
		V96I	14.6±0.8	1.59±0.13	7.1	1.16
		F97A	>5000	>5.04	>33.8	>2.08
		F97Y	725±74	3.9±2.5	47.8	2.29
		L99A	9.6±0.4	1.34±0.54	2.5	0.54
		P100A	13±0.6	1.5±0.3	2.5	0.54

Figure 18

VEGF residue alanine scan	Relative affinity (IC50mut/IC50wt)						
	G6 Fab	G6-23 Fab	B20-4	*A4.6.1	*Flt-1(1-3)	*Flt-1$_{D2}$	*KDR-IgG
20s helix							
WT	1.0	1.0	1.0	1.0	1	1	1.0
V14A	1.1	1.4	1.2	1.5			1.8
K16A	1.1	1.7	0.8	1.2	1	2	1.1
F17A	NB	NB	>100	0.8	54		NB
M18A	4.4±0.5	4.1±0.4	>100	1.2	9		5.0
D19A	1	0.9	>100	0.7	0.8	1	0.6
Y21A	22.6±4.3	1540±720	>100	2.6	29		2.8
Q22A	7.2±0.4	3.9±0.4	0.5	1.4	15	11	2.2
Y25A	20.5±2.9	131.5±15.2	>100	1.2	7	17	1.7
40s loop							
I43A	1	1.6	1.2	1.2	3		5.6
Y45A	3.7±0.2	1.8	1.1	2.2		4	1.6
I46A	2.2	1.7	1.3	2.1	4	3	NB
F47A	3.5±0.2	2.8	1.8	NB	3	1	3.6
K48A	0.9	1.5	1	0.7	1		0.7
60s loop							
N62A	0.5	1.1	1.7	2.2			2.0
D63A	5.2±0.6	13.4±1.4	1.7	0.9	8	7	0.5
E64A	2.4	3.2±0.1	1.7	1.7	5	5	8.6
G65A	0.6	1.5	1.6	1.7	1	1	1.3
L66A	0.7	2	1.8	1.6	10		0.3
80s loop							
M81A	1.3	4.2±1.2	1.8	NB	5	7	2.0
R82A	1.3	1.1	1.3	3.3			0.6
I83A	39.9±11.1	17.6±0.9	0.9	1.0	7	5	100.0
K84A	0.8	1.8	1.5	1.8			2.2
H86A	7.5±1.1	2.8	0.8	1.9	1	1	1.3
Q87A	2.3	3.2±0.1	1.1	1.3			1.8
G88A	2.7	1.6	1.1	NB			1.0
Q89A	91.5±19.2	8945±5815	>100	NB	1	1	2.5
H90A	1	1	1.2	1.8			1.7
I91A	6.9±0.4	2.8	1.3	1.2	1	1	1.0
G92A	0.9	1.6	1.4	4.5			1.2
E93A	2.9	1.4	1.2	6.6			0.5
M94A	1	0.8	1.1	NB			2.8
E103A	0.7	0.8	1	2.3			1.1
P106	1.8	1.2				6	
R105A	1.6	0.7	0.9	1.4		1	0.8
				>20			
				5<20			

Figure 19

A.


G6 HC Shotgun Scan
Alanine-scan
Homolog-scan
CDR-H1
CDR-H2
CDR-H3
S30
D31
Y32
W33
G50
I51
T52
P52a
A53
G54
G55
Y56
T57
Y58
F95
V96
F97
F98
L99
P100
Y100a
CDR-HC residues
0.01
0.1
1
10
100
1000
F(Wt/mut)

B.

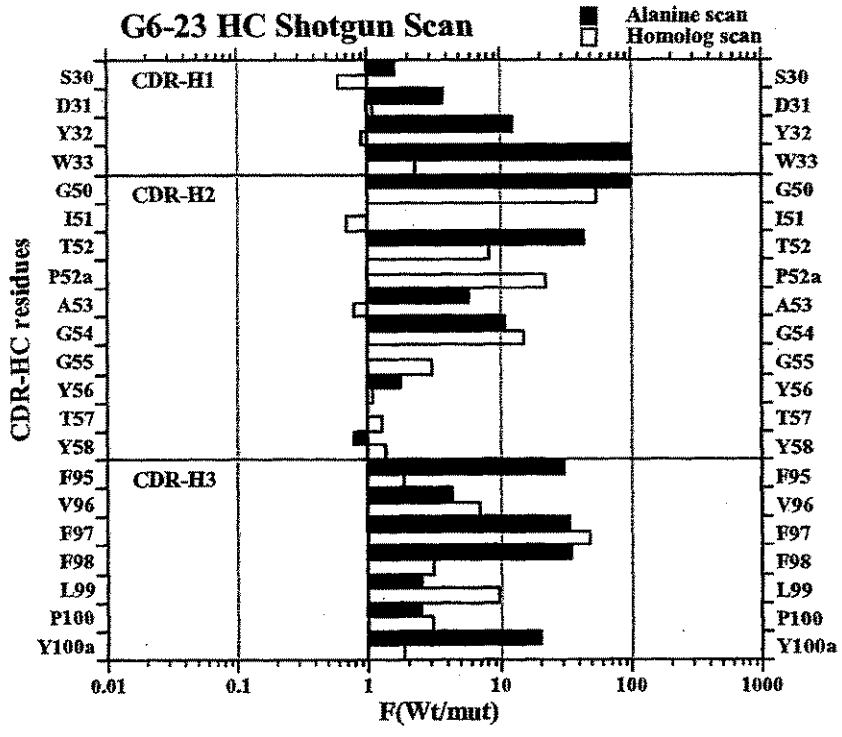
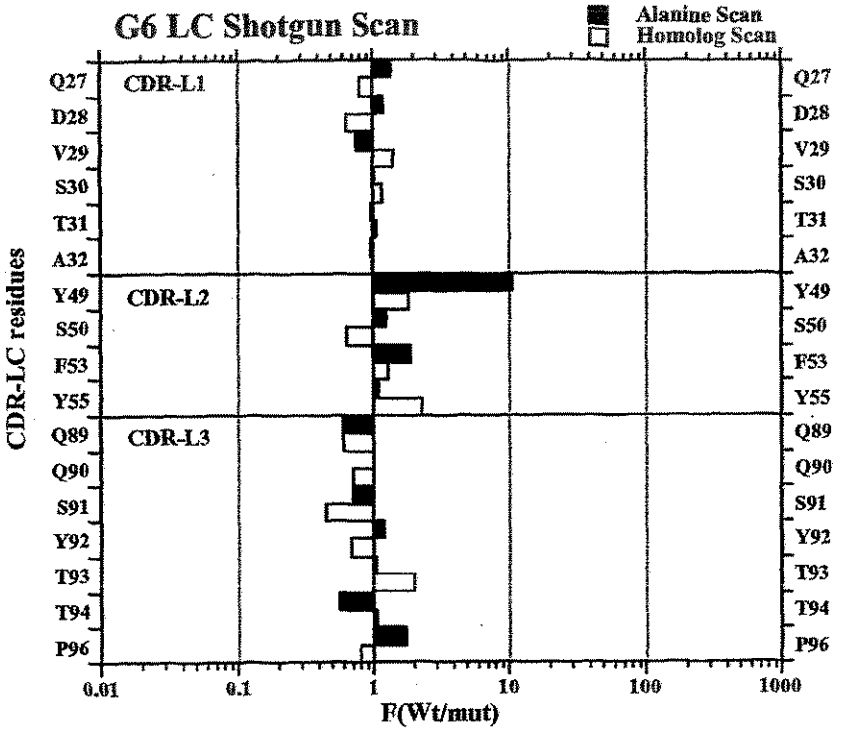

G6-23 HC Shotgun Scan
Alanine scan
Homolog scan
CDR-H1
CDR-H2
CDR-H3
S30
D31
Y32
W33
G50
I51
T52
P52a
A53
G54
G55
Y56
T57
Y58
F95
V96
F97
F98
L99
P100
Y100a
CDR-HC residues
0.01
0.1
1
10
100
1000
F(Wt/mut)

Figure 20

A.


G6 LC Shotgun Scan
Alanine Scan
Homolog Scan
CDR-L1
CDR-L2
CDR-L3
Q27
D28
V29
S30
T31
A32
Y49
S50
F53
Y55
Q89
Q90
S91
Y92
T93
T94
P96
CDR-LC residues
0.01
0.1
1
10
100
1000
F(Wt/mut)

B.

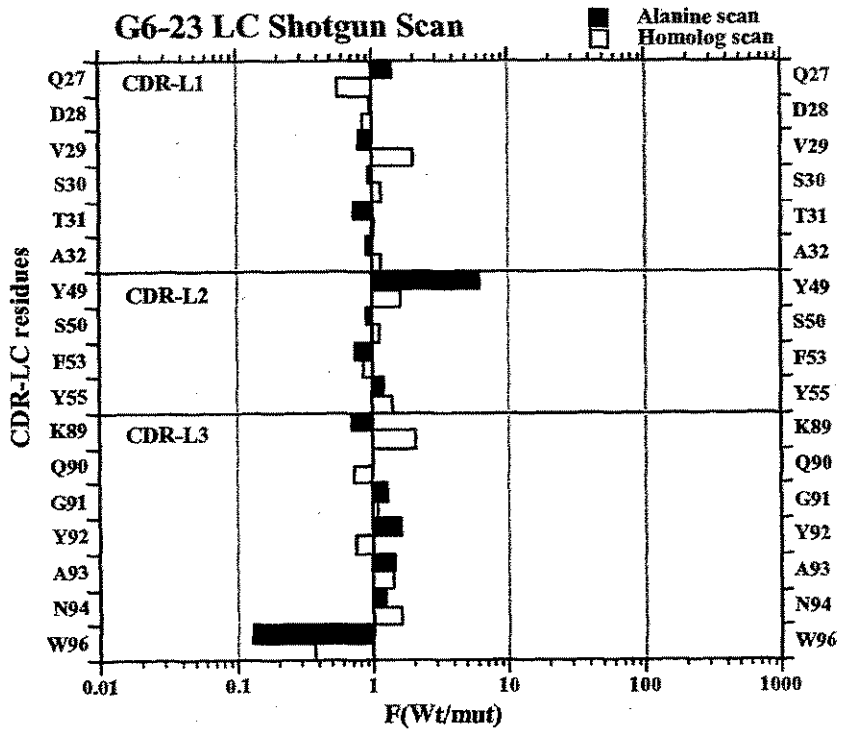
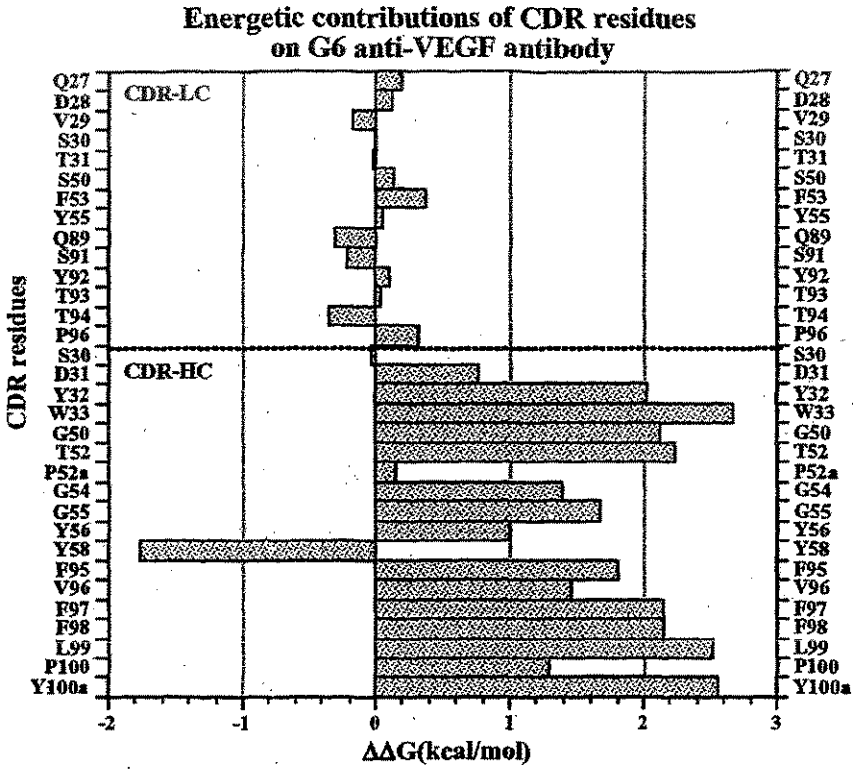

