FIGURE 1

Activity of c-Kit ADC on a panel of cell lines

ADC	GIST T-1		GIST882		GIST430		NCI-H526		NCI-H1048		Kasumi-6		Kasumi-1		MDA-MB-453 (c-Kit neg)	
	IC50 nM	Amax %	IC50 nM	Amax %	IC50 nM	Amax %	IC50 nM	Amax %	IC50 nM	Amax %	IC50 nM	Amax %	IC50 nM	Amax %	IC50 nM	Amax %
IgG-MCC-DM1	13.622	82	**>68**	27	33.121	53	35.738	99	12.141	98	19.200	78	8.300	99	11.383	95
NEG024-MCC-DM1	0.021	72	0.721	66	0.038	53	0.074	99	0.737	98	5.262	78	8.493	99	22.772	92
NEG085-MCC-DM1	0.009	76	0.471	71	0.036	59	0.026	99	0.547	98	0.637	80	4.824	99	46.827	90
NEG086-MCC-DM1	0.013	74	0.430	69	0.056	54			0.828	98	0.522	80	4.223	99	16.922	91
NEG087-MCC-DM1	0.008	78	0.411	70	0.046	57			1.503	98	3.242	80	6.073	99	45.431	90
20376-MCC-DM1	0.051	69	1.000	69	0.038	54	0.074	99	1.340	97	1.505	79	1.329	100	2.690	95

FIGURE 2

Comparison of different linkers/toxins on multiple cell lines

Cell Line	Indication	9P3-MCC-DM1 GI50 (nM)	9P3-MCC-DM1 Amax (%)	9P3-SPDB-DM4 GI50 (nM)	9P3-SPDB-DM4 Amax (%)	9P3-CX1-1-DM1 GI50 (nM)	9P3-CX1-1-DM1 Amax (%)	cKit status
CMK11-5	AML	0.05	91	0.07	92	nd	nd	WT
Hel92.1.7	AML	0.61	95	1.26	94	7.6	95	WT
Kasumi-1	AML	4	100	0.83	100	0.02	100	N822K/WT
Kasumi-6	AML	1.29	92	0.91	94	nd	nd	WT
M-O7e	AML	0.08	100	0.11	99	nd	nd	WT
OCI-M1	AML	0.11	99	0.13	100	nd	nd	WT
SKNO1	AML	3.6	99	1.6	99	5.2	100	N822K/N822K
UKE-1	AML	1.8	99	5.6	100	nd	nd	WT
GIST T1	GIST	0.02	75	0.045	75	nd	nd	Ex11del
GIST430	GIST	0.08	60	0.17	65	0.04	55	Ex11del, Ex13
NCI-H526	SCLC	0.05	98	0.17	98	nd	nd	WT
NCI-H889	SCLC	0.15	86	1.47	84	nd	nd	Amp
NCI-H1048	SCLC	4.3	99	2.77	100	1.45	100	WT
NCI-H1930	SCLC	0.09	87	0.3	88	nd	nd	Amp

FIGURE 3

Sensitivy to c-Kit ADC by indication

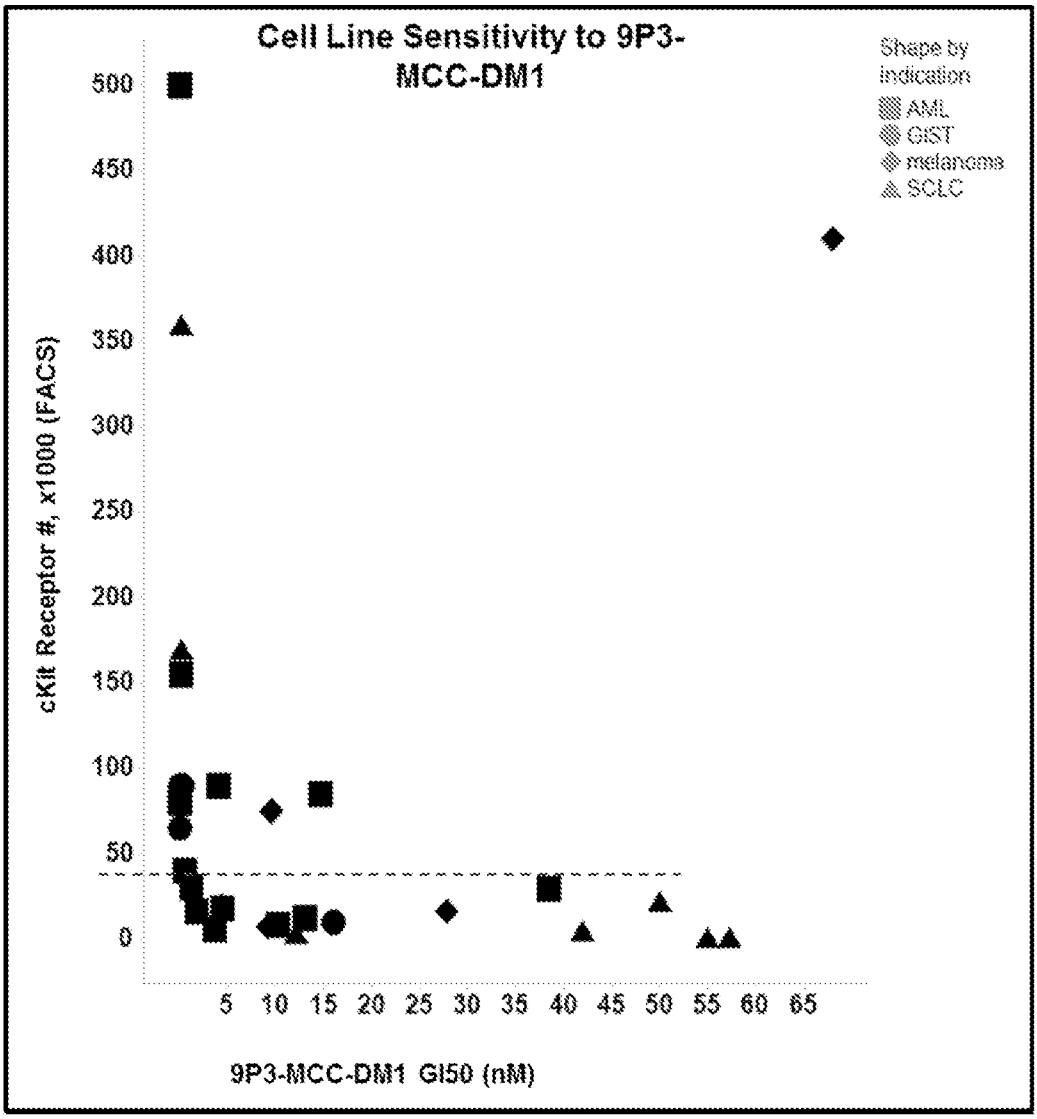


FIGURE 4

c-Kit ADC activity on GIST T1 cells

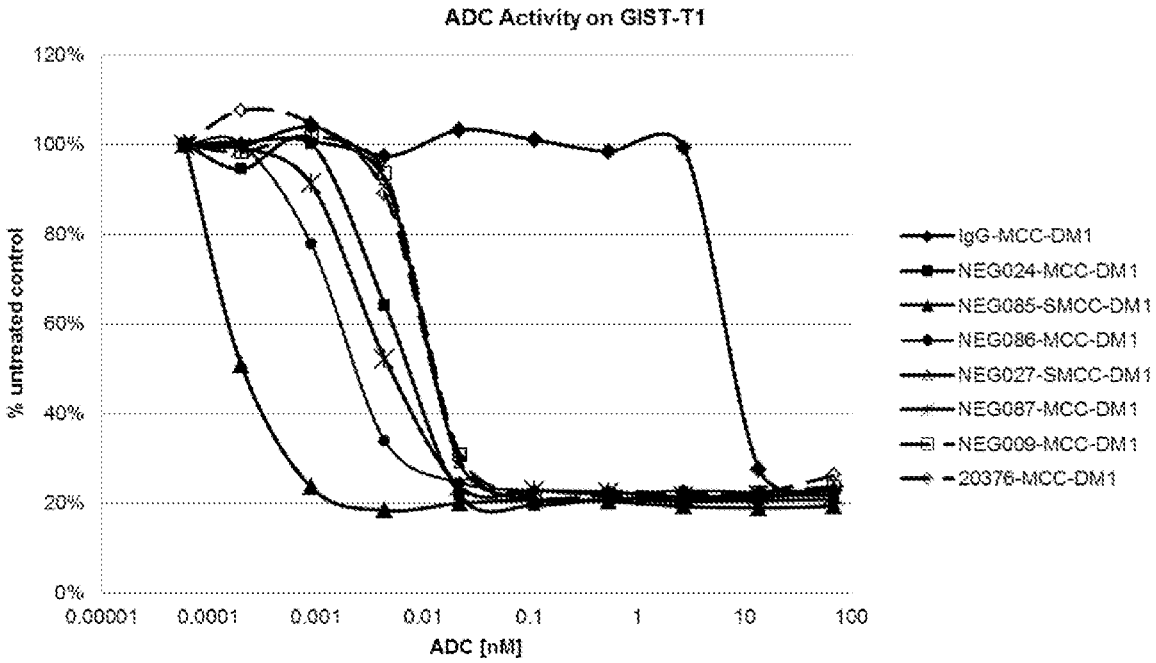


FIGURE 5

c-Kit ADC activity on GIST 430 cells

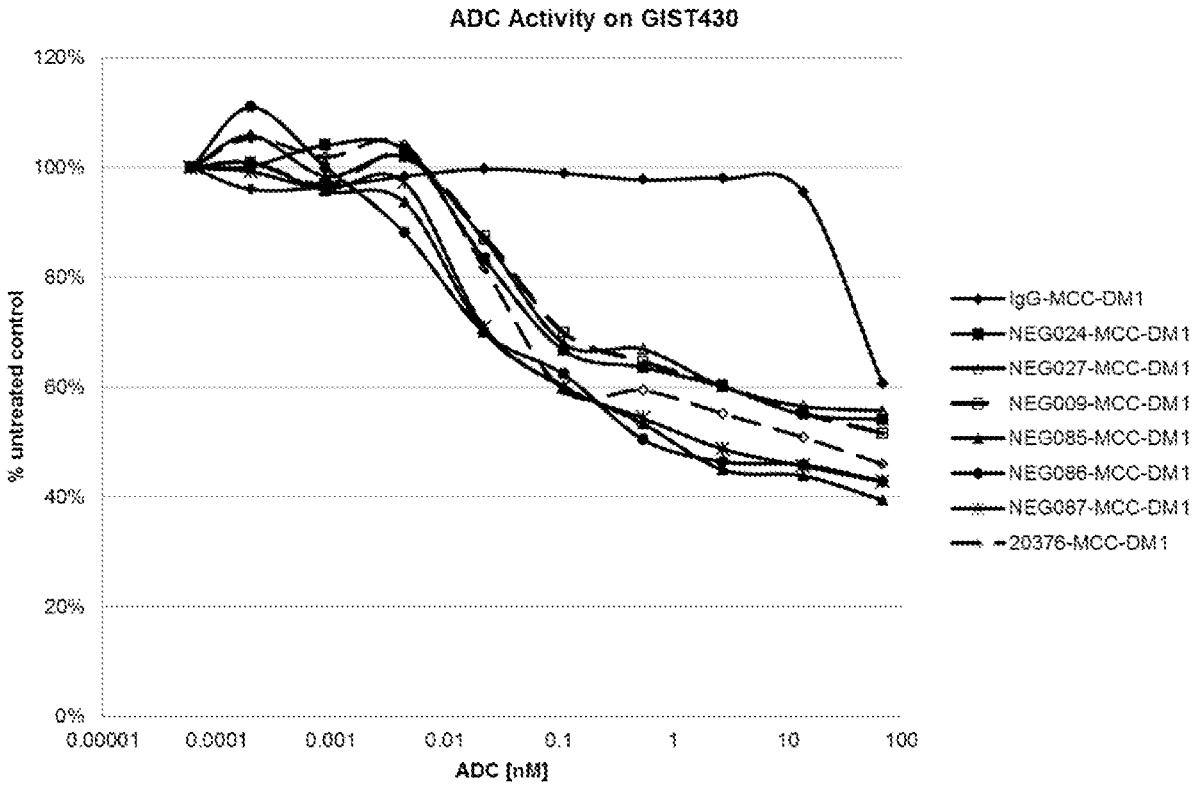


FIGURE 6

c-Kit ADC activity on NCI-H526 cells (small cell lung cancer (SCLC))

