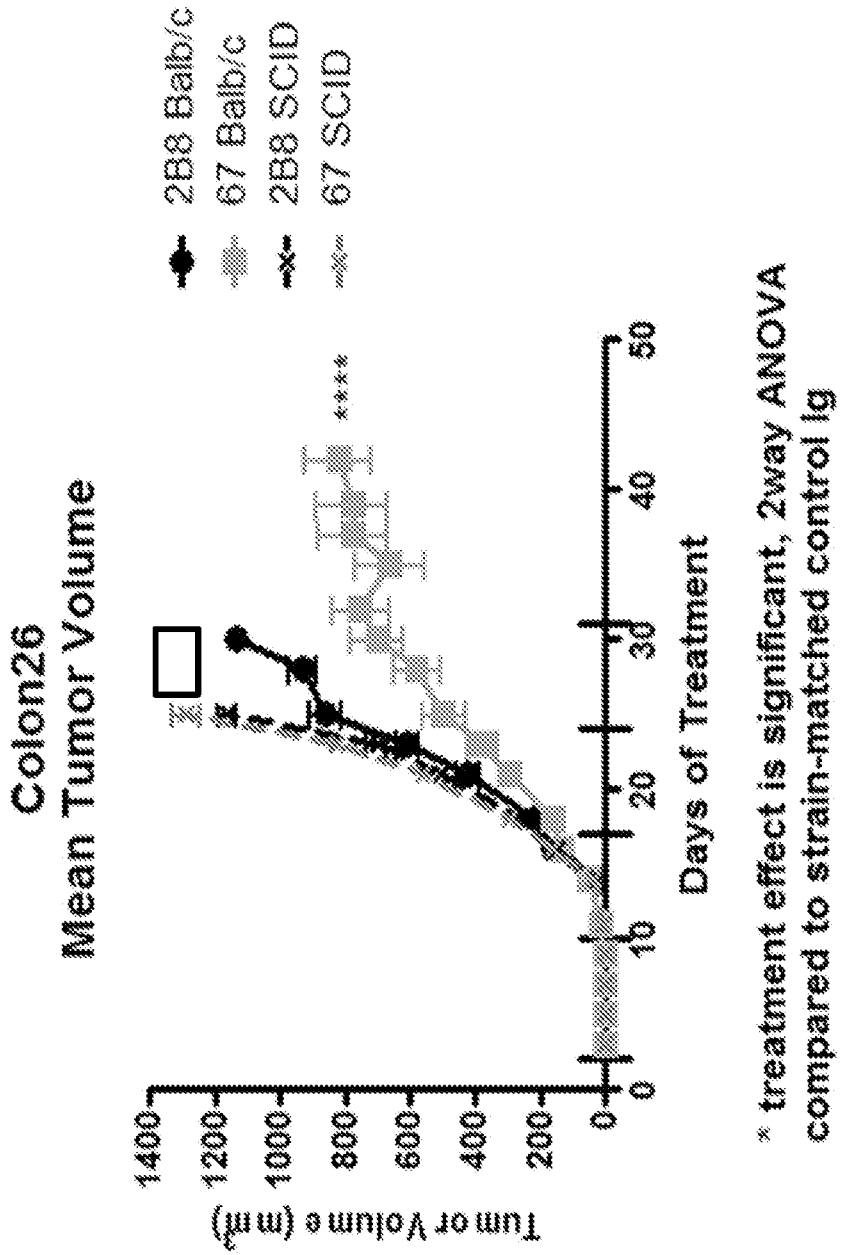

Colon26
Mean Tumor Volume
2B8 Balb/c
67 Balb/c
2B8 SCID
67 SCID
****
Tumor Volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Days of Treatment
* treatment effect is significant, 2way ANOVA
compared to strain-matched control Ig

FIG. 1A

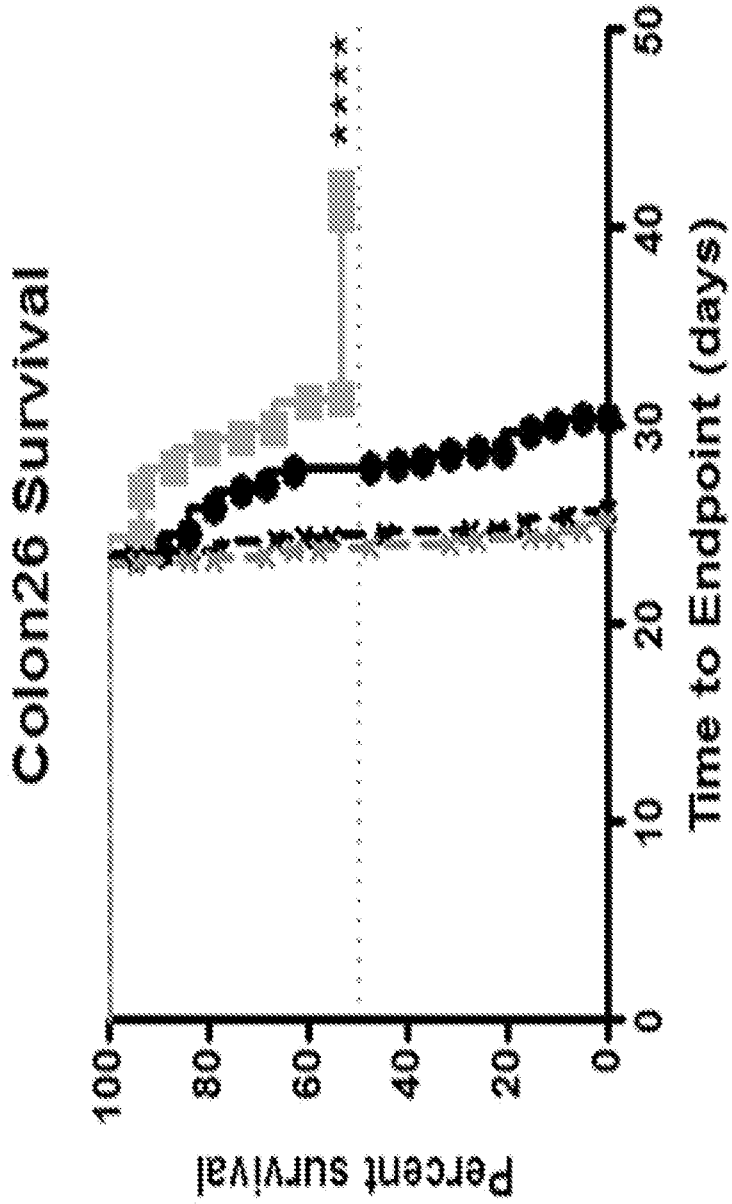

Colon26 Survival
Percent survival
100
80
60
40
20
0
0
10
20
30
40
50
Time to Endpoint (days)
****

FIG. 1B

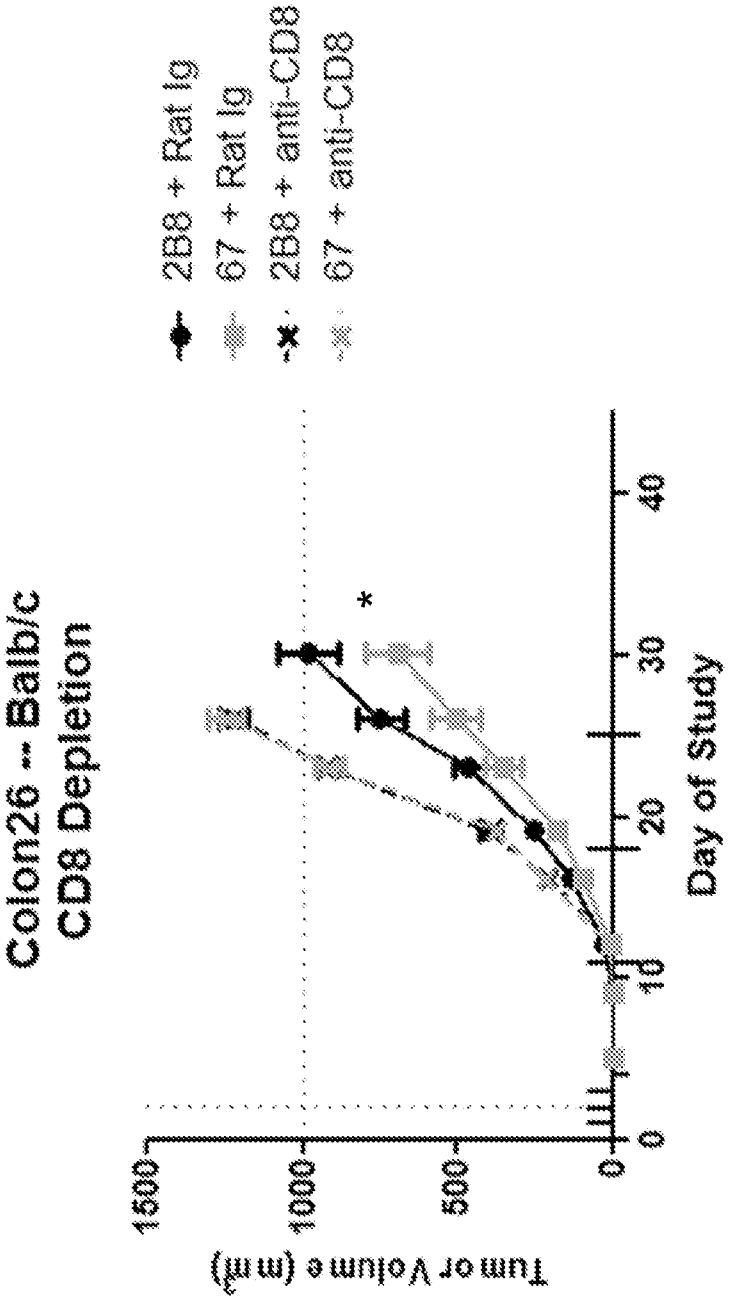

Colon26 -- Balb/c
CD8 Depletion
2B8 + Rat Ig
67 + Rat Ig
2B8 + anti-CD8
67 + anti-CD8
*
Tumor Volume (mm3)
1500
1000
500
0
0
10
20
30
40
Day of Study

FIG. 2

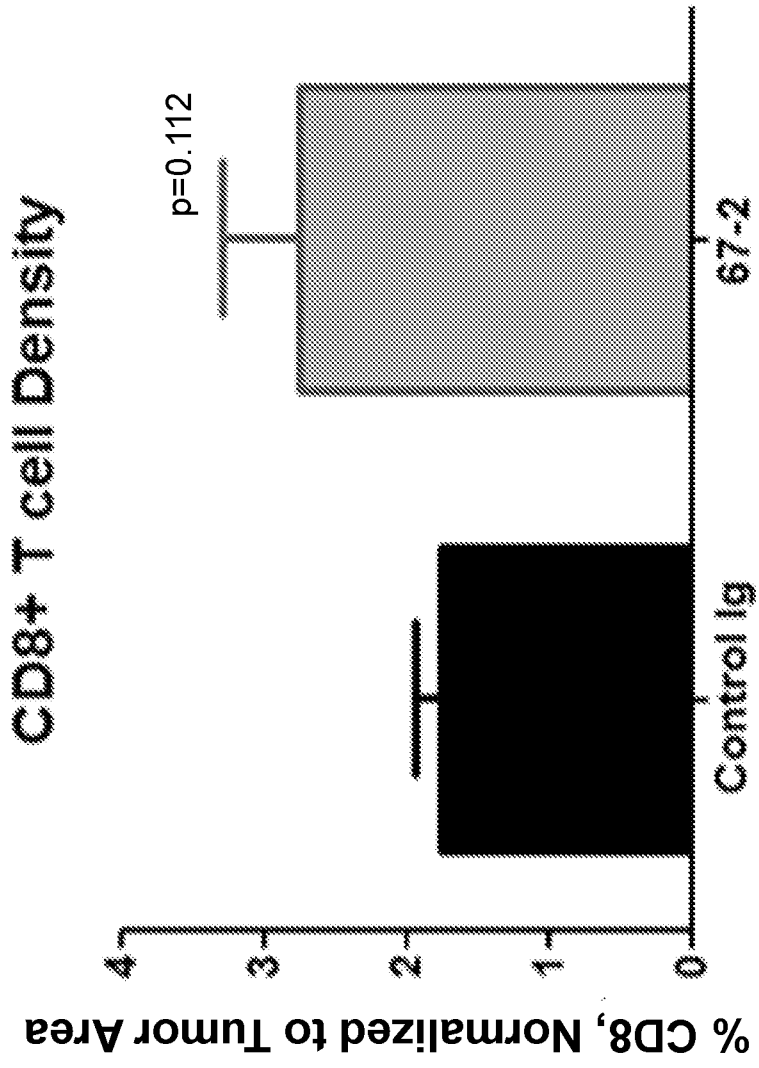

CD8+ T cell Density
p=0.112
67-2
Control Ig
0
1
2
3
4
% CD8, Normalized to Tumor Area

FIG. 3A

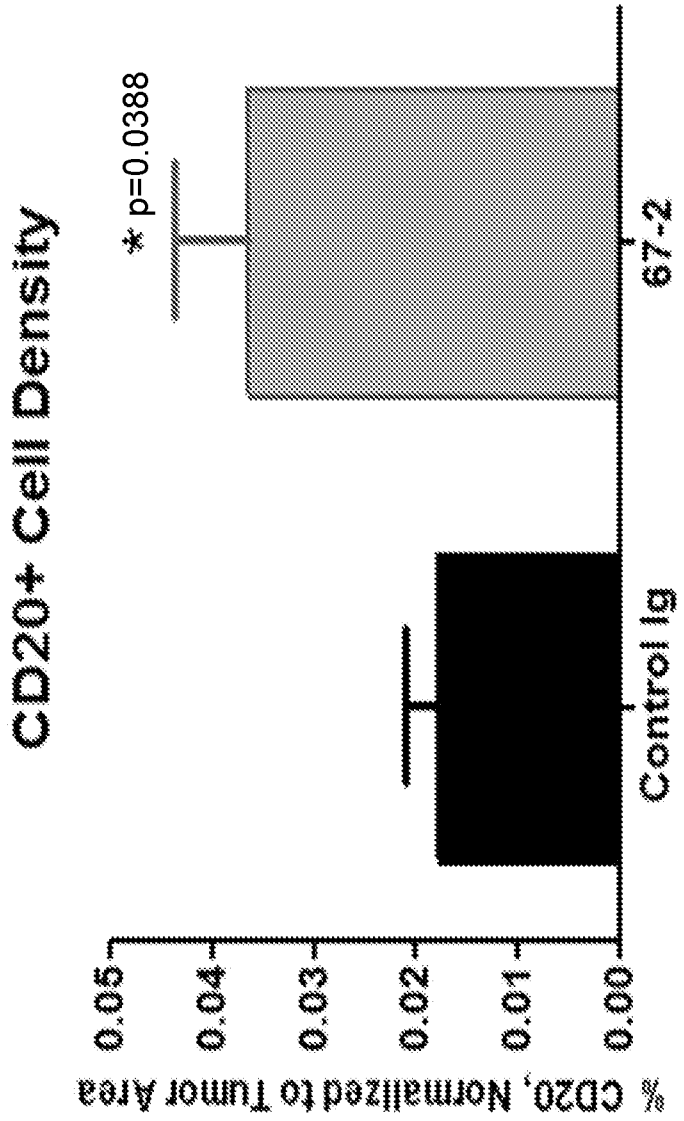

CD20+ Cell Density
* p=0.0388
67-2
Control Ig
0.05
0.04
0.03
0.02
0.01
0.00
% CD20, Normalized to Tumor Area