G6-23 LC Shotgun Scan
Alanine scan
Homolog scan
CDR-L1
CDR-L2
CDR-L3
Q27
D28
V29
S30
T31
A32
Y49
S50
F53
Y55
K89
Q90
G91
Y92
A93
N94
W96
CDR-LC residues
0.01
0.1
1
10
100
1000
F(Wt/mut)

Figure 21

A.


Energetic contributions of CDR residues
on G6 anti-VEGF antibody
CDR-LC
CDR-HC
CDR residues
Q27
D28
V29
S30
T31
S50
F53
Y55
Q89
S91
Y92
T93
T94
P96
D31
Y32
W33
G50
T52
P52a
G54
G55
Y56
Y58
F95
V96
F97
F98
L99
P100
Y100a
-2
-1
0
1
2
3
ΔΔG(kcal/mol)

B.

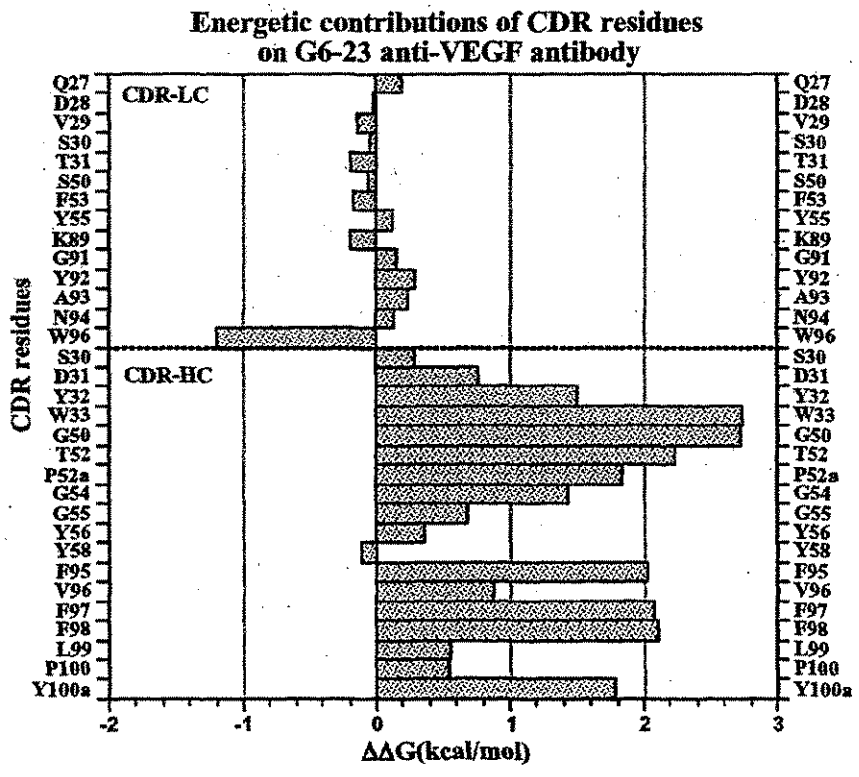

Energetic contributions of CDR residues
on G6-23 anti-VEGF antibody
CDR-LC
CDR-HC
CDR residues
Q27
D28
V29
S30
T31
S50
F53
Y55
K89
G91
Y92
A93
N94
W96
D31
Y32
W33
G50
T52
P52a
G54
G55
Y56
Y58
F95
V96
F97
F98
L99
P100
Y100a
-2
-1
0
1
2
3
ΔΔG(kcal/mol)

Figure 22

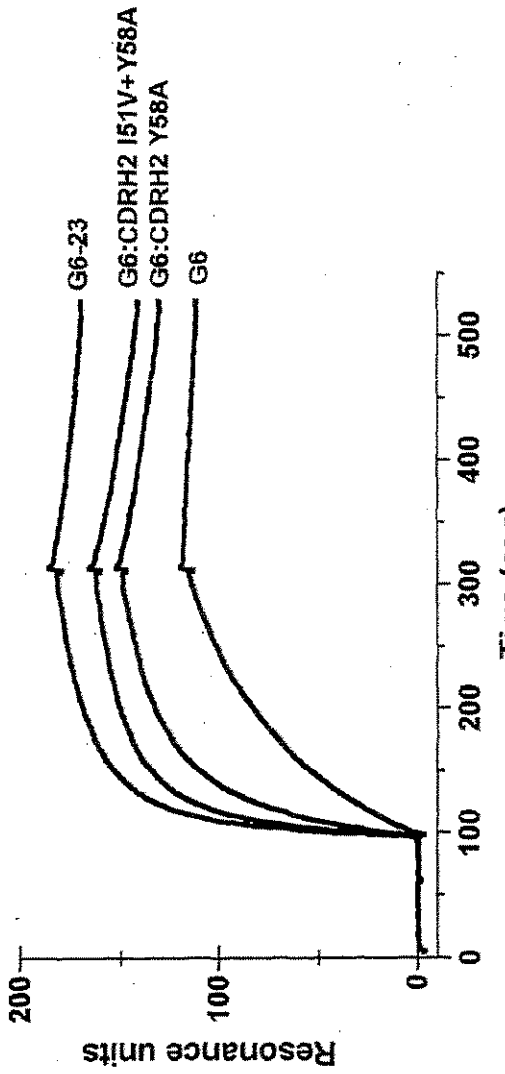

G6-23
G6:CDRH2 I51V+Y58A
G6:CDRH2 Y58A
G6
Resonance units
200
100
0
0
100
200
300
400
500
Time (sec)

Figure 23

G6 Fab Light chain

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVP

SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVEIKRTVAAPSVFIF

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS

TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

G6 Fab Heavy chain

a

EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIHWVRQAPGKGLEWVAGITPAGGYTY

YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPYAMDYWGQGTLVTV

SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL

QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH

Figure 24

G6-23 light chain

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCKQGYANPWTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

G6-23 Fab Heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIHWVRQAPGKGLEWVAGITPAGG
YTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPYAMDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTH

G6-31 light chain (heavy chain sequence same as G6)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGYGNPFTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

G6-8 light chain (heavy chain sequence same as G6)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGAGSPLTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 25

G6-23.1 light chain

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCKQGFANPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC

G6-23.1 Fab Heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIHWVRQAPGKGLEWVAGVTPAGGYTYYADSVKGRF
TISADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTH

G6-23.2 light chain (same as G6-23.1)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCKQGFANPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC

G6-23.2 Fab Heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFTISDYWIHWVRQAPGKGLEWVAGVTPAGGYTAYADSVKGRF
TISADTSKNTAYLQMNSLRAEDTAVYYCARFVFFLPYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTH

Figure 26

B20 (Fab) Light chain

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

B20 (Fab) Heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIHWVRQAPGKGLEWVGAIYPYSG
YTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARWGHSTSPWAMDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTH

Figure 27

Clone No.	CDR-L1						CDR-L2			CDR-L3					IC50 (nM)	
	28	29	30	31	32	33	50	53	55	91	92	93	94	96	mVEGF	hVEGF
B20	D	V	S	T	A	V	S	F	Y	S	Y	T	T	P	22	159
B20-50	D	V	S	T	A	V	S	F	Y	S	Y	G	A	L	8.8	44.7
B20-59	D	V	S	T	A	V	S	F	Y	S	Y	S	S	L	9.3	34.4
B20-73	G	V	R	T	S	L	G	T	A	S	Y	A	S	L	13.8	8.6
B20-3	V	I	G	R	S	L	S	F	Y	S	Y	A	S	L	6.2	6.3
B20-8	D	V	S	T	A	V	S	F	Y	S	Y	G	S	L	2.6	21.5
B20-2	N	V	R	S	S	L	S	N	E	S	Y	A	A	L	6.2	9.6
B20-4a	V	I	G	T	S	L	S	N	A	S	Y	G	S	L	7.7	24.8
B20-4	V	I	R	R	S	L	A	N	A	S	N	T	S	L	8.2	6
B20-6	D	V	S	T	A	V	S	T	A	S	T	T	S	L	6.8	43
B20-5	D	V	S	T	A	V	S	F	Y	S	T	N	S	L	8	57
B20-1a	G	V	K	N	S	L	S	T	E	S	N	A	S	L	7.3	11.6
B20-1	D	V	R	T	S	L	G	T	A	S	N	S	S	L	4.6	6
B20-13	N	V	R	N	F	L	S	T	A	H	T	N	S	L	2.8	28.5