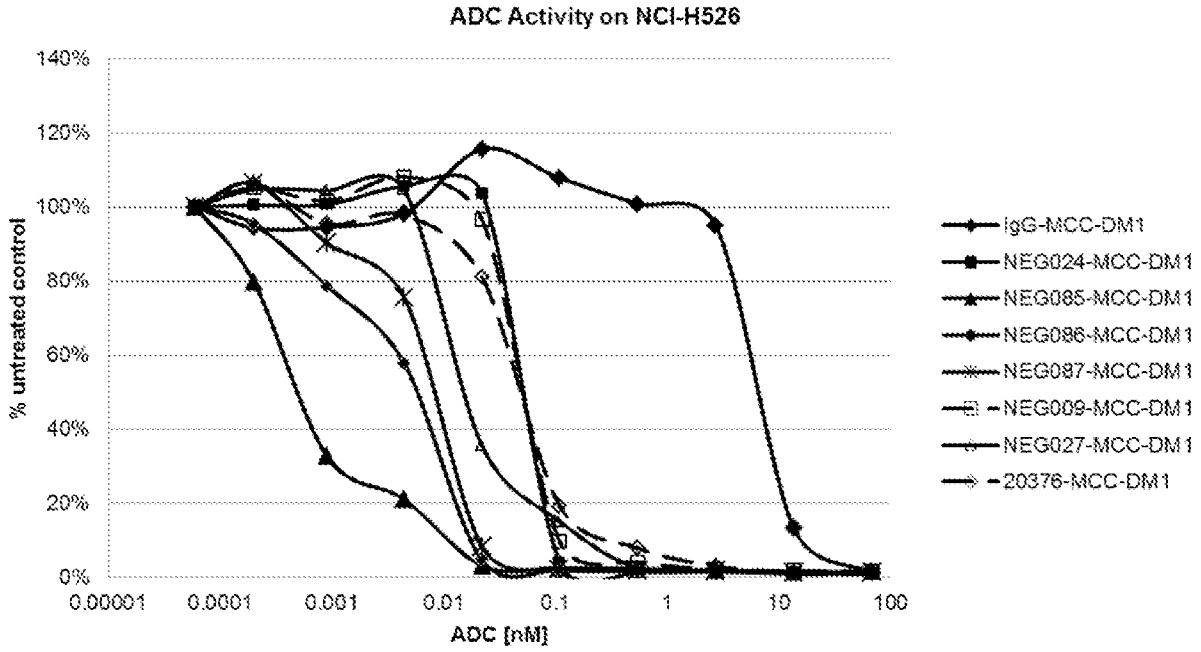


FIGURE 7

c-Kit ADC activity on NCI-H1048 cells

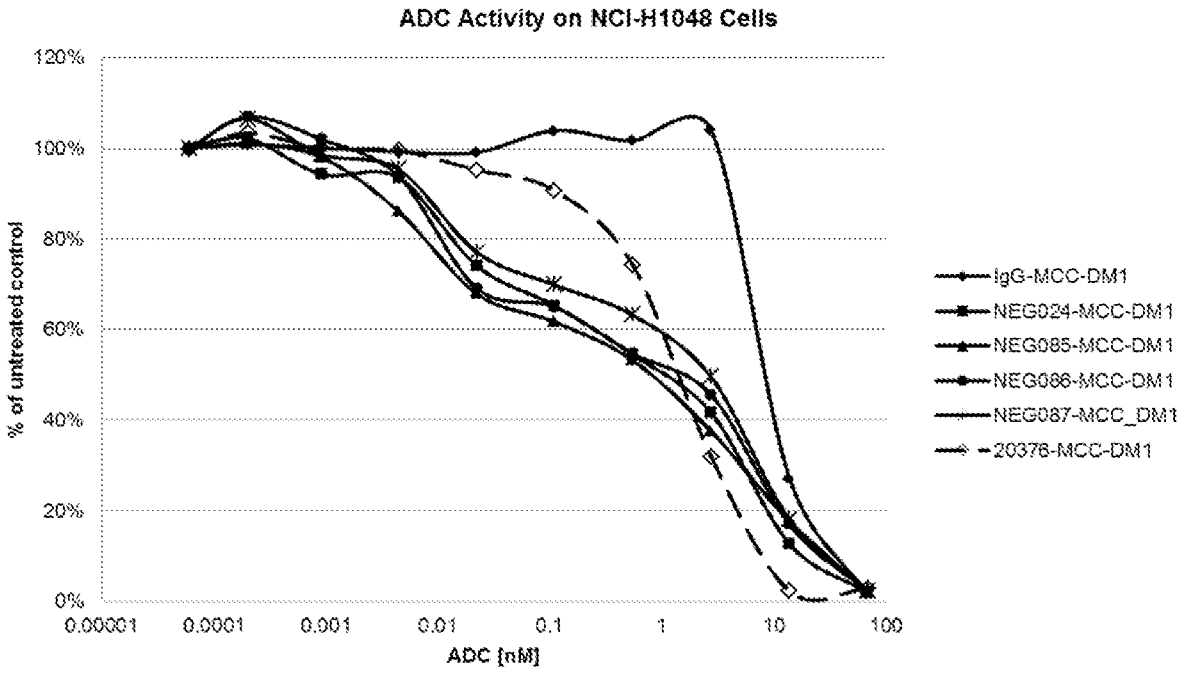


FIGURE 8

c-Kit ADC activity on CMK-11-5 cells

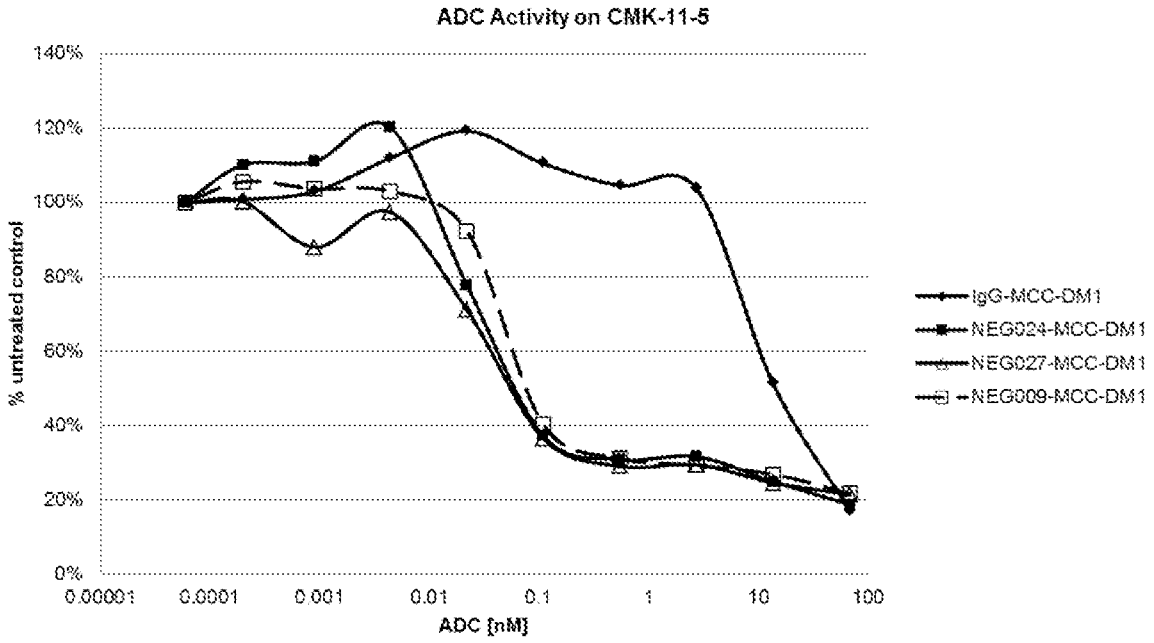


FIGURE 9

c-Kit ADC activity on Uke-1 cells

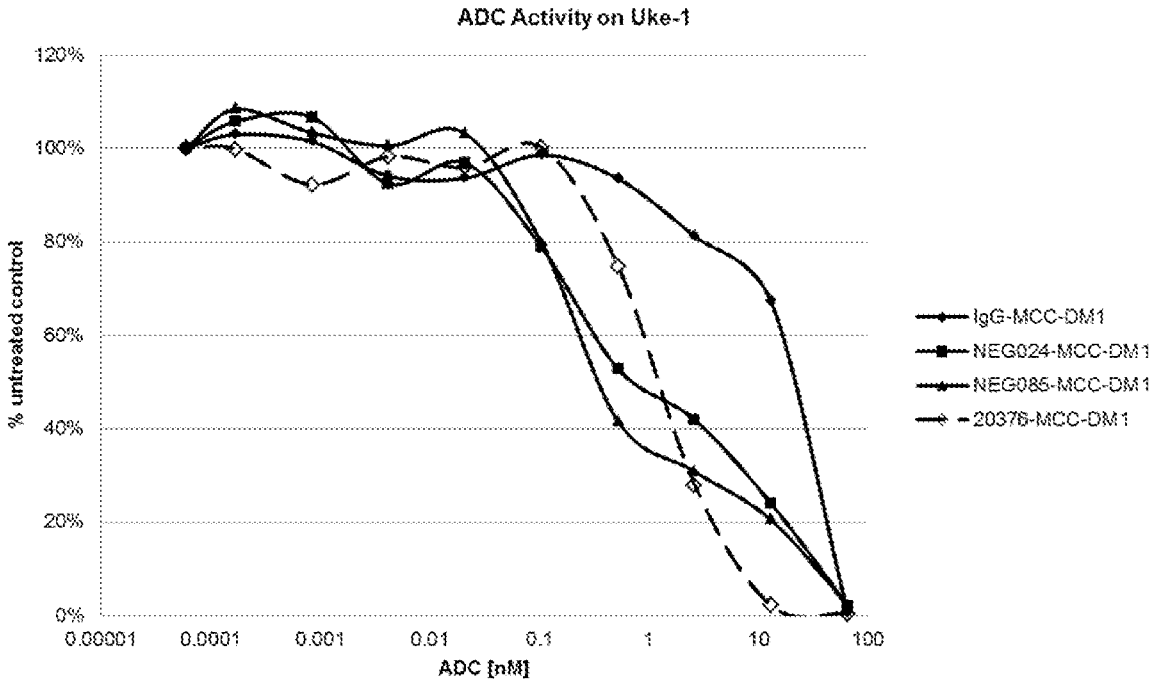


FIGURE 10

Mapping of anti-c-Kit binding to c-kit receptor

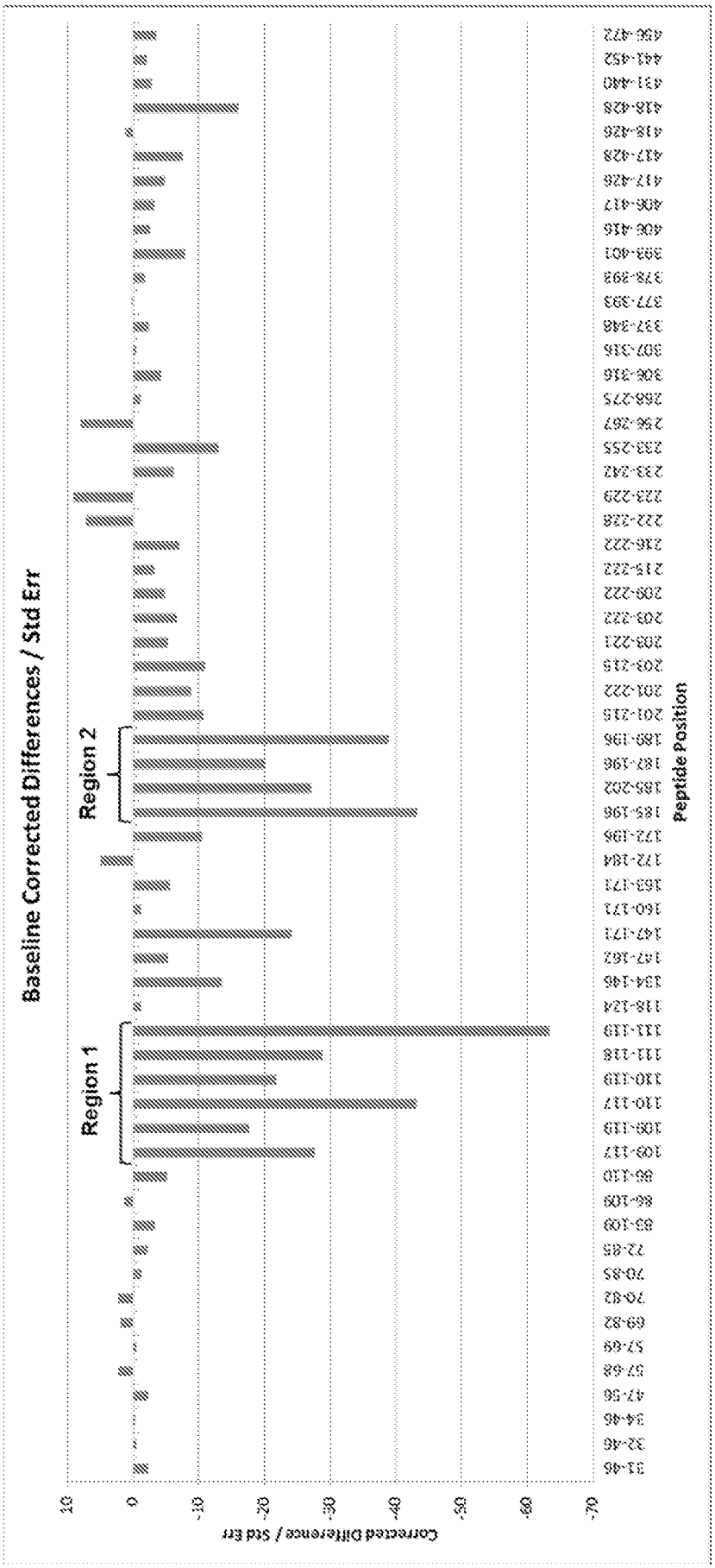


FIGURE 11

Mapping regions of c-Kit ADC interaction

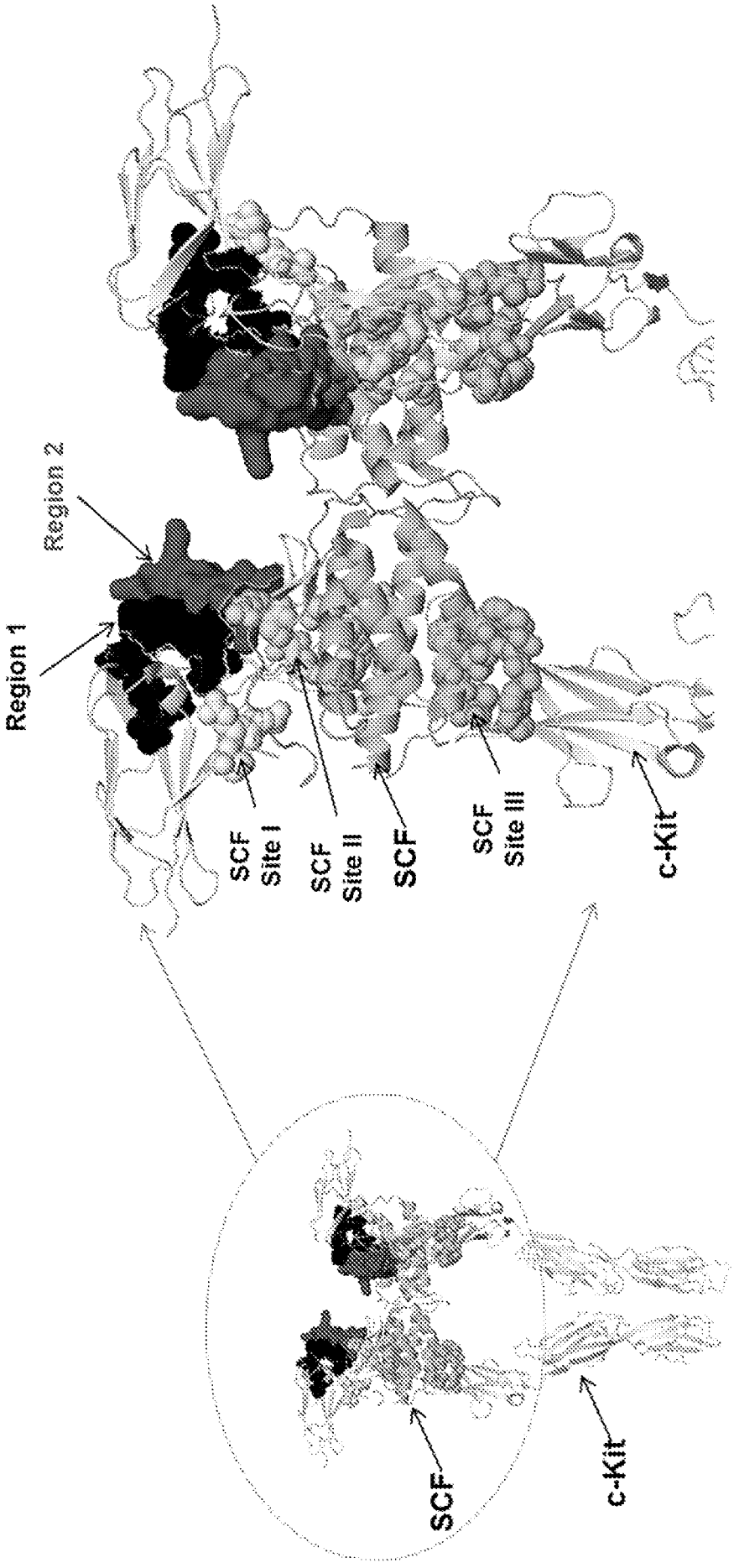


FIGURE 12

Anti-c-Kit ADCs can mediate c-Kit phosphorylation but not downstream pathways in wildtype cKit expressing cells. Anti-cKit ADCs do not affect signaling in c-Kit mutant cells