FIG. 3B

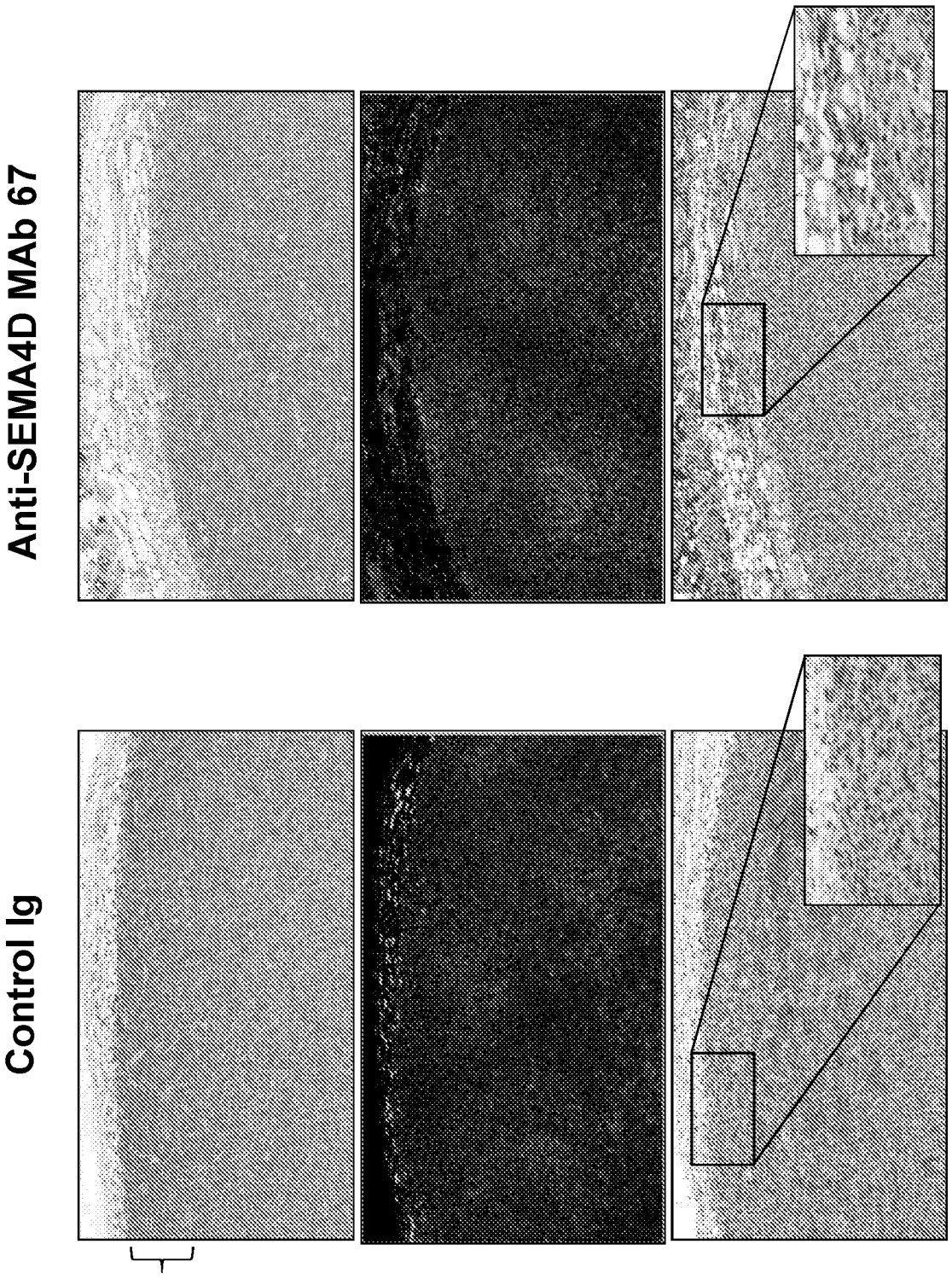


FIG. 4A

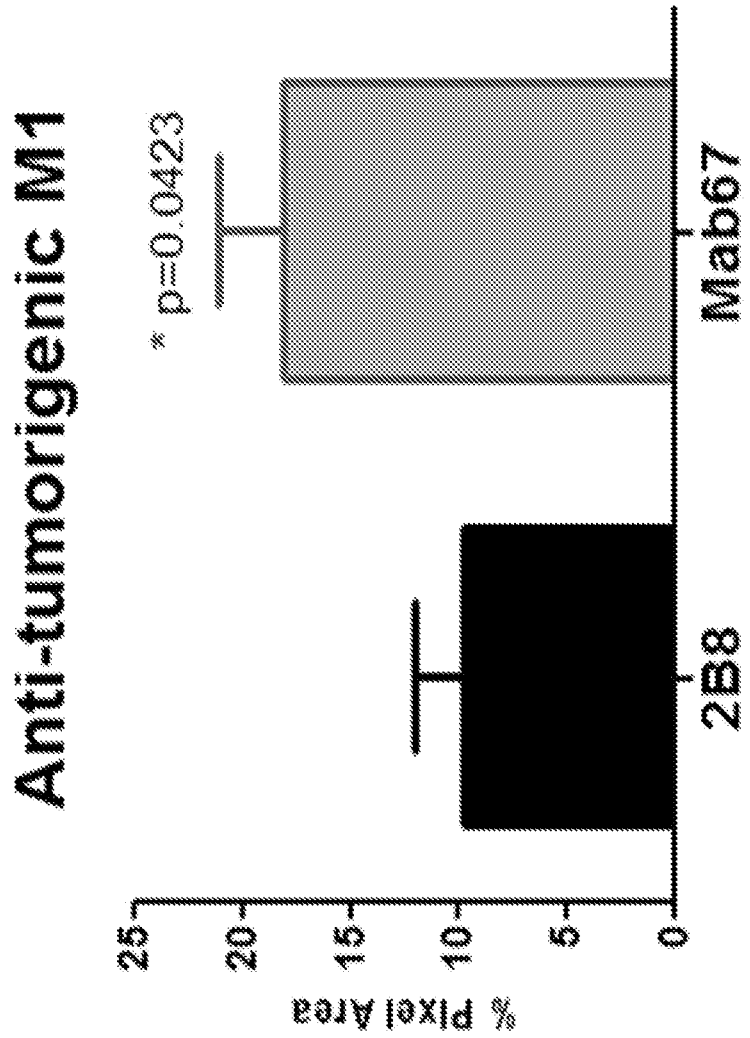

Anti-tumorigenic M1
* p=0.0423
% Pixel Area
25
20
15
10
5
0
2B8
Mab67

FIG. 4B

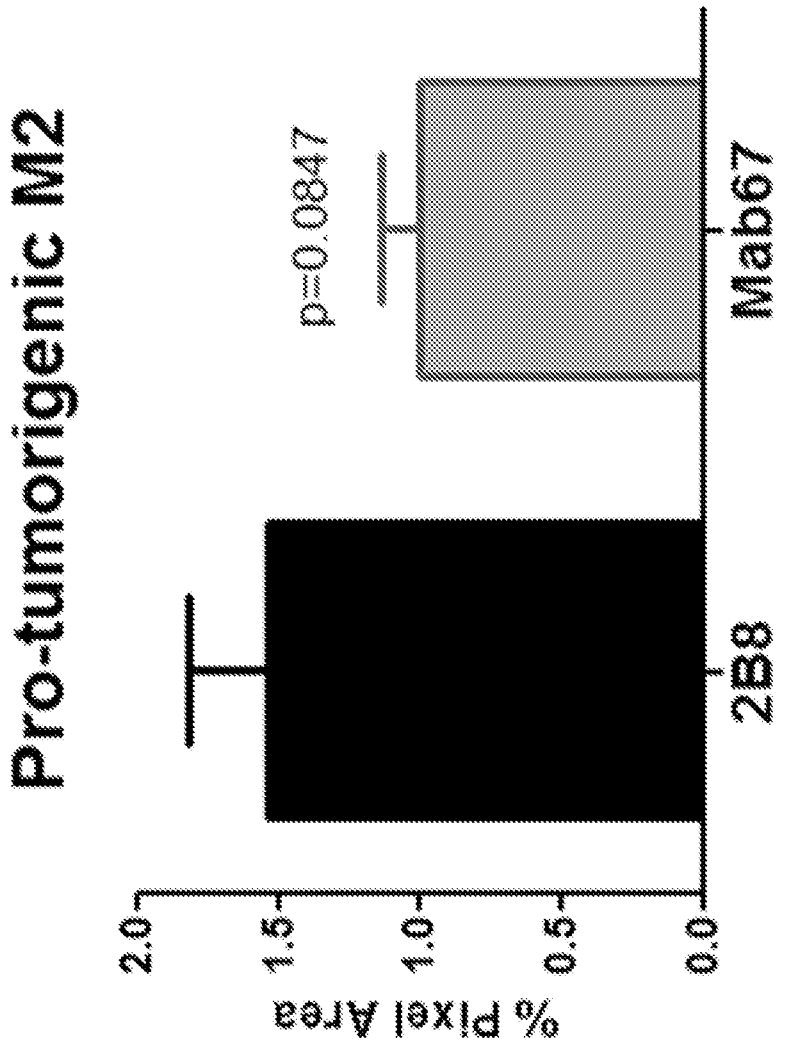


FIG. 4C

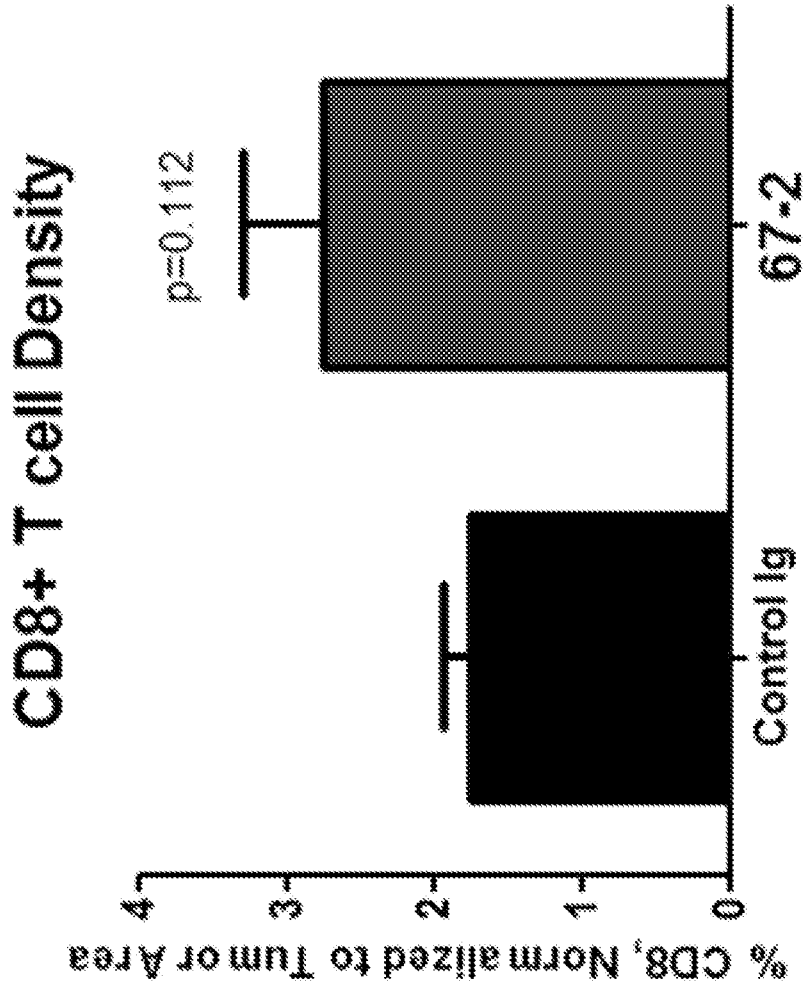

CD8+ T cell Density
p=0.112
67-2
Control Ig
% CD8+ Normalized to Tumor Area
0
1
2
3
4

FIG. 4D

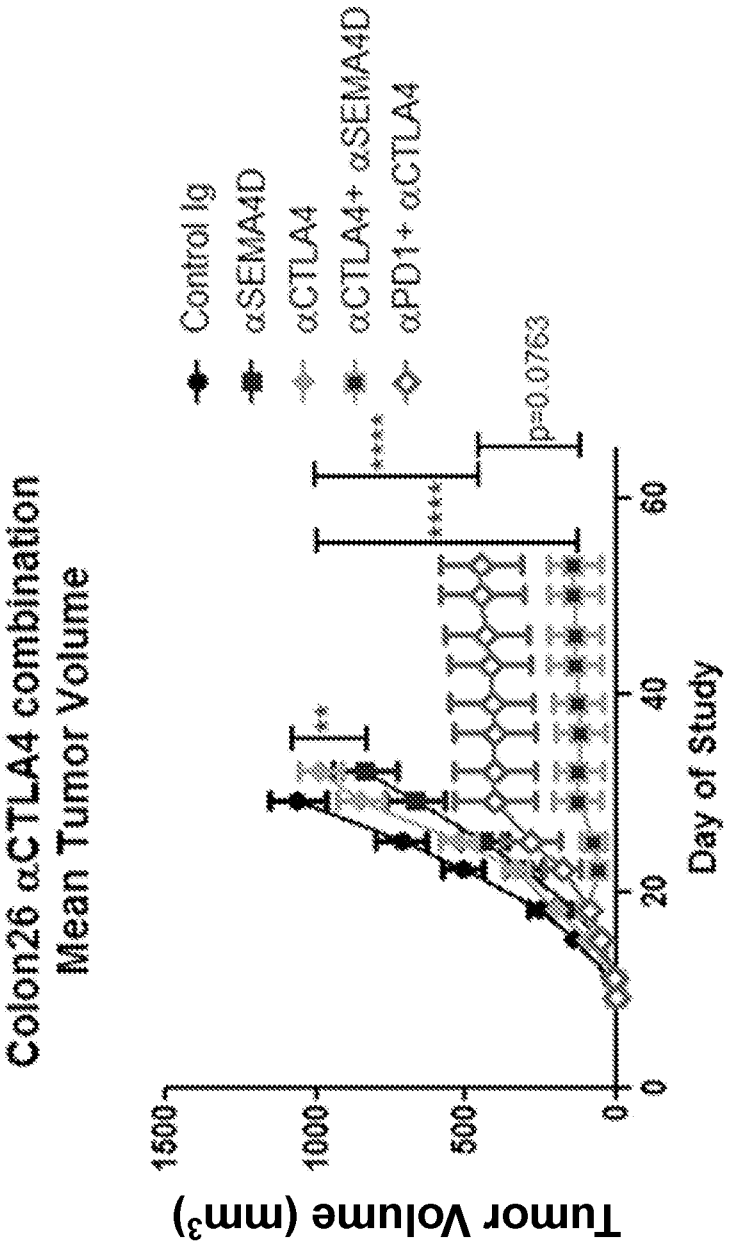

Colon26 αCTLA4 combination
Mean Tumor Volume
Control Ig
αSEMA4D
αCTLA4
αCTLA4+ αSEMA4D
αPD1+ αCTLA4
**
****
****
p=0.0763
Tumor Volume (mm3)
1500
1000
500
0
0
20
40
60
Day of Study

FIG. 5A

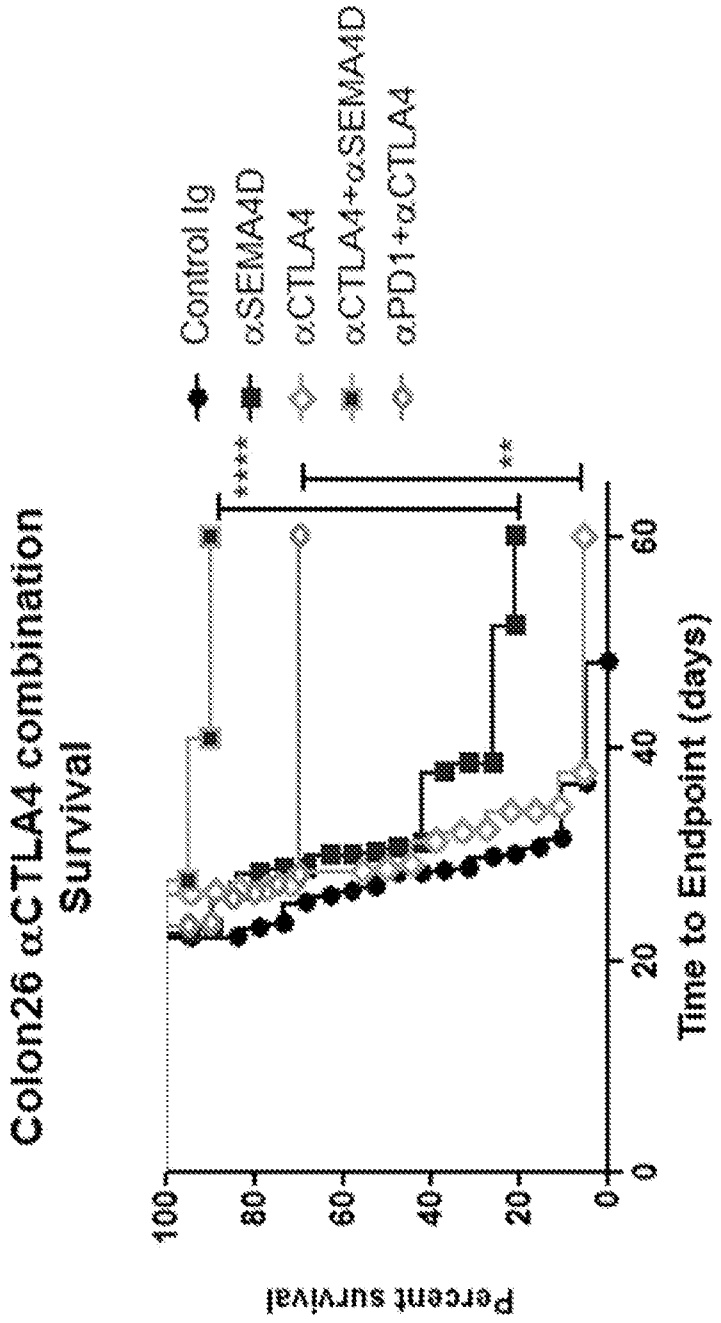

Colon26 αCTLA4 combination
Survival
Control Ig
αSEMA4D
αCTLA4
αCTLA4+αSEMA4D
αPD1+αCTLA4
****
**
Percent survival
100
80
60
40
20
0
0
20
40
60
Time to Endpoint (days)