Figure 28

B20-4 (+light chain randomization) Fab

Light chain
DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAWYQQKPGKAPKLLIYAASNLASGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQSNTSPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain (same as B20)
EVQLVESGGGLVQPGGSLRLSCAASGFTINASWIHWVRQAPGKGLEWVGAIYPYSGYTNYADSVKGRFTISADTS
KNTAYLQMNSLRAEDTAVYYCARWGHSTSPWAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTH

B20-4.1
Light chain
DIQMTQSPSSLSASVGDRVTITCRASQVIRRSLAWYQQKPGKAPKLLIYAASNLASGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQSNTSPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain
EVQLVESGGGLVQPGGSLRLSCAASGFSINGSWIFWVRQAPGKGLEWVGAIWPFGGYTHYADSVKGRFTISADTS
KNTAYLQMNSLRAEDTAVYYCARWGHSTSPWAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTH

Figure 29

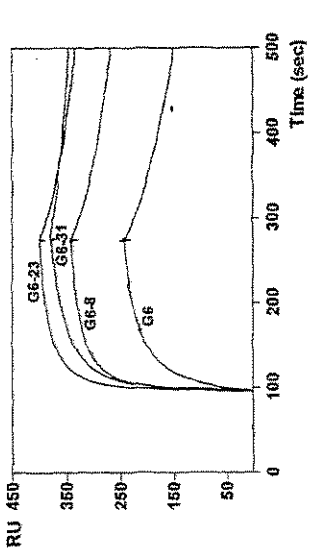

A.
RU
450
350
250
150
50
0
100
200
300
400
500
Time (sec)
G6-23
G6-31
G6-8
G6
B.
G6
G6-8
G6-23
G6-31
1.5
1
0.5
0
50
100
150
200
250
300
350
[mVEGF], nM (monomer)

Figure 30

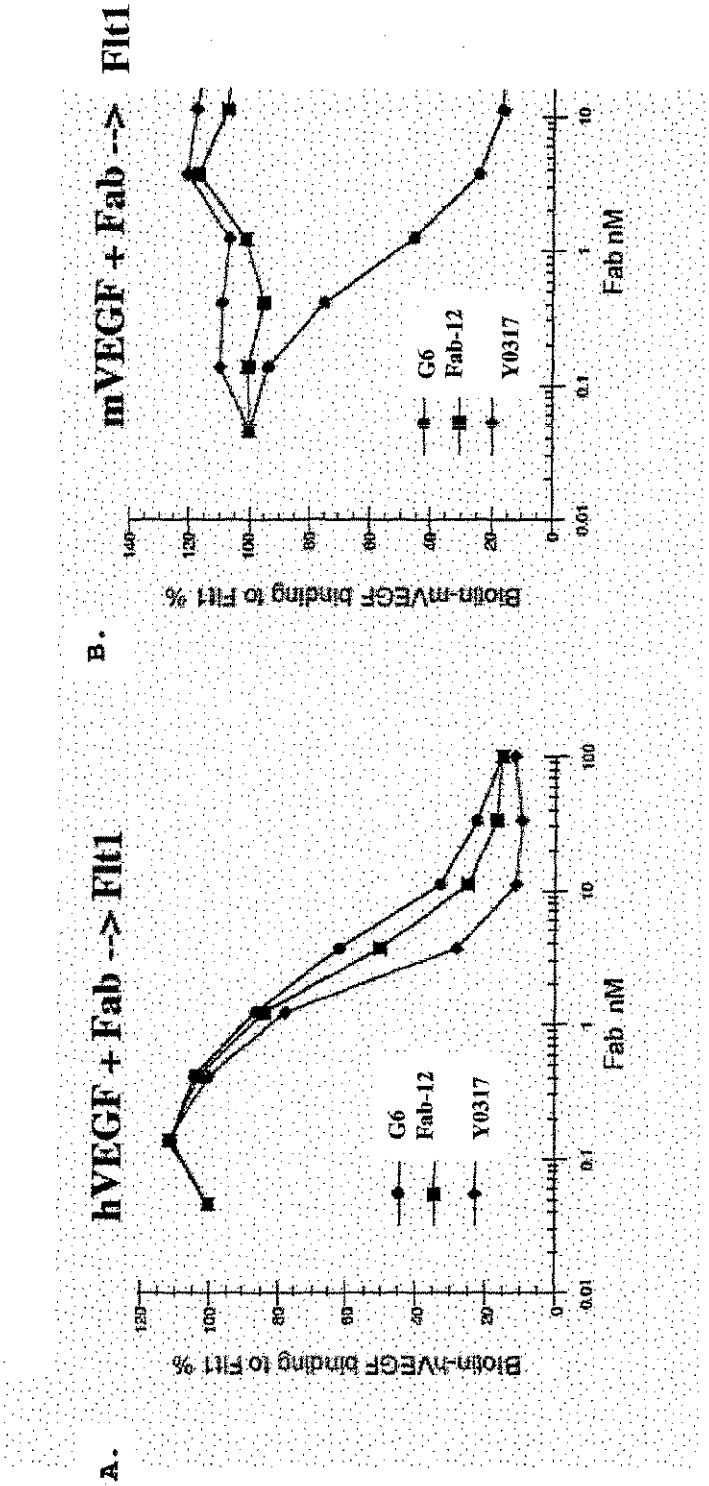

A.
hVEGF + Fab --> Flt1
Biotin-hVEGF binding to Flt1 %
Fab nM
G6
Fab-12
Y0317
B.
mVEGF + Fab --> Flt1
Biotin-mVEGF binding to Flt1 %
Fab nM
G6
Fab-12
Y0317

Figure 31

	25 C							
	$k_{on}(x10^4)\ M^{-1}s^{-1}$		$k_{off}(x10^{-4})s^{-1}$		$Kd(k_{off}/k_{on})$ nM		IC_{50} nM	
	hVEGF	mVEGF	hVEGF	mVEGF	hVEGF	mVEGF	hVEGF	mVEGF
G6	15.2	19.3	2.5	1.3	1.6	0.7	2.1	0.6
G6-8	~50	~50	2.6	1.2	~0.5	~0.3	0.07	0.06
G6-31	~50	~50	0.9	0.3	~0.2	~0.08	0.02	0.03
G6-23	~126	~114	2.6	1.2	~0.2	~0.1	0.01	0.01
Fab-12	4.1	NB	1.2	NB	2.9	NB	1.1	NB
Y0317	3.6	ND	≤0.05	ND	~0.2	ND	0.02	~300
	37 C							
G6	24.4	25.5	21.1	8	8.6	3.1	3.5	ND
G6-8	~80	~100	25.3	12.4	~3.2	~1.2	0.13	ND
G6-31	~80	~100	3.4	0.6	~0.4	~0.06	0.04	ND
G6-23	~120	~138	7.8	2.8	~0.6	~0.2	0.04	ND
Fab-12	5.1	NB	6.6	NB	12.9	NB	2.5	NB
Y0317	5.4	ND	0.06	ND	0.11	ND	ND	ND

Figure 32

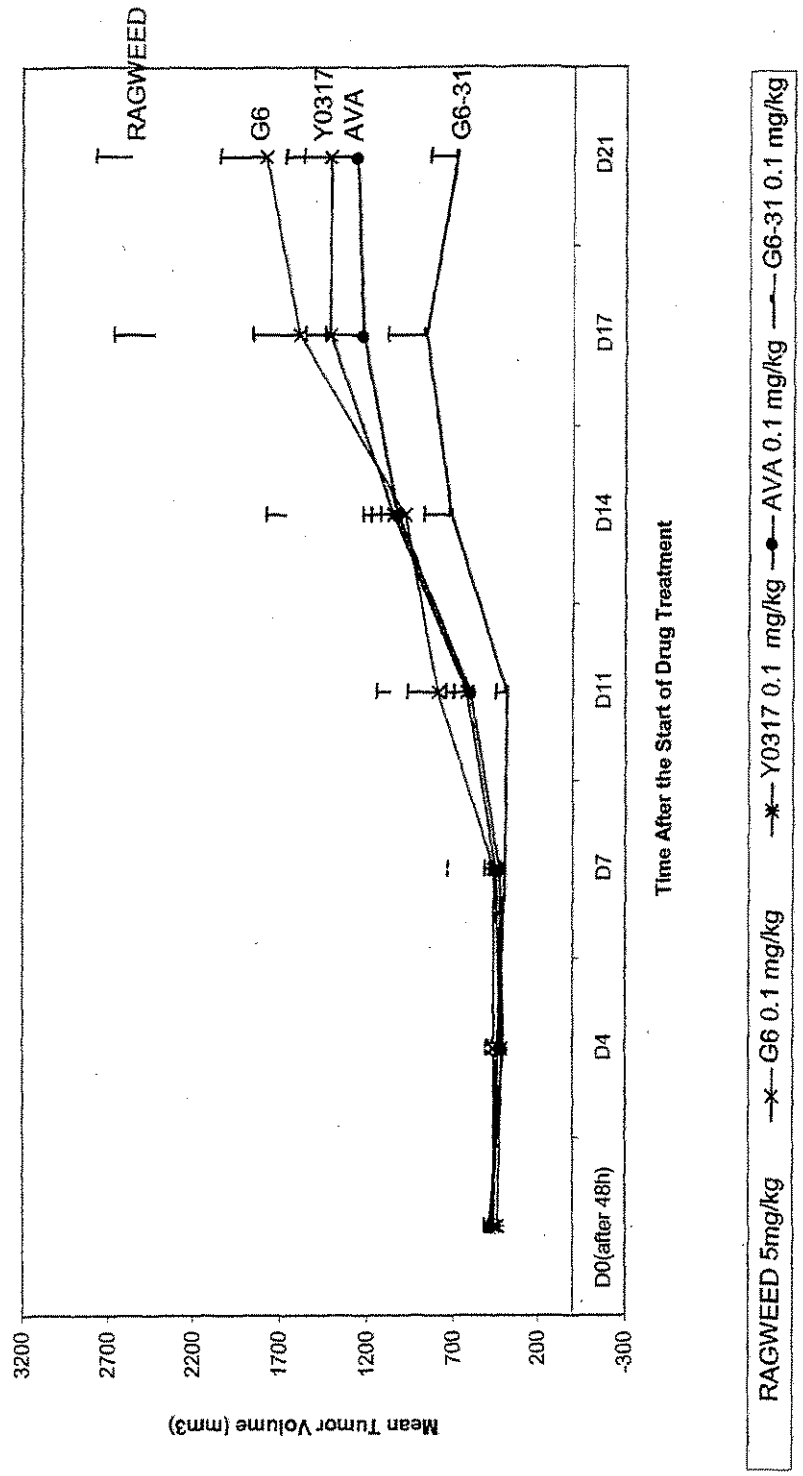

G6 and G6-31 at dose 0.1 mg/kg suppresses HM7 Human Tumor Growth in Mice
Mean Tumor Volume (mm3)
3200
2700
2200
1700
1200
700
200
-300
RAGWEED
G6
Y0317
AVA
G6-31
D0(after 48h)
D4
D7
D11
D14
D17
D21
Time After the Start of Drug Treatment
RAGWEED 5mg/kg
G6 0.1 mg/kg
Y0317 0.1 mg/kg
AVA 0.1 mg/kg
G6-31 0.1 mg/kg