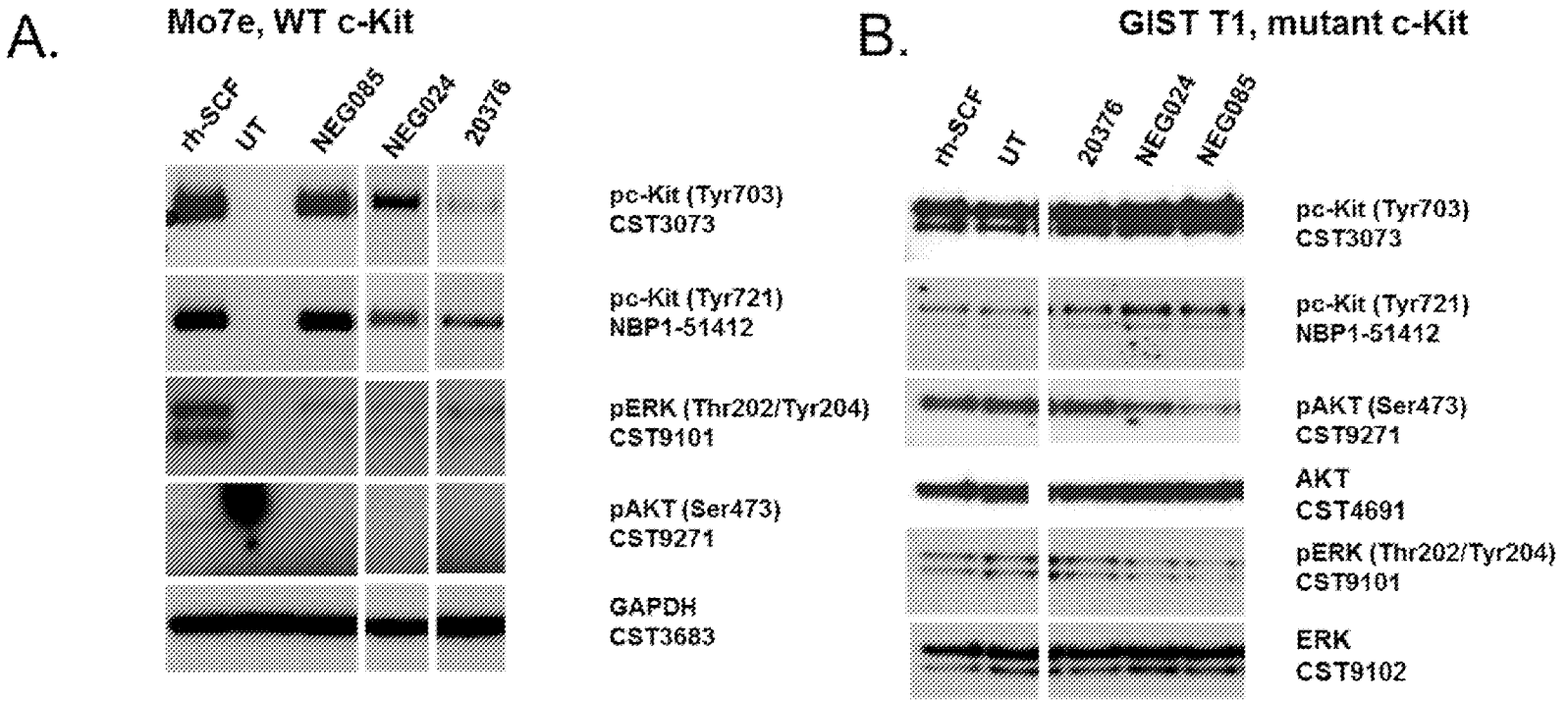


FIGURE 13

NEG085 and 20376 mediate rapid internalization of c-Kit on cells

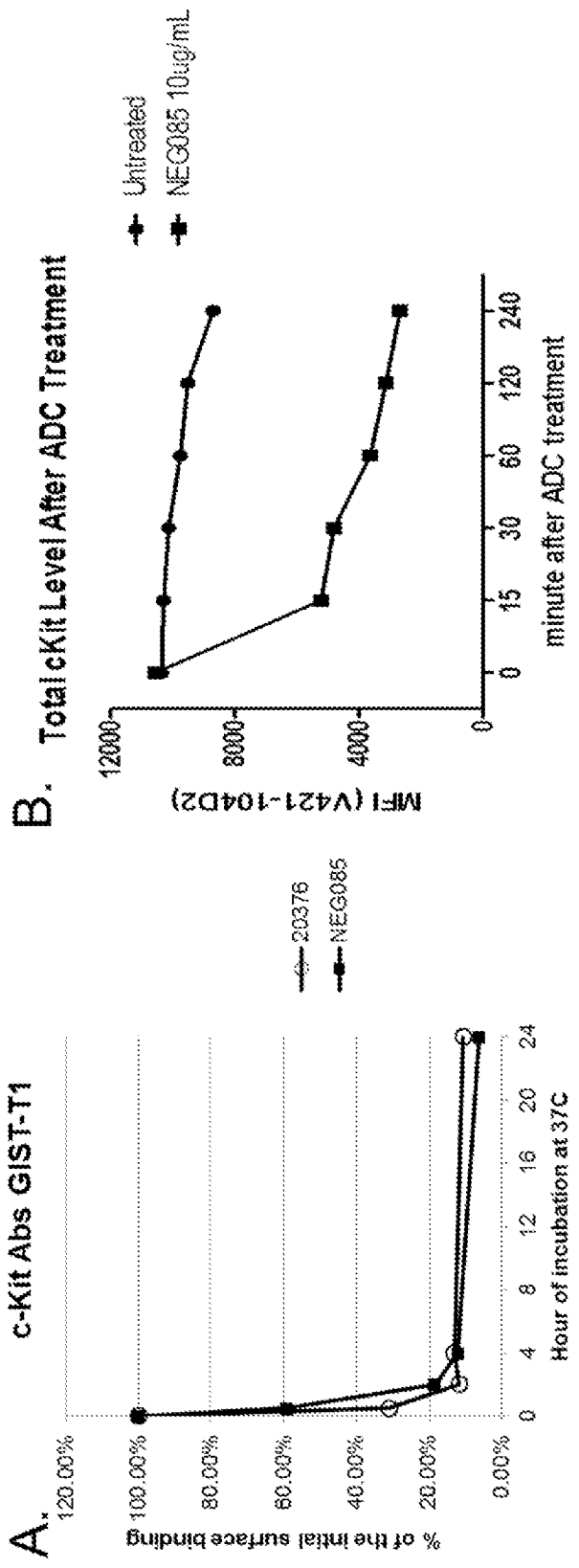


FIGURE 14

NEG085-MCC-DM1 enhances degradation of cKIT

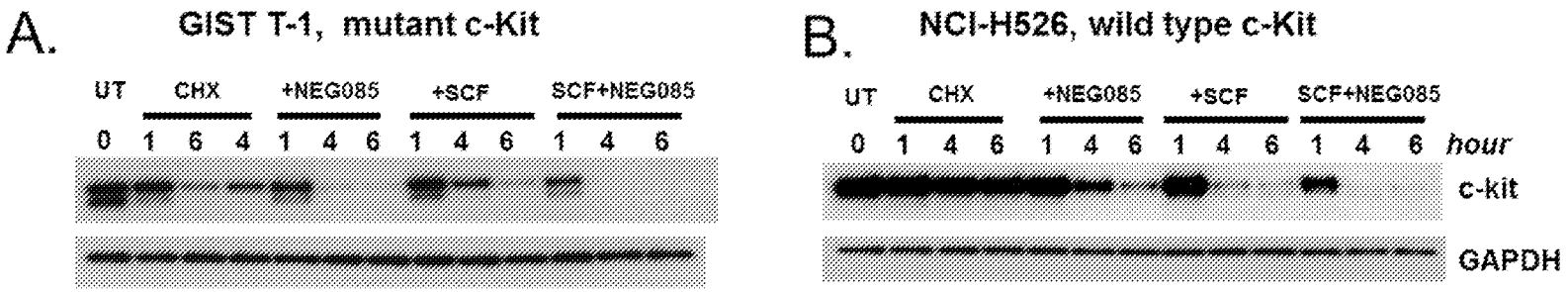


FIGURE 15

Activity of NEG085, NEG024, 20376 in Proliferation Assays In SCF-Dependent Cell Line Mo7e

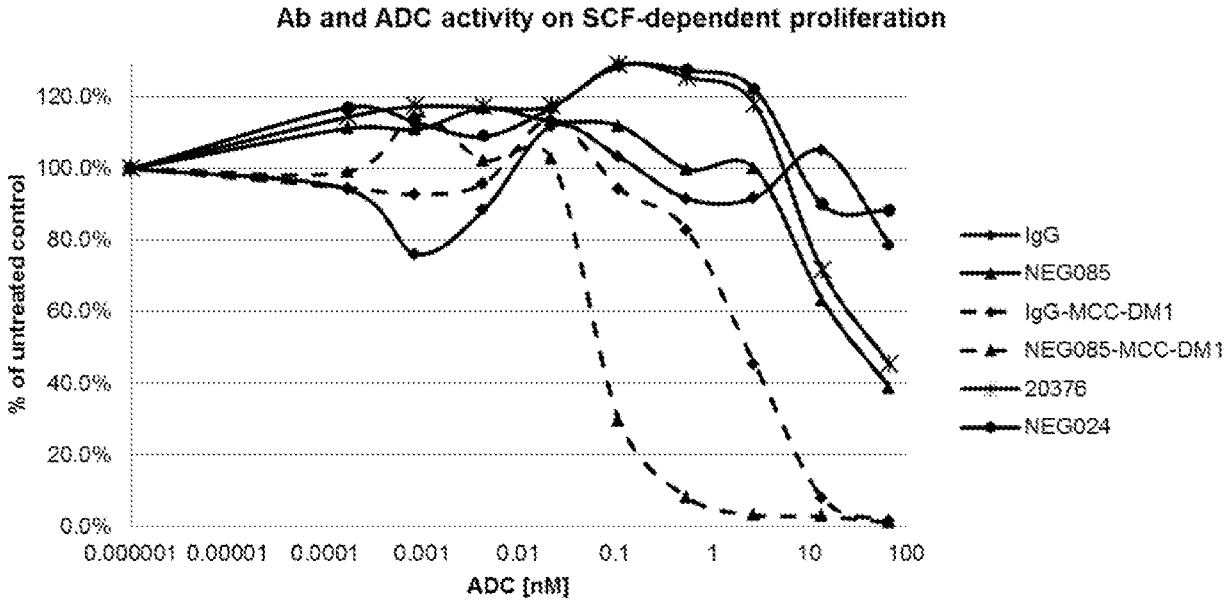


FIGURE 16

Activity of NEG085 in Proliferation Assays in Cell Line Mo7e, treated with GM-CSF

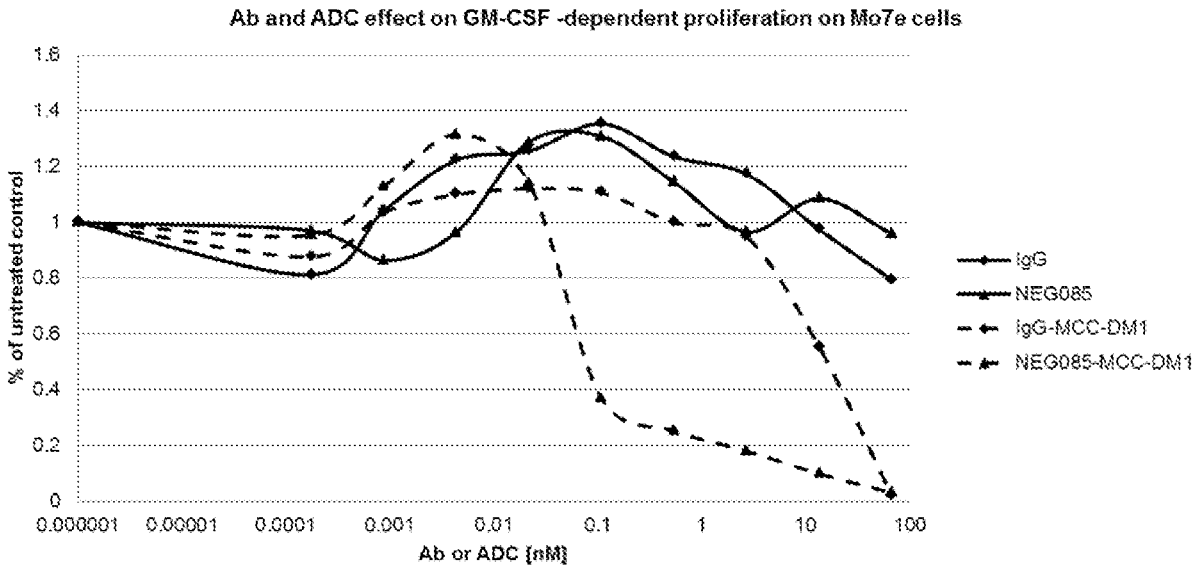


FIGURE 17

NEG085 and 20376 do not mediate in vitro ADCC

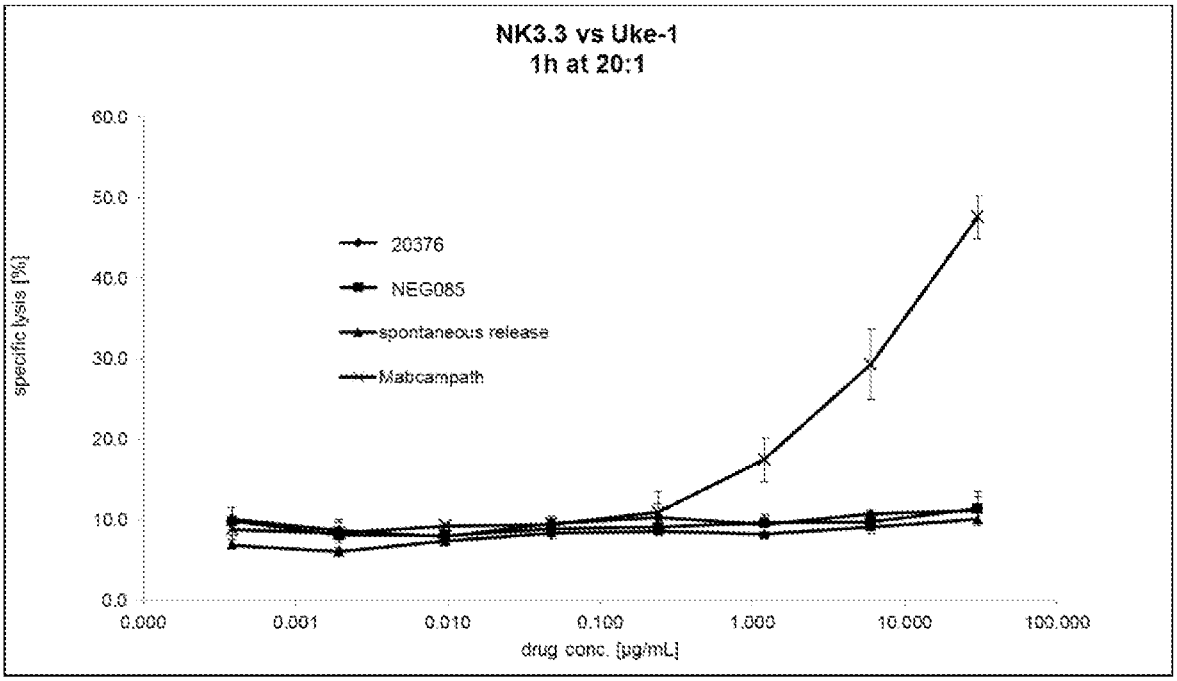


FIGURE 18

cKIT antibodies do not lead to apoptosis of human primary mast cells

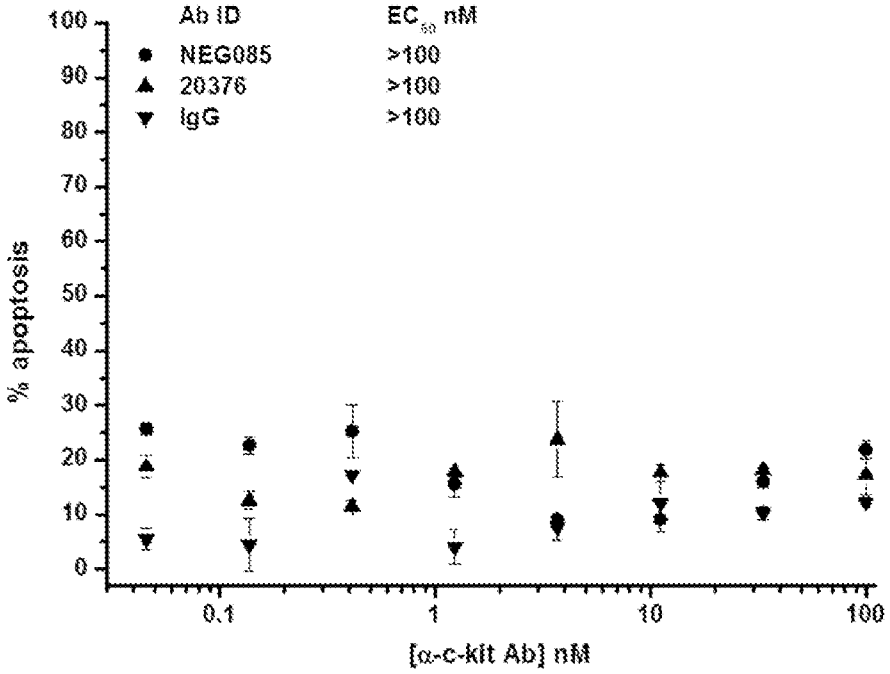


FIGURE 19

NEG085 and 20376 do not mediate mast cell degranulation on ex vivo primary human mast cells

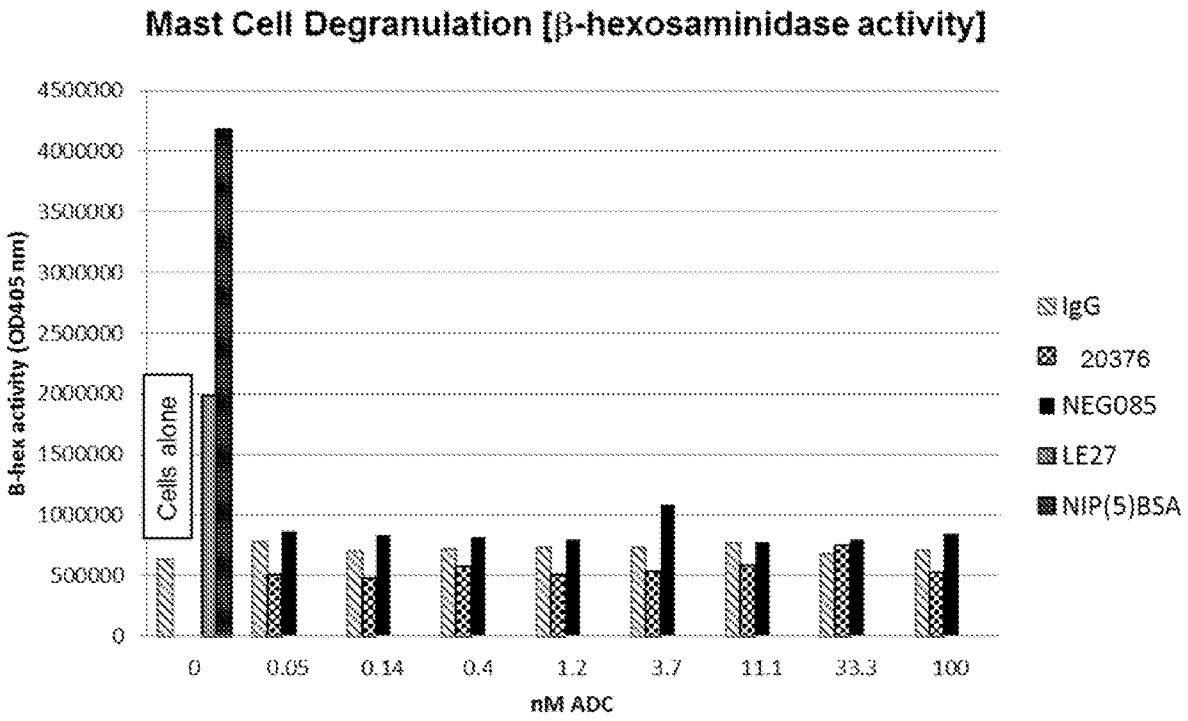


FIGURE 20A

Co-localization of IgG1 and mitotic arrest of NEG027-MCC-DM1 in GIST T1 xenograft model