FIG. 5B

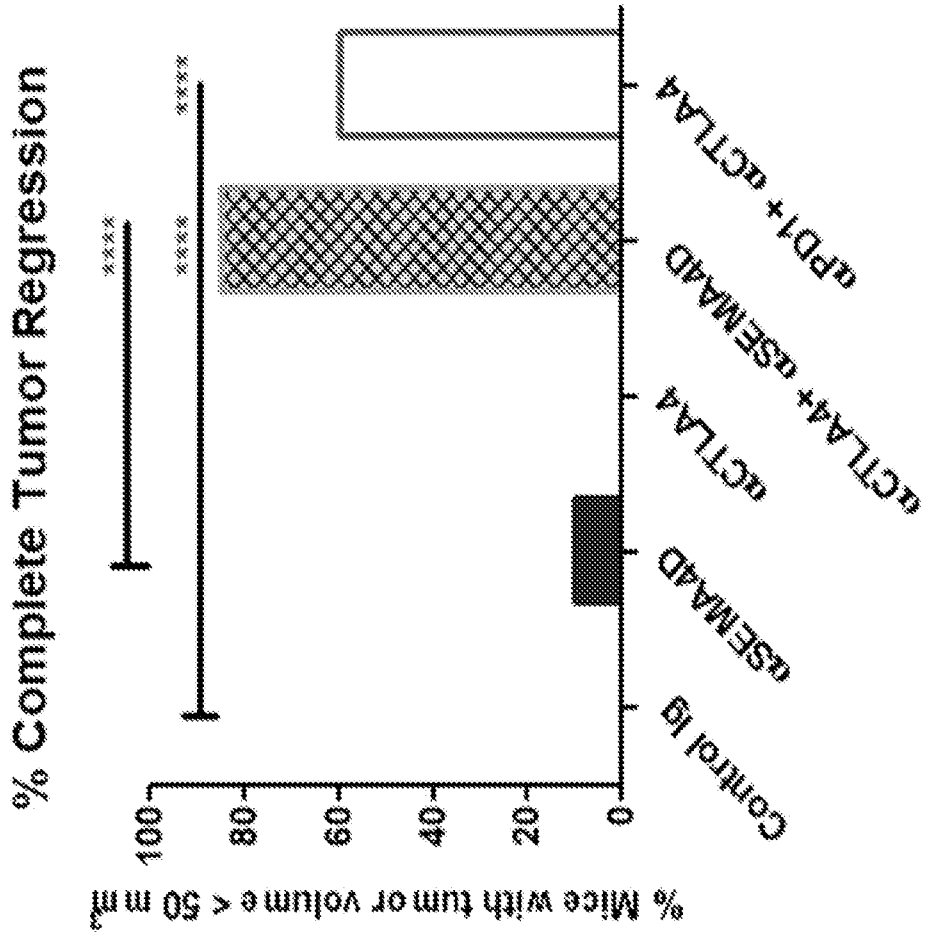

% Complete Tumor Regression
% Mice with tumor volume < 50 mm³
100
80
60
40
20
0
****
****
****
Control Ig
αSEMA4D
αCTLA4
αCTLA4+ αSEMA4D
αPD1+ αCTLA4

FIG. 5C

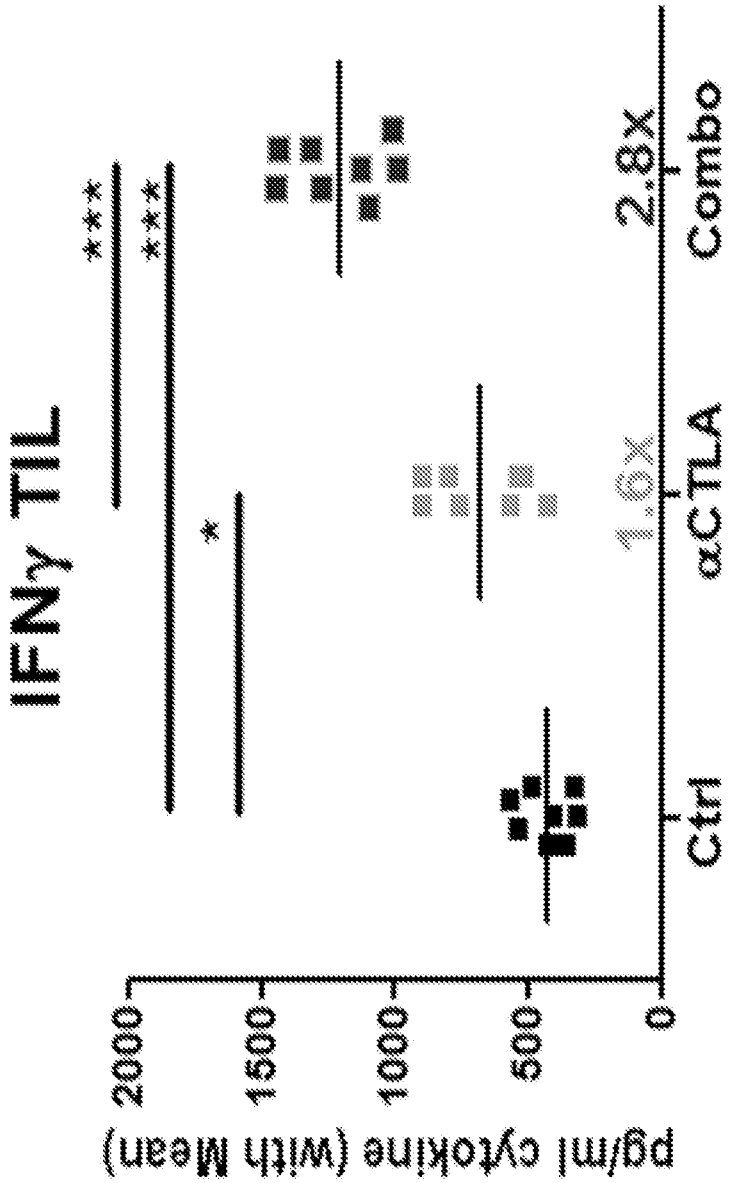

IFNγ TIL
pg/ml cytokine (with Mean)
2000
1500
1000
500
0
Ctrl
αCTLA
Combo
1.6x
2.8x
*
***
***

FIG. 5D

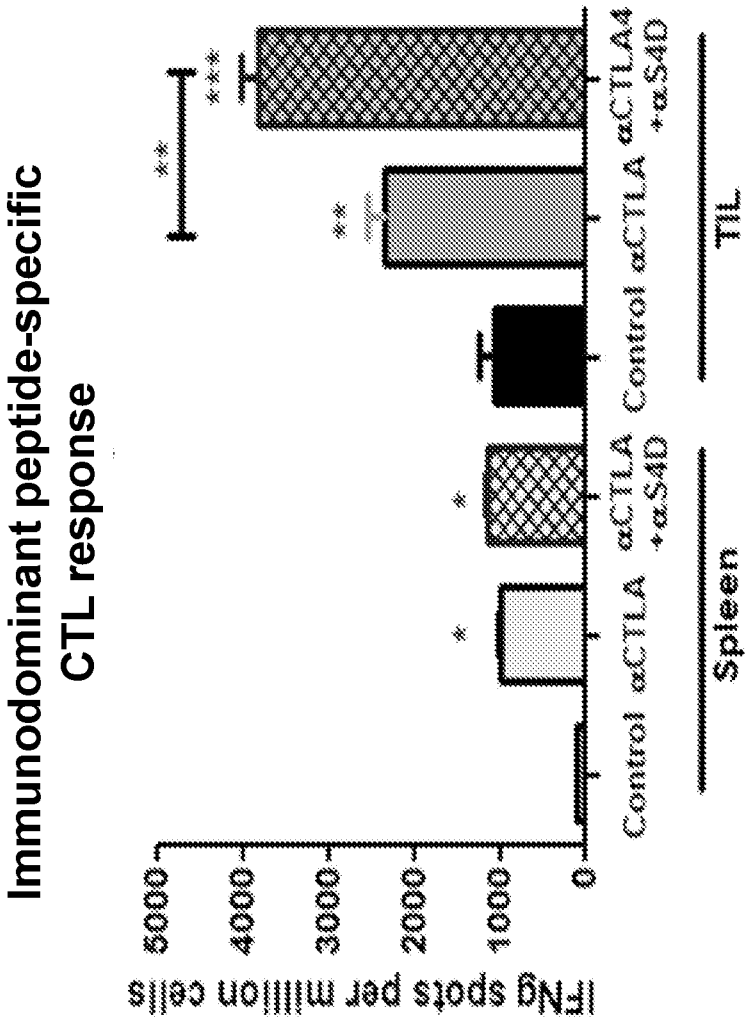

Immunodominant peptide-specific
CTL response
IFNg spots per million cells
5000
4000
3000
2000
1000
0
*
*
**
**
***
Control αCTLA
αCTLA +αS4D
Control αCTLA
αCTLA4 +αS4D
Spleen
TIL

FIG. 5E

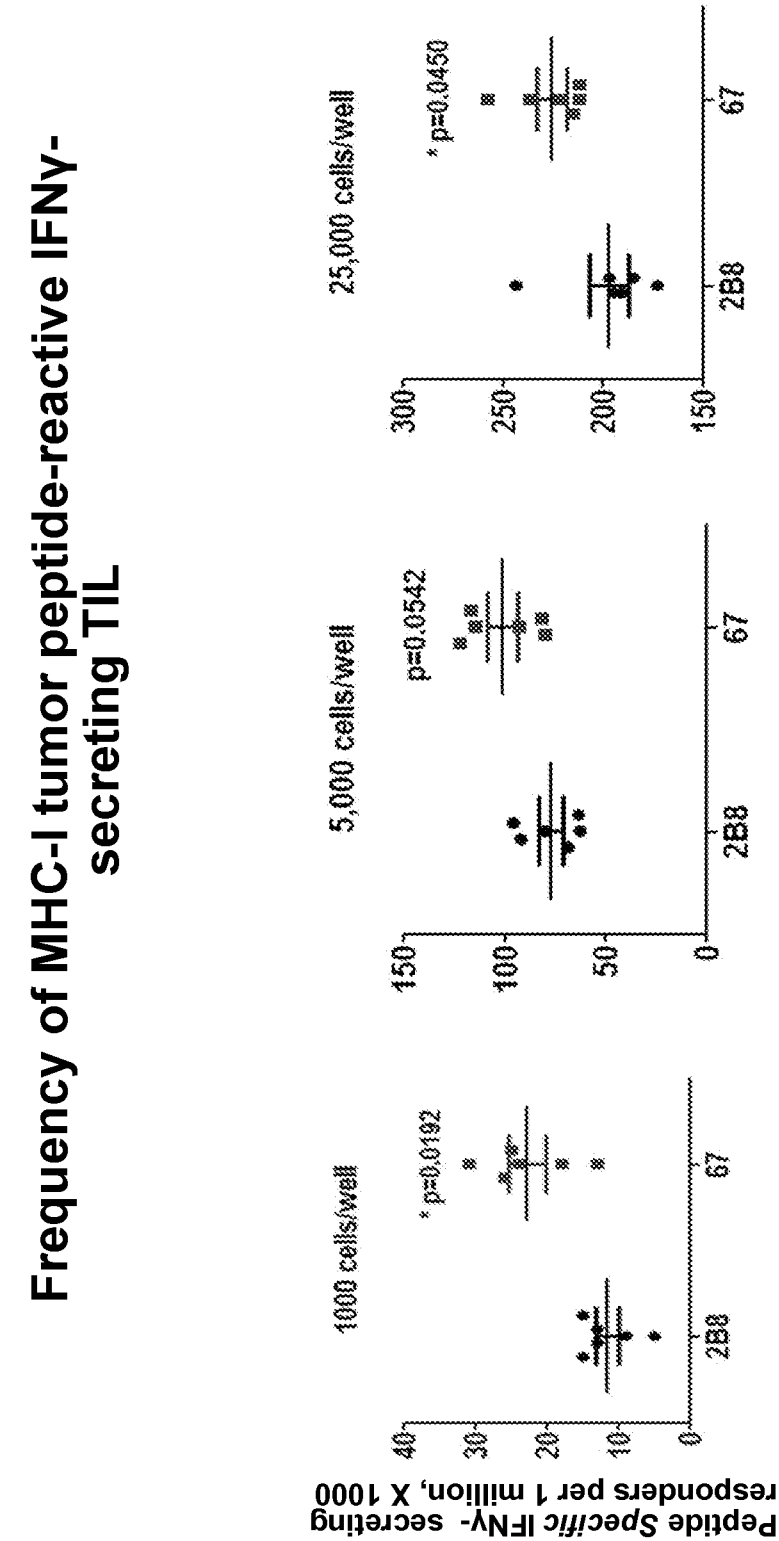

Frequency of MHC-I tumor peptide-reactive IFNγ-secreting TIL
Peptide Specific IFNγ- secreting responders per 1 million, X 1000
1000 cells/well
5,000 cells/well
25,000 cells/well
* p=0.0192
p=0.0542
* p=0.0450
2B8
67