Figure 33A

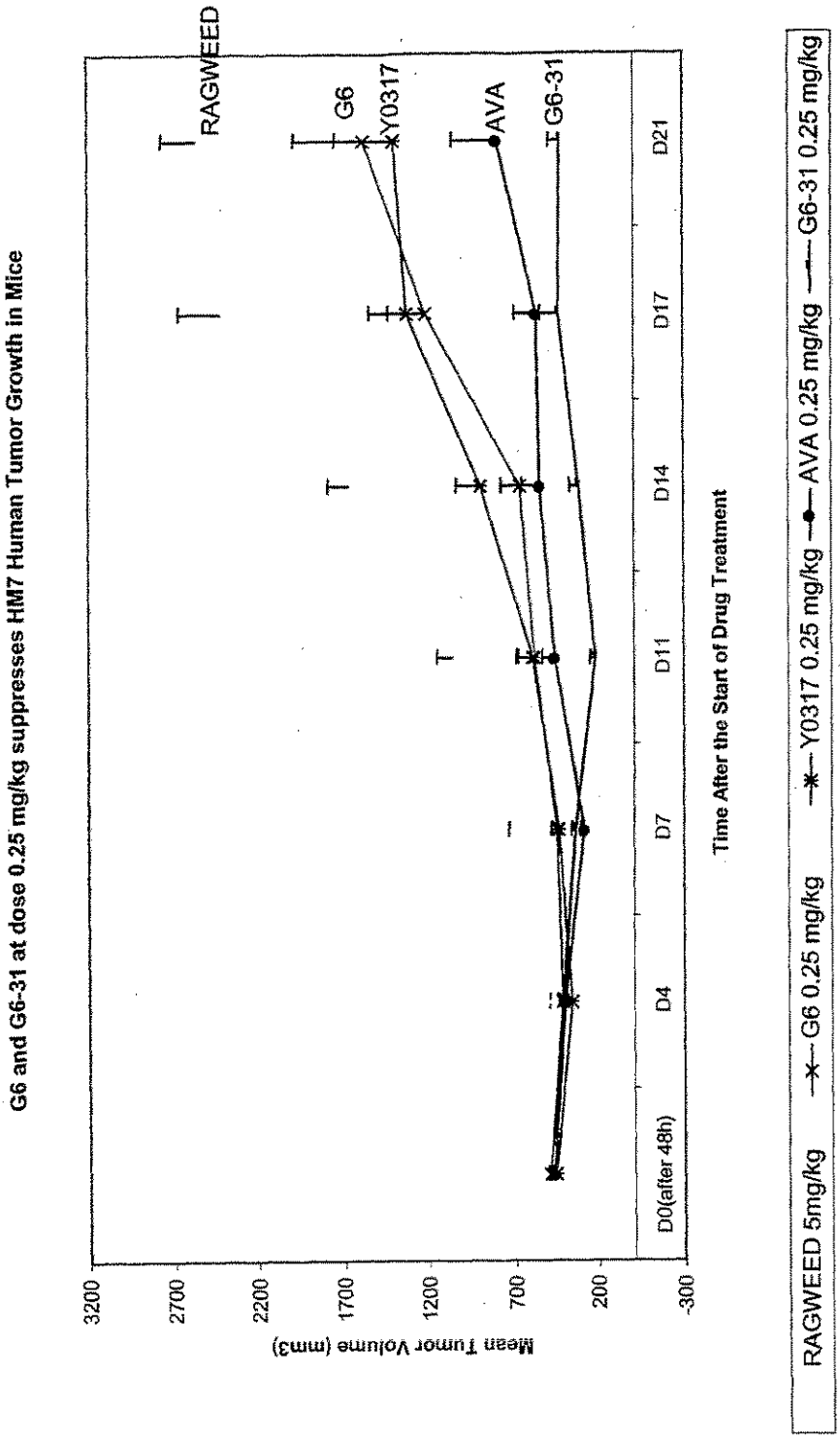

G6 and G6-31 at dose 0.25 mg/kg suppresses HM7 Human Tumor Growth in Mice
Mean Tumor Volume (mm3)
3200
2700
2200
1700
1200
700
200
-300
RAGWEED
G6
Y0317
AVA
G6-31
D0(after 48h)
D4
D7
D11
D14
D17
D21
Time After the Start of Drug Treatment
RAGWEED 5mg/kg
G6 0.25 mg/kg
Y0317 0.25 mg/kg
AVA 0.25 mg/kg
G6-31 0.25 mg/kg

Figure 33B

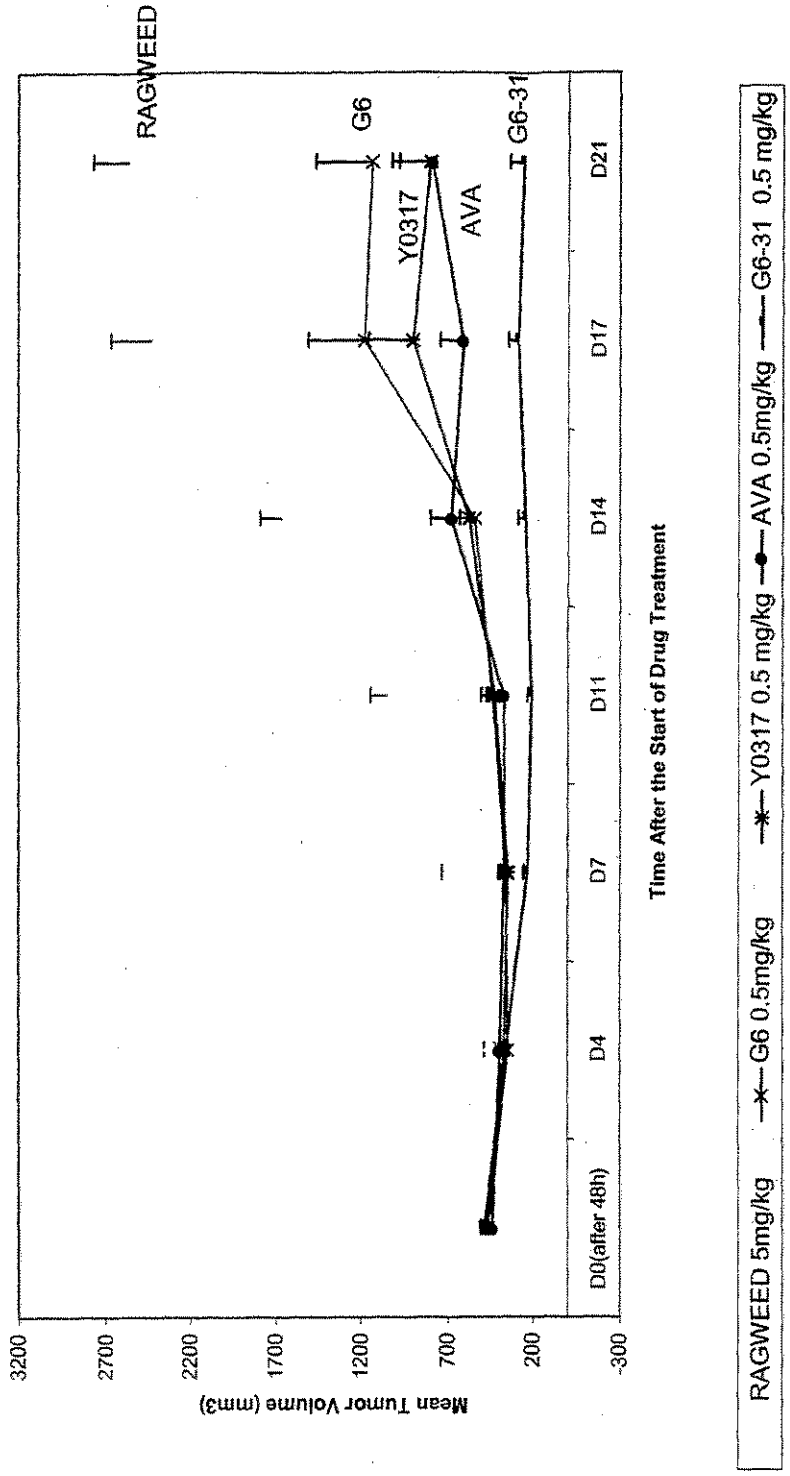

G6 and G6-31 at dose 0.5 mg/kg suppresses HM7 Human Tumor Growth in Mice
Mean Tumor Volume (mm3)
3200
2700
2200
1700
1200
700
200
-300
RAGWEED
G6
Y0317
AVA
G6-31
D0(after 48h)
D4
D7
D11
D14
D17
D21
Time After the Start of Drug Treatment
RAGWEED 5mg/kg
G6 0.5mg/kg
Y0317 0.5 mg/kg
AVA 0.5mg/kg
G6-31 0.5 mg/kg