Hu IgG

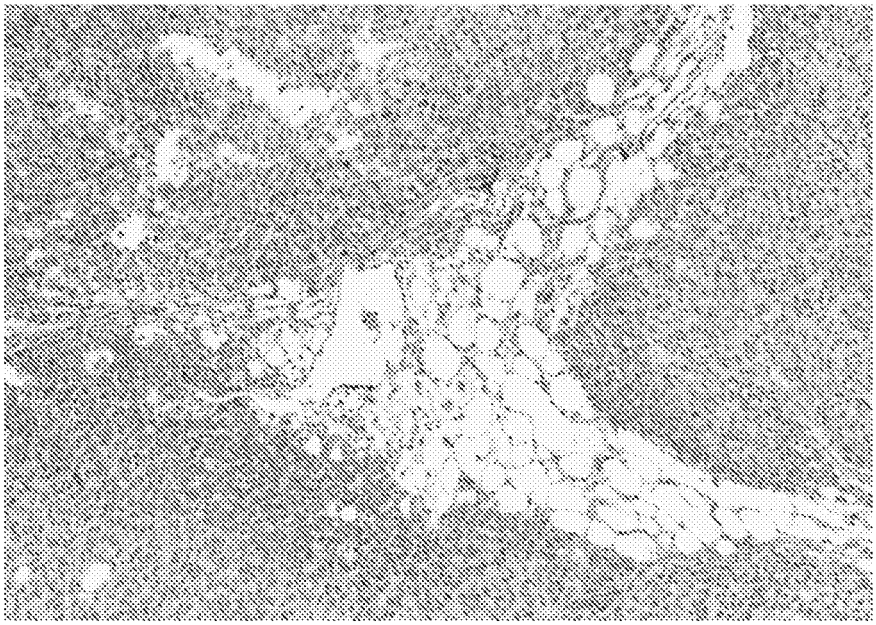


FIGURE 20B

P-Histone H3

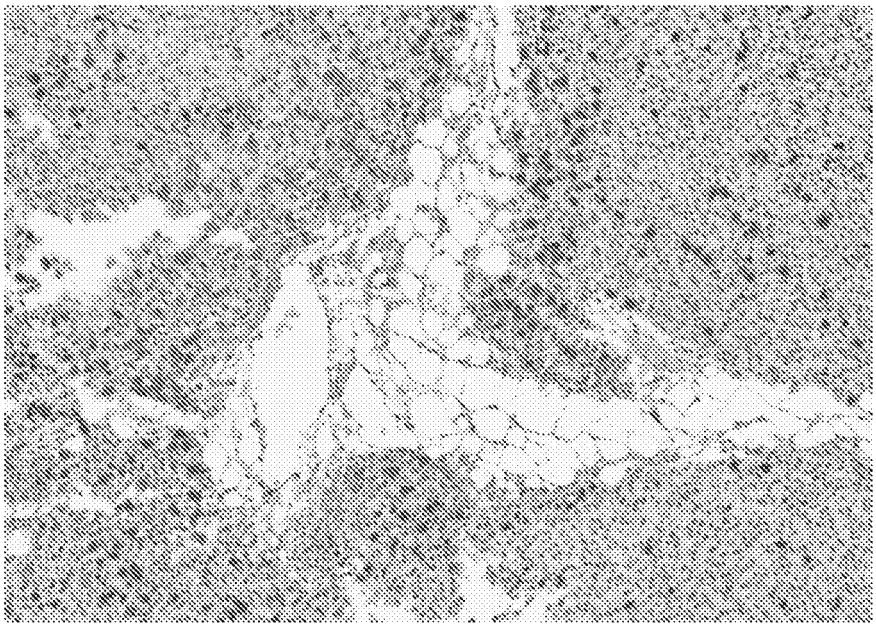


FIGURE 21

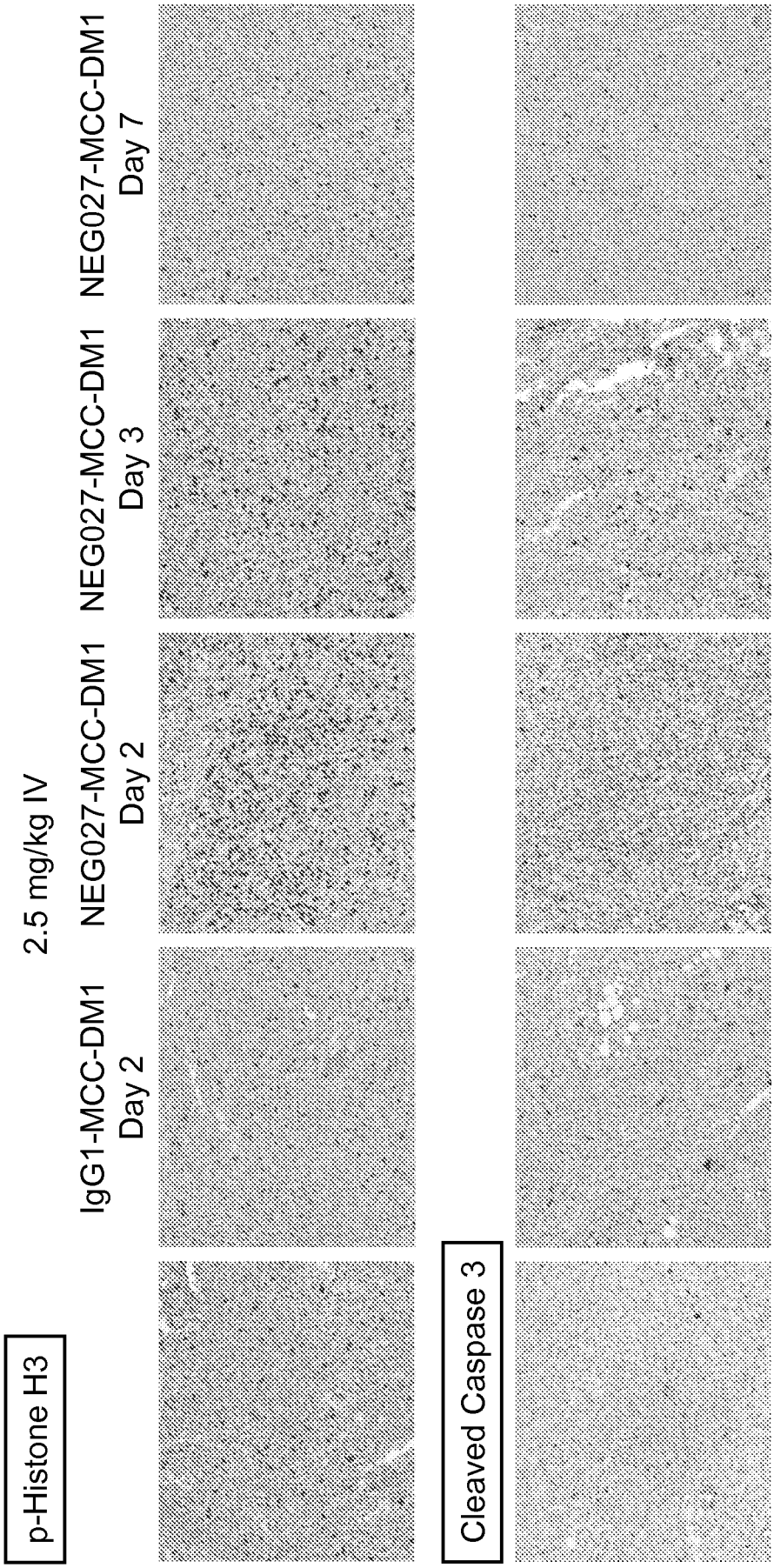

Mitotic arrest (p-histone H3) and apoptosis (caspase 3) after single dose of cKIT ADC
2.5 mg/kg IV
IgG1-MCC-DM1 Day 2
NEG027-MCC-DM1 Day 2
NEG027-MCC-DM1 Day 3
NEG027-MCC-DM1 Day 7
p-Histone H3
Cleaved Caspase 3

FIGURE 22

Mitotic arrest and apoptosis induction 8 days post single dose of cKIT ADC

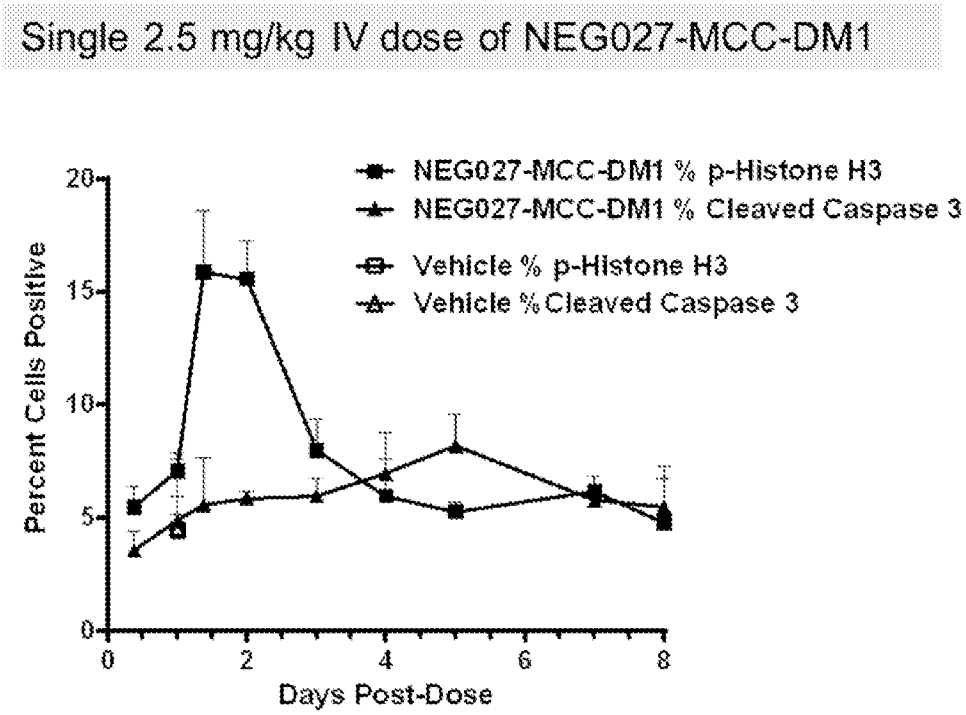
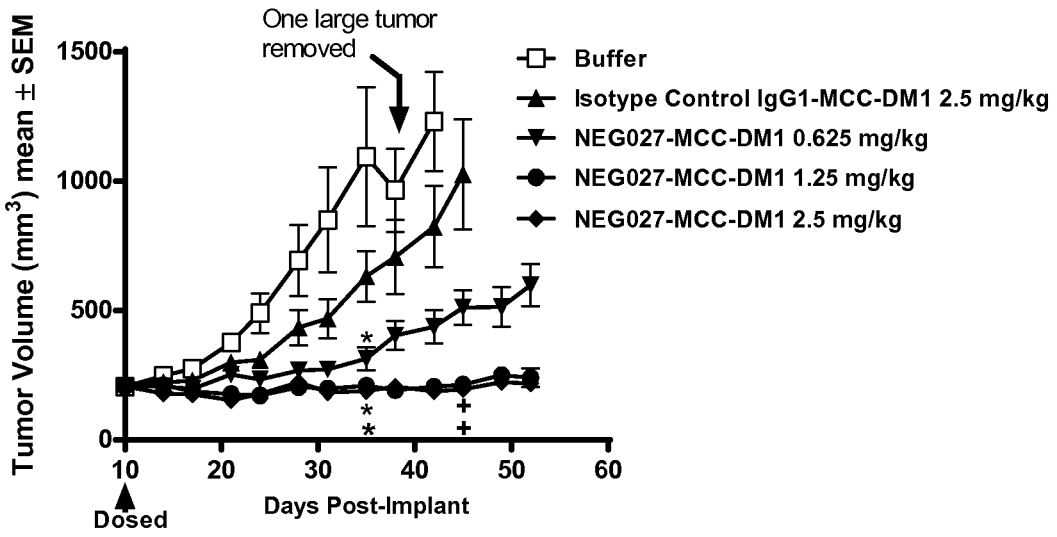


FIGURE 23

(A) Dose response efficacy in GIST T1 mouse xenograft



(B) Change in body weight over course of treatment

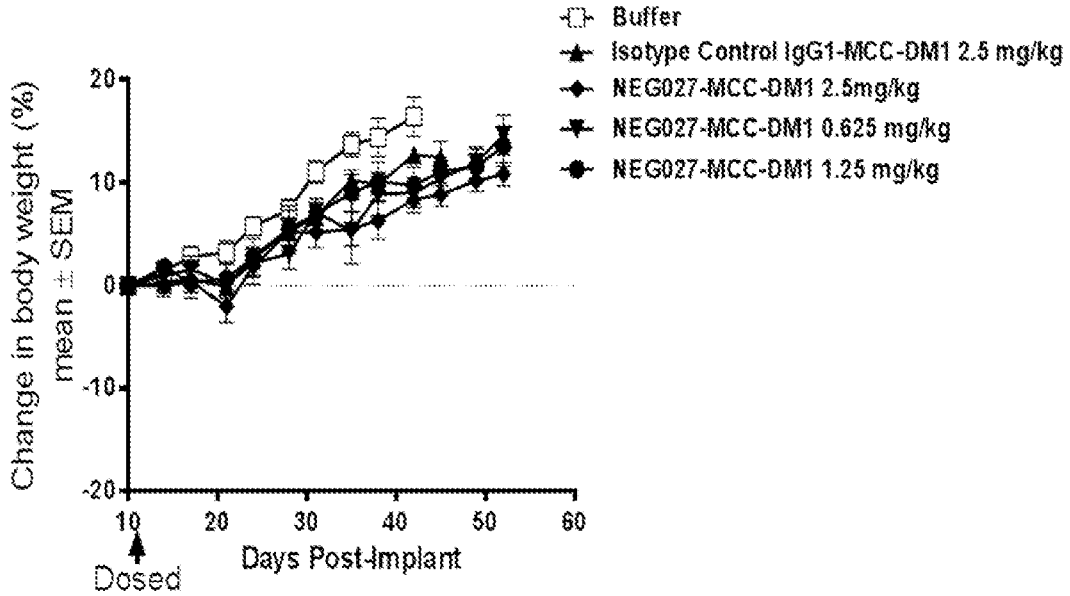
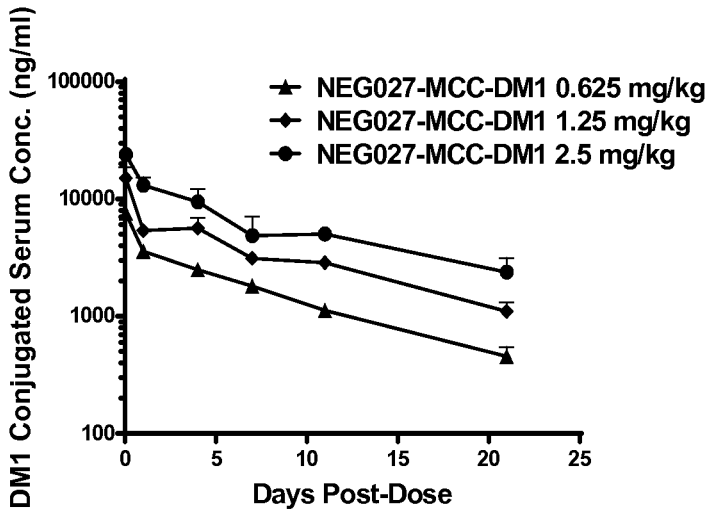


FIGURE 24

(A) anti-DM1 ELISA after dosing in a GIST T1 xenograft model



(B) anti-human IgG1 ELISA after dosing in a GIST T1 xenograft model

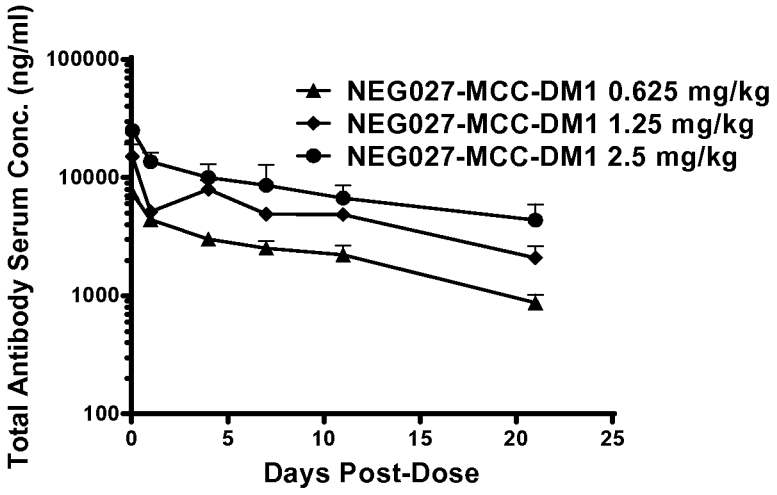


FIGURE 25

NEG027-MCC-DM1 dose response in a GIST T1 xenograft mouse model

(Top graph)Day 35 data table ($p < 0.05$ vs vehicle, ANOVA/Tukey's Test) (Bottom graph Day 45 data table ($p < 0.05$ vs Grp 2 (IgG-DM1 control), ANOVA/Dunn's Method)

Group	Drug	Dose	Schedule	Mean change of tumor volume vs control (T/C) (%)	Regression (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ Total)
1	TBS	0 mg/kg	single IV dose	100	-	890 ± 270	13.7 ± 1.2	9/9
2	IgG5-MCC-DM1	2.5 mg/kg	single IV dose	47	-	417 ± 95	10.2 ± 1.0	9/9
3	NEG027-MCC-DM1	0.625 mg/kg	single IV dose	12*	-	109 ± 39	5.3 ± 3.2	9/9
4	NEG027-MCC-DM1	1.25 mg/kg	single IV dose	0*	-	4 ± 15	9.0 ± 1.8	9/9
5	NEG027-MCC-DM1	2.5 mg/kg	single IV dose	-*	-8.71	-18 ± 21	5.5 ± 1.6	9/9

Group	Drug	Dose	Schedule	Mean change of tumor volume vs IgG control (T/C) (%)	Regression (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ Total)
1	TBS	0 mg/kg		Down	Down	0 ± 0	0.0 ± 0.0	9/9
2	IgG-MCC-DM1	2.5 mg/kg	single IV dose	-	-	811 ± 214	12.5 ± 1.5	9/9
3	NEG027-MCC-DM1	0.625 mg/kg	single IV dose	50	-	301 ± 62	10.4 ± 1.7	8/9 (one removed from study on D38)
4	NEG027-MCC-DM1	1.25 mg/kg	single IV dose	21*	-	8 ± 19	11.1 ± 1.4	9/9
5	NEG027-MCC-DM1	2.5 mg/kg	single IV dose	20*	-	-14 ± 28	8.8 ± 1.1	8/9 (one removed from study D38)