FIG. 6A

Frequency of MHC-I tumor peptide-reactive IFNγ-secreting TIL

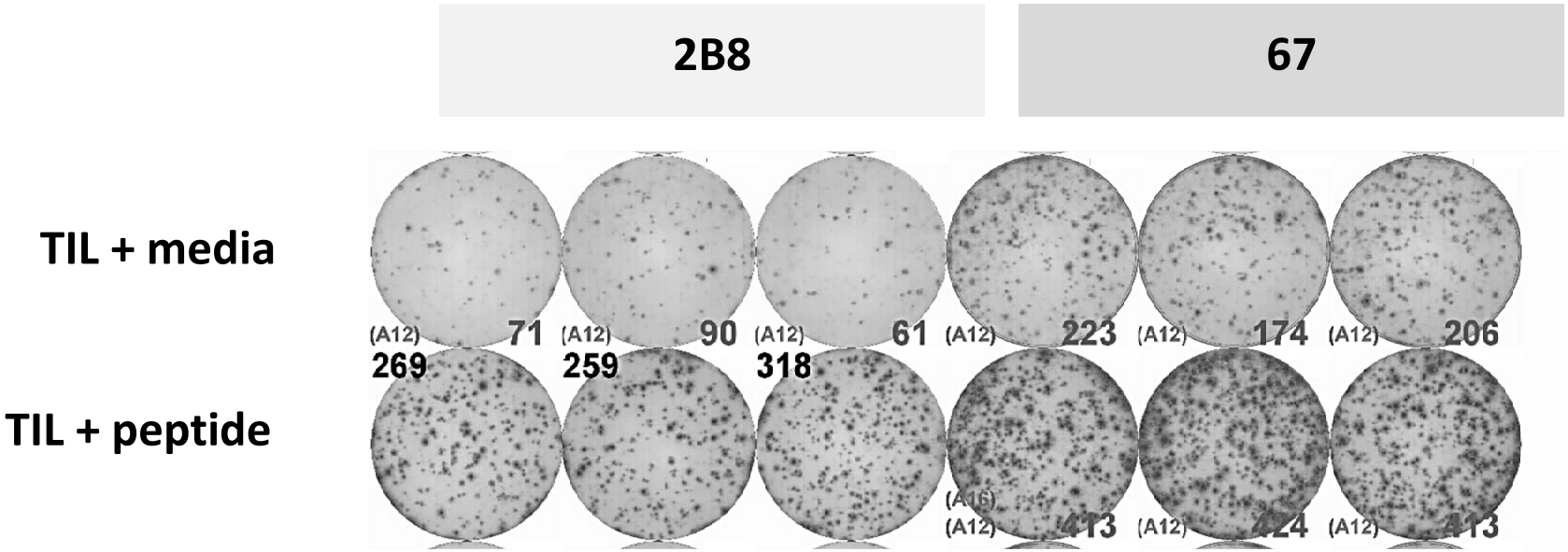


FIG. 6B

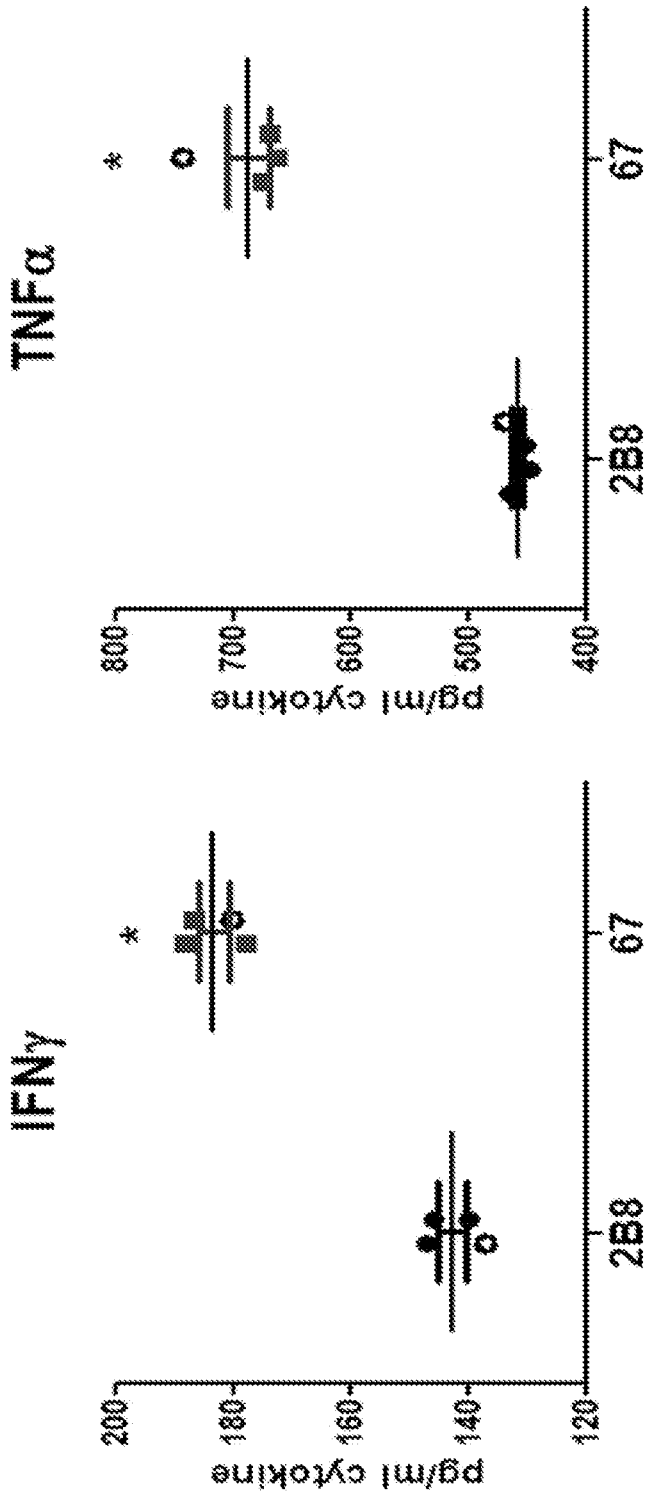

TNFα
IFNγ
pg/ml cytokine
800
700
600
500
400
200
180
160
140
120
2B8
67
*

FIG. 6C

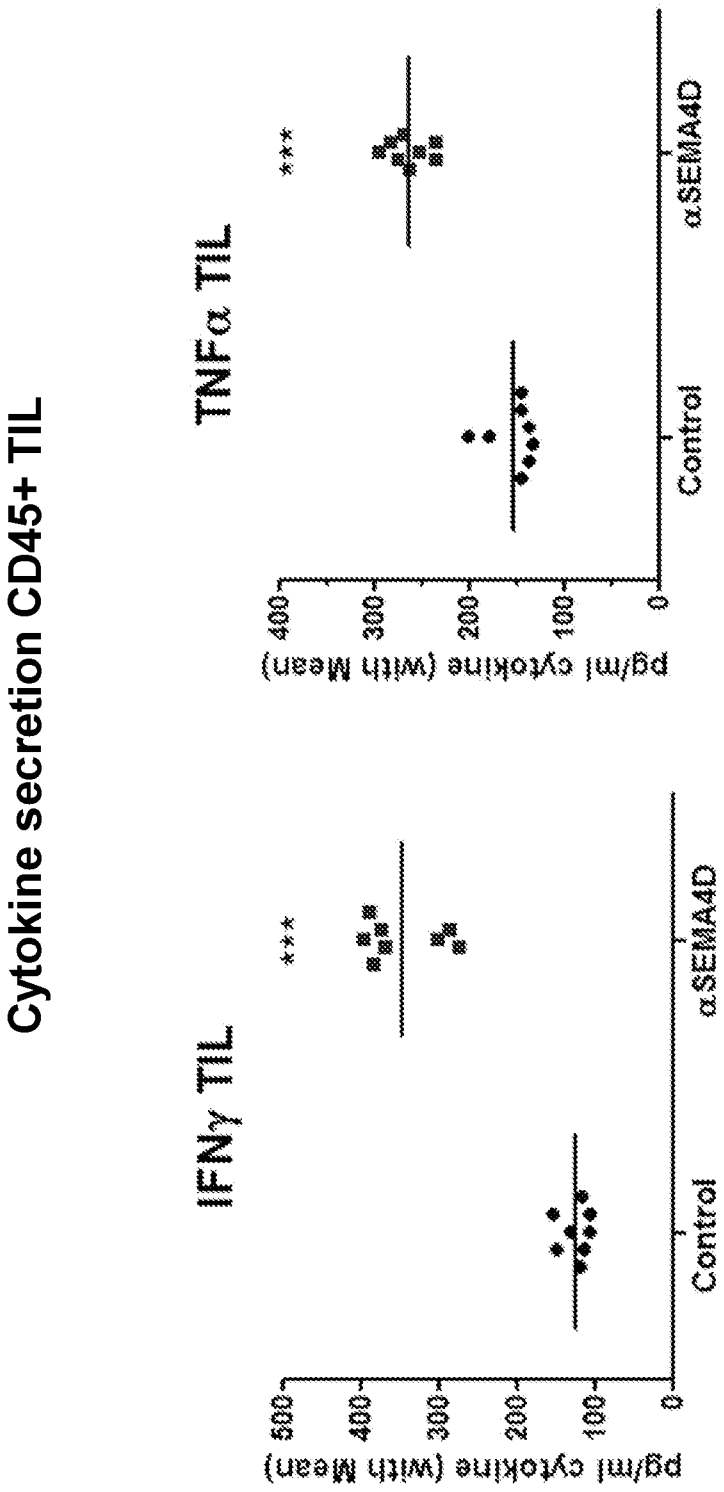

Cytokine secretion CD45+ TIL
IFNγ TIL
TNFα TIL
pg/ml cytokine (with Mean)
500
400
300
200
100
0
***
***
Control
αSEMA4D
Control
αSEMA4D

FIG. 6D

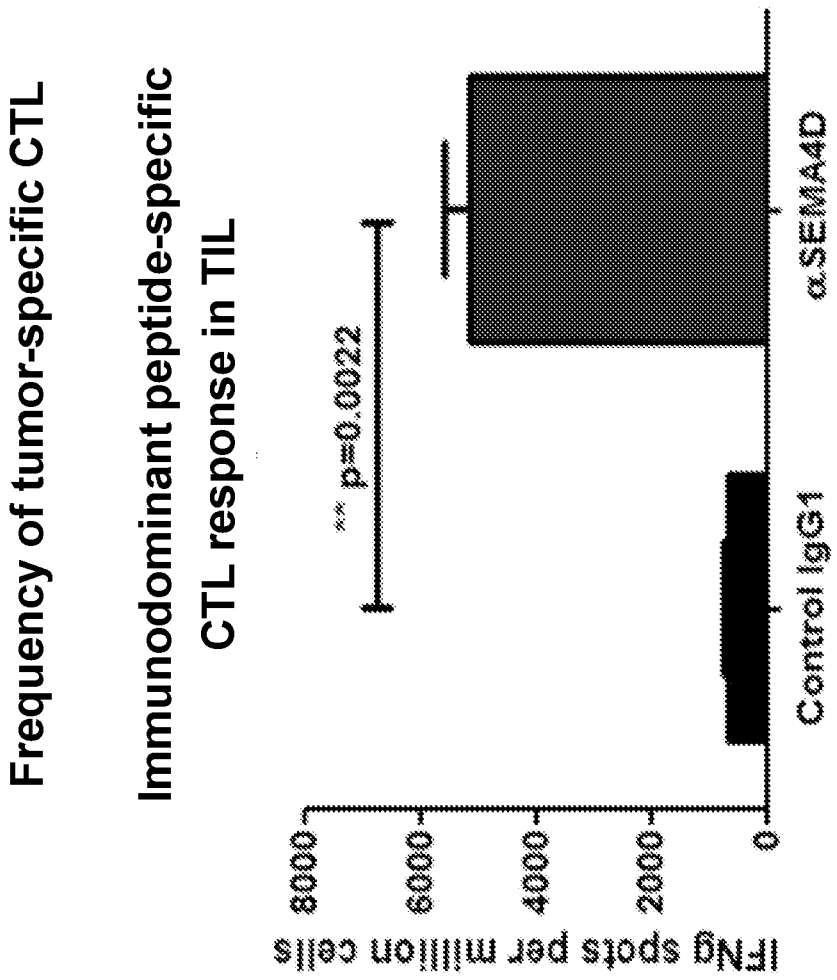

Frequency of tumor-specific CTL
Immunodominant peptide-specific
CTL response in TIL
IFNg spots per million cells
8000
6000
4000
2000
0
** p=0.0022
Control IgG1
αSEMA4D

FIG. 6E

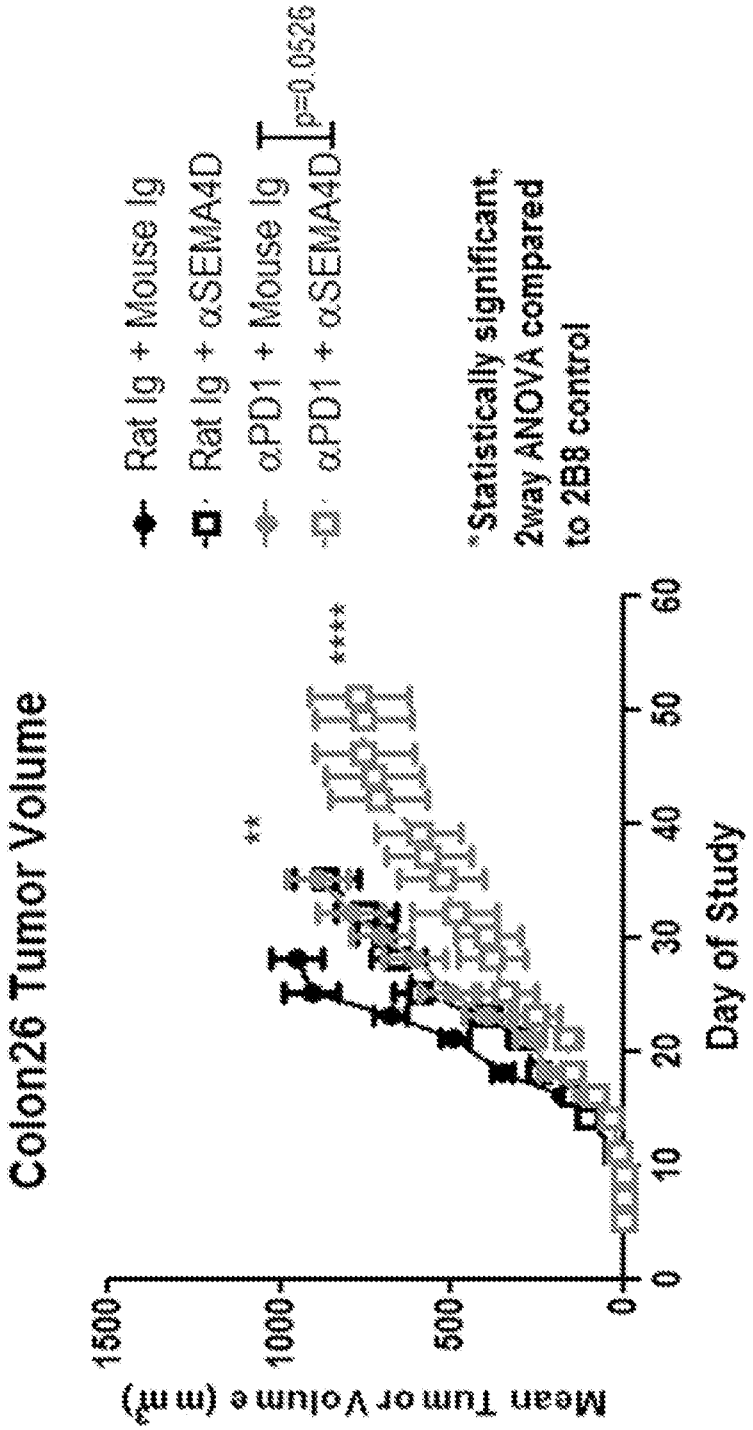

Colon26 Tumor Volume
Rat Ig + Mouse Ig
Rat Ig + αSEMA4D
αPD1 + Mouse Ig
αPD1 + αSEMA4D
p=0.0526
*Statistically significant, 2way ANOVA compared to 2B8 control
**
****
Mean Tumor Volume (mm³)
1500
1000
500
0
0
10
20
30
40
50
60
Day of Study

FIG. 7A

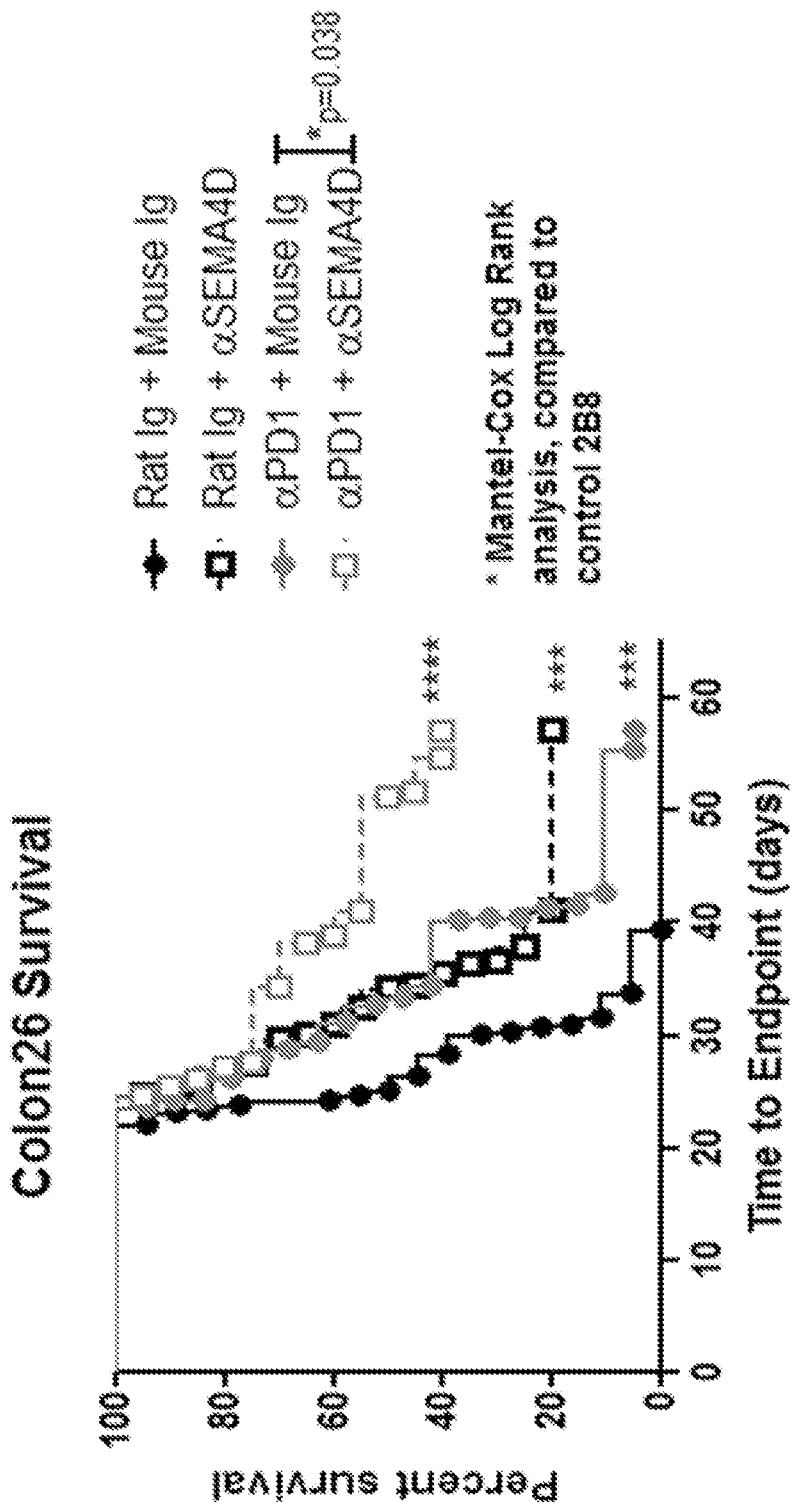

Colon26 Survival
Rat Ig + Mouse Ig
Rat Ig + αSEMA4D
αPD1 + Mouse Ig
αPD1 + αSEMA4D
*p=0.038
* Mantel-Cox Log Rank analysis, compared to control 2B8
Percent survival
Time to Endpoint (days)
0
20
40
60
80
100
0
10
20
30
40
50
60
***
***
****