Figure 33C

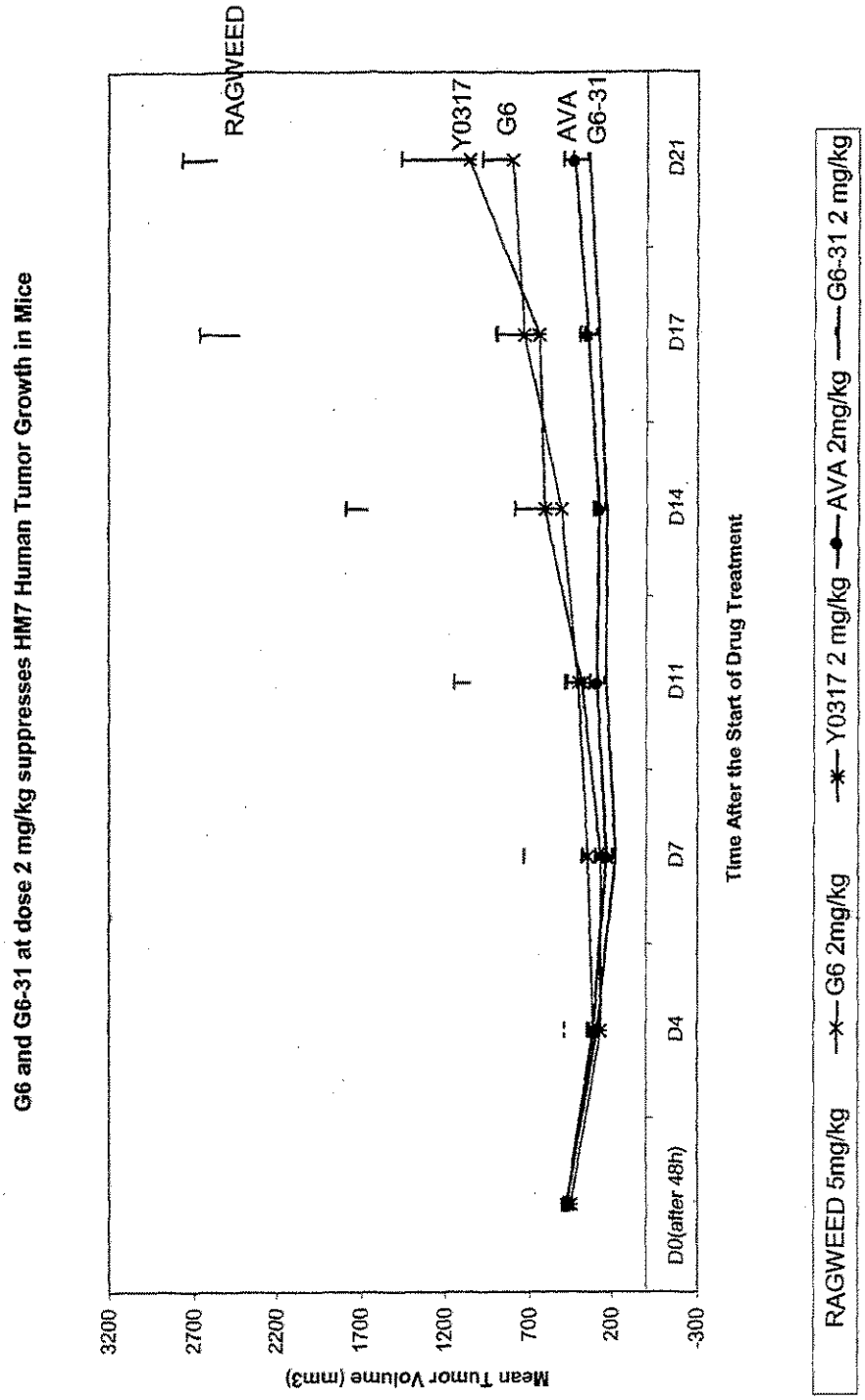

G6 and G6-31 at dose 2 mg/kg suppresses HM7 Human Tumor Growth in Mice
Mean Tumor Volume (mm3)
3200
2700
2200
1700
1200
700
200
-300
RAGWEED
Y0317
G6
AVA
G6-31
D0(after 48h)
D4
D7
D11
D14
D17
D21
Time After the Start of Drug Treatment
RAGWEED 5mg/kg
G6 2mg/kg
Y0317 2 mg/kg
AVA 2mg/kg
G6-31 2 mg/kg

Figure 33D

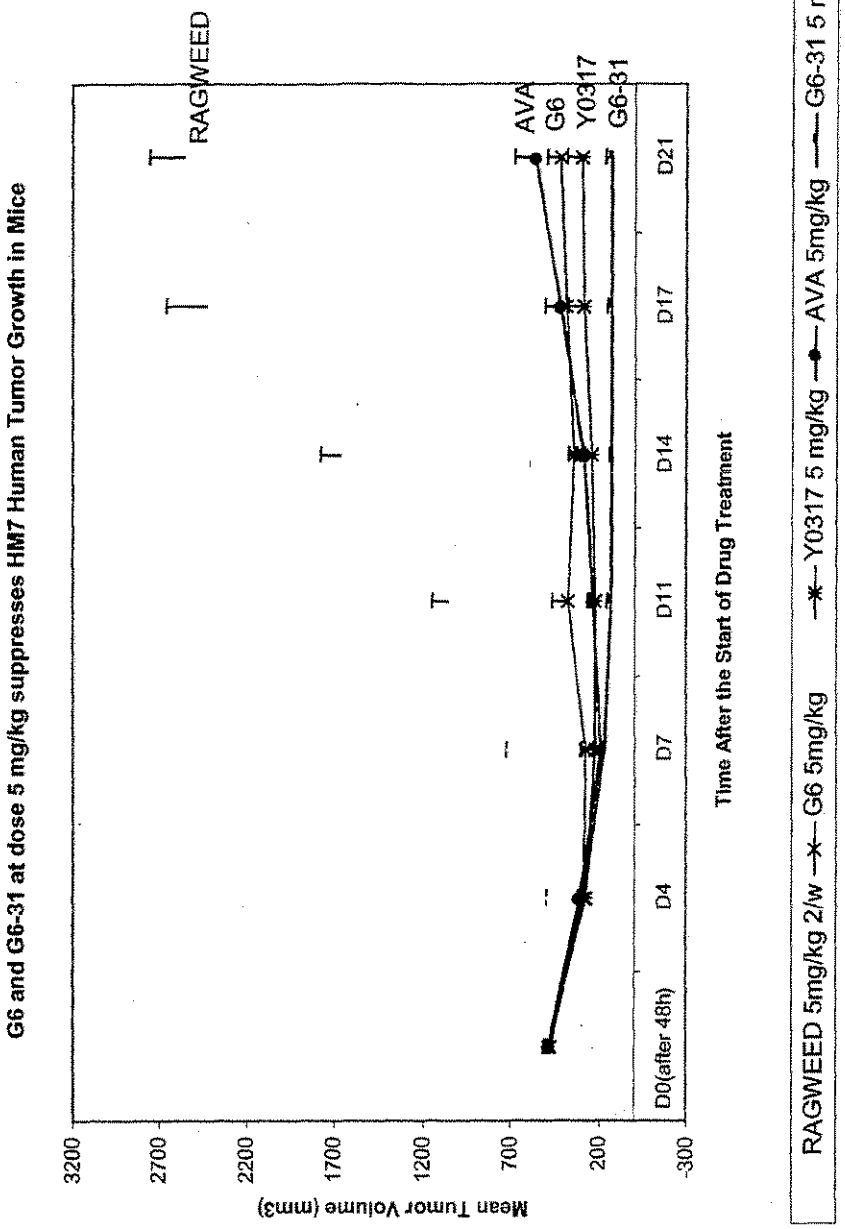

G6 and G6-31 at dose 5 mg/kg suppresses HM7 Human Tumor Growth in Mice
Mean Tumor Volume (mm3)
3200
2700
2200
1700
1200
700
200
-300
RAGWEED
AVA
G6
Y0317
G6-31
D0(after 48h)
D4
D7
D11
D14
D17
D21
Time After the Start of Drug Treatment
RAGWEED 5mg/kg 2/w
G6 5mg/kg
Y0317 5 mg/kg
AVA 5mg/kg
G6-31 5 mg/kg