FIGURE 26A

NEG027-MMC-DM1 dose response efficacy in GIST T1

Group 4 pooled tumors

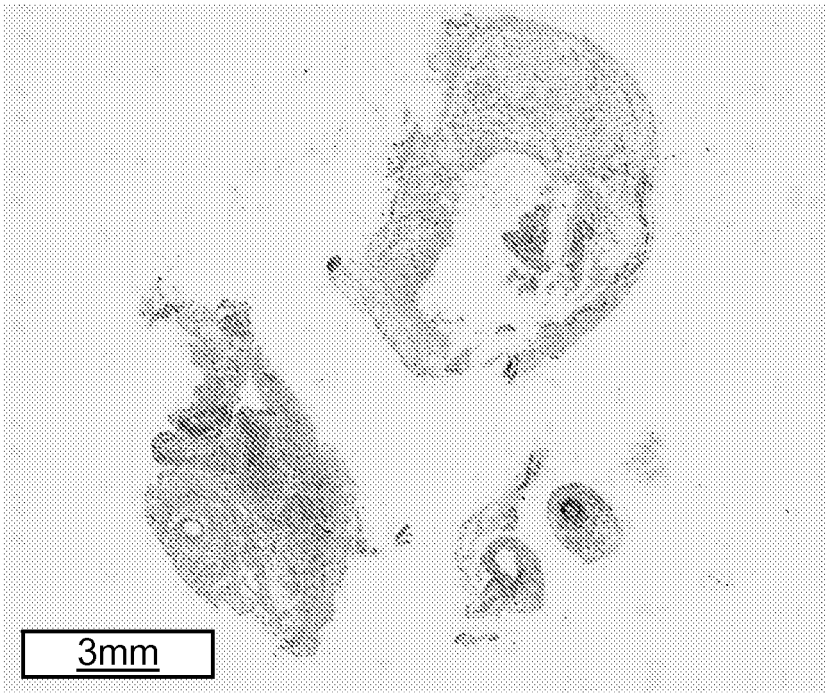


FIGURE 26B

NEG027-MMC-DM1 dose response efficacy in GIST T1

Group 5 pooled tumors

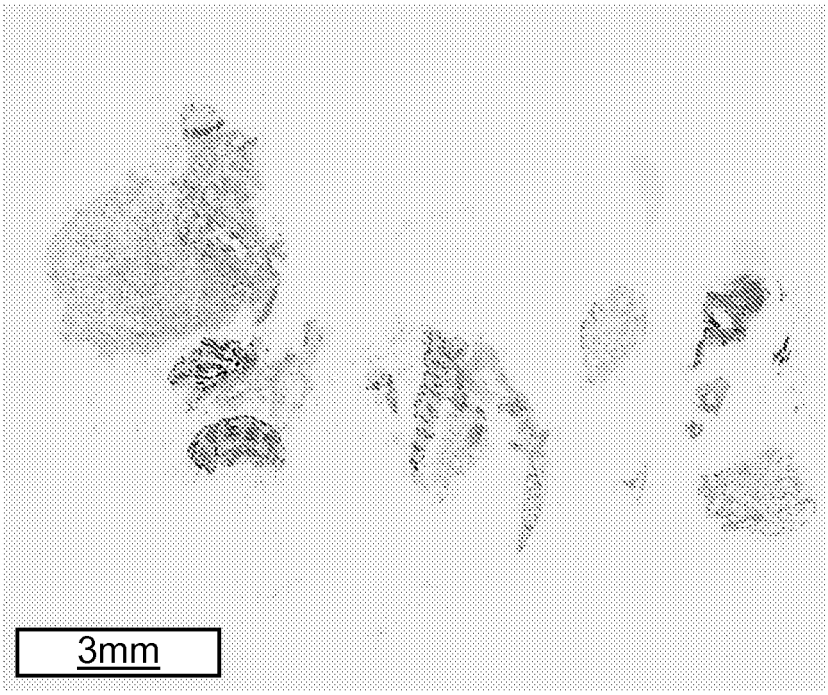
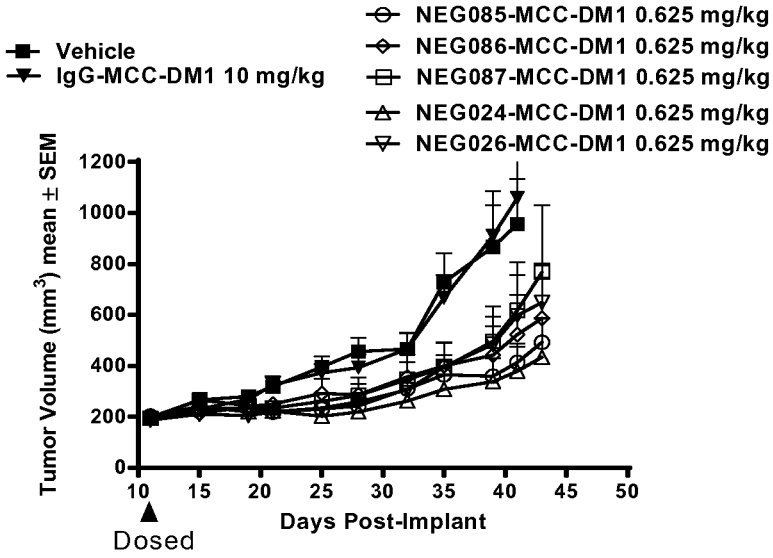


FIGURE 27

(A) Efficacy with 0.625mg/kg in a GIST T1 xenograft mouse model



(B) change of tumor volume vs control (% T/C)

ADC	% ΔT/ΔC (D41)
IgG1-DM1	114
NEG085-DM1	**28**
NEG086-DM1	43
NEG087-DM1	56
NEG024-DM1	**23**
NEG026-DM1	54

(C) Change in body weight over course of treatment

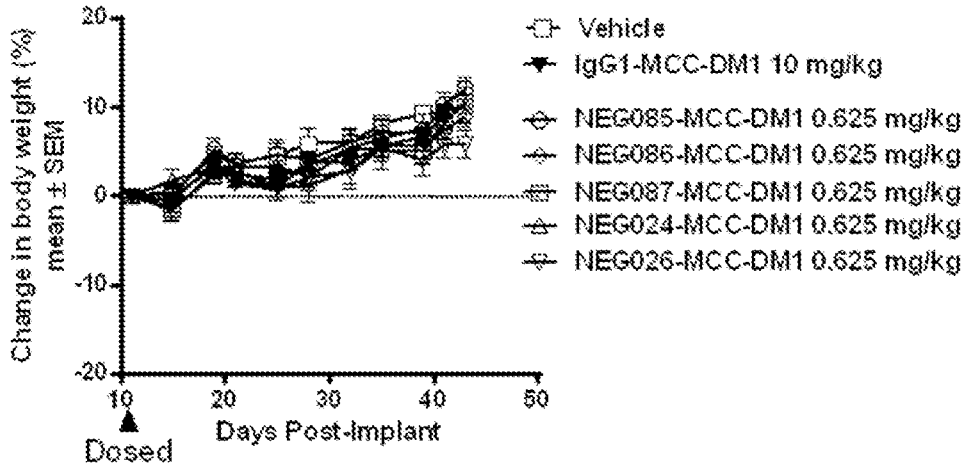


FIGURE 28

Day 41 after administration of single dose of anti-cKIT ADC to a GIST T1 xenograft mouse

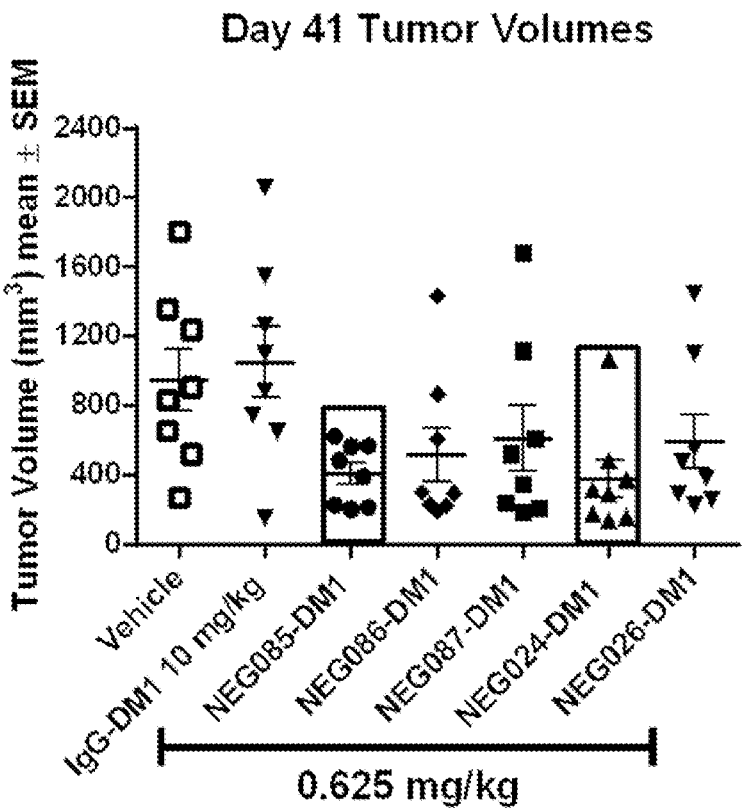


FIGURE 29

Anti-cKIT-ADC Efficacy Study (low dose) in GIST T1 Model in SCID-beige Mice

Drug	Dose	Schedule	Tumor Response		Host Response	
			Mean change of tumor volume vs control (T/C) (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/total)
TBS	0mg/kg	single dose IV	100	757 ± 175	9.3 ± 1.0	8/8
IgG-SMCC-DM1	10mg/kg	single dose IV	114	861 ± 206	9.1 ± 1.0	8/8
NEG085-SMCC-DM1	0.625mg/kg	single dose IV	28	210 ± 56	10.3 ± 1.3	8/8
NEG086-SMCC-DM1	0.625mg/kg	single dose IV	43	325 ± 146	7.4 ± 1.4	8/8
NEG087-SMCC-DM1	0.625mg/kg	single dose IV	56	423 ± 188	8.4 ± 2.1	8/8
NEG024-SMCC-DM1	0.625mg/kg	single dose IV	23	176 ± 104	9.6 ± 2.0	8/8
NEG026-SMCC-DM1	0.625mg/kg	single dose IV	54	410 ± 166	5.7 ± 1.4	8/8

FIGURE 30

(A) Anti-cKIT PK in a GIST T1 xenograft mouse model, (left panel is anti-DM1 ELISA)

(B) Right panel is anti-human IgG1 ELISA

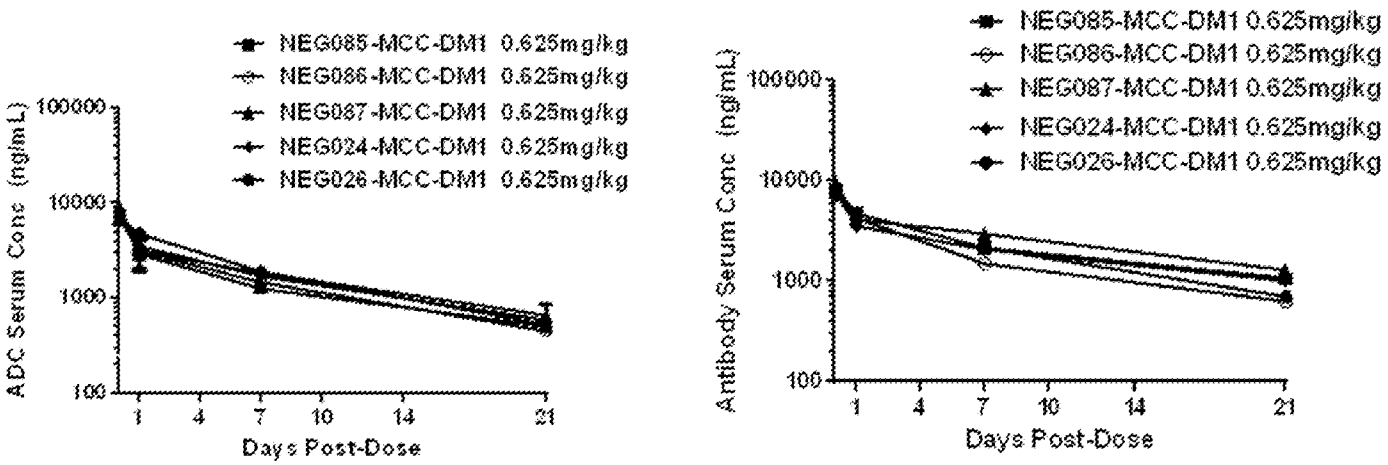


FIGURE 31

(A) NEG085, NEG024 and NEG086 activity in a SCLC model

(B) change in body weight over course of treatment

(C) expression of cKIT on tumor sample

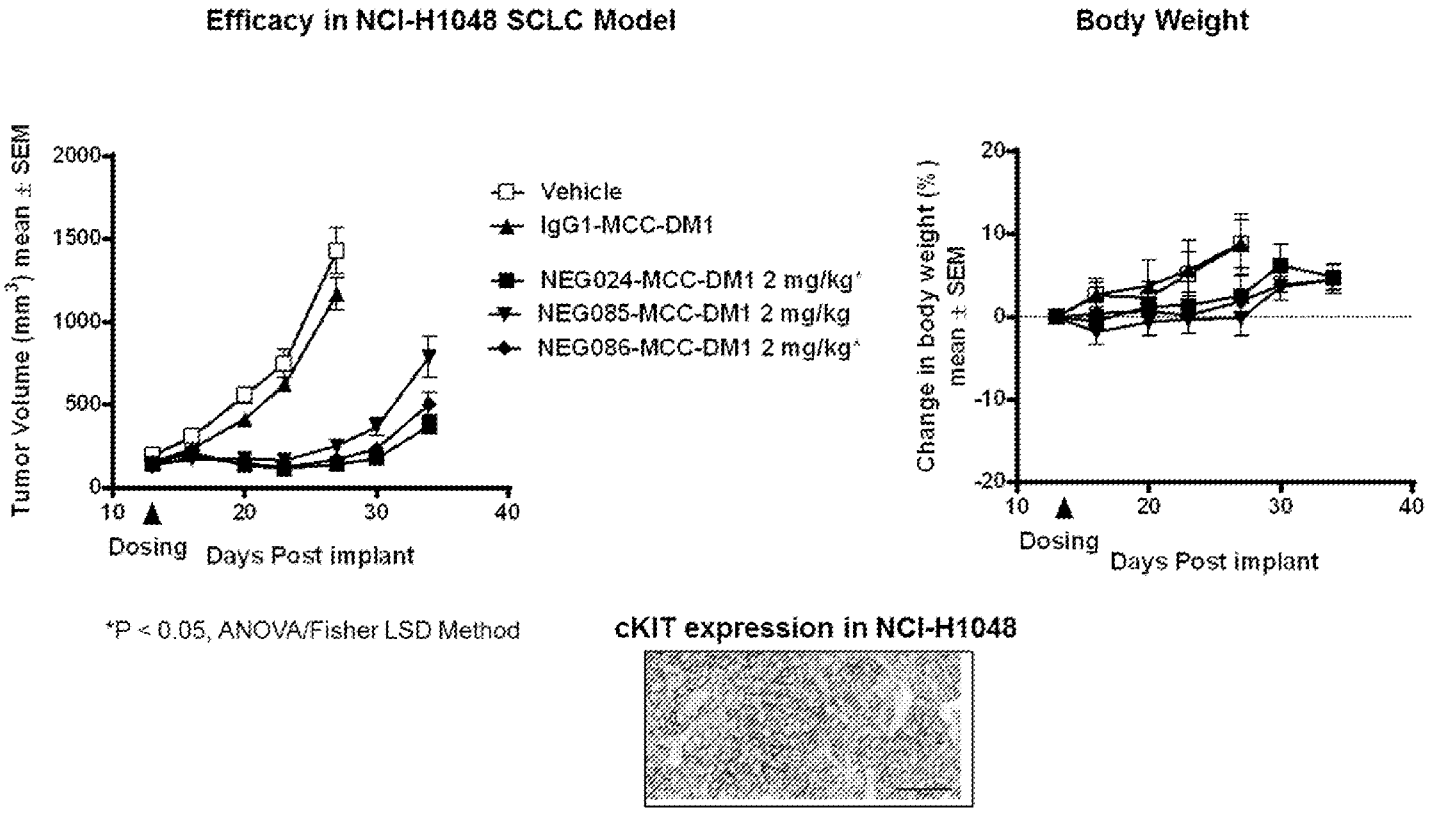


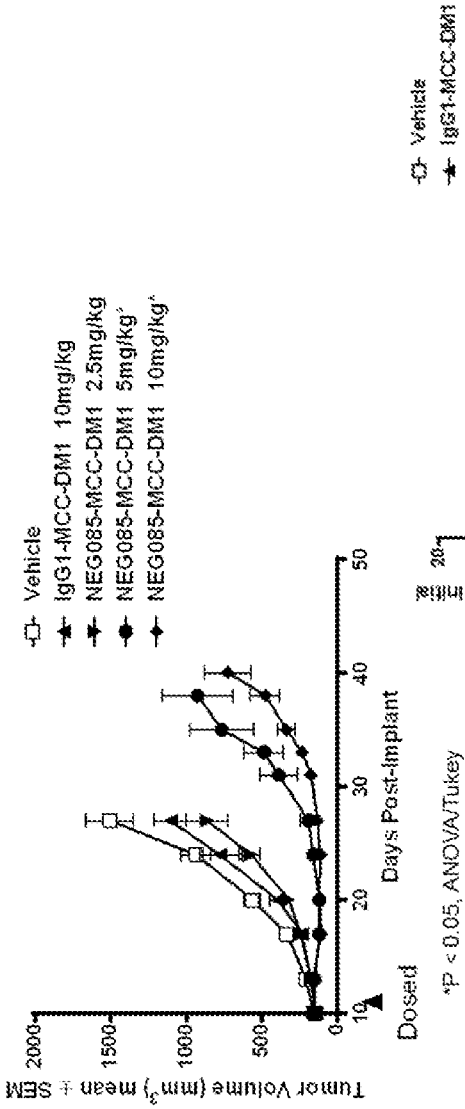
FIGURE 32

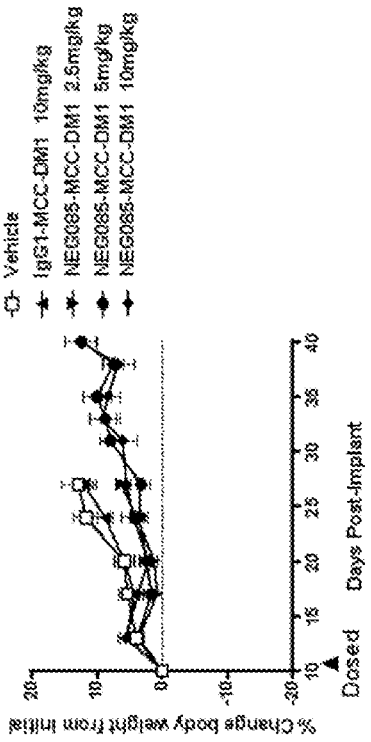
anti-cKIT-ADC Efficacy Study in NCI-H1048 SCLC

Drug	Dose	Schedule	Tumor Response			Host Response	
			Mean change of tumor volume vs control (T/C) (%)	Regression (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ total)
Vehicle	0mg/kg	single dose IV	100	-	1234 ± 125	8.8 ± 3.7	8/8
IgG1-MCC-DM1	2mg/kg	single dose IV	82	-	1013 ± 91	8.8 ± 2.9	8/8
NEG024-MCC-DM1	2mg/kg	single dose IV	-	-2.16*	-3 ± 18	2.5 ± 2.4	8/8
NEG085-MCC-DM1	2mg/kg	single dose IV	9	-	114 ± 42	-0.2 ± 2.1	8/8
NEG086-MCC-DM1	2mg/kg	single dose IV	4*	-	44 ± 22	1.8 ± 0.7	8/8