FIG. 7B

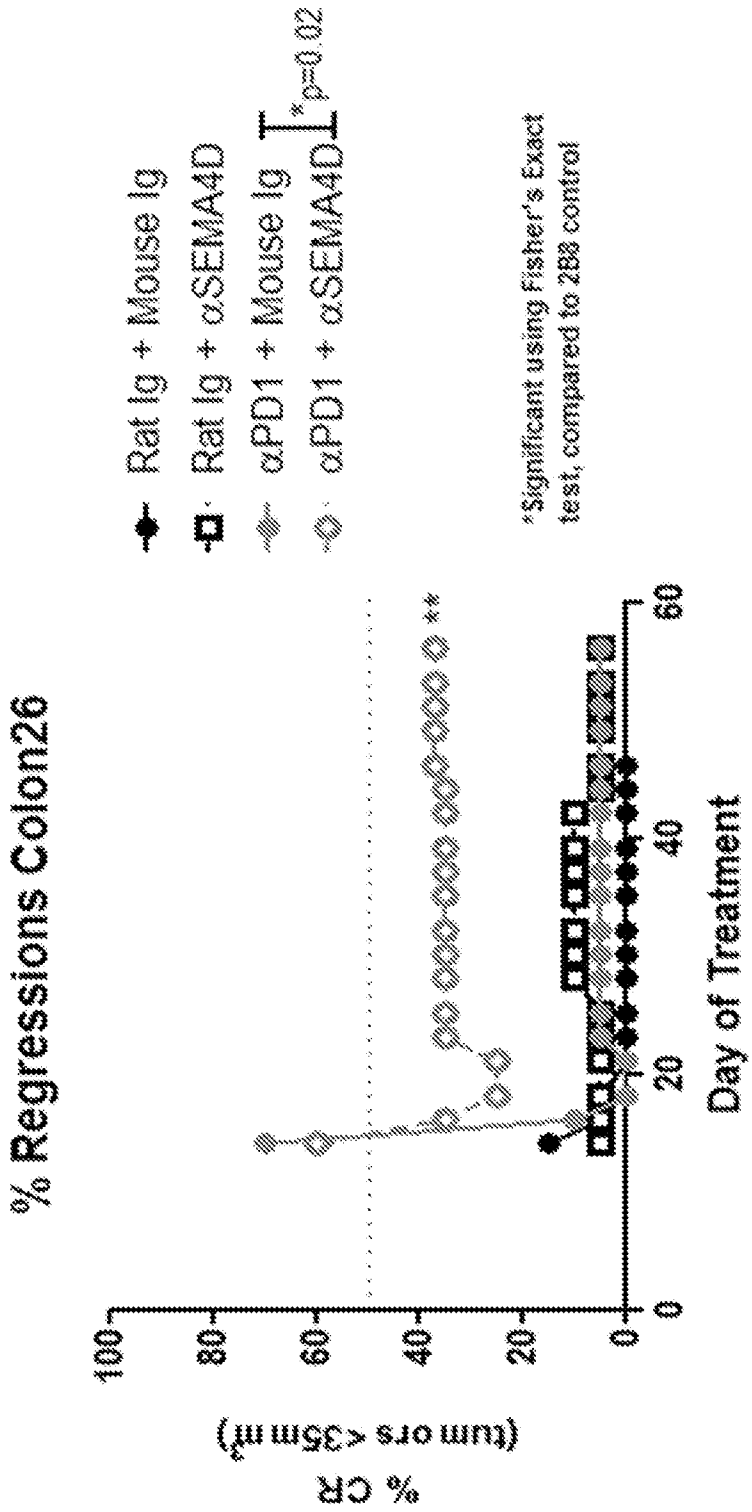

% Regressions Colon26
Rat Ig + Mouse Ig
Rat Ig + αSEMA4D
αPD1 + Mouse Ig
αPD1 + αSEMA4D
*p=0.02
*Significant using Fisher's Exact test, compared to 2B8 control
% CR
(tumors <35m m²)
100
80
60
40
20
0
0
20
40
60
Day of Treatment

FIG. 7C

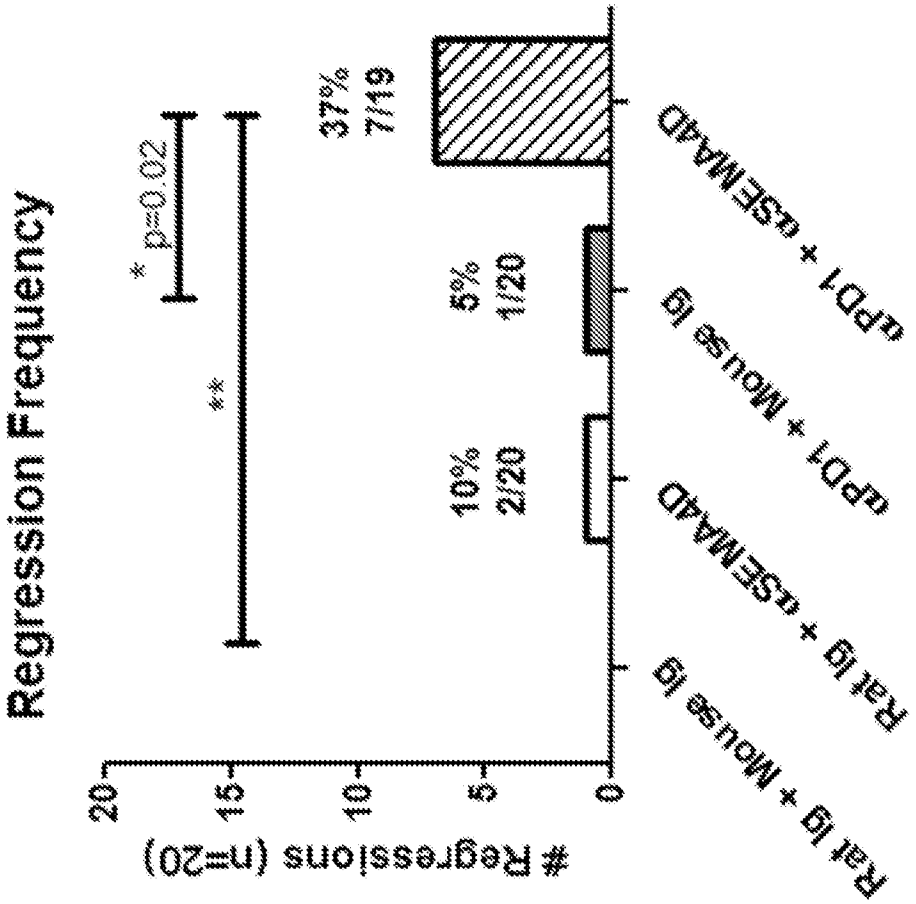

Regression Frequency
Regressions (n=20)
0
5
10
15
20
* p=0.02
**
37%
7/19
5%
1/20
10%
2/20
Rat Ig + Mouse Ig
Rat Ig + αSEMA4D
αPD1 + Mouse Ig
αPD1 + αSEMA4D

FIG. 7D

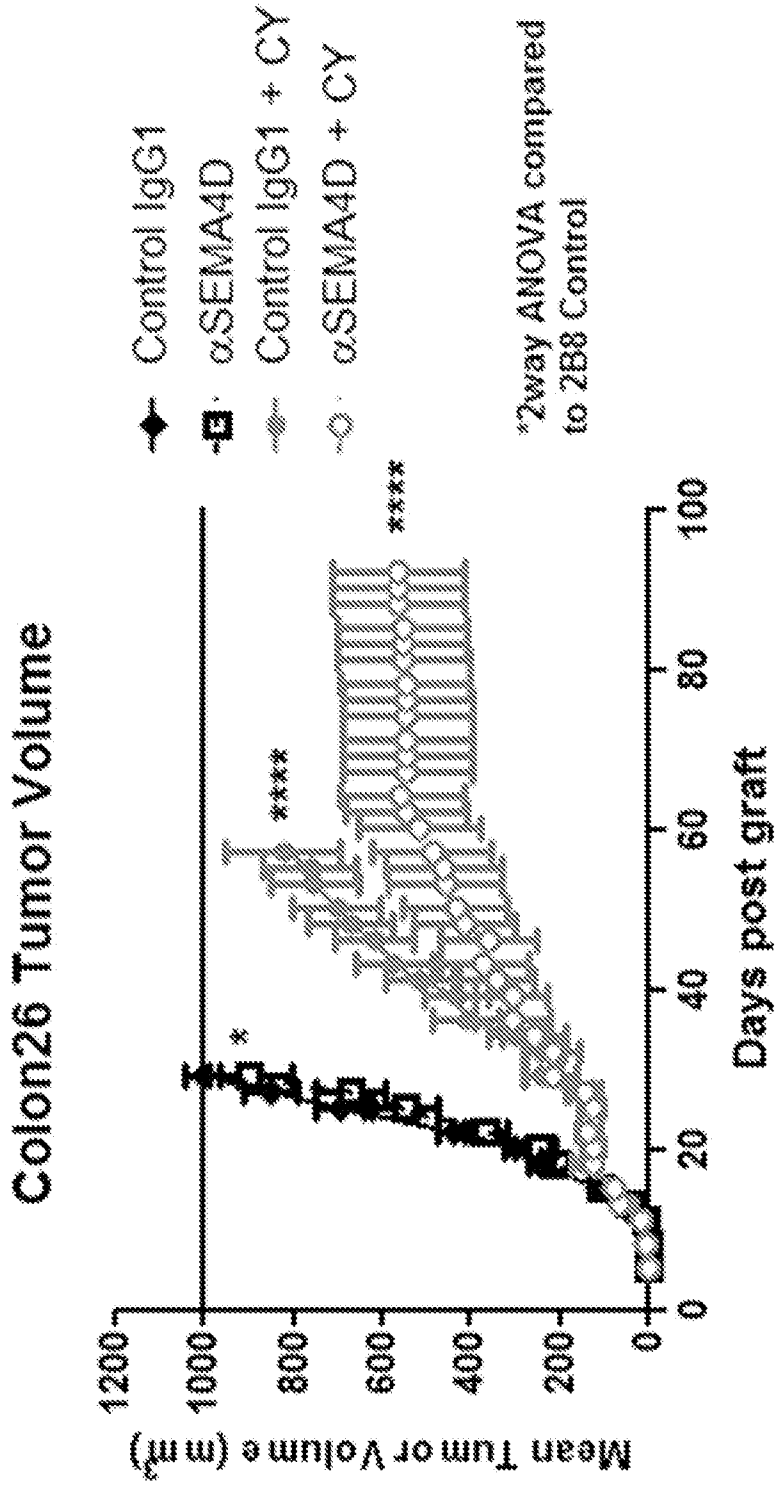

Colon26 Tumor Volume
Control IgG1
αSEMA4D
Control IgG1 + CY
αSEMA4D + CY
*2way ANOVA compared to 2B8 Control
****
****
*
Mean Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
20
40
60
80
100
Days post graft

FIG. 8A

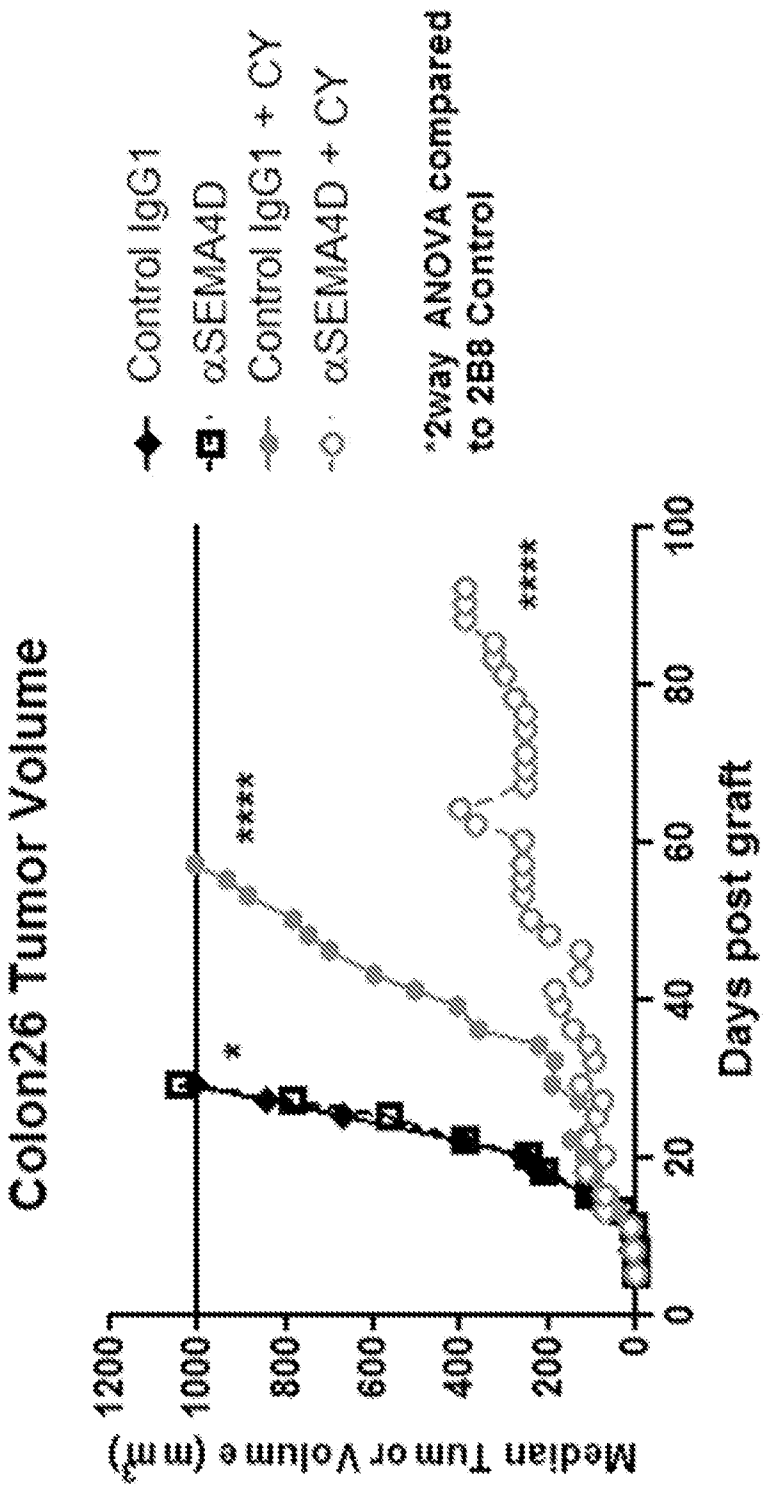

Colon26 Tumor Volume
Control IgG1
αSEMA4D
Control IgG1 + CY
αSEMA4D + CY
*2way ANOVA compared to 2B8 Control
****
*
****
Median Tumor Volume (mm)
1200
1000
800
600
400
200
0
0
20
40
60
80
100
Days post graft

FIG. 8B

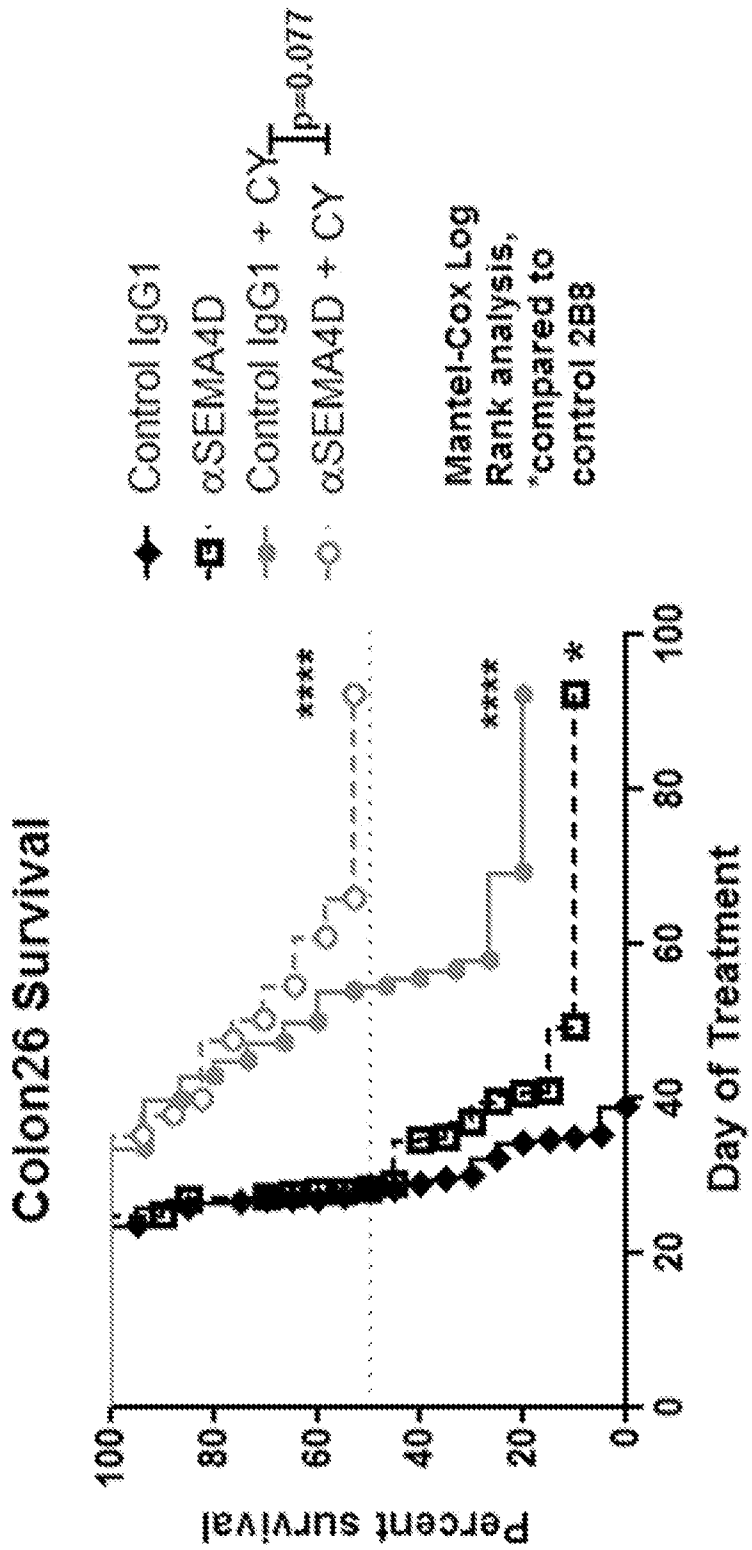

Colon26 Survival
Percent survival
Day of Treatment
Control IgG1
αSEMA4D
Control IgG1 + CY
αSEMA4D + CY
p=0.077
Mantel-Cox Log Rank analysis, *compared to control 2B8
****
****
*
0
20
40
60
80
100