Figure 33E

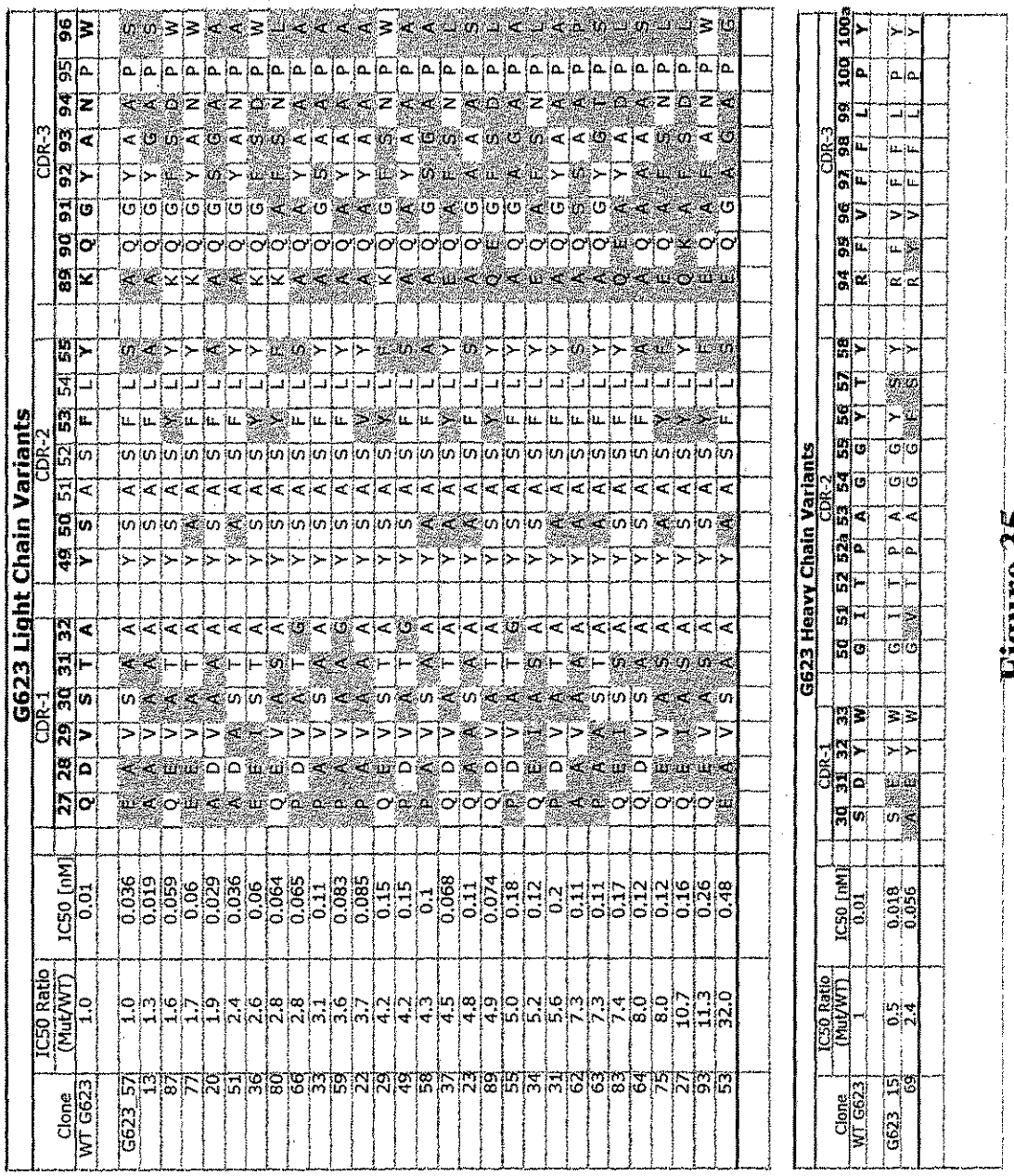
G6 Light Chain Variants

Clone	IC50 Ratio (Mut/WT)	IC50 [nM]	CDR-1						CDR-2							CDR-3							
			27	28	29	30	31	32	49	50	51	52	53	54	55	89	90	91	92	93	94	95	96
WT G6	1.0	2	Q	D	V	S	T	A	Y	S	A	S	F	L	Y	Q	Q	S	Y	T	T	P	P
G6_72	0.001	0.001	Q	D	V	A	S	S	Y	A	A	S	F	L	Y	K	Q	A	F	S	S	P	P
14	0.01	0.03	Q	D	V	A	T	S	Y	A	A	S	F	L	Y	E	Q	A	F	T	S	P	P
43	0.01	0.03	Q	D	V	S	S	A	Y	S	A	S	Y	L	Y	Q	E	A	F	T	S	P	P
54	0.02	0.02	E	E	V	A	T	S	Y	A	A	S	Y	L	Y	E	E	A	F	T	S	P	P
5	0.02	0.05	Q	D	V	A	T	A	Y	S	A	S	Y	L	Y	E	E	A	Y	T	S	P	P
44	0.02	0.04	Q	D	V	A	S	S	Y	A	A	S	Y	L	Y	Q	E	A	F	T	S	P	P
83	0.02	0.07	Q	E	V	A	S	A	Y	S	A	S	F	L	Y	Q	Q	A	Y	S	S	P	A
78	0.02	0.04	Q	D	V	S	T	A	Y	S	A	S	F	L	Y	K	Q	A	F	S	S	P	P
46	0.02	0.05	E	D	L	S	T	A	Y	S	A	S	F	L	F	Q	Q	A	F	T	T	P	P
57	0.03	0.09	Q	E	L	S	T	S	Y	A	A	S	Y	L	Y	Q	Q	A	F	S	S	P	P
61	0.03	0.05	Q	D	V	A	S	S	Y	A	A	S	Y	L	Y	E	Q	A	Y	S	S	P	P
16	0.03	0.03	E	D	V	S	T	S	Y	A	A	S	Y	L	Y	E	Q	A	F	T	N	P	P
71	0.03	0.10	Q	D	V	A	T	A	Y	S	A	S	F	L	F	E	E	A	Y	T	S	P	P
85	0.03	0.10	E	D	L	A	S	S	Y	A	A	S	Y	L	F	Q	Q	A	F	S	S	P	P
58	0.04	0.09	E	E	V	S	T	A	Y	A	A	S	F	L	Y	Q	Q	A	F	T	S	P	A
67	0.05	0.09	Q	E	L	S	S	S	Y	S	A	S	Y	L	Y	Q	E	A	F	T	T	P	P
53	0.05	0.10	E	D	L	A	S	S	Y	S	A	S	Y	L	Y	E	E	S	F	S	T	P	P
50	0.05	0.17	Q	D	L	S	S	S	Y	A	A	S	F	L	Y	Q	Q	A	F	T	S	P	P
56	0.06	0.13	E	D	V	S	T	S	Y	A	A	S	Y	L	Y	E	E	A	F	T	T	P	P
32	0.07	0.13	Q	D	L	S	S	S	Y	S	A	S	Y	L	Y	K	Q	S	Y	S	S	P	P
15	0.08	0.12	E	D	L	S	S	A	Y	S	A	S	F	L	Y	E	E	A	Y	T	S	P	P
62	0.10	0.19	E	E	L	S	S	S	Y	S	A	S	F	L	Y	E	E	S	F	T	T	P	P
64	0.10	0.09	Q	E	V	A	T	A	F	S	A	S	F	L	Y	Q	Q	A	F	S	S	P	A
4	0.12	0.18	E	D	V	A	S	A	Y	A	A	S	Y	L	Y	Q	E	S	Y	S	S	P	P
25	0.13	0.20	Q	D	V	S	S	S	Y	A	A	S	Y	L	Y	Q	E	A	F	S	S	P	A
86	0.21	0.72	Q	D	L	A	T	S	Y	A	A	S	F	L	F	Q	Q	S	Y	T	N	P	P
74	0.24	0.23	E	E	V	A	S	S	Y	S	A	S	Y	L	Y	Q	K	A	Y	S	T	P	P

G6 Heavy Chain Variants

Clone	IC50 Ratio (Mut/WT)	IC50 [nM]	CDR-1				CDR-2											CDR-3							
			30	31	32	33	49	50	51	52	52a	53	54	55	56	57	58	94	95	96	97	98	99	100	100a
WT G6	1	2	S	D	Y	W	A	G	I	T	P	A	G	G	Y	T	Y	R	F	V	F	F	L	P	Y
G6_87	0.10	0.34	S	D	F	W	A	G	V	T	P	A	G	G	Y	S	Y	R	F	V	F	F	L	P	Y
88	0.34	1.20	A	E	Y	W	A	G	V	T	P	A	G	G	Y	T	F	R	F	V	F	F	L	P	Y
89	1.14	4.00	S	D	F	W	A	G	I	T	A	A	G	G	F	S	Y	R	F	V	F	F	L	P	Y

Figure 34



G623 Light Chain Variants

Clone	IC50 Ratio (Mut/WT)	IC50 [nM]	CDR-1 27	28	29	30	31	32	CDR-2 49	50	51	52	53	54	55	CDR-3 89	90	91	92	93	94	95	96
WT G623	1.0	0.01	Q	D	V	S	T	A	Y	S	A	S	F	L	Y	K	Q	G	Y	A	N	P	W
G623_57	1.0	0.036	F	A	V	S	A	A	Y	S	A	S	F	L	S	A	Q	G	Y	A	A	P	S
13	1.3	0.019	F	A	V	A	A	A	Y	S	A	S	F	L	A	A	Q	G	Y	G	K	P	S
87	1.6	0.059	Q	E	V	A	T	A	Y	S	A	S	M	L	Y	K	Q	G	F	S	D	P	W
77	1.7	0.06	F	E	V	A	T	A	Y	A	A	S	F	L	Y	K	Q	G	Y	A	N	P	W
20	1.9	0.029	A	D	V	A	A	A	Y	S	A	S	F	L	A	A	Q	G	S	G	A	P	A
51	2.4	0.036	A	D	A	S	T	A	Y	A	A	S	F	L	Y	A	Q	G	Y	A	N	P	A
36	2.6	0.06	A	E	T	S	T	A	Y	S	A	S	Y	L	Y	K	Q	G	F	S	D	P	W
80	2.8	0.064	Q	E	V	A	S	A	Y	S	A	S	Y	L	F	K	Q	A	F	S	N	P	L
66	2.8	0.065	P	D	V	A	T	G	Y	S	A	S	F	L	S	A	Q	A	Y	A	A	P	A
33	3.1	0.11	R	A	V	S	A	A	Y	S	A	S	F	L	Y	A	Q	G	S	A	A	P	A
59	3.6	0.083	P	A	V	A	A	G	Y	S	A	S	F	L	Y	A	Q	A	Y	A	A	P	A
22	3.7	0.085	P	A	V	A	A	A	Y	S	A	S	V	L	Y	A	Q	A	Y	A	A	P	A
29	4.2	0.15	Q	E	V	S	T	A	Y	S	A	S	Y	L	F	K	Q	G	E	S	N	P	W
49	4.2	0.15	R	D	V	A	T	G	Y	S	A	S	F	L	S	A	Q	A	Y	A	A	P	A
58	4.3	0.1	P	A	V	S	A	A	Y	A	A	S	F	L	A	A	Q	G	S	G	A	P	A
37	4.5	0.068	Q	D	V	A	T	A	Y	A	A	S	Y	L	Y	E	Q	A	F	S	N	P	L
23	4.8	0.11	Q	A	A	S	A	A	Y	S	A	S	F	L	S	A	Q	G	A	A	A	P	S
89	4.9	0.074	Q	D	V	A	T	A	Y	S	A	S	Y	L	Y	Q	E	G	F	S	D	P	L
55	5.0	0.18	P	D	V	A	T	G	Y	S	A	S	F	L	Y	A	Q	G	A	G	A	P	A
34	5.2	0.12	Q	E	L	A	S	A	Y	S	A	S	F	L	Y	E	Q	A	F	S	N	P	L
31	5.6	0.2	P	D	V	A	T	A	Y	A	A	S	F	L	Y	A	Q	G	Y	A	A	P	A
62	7.3	0.11	A	K	V	A	A	A	Y	A	A	S	F	L	S	A	Q	G	S	S	A	P	S
63	7.3	0.11	P	A	A	S	T	A	Y	A	A	S	F	L	Y	A	Q	G	Y	G	T	P	S
83	7.4	0.17	Q	E	T	S	S	A	Y	S	A	S	F	L	Y	Q	E	A	Y	A	D	P	L
64	8.0	0.12	Q	D	V	S	A	A	Y	S	A	S	F	L	A	A	Q	A	A	A	A	P	S
75	8.0	0.12	Q	E	V	A	S	A	Y	A	A	S	Y	L	F	E	Q	A	F	S	N	P	L
27	10.7	0.16	Q	E	T	A	S	A	Y	S	A	S	Y	L	Y	Q	K	A	F	S	D	P	L
93	11.3	0.26	Q	E	V	A	S	A	Y	S	A	S	Y	L	F	E	Q	A	F	A	N	P	W
53	32.0	0.48	E	A	V	S	A	A	Y	A	A	S	F	L	S	E	Q	G	A	G	A	P	G

G623 Heavy Chain Variants

Clone	IC50 Ratio (Mut/WT)	IC50 [nM]	CDR-1 30	31	32	33	CDR-2 50	51	52	52a	53	54	55	56	57	58	CDR-3 94	95	96	97	98	99	100	100a
WT G623	1	0.01	S	D	Y	W	G	I	T	P	A	G	G	Y	T	Y	R	F	V	F	F	L	P	Y
G623_15	0.5	0.018	S	E	Y	W	G	I	T	P	A	G	G	Y	S	Y	R	F	V	F	F	L	P	Y
69	2.4	0.056	A	E	Y	W	G	V	T	P	A	G	G	F	S	Y	R	W	V	F	F	L	P	Y