FIGURE 33

(A) NEG085-MCC-DM1 dose response in NCI-H1048 (SCLC) xenograft model





(B) Change in body weight over course of treatment

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FIGURE 34

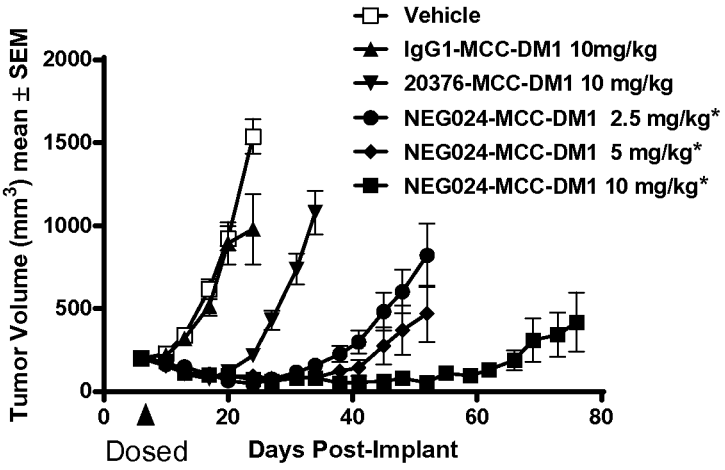
NEG085-MCC-DM1 efficacy study in NCI-1048 (SCLC) xenograft mouse model

Data Table Day 27

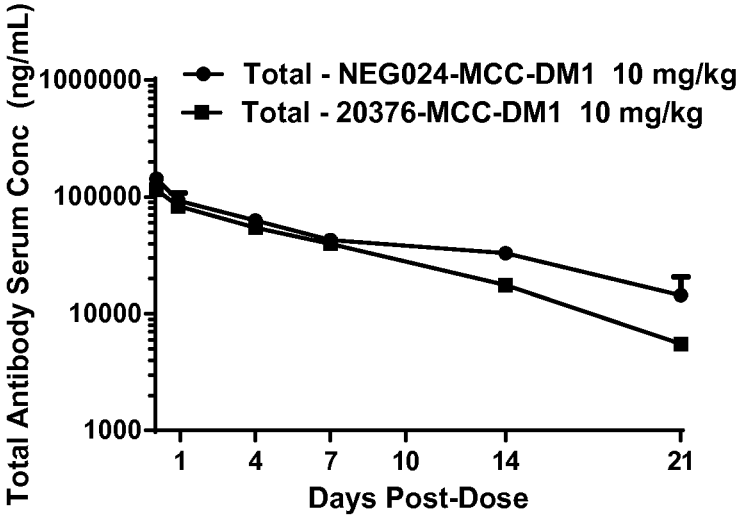
Drug	Dose	Schedule	Tumor Response			Host Response	
			Mean change of tumor volume vs control (T/C) (%)	Regression (%)	Mean change of tumor volume (mm 3± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ total)
TBS	0mg/kg	Single Dose IV	100	–	1347 ± 164	12.9 ± 2.6	8/8
IgG1-MCC-DM1	10mg/kg	Single Dose IV	72	–	963 ± 113	12.0 ± 1.3	8/8
NEG085-MCC-DM1	2.5mg/kg	Single Dose IV	53	–	716 ± 141	6.3 ± 1.7	8/8
NEG085-MCC-DM1	5mg/kg	Single Dose IV	3*	–	37 ± 55	6.0 ± 1.8	8/8
NEG085-MCC-DM1	10mg/kg	Single Dose IV	–	-14.92*	-23 ± 14	5.5 ± 1.7	8/8

FIGURE 35

(A) Efficacy of 20376 and NEG024 in NCI-H526 (SCLC) xenograft mouse model



(B) Antibody serum concentration after dosing



(C) IHC for c-Kit shows expression of cKIT levels on H526 tumor

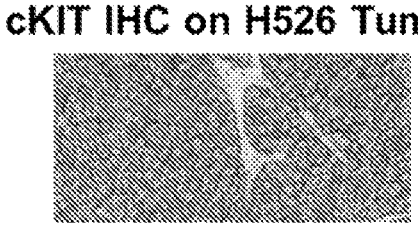


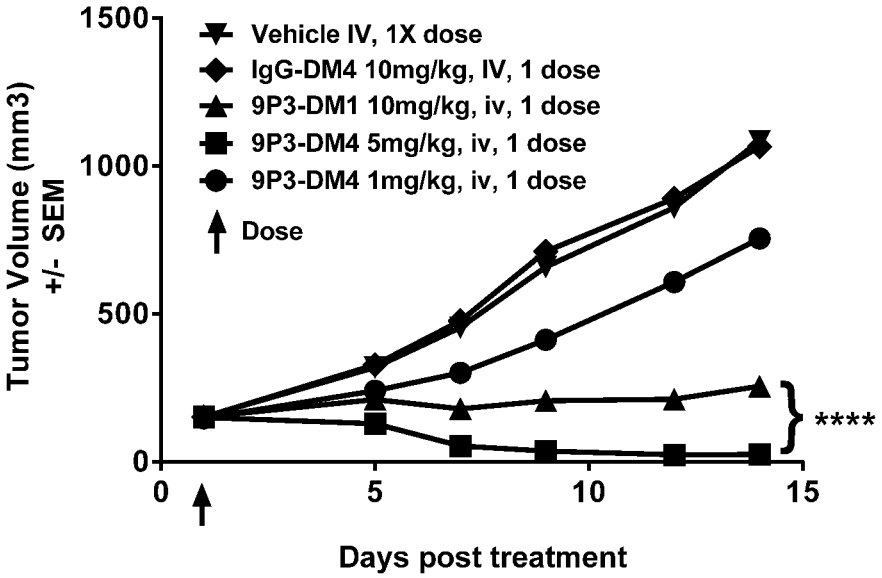
FIGURE 36

Anti-cKITADC in a small cell lung cancer (SCLC) xenograft model (*p < 0.05, ANOVA/Dunn's)

Drug	Dose	Schedule	Tumor Response			Host Response	
			Mean change of tumor volume vs control (T/C) (%)	Regression (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ total)
TBS	0mg/kg	Single Dose IV	100	-	1338 ± 107	13.5 ± 3.6	9/9
IgG1-MCC-DM1	10mg/kg	Single Dose IV	57	-	766 ± 208	6.6 ± 0.7	9/9
20376-MCC-DM1	10mg/kg	Single Dose IV	1	-	18 ± 28	4.1 ± 1.9	9/9
NEG024-MCC-DM1	2.5mg/kg	Single Dose IV	-	-76.14*	-148 ± 17	3.0 ± 1.1	9/9
NEG024-MCC-DM1	5mg/kg	Single Dose IV	-	-52.94*	-106 ± 15	3.3 ± 1.1	9/9
NEG024-MCC-DM1	10mg/kg	Single Dose IV	-	-63.04*	-123 ± 14	-0.1 ± 2.2	9/9

FIGURE 37

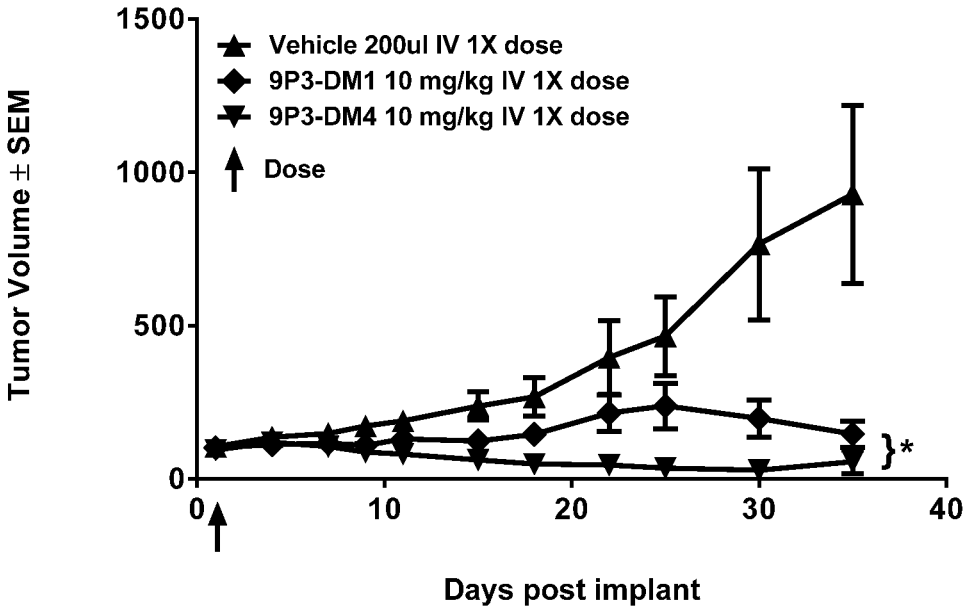
Efficacy in an AML xenograft model (Kasumi-1)



Control vs Treatment groups: Significant differences were measured using one-way ANOVA, posttest Tukey multiple comparison test, (**p<0.0001)**

FIGURE 38

Efficacy in a HMC-1 mastocytosis xenograft mouse model

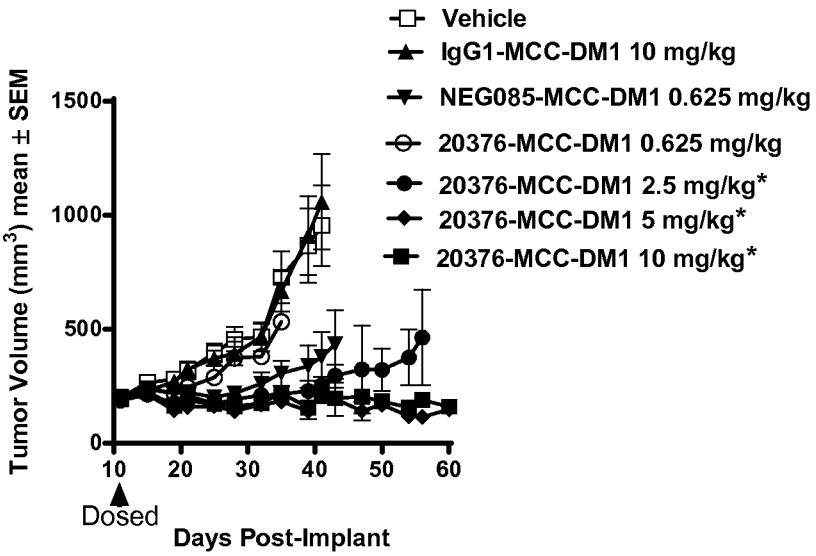


Control vs Treatment groups: Significant differences were measured using one-way ANOVA, posttest Tukey multiple comparison test, (*p<0.05)

FIGURE 39

Efficacy of mouse cross reactive 20376-MCC-DM1 in GIST T1 xenograft mouse model

A- Dosage and tumor volume



B- Change in body weight over course of treatment

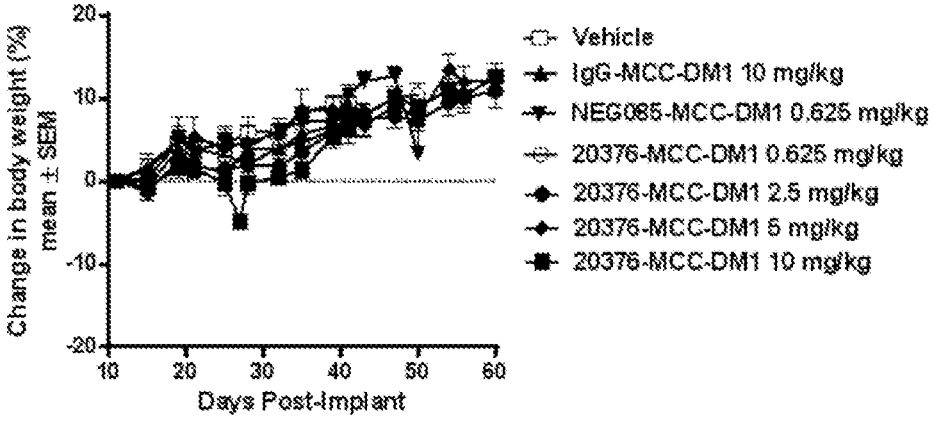
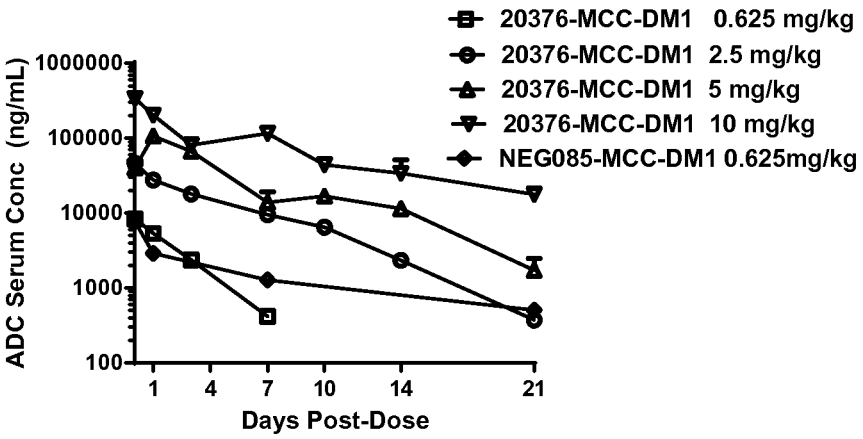
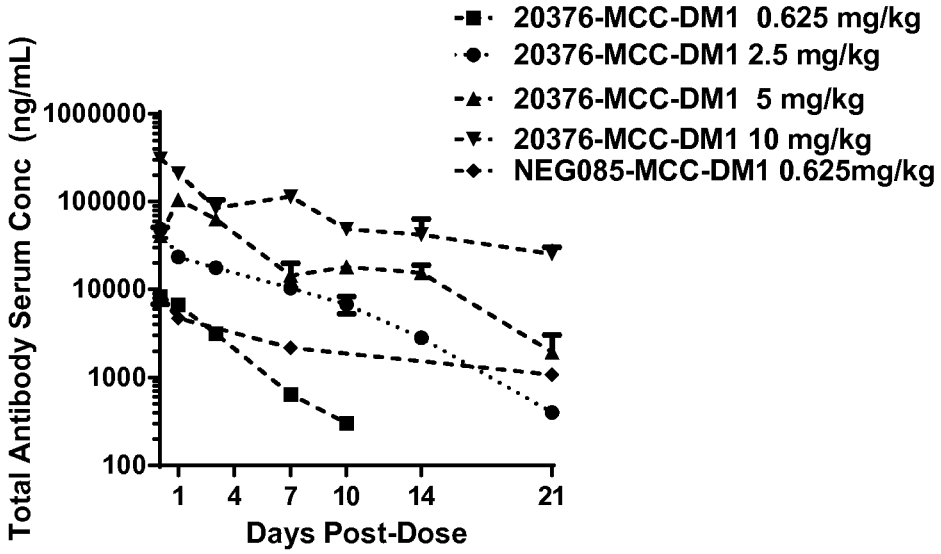


FIGURE 40

(A) Efficacy of mouse cross reactive 20376-MCC-DM1 in GIST T1 xenograft mouse model -PK



(B) Antibody serum concentration post dosing



SUBSTITUTE SHEET (RULE 26)

FIGURE 41

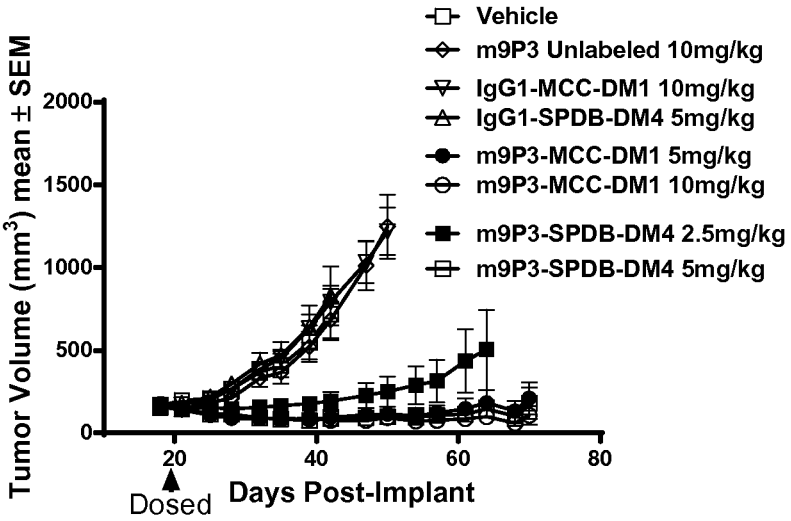
Dose response efficacy study in GIST T1 SCID–beige mice (Data Table 41)

Data Table Day 27

			Tumor Response		Host Response	
Drug	Dose	Schedule	Mean change of tumor volume vs control (T/C) (%)	Mean change of tumor volume (mm 3± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/total)
Vehicle	0mg/kg	Single Dose IV	100	757 ± 175	9.3 ± 1.0	8/8
IgG–SMCC–DM1	10mg/kg	Single Dose IV	114	861 ± 206	9.1 ± 1.0	8/8
NEG085–SMCC–DM1	0.625mg/kg	Single Dose IV	28	210 ± 56	10.3 ± 1.3	8/8
20376–SMCC–DM1	0.625mg/kg	Single Dose IV	51	385 ± 106	8.1 ± 1.0	8/9 (one mouse removed from study)
20376–SMCC–DM1	2.5mg/kg	Single Dose IV	9*	66 ± 41	7.2 ± 1.6	9/9
20376–SMCC–DM1	5mg/kg	Single Dose IV	4*	34 ± 62	7.5 ± 1.2	9/9
20376–SMCC–DM1	10mg/kg	Single Dose IV	3*	22 ± 16	6.1 ± 1.5	8/9 (one mouse removed from study)