FIG. 8C

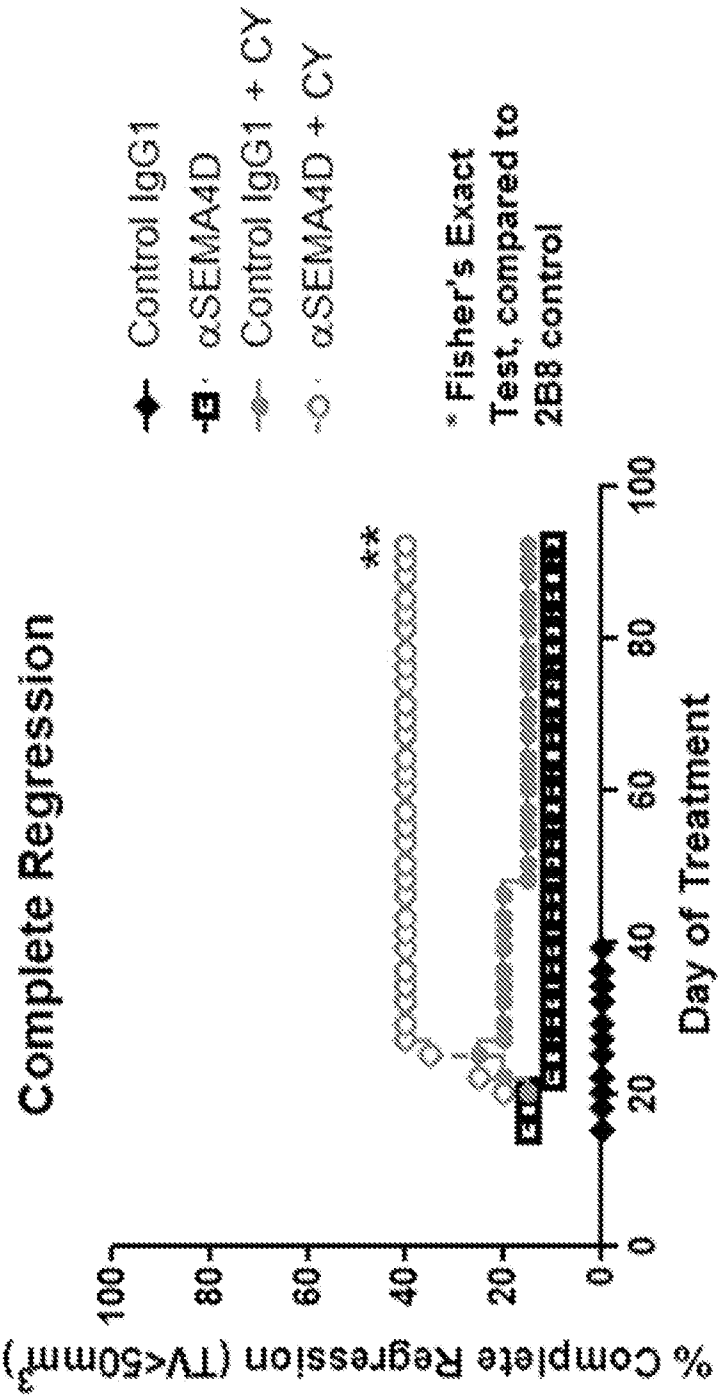

Complete Regression
Control IgG1
αSEMA4D
Control IgG1 + CY
αSEMA4D + CY
* Fisher's Exact Test, compared to 2B8 control
**
% Complete Regression (TV<50mm³)
0
20
40
60
80
100
Day of Treatment
0
20
40
60
80
100

FIG. 8D

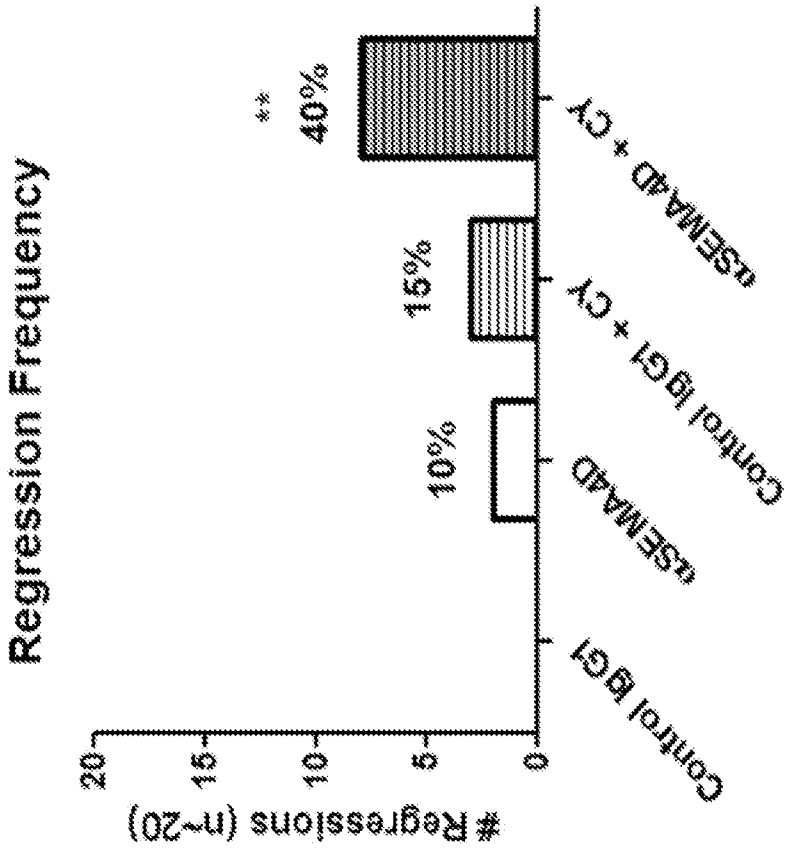

Regression Frequency
Regressions (n~20)
20
15
10
5
0
**
40%
15%
10%
αSEMA4D + CY
Control IgG1 + CY
αSEMA4D
Control IgG1

FIG. 8E

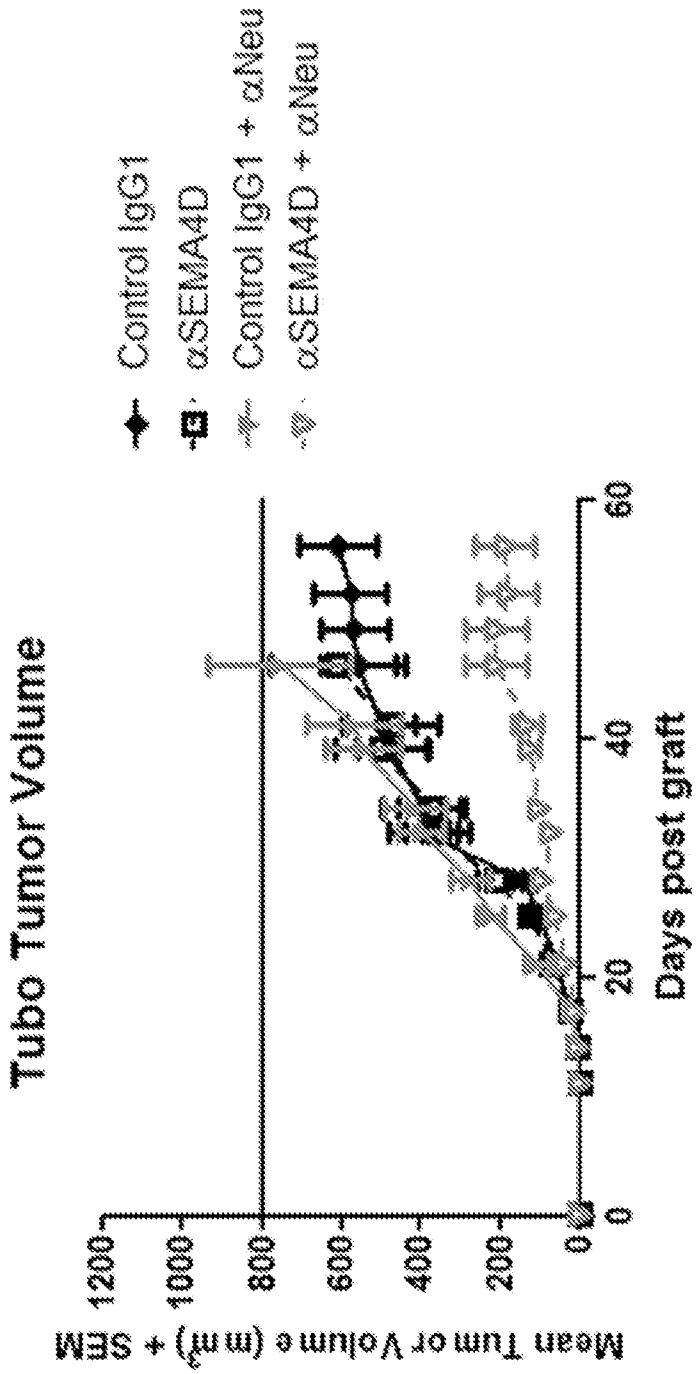

Tubo Tumor Volume
Control IgG1
αSEMA4D
Control IgG1 + αNeu
αSEMA4D + αNeu
Mean Tumor Volume (mm³) + SEM
1200
1000
800
600
400
200
0
0
20
40
60
Days post graft

FIG. 9A

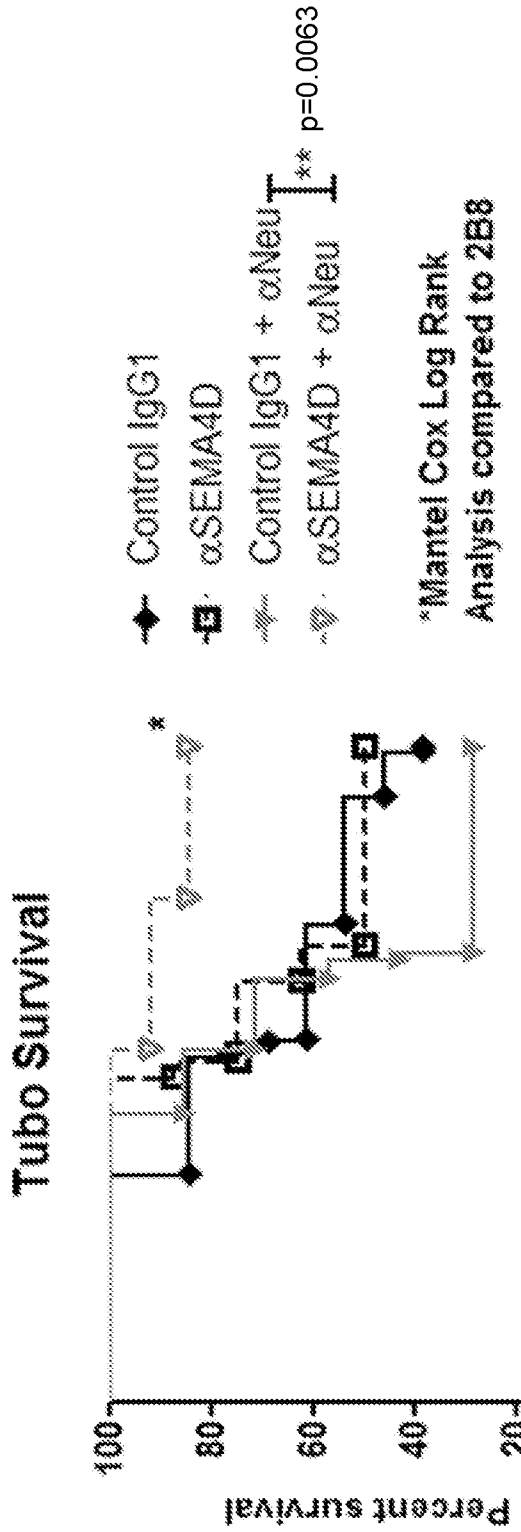

Tubo Survival
Percent survival
TTE (days)
0
10
20
30
40
50
60
20
40
60
80
100
*
Control IgG1
αSEMA4D
Control IgG1 + αNeu
αSEMA4D + αNeu
** p=0.0063
*Mantel Cox Log Rank
Analysis compared to 2B8

FIG. 9B

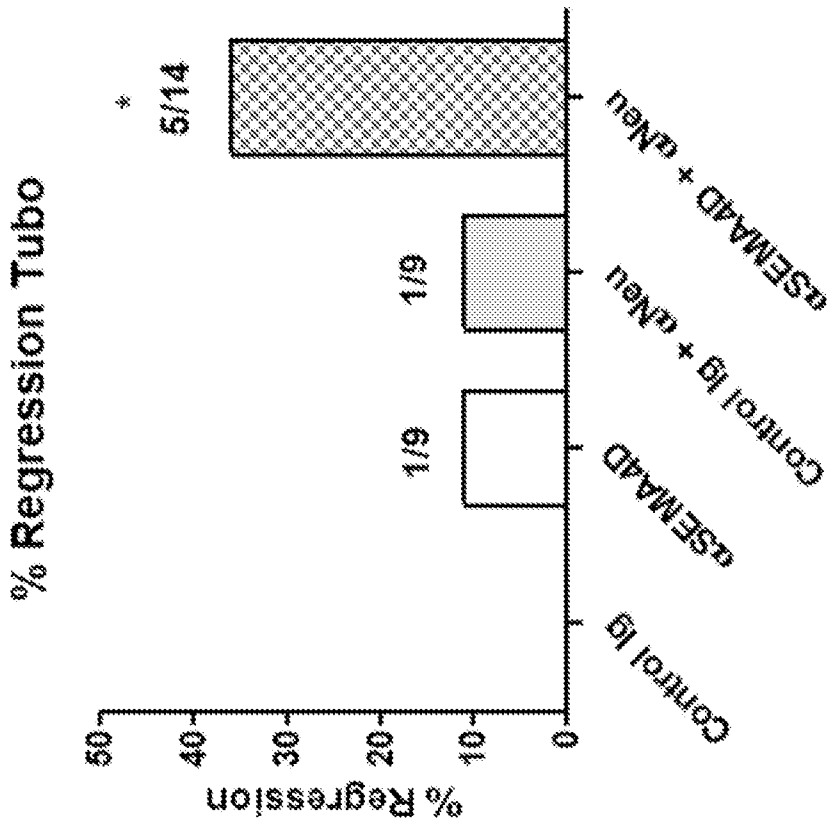

% Regression Tubo
% Regression
50
40
30
20
10
0
Control Ig
αSEMA4D
Control Ig + αNeu
αSEMA4D + αNeu
1/9
1/9
5/14
*