Figure 35

A

	CDR-H1						CDR-HS										CDR-H3										
Fab	28	29	30	31	32	33	50	51	52	52A	53	54	55	56	57	58	94	95	96	97	98	99	100	100A			100
YADS1	D	I	Y	D	D	D	Y	I	A	P	S	Y	G	Y	T	D	R	S	S	D	A	S	Y	S	Y	S	A
YADS2	A	I	Y	D	Y	D	D	I	A	P	Y	A	G	A	T	A	R	S	S	Y	A	Y	Y	A			A
YADS3	S	I	S	D	Y	D	A	I	A	P	Y	S	G	Y	T	Y	R	S	S	Y	A	Y	Y	S			A
4	D	I	Y	D	Y	A	S	I	A	P	Y	A	G	S	T	Y	R	D	S	Y	A	Y	Y	A			A
5	S	I	A	Y	Y	D	S	I	A	P	Y	A	G	A	T	D	R	S	S	Y	A	Y	A	S			A
6	A	I	A	Y	D	D	S	I	A	P	Y	A	G	A	T	A	R	A	A	A	Y	A	A	Y			A
7	A	I	D	Y	Y	D	S	I	A	P	Y	A	G	S	T	Y	R	S	A	A	Y	Y	A	Y			A
8	A	I	A	A	Y	D	S	I	S	P	Y	A	G	A	T	S	R	A	A	A	Y	Y	Y	D			A
9	D	I	S	A	Y	A	Y	I	S	P	S	S	G	A	T	A	R	S	A	Y	Y	S	Y	A			A
10	D	I	Y	D	Y	S	Y	I	S	P	Y	S	G	A	T	A	R	S	S	Y	A	Y	Y	D			A
11	D	I	S	A	S	D	S	I	S	P	Y	A	G	Y	T	A	R	S	A	Y	Y	A	A	S			A
12	D	I	A	Y	Y	D	S	I	A	P	Y	A	G	S	T	A	R	A	A	A	A	Y	A	Y			A
13	S	I	D	Y	Y	A	S	I	A	P	Y	A	G	Y	T	Y	R	A	A	A	Y	Y	Y	D			A
14	D	I	S	Y	Y	D	S	I	A	P	Y	A	G	A	T	Y	R	A	A	D	Y	A	Y	D			A
15	A	I	A	A	Y	A	D	I	Y	P	A	A	G	D	T	A	R	S	Y	Y	Y	S	Y	S	S	Y	A

B

	CDR-L1					CDR-L2				CDR-L3						
Fab	28	29	30	31	32	50	51	52	53	90	91	92	93	94	95	96
YADS1	A	S	Y	S	S	A	A	S	Y		S	S	A	S	P	A
YADS2	S	Y	A	Y	A	D	A	S	Y	Q	A	Y	S	S	P	D
YADS3	A	S	Y	Y	D	A	A	S	Y	Q	Y	Y	Y	A	P	A

Figure 36

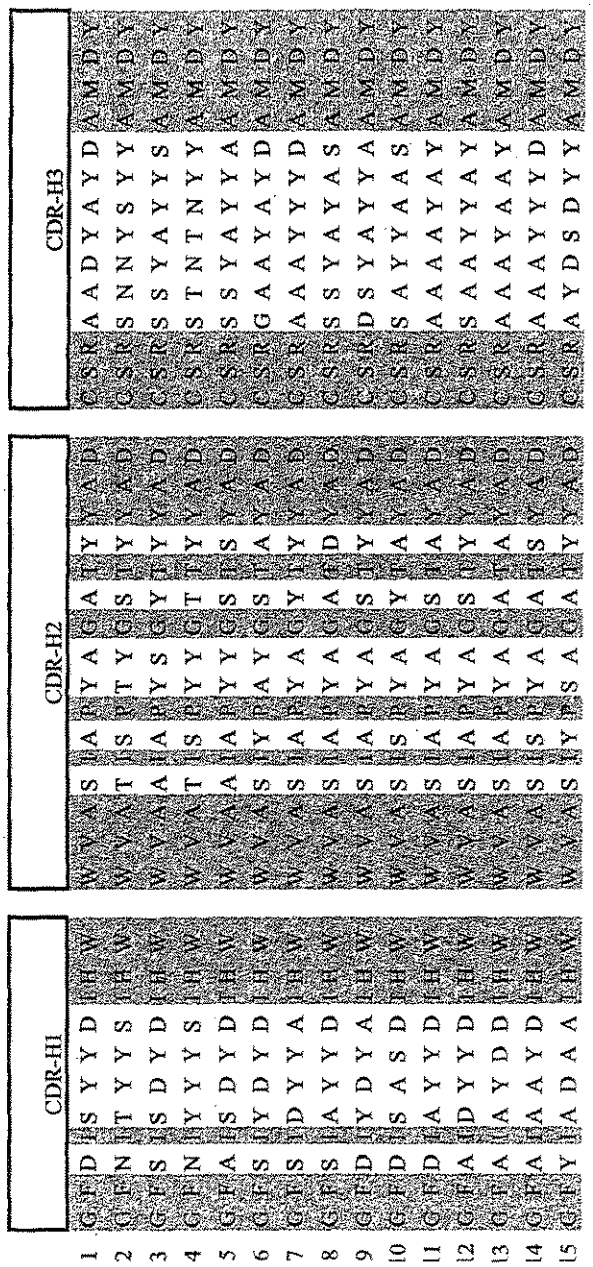


Figure 37

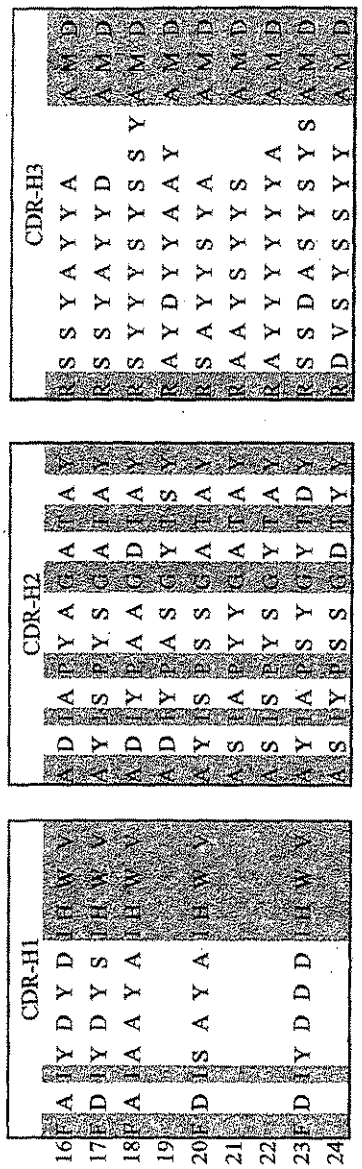


Figure 38

YADS2 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSYAYAVAWYQQKPGKAPKLLIYDASYLYSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQAYSSPDTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

YADS2 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVESGGGLVQPGGSLRLSCAASGFAISDYDIHWV
RQAPGKGLEWVADIAPYAGATAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY
CSRSSYAYYAAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTH

YADS3 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQASYYDVAWYQQKPGKAPKLLIYAASYLYSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYYAPATFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

YADS3 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVESGGGLVQPGGSLRLSCAASGFSISDYDIHWV
RQAPGKGLEWVAAIAPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY
CSRSSYAYYSAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTH

Figure 39

	H1								H2										H3														
clone	27							36	49	50								59		95		97		99	100	100a	102					
NNK-1	F	A	I	Y	D	Y	D	I	A	S	I	A	P	Y	A	G	A	T	A	Y	R	E	S	L	A	H	W	S	A	M
NNK-2	F	A	I	Y	D	Y	D	I	A	L	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	F	W	L	A	M
NNK-3	F	A	I	Y	D	Y	D	I	A	M	I	A	P	Y	A	G	A	T	A	Y	R	T	S	F	A	F	W	N	A	M
NNK-4	F	A	I	Y	D	Y	D	I	A	R	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	F	F	S	A	M
NNK-5	F	A	I	Y	D	Y	D	I	A	S	I	A	P	Y	A	G	A	T	A	Y	R	A	S	F	A	F	F	S	A	M
NNK-6	F	A	I	Y	D	Y	D	I	A	M	I	A	P	Y	A	G	A	T	A	Y	R	x	S	L	A	L	Y	D	A	M
NNK-7	F	A	I	Y	D	Y	D	I	A	Y	I	A	P	Y	A	G	A	T	A	Y	R	F	S	F	A	F	R	M	A	M
NNK-8	F	A	I	Y	D	Y	D	I	A	G	I	A	P	Y	A	G	A	T	A	Y	R	G	S	F	A	F	F	G	A	M
NNK-9	F	A	I	Y	D	Y	D	I	A	L	I	A	P	Y	A	D	A	T	A	Y	R	S	S	Y	A	R	W	S	A	M
NNK-10	F	A	I	Y	D	Y	D	I	A	Y	I	A	P	Y	A	G	A	T	A	Y	R	S	S	Y	A	F	F	R	A	M
NNK-11	F	A	I	Y	D	Y	D	I	H	I	I	A	P	Y	A	G	A	T	A	Y	A	S	S	Y	A	F	F	R	A	M
NNK-12	F	A	I	Y	D	Y	D	I	A	V	I	A	P	Y	A	G	A	T	A	Y	R	N	S	F	A	F	F	G	A	M
NNK-13	F	A	I	Y	D	Y	D	I	A	F	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	F	W	M	A	M
NNK-14	F	A	I	Y	D	Y	D	I	A	F	I	A	P	Y	A	G	A	T	A	Y	R	G	S	F	A	F	W	S	A	M
NNK-15	F	A	I	Y	D	Y	D	I	A	V	I	A	P	Y	A	G	A	T	A	Y	R	N	S	F	A	F	F	G	A	M
NNK-16	F	A	I	Y	D	Y	D	I	A	N	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	F	F	M	A	M
NNK-17	F	A	I	Y	D	Y	D	I	A	V	I	A	P	Y	A	G	A	T	A	Y	R	N	S	F	A	F	F	G	A	M
NNK-18	F	A	I	Y	D	Y	D	I	A	F	I	A	P	Y	A	G	A	T	A	Y	R	R	S	F	A	F	Y	N	A	M
NNK-19	F	A	I	Y	D	Y	D	I	A	F	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	F	Y	G	A	M
NNK-20	F	A	I	Y	D	Y	D	I	A	F	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	F	K	A	A	M
NNK-21	F	A	I	Y	D	Y	D	I	A	A	I	A	P	Y	A	G	A	T	A	Y	R	L	S	F	A	L	Y	N	A	M
NNK-22	F	A	I	Y	D	Y	D	I	A	Y	I	A	P	Y	A	G	A	T	A	Y	R	V	S	W	A	F	F	Q	A	M
NNK-23	F	A	I	Y	D	Y	D	I	A	W	I	A	P	Y	A	G	A	T	A	Y	R	L	S	L	A	Y	F	H	A	M
NNK-24	F	A	I	Y	D	Y	D	I	A	V	I	A	P	Y	A	G	A	T	A	Y	R	N	S	F	A	F	F	G	A	M
NNK-25	F	A	I	Y	D	Y	D	I	A	V	I	A	P	Y	A	G	A	T	A	Y	R	I	S	F	A	F	F	G	A	M
NNK-26	F	A	I	Y	D	Y	D	I	A	S	I	A	P	Y	A	G	A	T	A	Y	R	S	S	F	A	H	M	H	A	M
NNK-27	F	A	I	Y	D	Y	D	I	A	V	I	A	P	Y	A	G	A	T	A	Y	R	N	S	F	A	F	F	G	D	M