FIGURE 42

(A) Efficacy in GIST T1 xenograft mouse model (no efficacy with unconjugated)



(B) Change in body weight over course of treatment

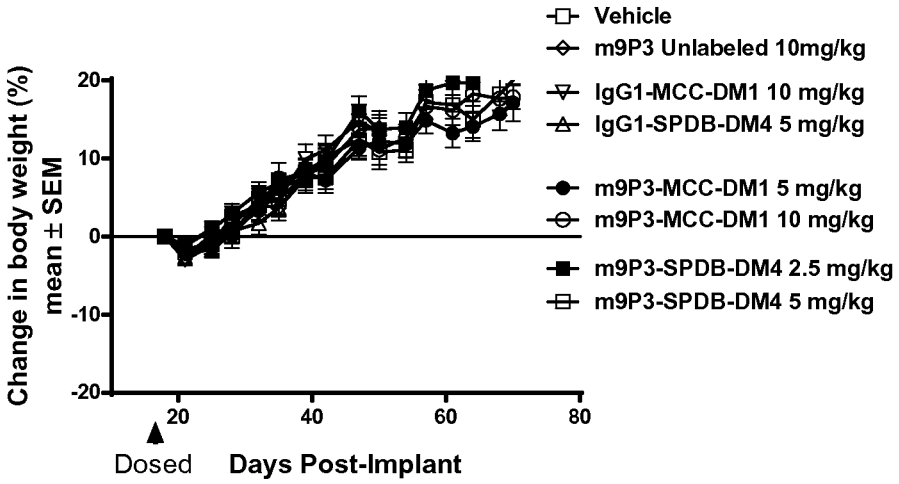


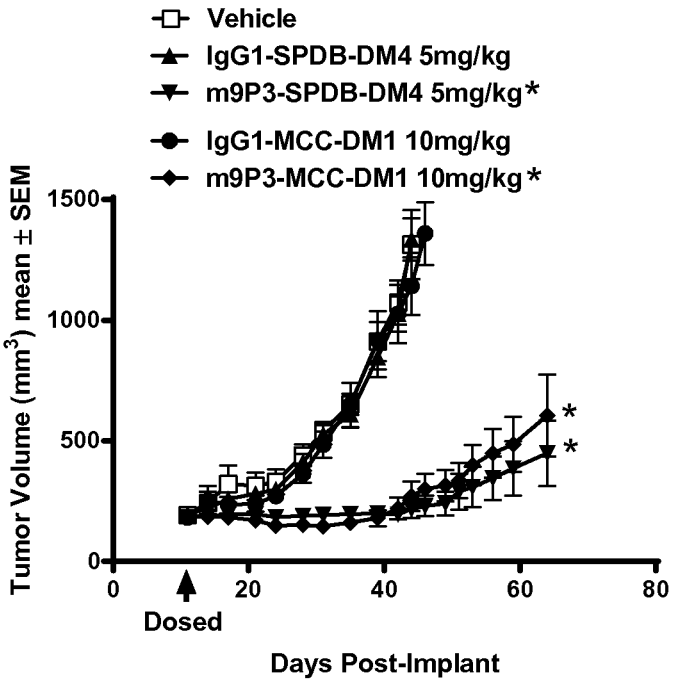
FIGURE 43

Efficacy in a GIST T1 mouse xenograft model (unlabeled/MCC-DM1/SPDB-DM4) (Data Table Day 42, $p<0.05$, ANOVA/Tukey

Drug	Dose	Schedule	Tumor Response			Host Response	
			Mean change of tumor volume vs control (T/C) (%)	Regression (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ total)
Vehicle	0mg/kg	single dose IV	100	-	554 ± 148	9.5 ± 2.2	8/8
9P3 unlabeled	10mg/kg	single dose IV	95	-	525 ± 103	9.0 ± 1.9	8/8
IgG1-MCC-DM1	5mg/kg	single dose IV	113	-	624 ± 96	11.1 ± 1.9	9/9
IgG1-SPDB-DM4	10mg/kg	single dose IV	119	-	657 ± 176	10.0 ± 1.3	9/9
9P3-MCC-DM1	10mg/kg	single dose IV	-	-55.80	-97 ± 23	10.2 ± 1.1	9/9
9P3-MCC-DM1	5mg/kg	single dose IV	-	-43.84	-74 ± 28	7.2 ± 1.1	9/9
9P3-SPDB-DM4	5mg/kg	single dose IV	-	-43.24	-67 ± 14	7.6 ± 2.0	9/9
9P3-SPDB-DM4	2.5mg/kg	single dose IV	7	-	36 ± 58	9.6 ± 1.6	9/9

FIGURE 44

(A) Efficacy in a GIST 430 xenograft model comparing SPDB-DM4 and MCC-DM1



(B) Change in body weight over course of treatment

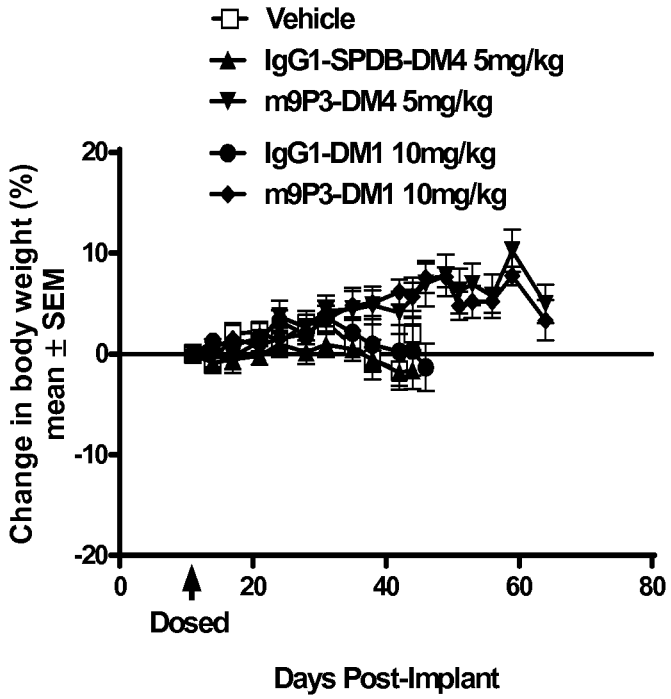


FIGURE 45

Efficacy in GIST 430 SCID-beige mouse model

Drug	Dose (mg/kg)	Sche-dule	Tumor Response			Host Response	
			Mean change of tumor volume vs control (T/C) (%)	Regres-sion (%)	Mean change of tumor volume (mm3 ± SEM)	Mean change of body weight (% ± SEM)	Survival (Survivors/ total)
Vehicle	0	single dose IV	100	-	1127 ± 161	2.1 ± 1.7	8/9 (cachexia w/ larger tumors)
IgG1-SPDB-DM4	5	single dose IV	101	-	1139 ± 91	-1.6 ± 1.9	9/9
9P3-SPDB-DM4	5	single dose IV	3*	-	34 ± 38	5.6 ± 2.0	9/9
IgG1-MCC-DM1	10	single dose IV	85	-	959 ± 112	0.3 ± 2.5	9/9
9P3-MCC-DM1	10	single dose IV	7*	- (22% on D24)	80 ± 51	5.7 ± 1.4	9/9

FIGURE 46

Mitotic arrest (p-histone H3) after single dose of c-Kit ADC NEG085-MCC-DM1

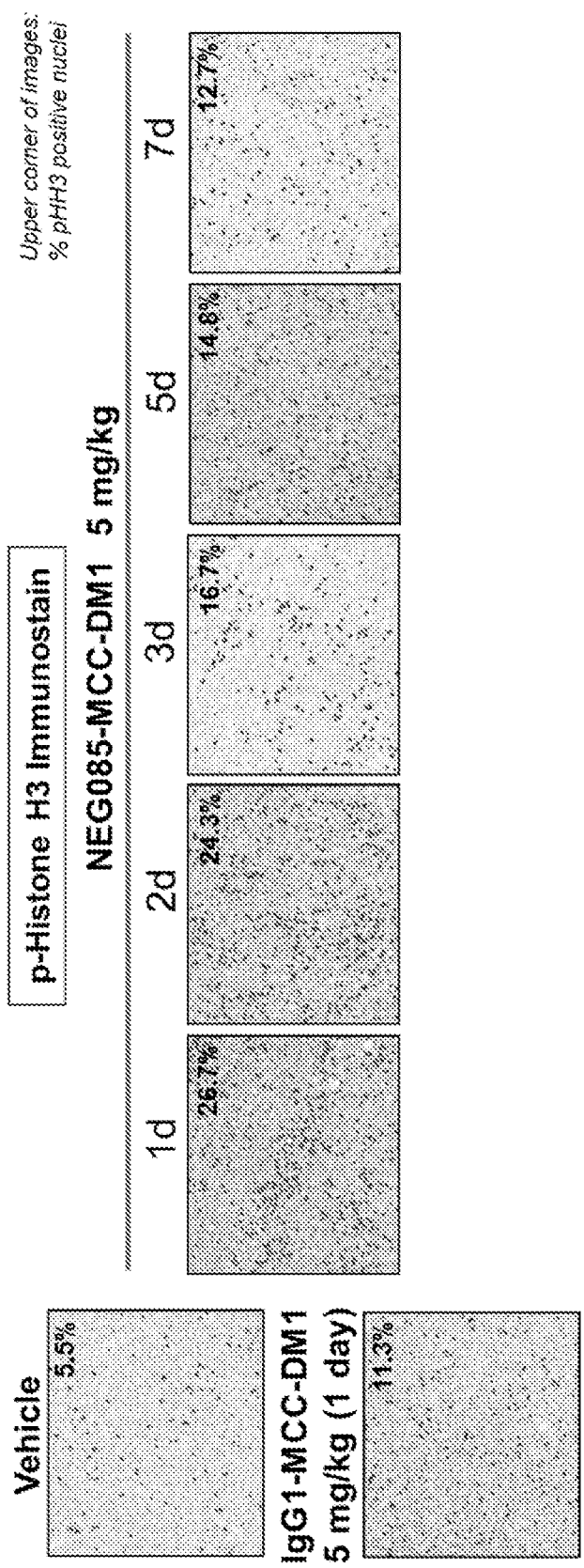


FIGURE 47

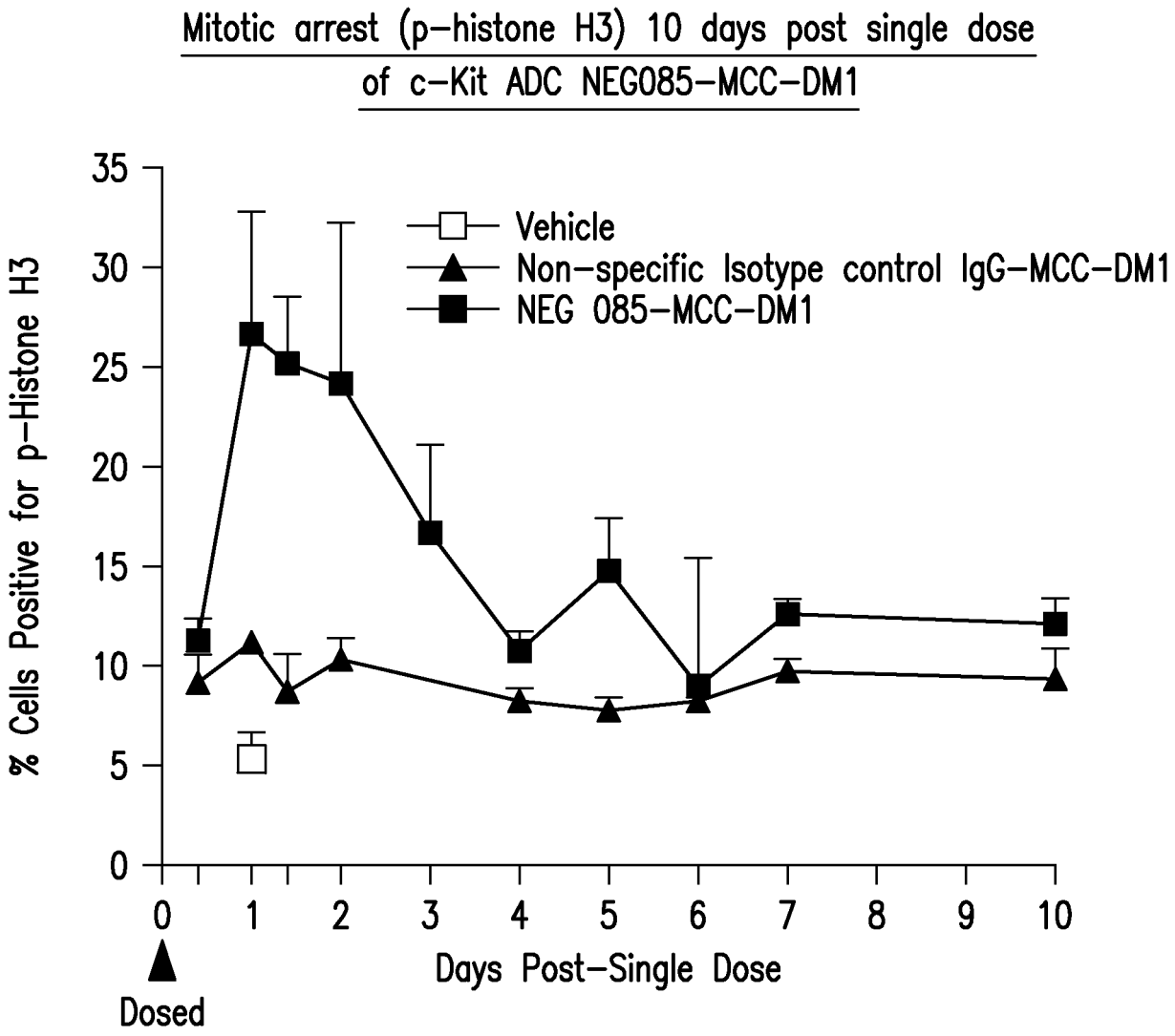

Mitotic arrest (p-histone H3) 10 days post single dose of c-Kit ADC NEG085-MCC-DM1
% Cells Positive for p-Histone H3
35
30
25
20
15
10
5
0
Vehicle
Non-specific Isotype control IgG-MCC-DM1
NEG 085-MCC-DM1
0
1
2
3
4
5
6
7
8
9
10
Dosed
Days Post-Single Dose