FIG. 9C

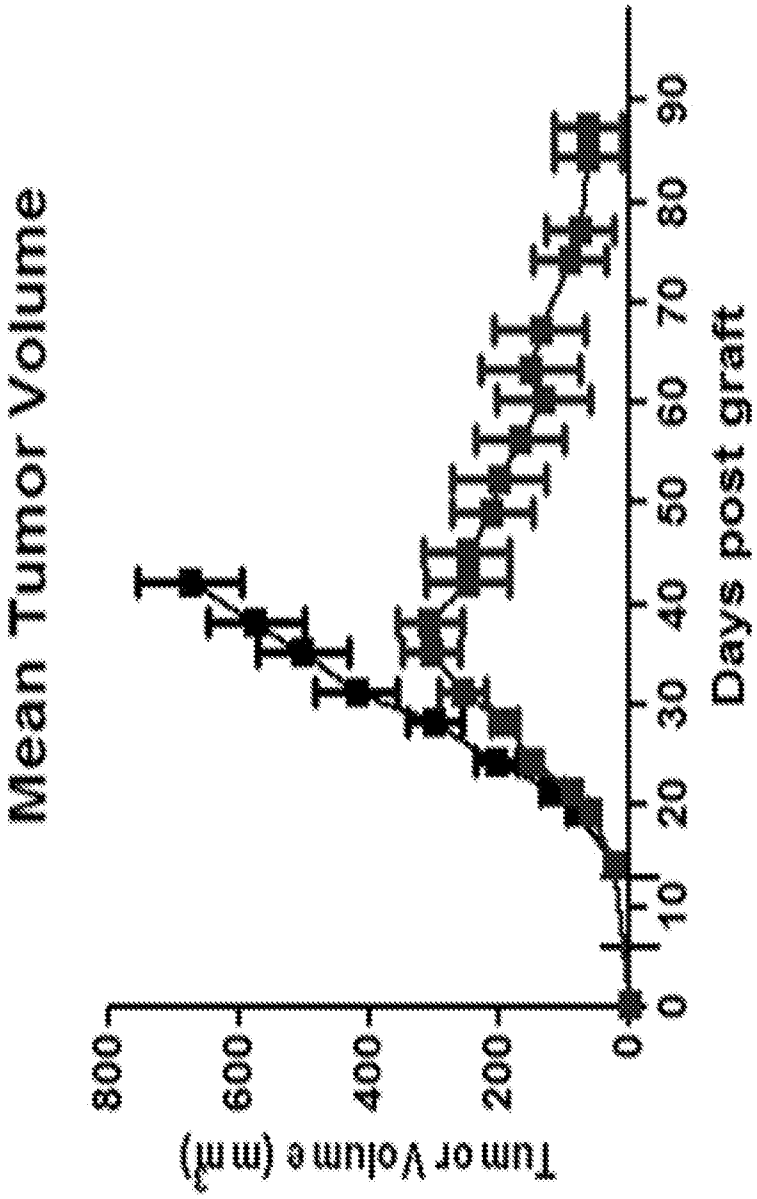

Mean Tumor Volume
Tumor Volume (mm³)
800
600
400
200
0
0
10
20
30
40
50
60
70
80
90
Days post graft

FIG. 10A

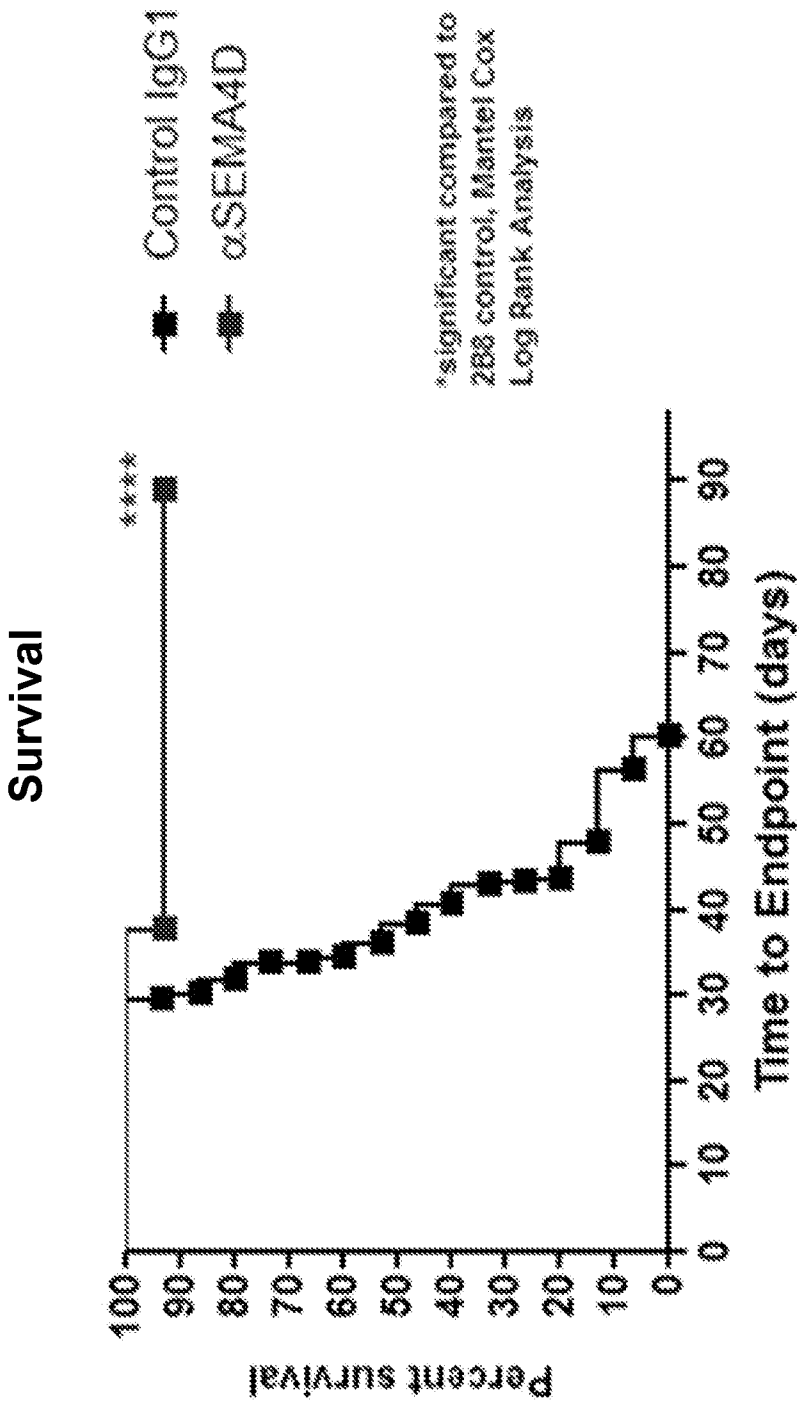

Survival
Control IgG1
αSEMA4D
*significant compared to 2B8 control, Mantel Cox Log Rank Analysis
****
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
Time to Endpoint (days)

FIG. 10B

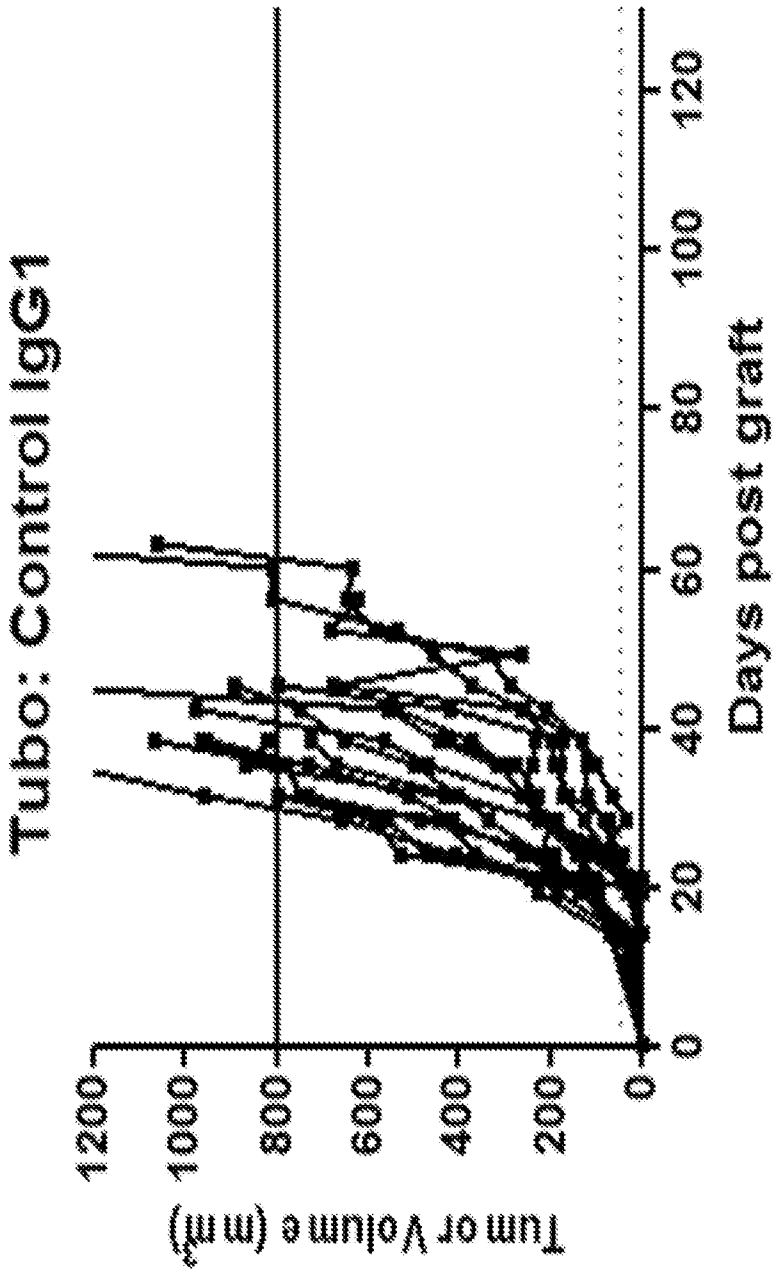

Tubo: Control IgG1
Tum or Volum e (mm³)
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
Days post graft

FIG. 10C

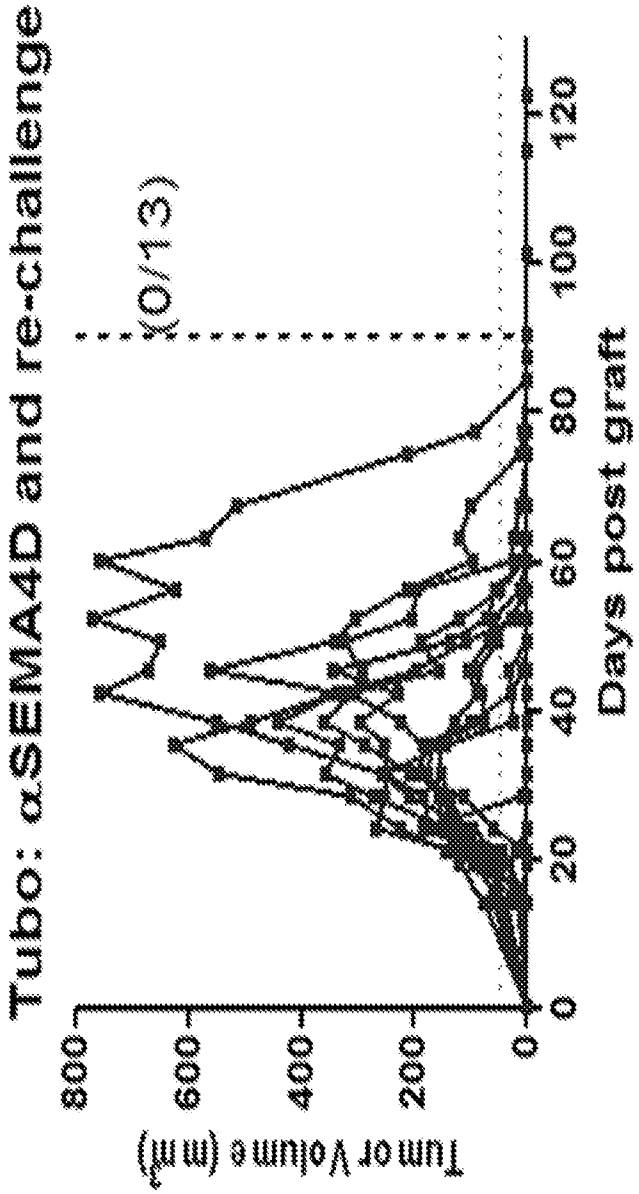

Tubo: αSEMA4D and re-challenge
(0/13)
Tumor Volume (mm3)
800
600
400
200
0
0
20
40
60
80
100
120
Days post graft

FIG. 10D

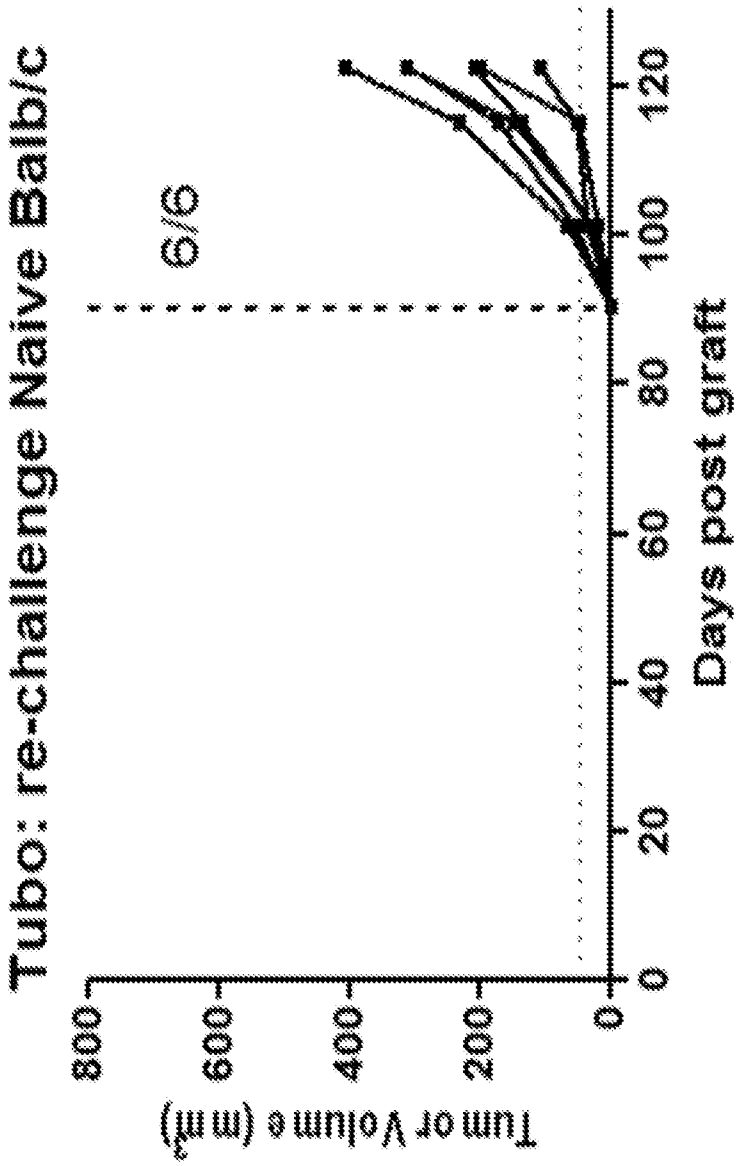

Tubo: re-challenge Naive Balb/c
6/6
Tumor Volume (mm³)
800
600
400
200
0
0
20
40
60
80
100
120
Days post graft