Figure 40

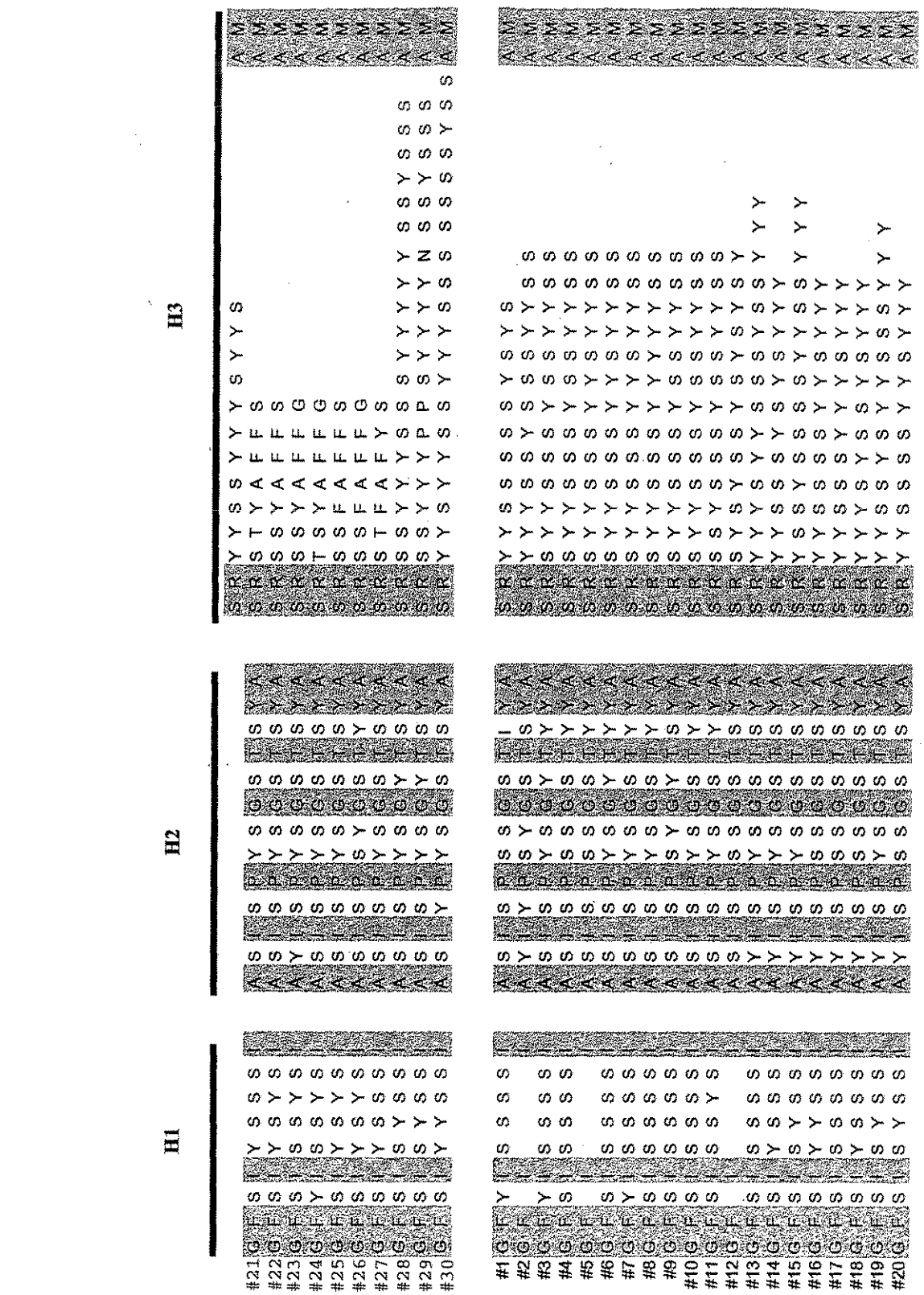


Figure 41

Clone

	CDR-L3	CDR-H1	CDR-H2	CDR-H3
1	SSYSY	SSSSS	YSSSSS	YYSSSYYYSYYY
2	SSYSY	SYYSS	YSSSSS	YYSSSSYYSYYY
3	SYYSY	SSYSS	YSYSSS	YSSYSSSSYYSSYYY
4	SYYSY	SSSSS	YSYSSS	YYYSYYSSSYSSYYY
5	SSYYS	SSSSS	YSYSSS	YSSYSSSYSSYSYYY
6	SYYSY	SSYSS	YSSSSS	YSSYSSYSSSYSYYY
7	SSYSY	SSSSS	YSYSYS	YSYSYSSSSSSSYYY
8	SYYSY	SSSSS	YSSSYS	YYSSYSSYSSYSYYY
9	SYYSY	SSYSS	YSYSSS	YYSSYSYYSSSYYY
10	SYSSY	SSSSS	YSSSSS	YSSYSYSSSYSYYY
11	SYYSY	SSSSS	YSSSYS	YSSSSYSSYSSYYY
12	SYSSY	YSSSS	YSSSSS	YSSSSSYYSSSYYY
13	SYYSY	SSSSS	YSSSSS	YSYSSSSYYYSYYY
14	SYYSY	SSSSS	YSYSSS	YSSSSYSSYYSYYY
15	SYSYS	SSSSS	YSYSSY	YSSYSYSSSYSYYY
16	SYSSY	SSSSS	YSYSSY	YSYYSYSSSYSSYYY
17	SYYYS	SSSSS	SSYYYS	YSSYSSYSSYSYYY
18	SYSSY	SYSSS	YSYSSS	YSYSSSSSSYSSYYY
19	SYSSY	SSSSS	YSYSYS	YSSSYYSSYSSYYY
20	SYSSY	SSSSS	YSYSSS	YSYSSSSSSYSSYYY
21	SYSSY	SYSSS	YSSSSS	YSYYSYSSSYSYYY
22	SSSSY	SYSSS	YSYSYS	YYSSSYSYSSSYYY
23	SYSSY	SSSSS	YSYSYS	YYYSYYSSSSYSYYY
24	SYYSY	SSSSS	YSSSSS	YYSYSSYSSSSYYY
25	SYSSY	SYYSS	YSSSSS	YYSSSYSSYSSYYY
26	SSSSY	SSSSS	YSYSSS	YSYSSSSSSYSYYY
27	SYYSY	SYYSS	SSYSYS	YSSSSYSYSSSYYY
28	SSSSY	SSSSS	YSSYSS	YYSSSSYSYYSYYY
29	SYYSY	SSSSS	YSYSYS	YSSSSSSSYYSYYY
30	SSSSY	SSSSS	YSSSSS	YSSSSYYSSYSYYY
31	SYYSY	SYSSS	YSSSSS	YSSSSSYSYSSYYY
32	SSSSY	SYSSS	YSSSSS	YYSSSSSSYYSYYY
33	SSYSY	YSSSS	YSSSYS	YSSYSSSSYSSYYY
34	SYSSY	SSSSS	YSYSSS	YSSSSYSSSSYYY
35	SYYSY	SSSSS	YSSSSS	YSSSSSYSYSSYYY
36	SYSSY	SYSSS	YSYSSY	YSSSSSSSYSSYYY
37	SYYSY	SSSSS	YSYSSY	YYSSSSSSYSSYYY
38	SYYSY	SYSSS	YSSSSS	YSYSSSYSSYSSYY
39	SYYSY	SYSSS	YSYSSS	YYSSSSYSYSYYY
40	SSYSY	SYSSS	YSSSSS	YYYSYSSYSYYY
41	SYYSY	SSSSS	YSSSSS	YSYSYSSYSYYY
42	SSSSY	SSYSS	YSYSSS	YSSSSSYSSYYY
43	SYSSY	SSSSS	YSYSSS	YSSSSYYYSYYY
44	SYSSY	SYSSS	YSSSSS	YSSSSSYYSYYY
45	SYSSY	SSSSS	SSYSYS	YSSYSYYYSYYY
46	SYYSY	SYSSS	YSSSYS	YSSSSYSYSYYY
47	SSSSY	SSSSS	YSYSSS	YSSSYSSYSYYY
48	SYYYS	SSSSS	YSYSYS	YSYSSSSSSYYY
49	SSYSY	SYSSS	YSYSSS	YSSSYYYYSYSY
50	SYYSY	SSSSS	YSSSSS	YSSSYYYSYYY
51	SYYSY	SSSSS	YSYSSS	YSSSYSYSYYY
52	SYYYS	SSSSY	SYYYYS	SSSSSYYS
53		YSSSS	SSYSYY	SYYSSSYSSYYSS
54		SSSSS	SSSSSY	SYYSSSYSSYYSS
55		SSSSS	SSSSSY	SYYSSSYYSYYSS
56		SSSSS	SSYSYY	SYYSSSYYSYYSS
57		YSSSS	SSYSSY	SYYSSSYYSYYSS
58		SSSSS	SSYSSY	SYYSSSYYYYYSS
59		SSSSS	SSSYYS	SYYSSSYSSYYSS
60		SSSSS	SSYSSY	SYYSSSYSSYYSS
61		SSSYS	SSYSSY	SYYYSSYSSYYSS
62		SSYSS	SSSSYY	SYYSSSYSSYYSS
63		SSSSS	SSYSYY	SSYSSSYYSYYSS

Figure 42

YS1 Fab Sequence

Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSYAYAVAWYQQKPGKAPKLLIYDASYLYSGVPSRFSGSGSGTDFTLT
ISSLQPEDFATYYCQQSYYSPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGFSISSSSIHWVRQAPGKGLEWVAYISPSSGSTSYADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCSRYYSSSYYYSYYYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDKTH

Figure 43