FIGURE 48A

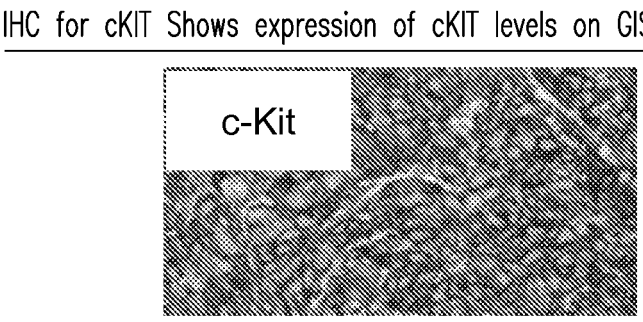


FIGURE 48B

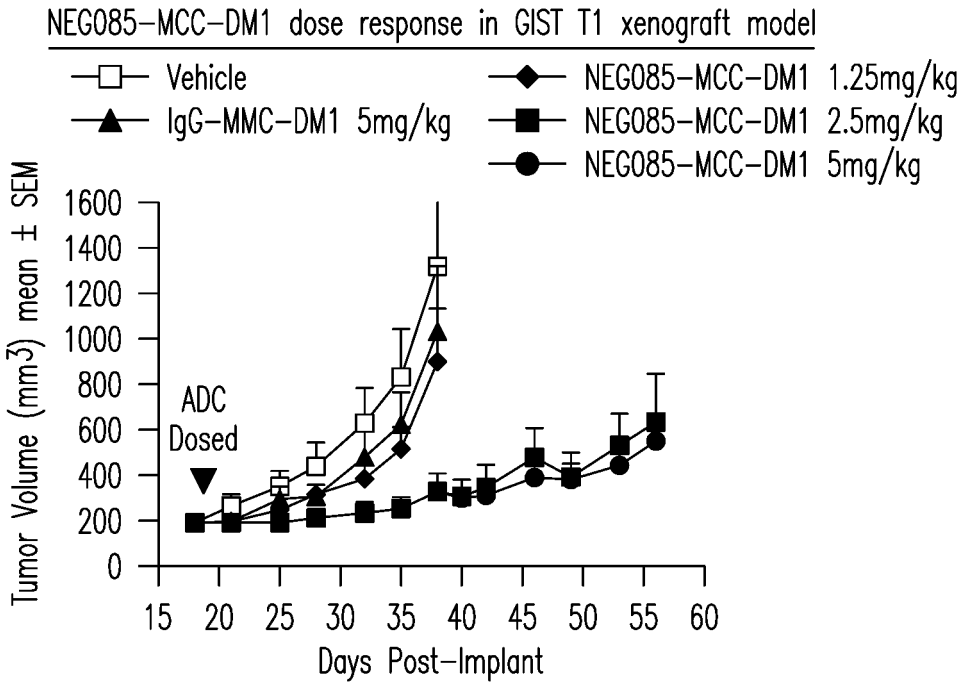


FIGURE 48C

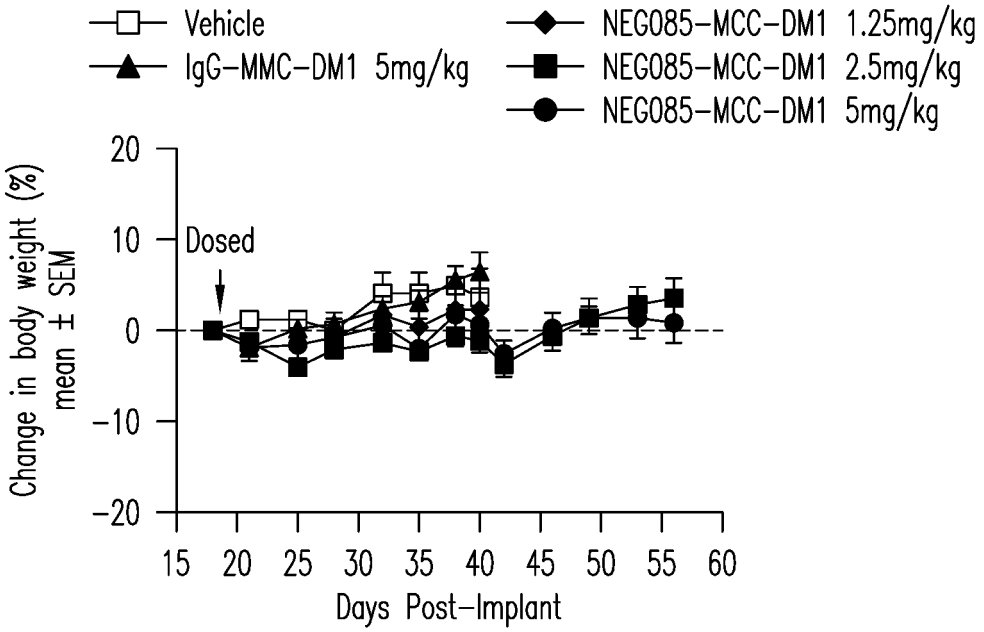


FIGURE 49A

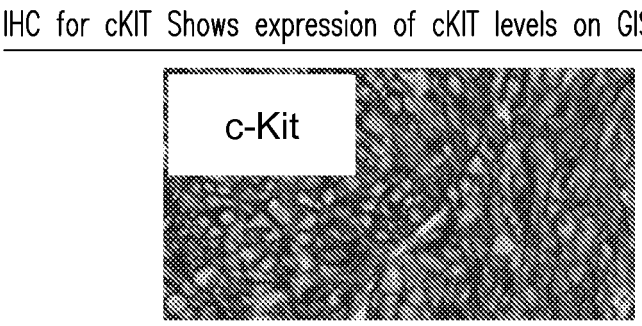


FIGURE 49B

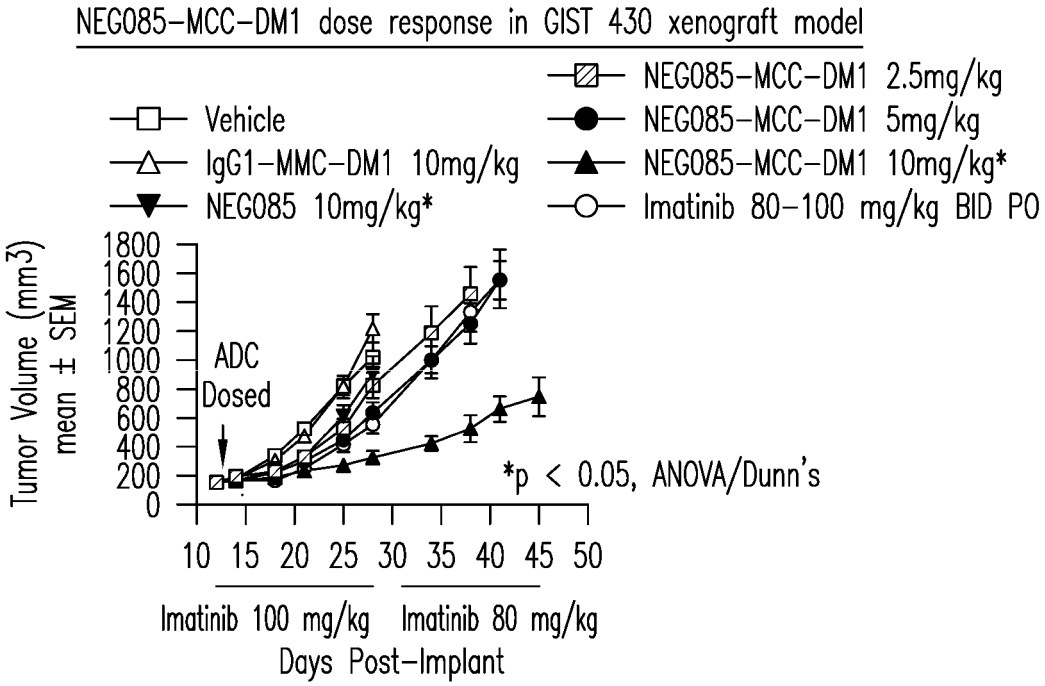


FIGURE 49C

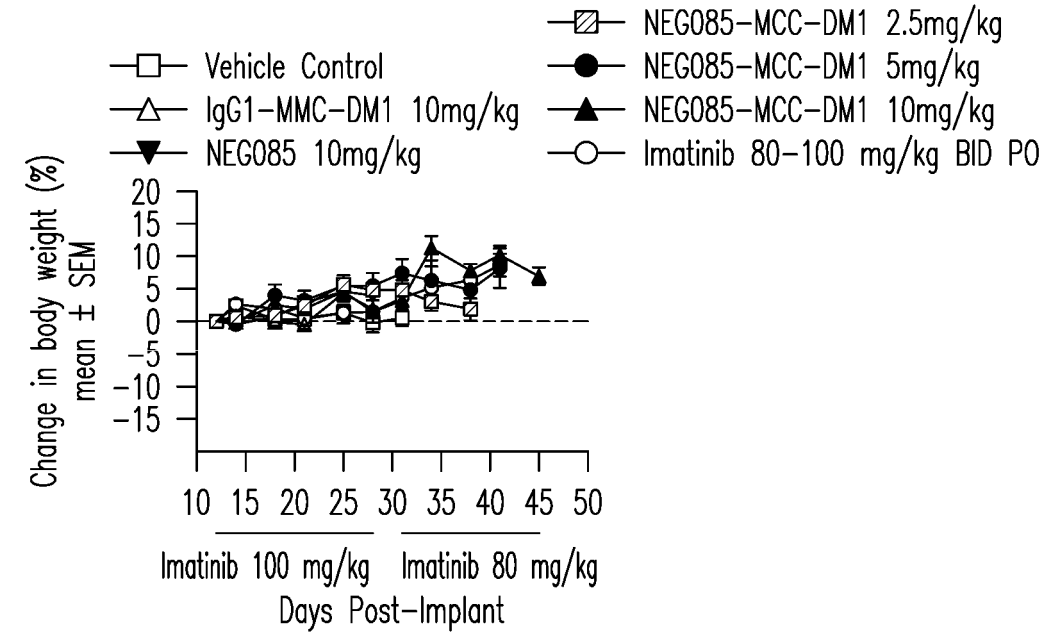


FIGURE 50A

IHC for c-KIT shows expression of cKIT levels on NCI-H526 tumor

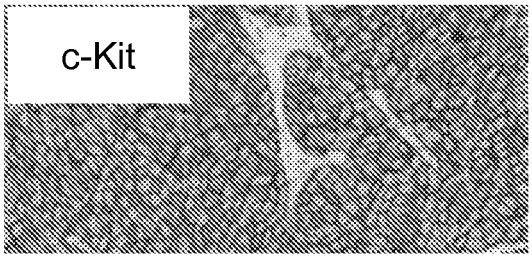


FIGURE 50B

NEG085-MCC-DM1 dose response in NCI-H526 (SCLC) xenograft model

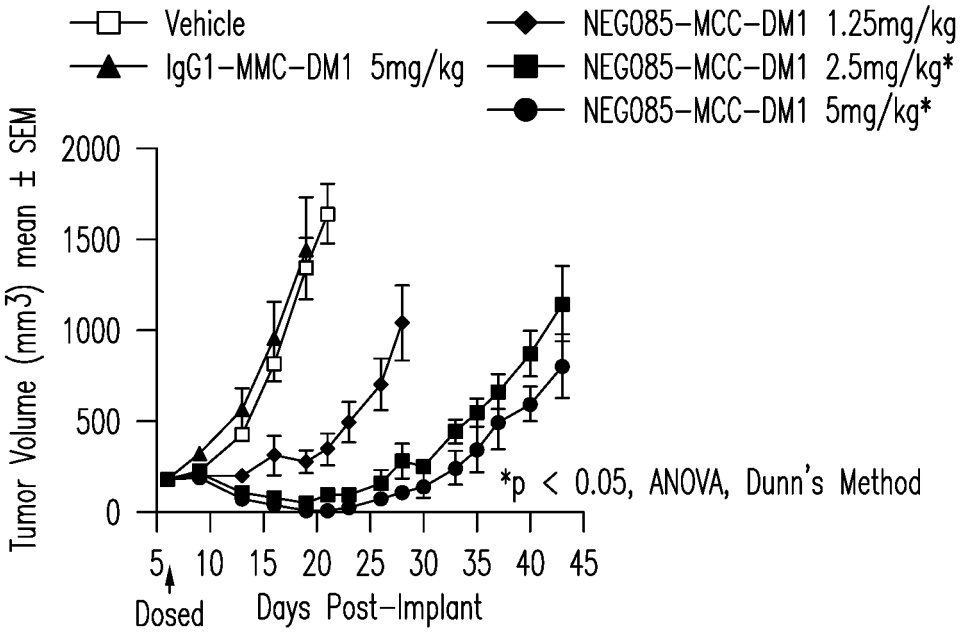


FIGURE 50C

NEG085-MCC-DM1 change in body weight over course of treatment

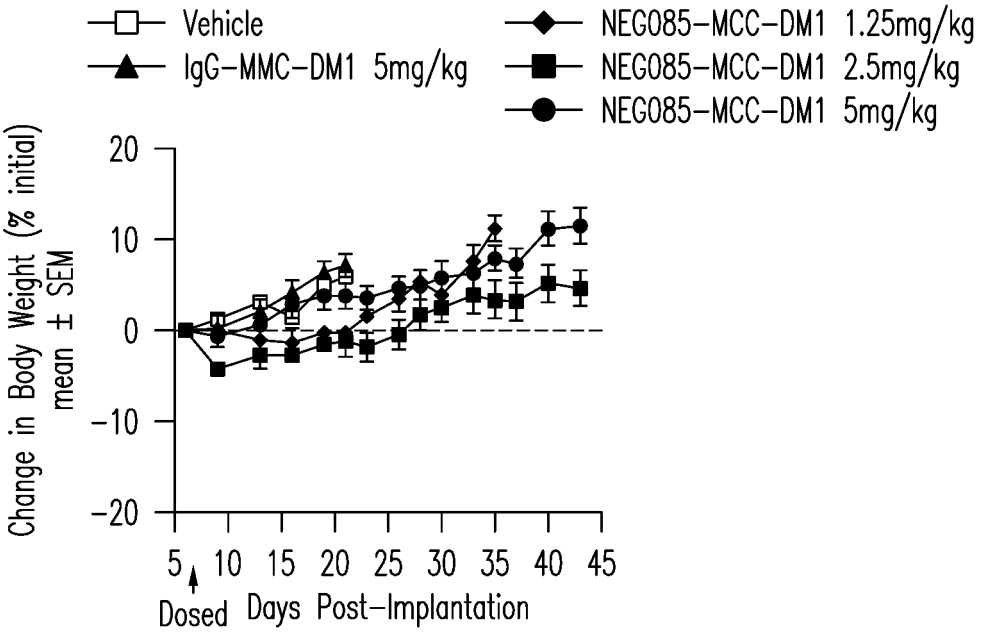
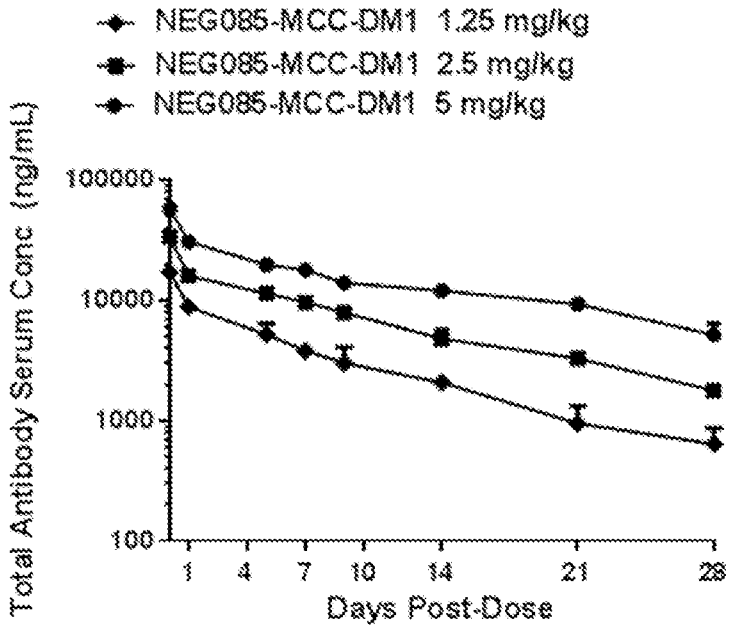


FIGURE 51

(A) anti-human IgG1 ELISA after dosing in a NCI-H526 xenograft model



(B) anti-DM1 ELISA after dosing in a NCI-H526 xenograft model

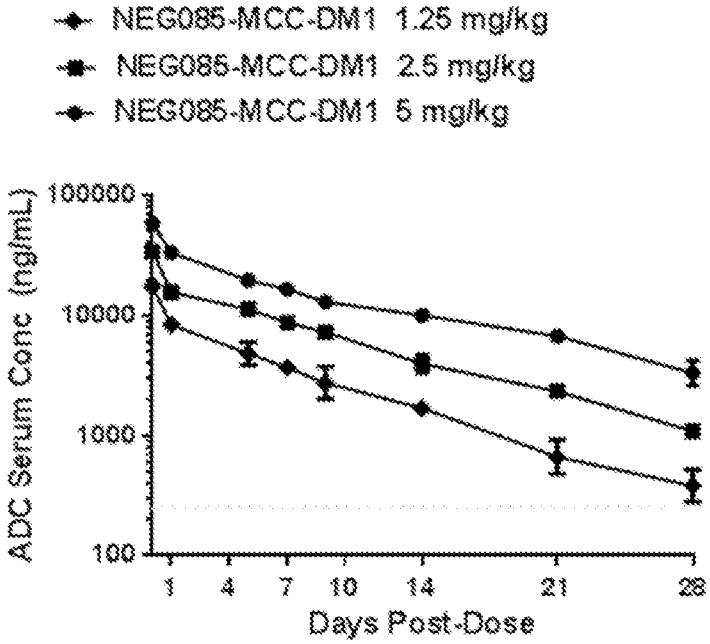
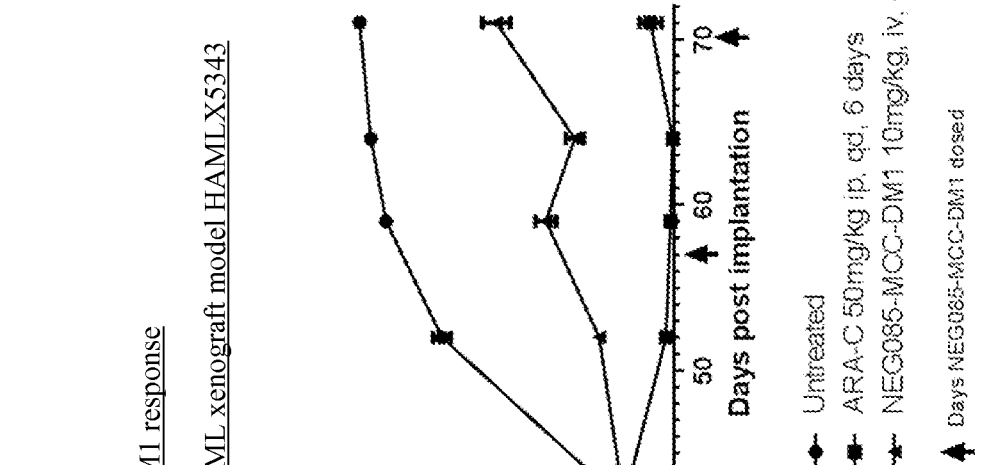


FIGURE 52

(A) NEG085-MCC-DM1 response in systemic primary AML xenograft model HAMLX5343



(B) Change in body weight over course of treatment

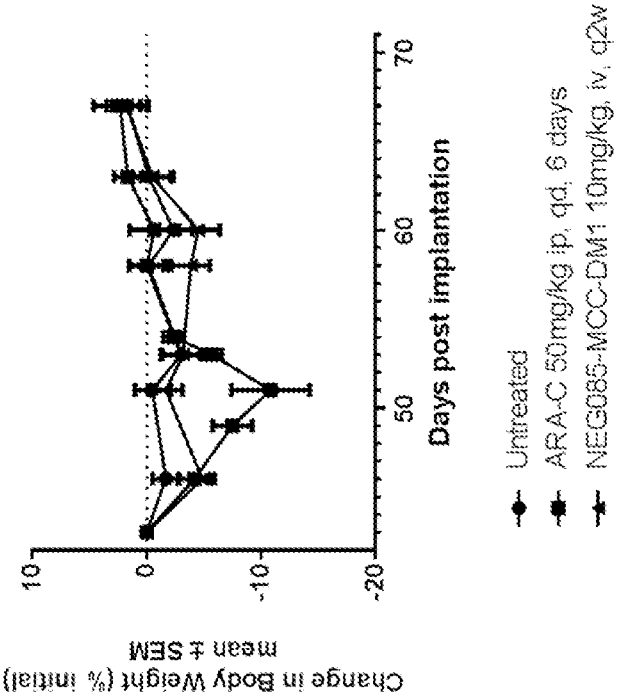


FIGURE 53

Representation of the 3.1-Å crystal structure of the Kit D1-D2/NEG085 Fab complex showing the Fab heavy chains (dark grey), Fab light chains (white), and Kit D1-D2 (light grey) domains. Epitopes and paratopes are colored black.