FIG. 10E

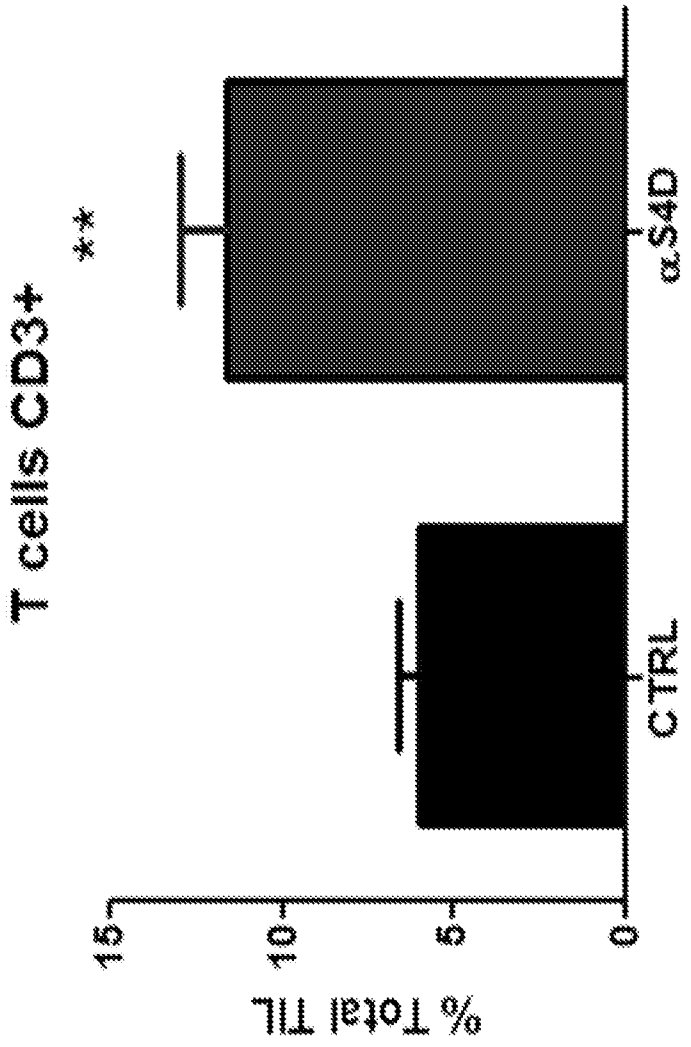


FIG. 11A

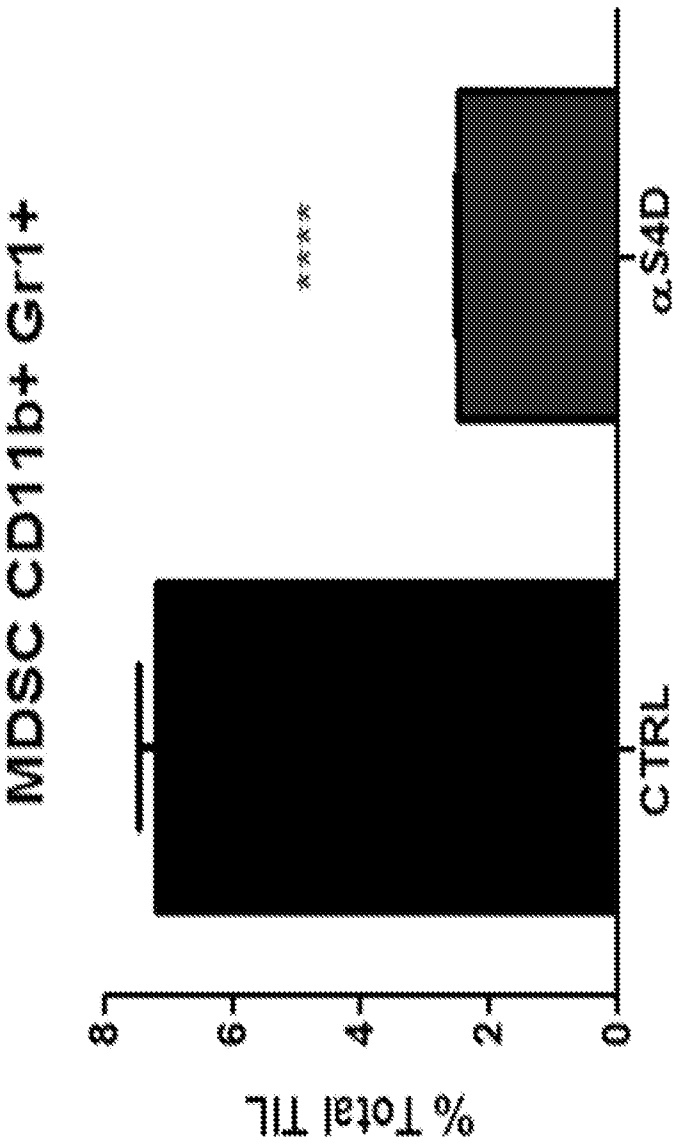


FIG. 11B

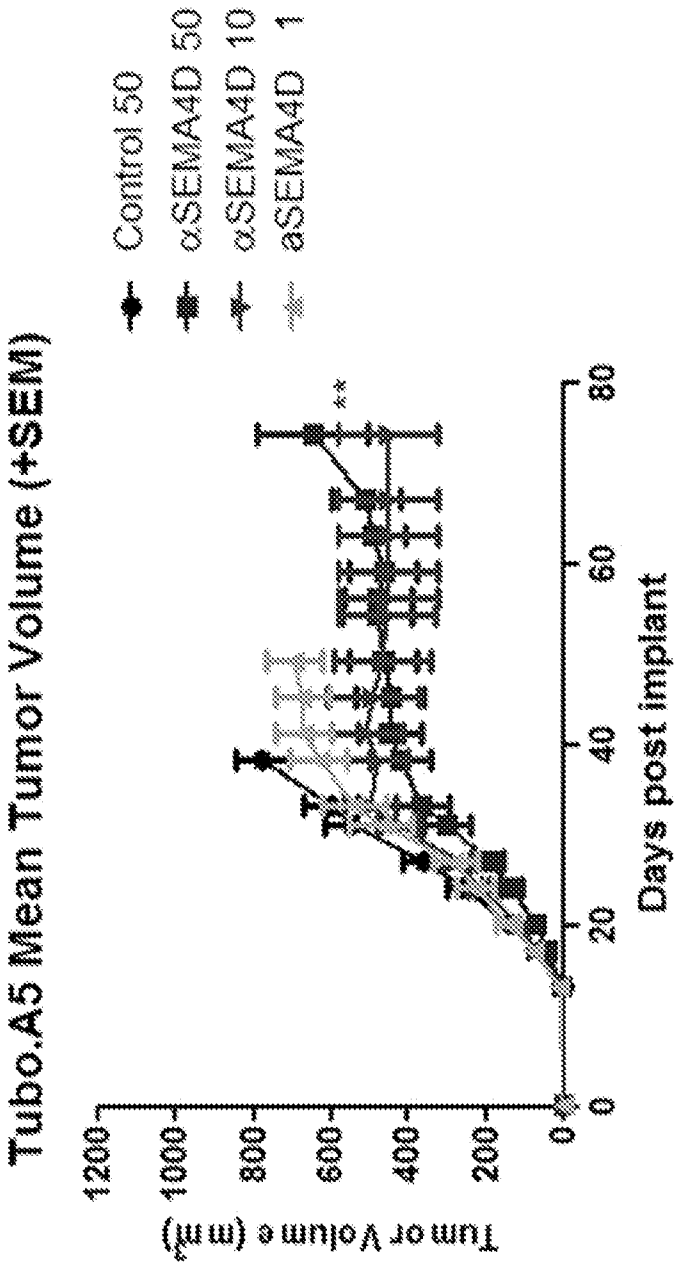

Tubo.A5 Mean Tumor Volume (+SEM)
Control 50
αSEMA4D 50
αSEMA4D 10
αSEMA4D 1
**
Tumor Volume (mm)
1200
1000
800
600
400
200
0
0
20
40
60
80
Days post implant

FIG. 12A

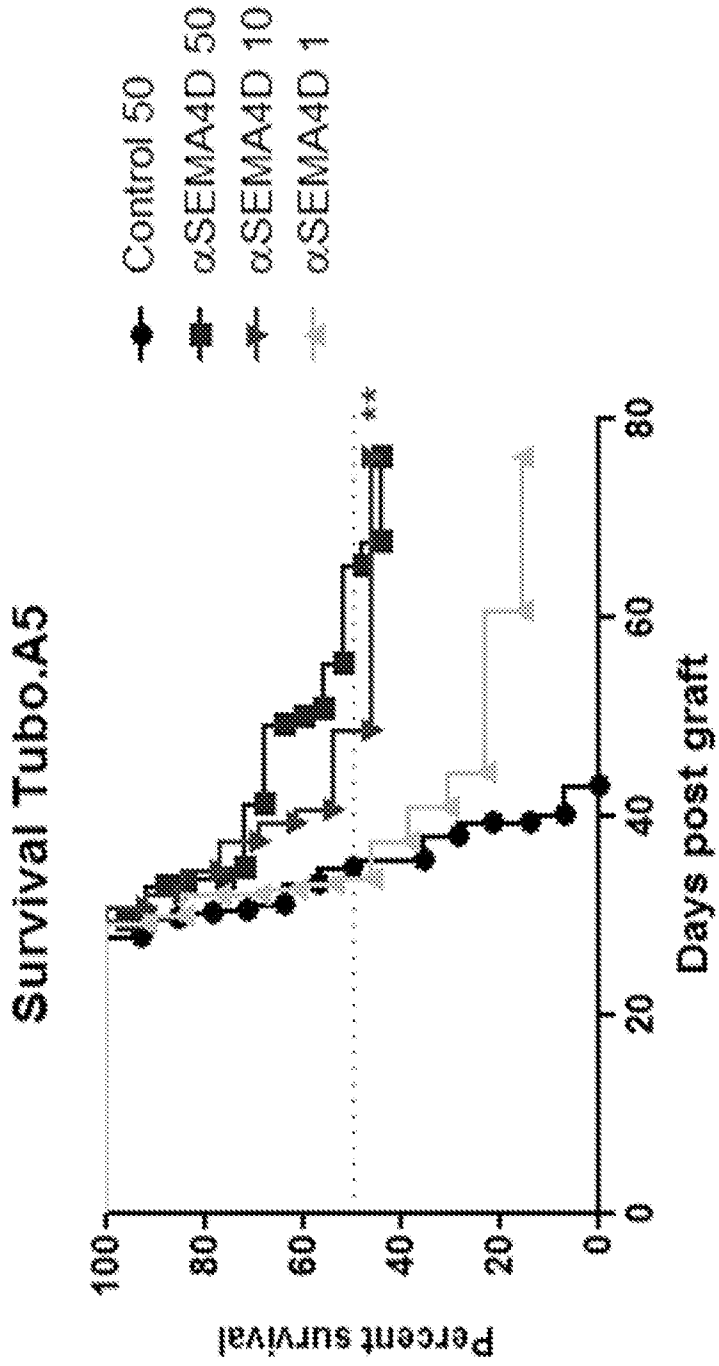

Survival Tubo.A5
Control 50
αSEMA4D 50
αSEMA4D 10
αSEMA4D 1
**
Percent survival
100
80
60
40
20
0
0
20
40
60
80
Days post graft

FIG. 12B

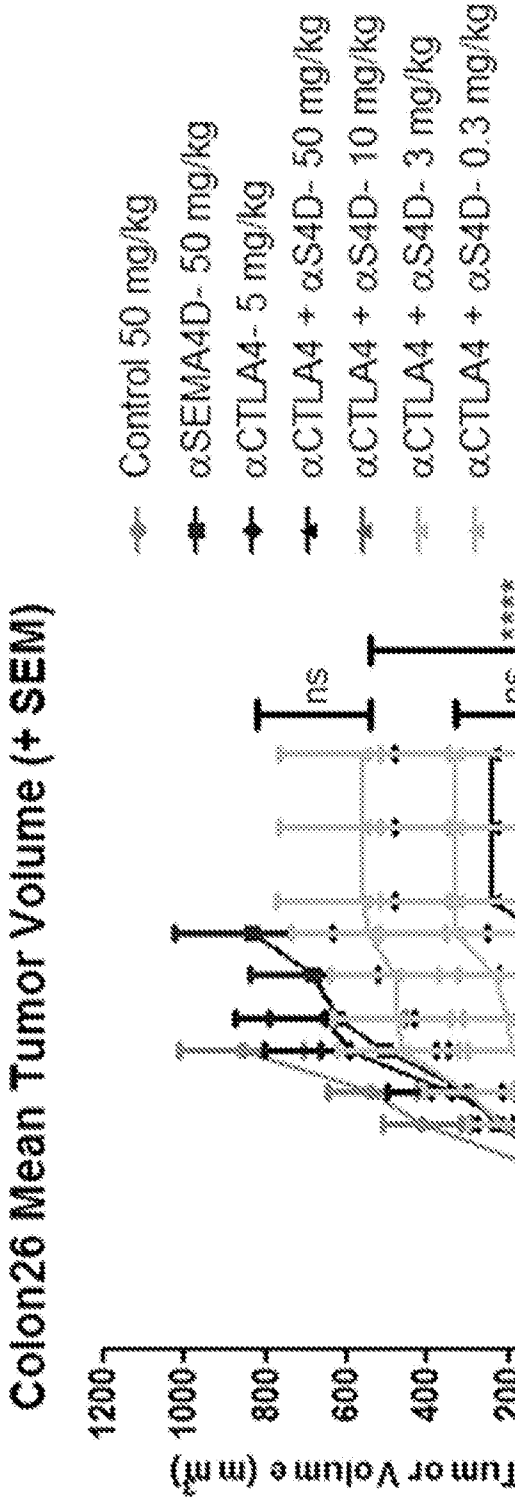

Colon26 Mean Tumor Volume (+ SEM)
Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
20
40
60
Days post graft
ns
ns
****
Control 50 mg/kg
αSEMA4D- 50 mg/kg
αCTLA4- 5 mg/kg
αCTLA4 + αS4D- 50 mg/kg
αCTLA4 + αS4D- 10 mg/kg
αCTLA4 + αS4D- 3 mg/kg
αCTLA4 + αS4D- 0.3 mg/kg

FIG. 12C

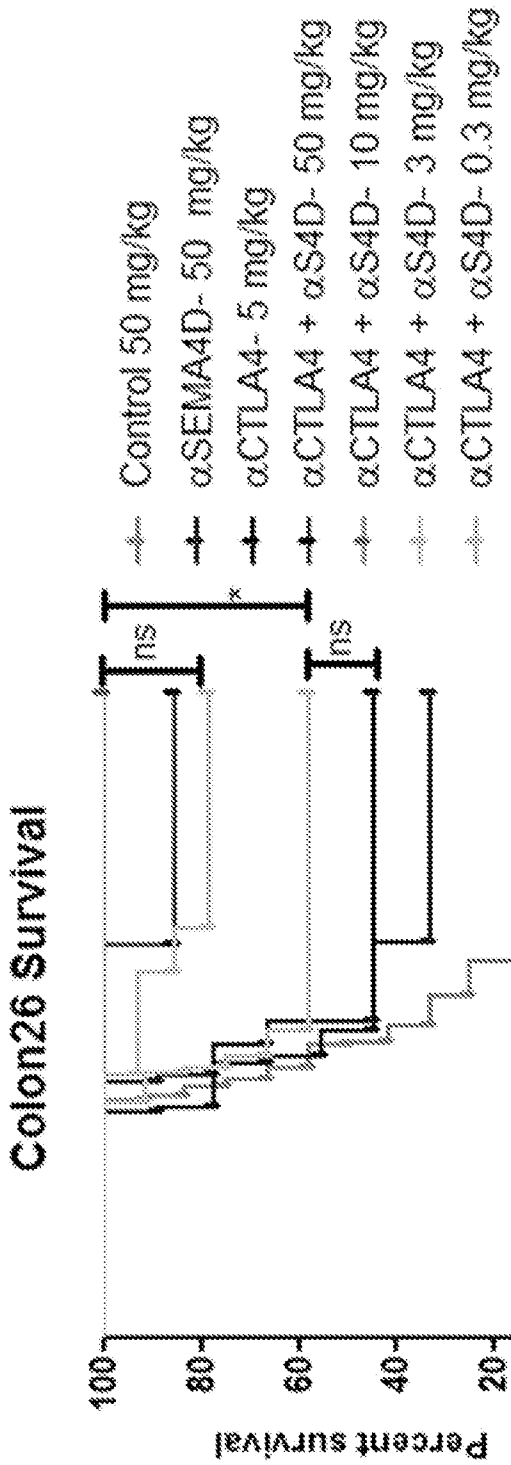

Colon26 Survival
Percent survival
Time To Endpoint (days)
0
20
40
60
80
100
0
10
20
30
40
50
60
ns
*
ns
Control 50 mg/kg
αSEMA4D- 50 mg/kg
αCTLA4- 5 mg/kg
αCTLA4 + αS4D- 50 mg/kg
αCTLA4 + αS4D- 10 mg/kg
αCTLA4 + αS4D- 3 mg/kg
αCTLA4 + αS4D- 0.3 mg/kg

FIG. 12D

Model	Treatment	% Complete Tumor Regression				Growth after tumor re-challenge
		% sig*	# mice	# studies	Stat	
Colon26	Control	1%	(3/266)	18	--	ND
	αSEMA4D	7%	(21/289)	18	***	0/2
	αPD1	8%	(3/40)	2	p=0.065	ND
	αPD1 + αSEMA4D	28%	(11/40)	2	****	ND
	αCTLA4	20%	(14/69)	4	***	* 1/8
	αCTLA4 + αSEMA4D	74%	(43/58)	3	****	0/21
	αCTLA4 + aPD1	60%	(12/20)	1	****	**** ND
	Cyclophosphamide	10%	(3/30)	2	ns	0/3
	Cyclophosphamide + αSEMA4D	40%	(8/20)	1	**	0/8
Tubo Mammary Carcinoma	Control	0	(0/27)	2	--	ND
	αSEMA4D	85%	(23/27)	2	****	0/13
	αCTLA4	23%	(3/13)	1	*	ND
	αPD1	0	(0/10)	1	ns	ND
	αCTLA4 + aPD1	14%	(2/14)	1	*	ND

*** Statistically significant, Fisher's Exact Test. Prism reports results as non-significant (ns) at P > 0.05, significant at 0.01 < P ≤ 0.05 (symbolized as *), very significant (**) at 0.001 < P ≤0.01, and extremely significant (***) at P ≤ 0.001.**

FIG. 13