

Heavy chain framework variants

AH1

QVQLVQSGVEVKKPGASVKVSKASGNTFTSYVMHWVRQAPCQGLEWM
 AYINPYNDGTYNEKFKGRVTLTDSSTTAYMELKSLQFDDTAVYYC
 ARSTARATPFYFAMDYWGQGTIVTVSS SEQ ID NO:7

AH2

EVQLVESGGGLVQPGGSLRLSCAASGNTFTSYVMHWVRQAPCKGLEWV
 AYINPYNDGTYNEKFKGRFTLSVDRSKNTLYLQMNSLRRAEDTAVYYC
 ARSTARATPFYFAMDYWGQGTIVTVSS SEQ ID NO:8

AH3

QVQLKQSGPGLVQPSSLSITCTVSGNTFTSYVMHWVRQPPCGLEWI
 AYINPYNDGTYNEKFKGRVTMLVDTSKNQFSLRLSSVTAADTAVYYC
 ARSTARATPFYFAMDYWGQGTIVTVSS SEQ ID NO:9

LPH1

QVQLVQSGAEVKKPGASVKVSKASGNTFTSYVMHWVRQAPCQGLEWM
 GYINPYNDGTYNEKFKGRVTMTTRDTSTSTVYMESSLRSED TAVYYC
 ARSTARATPFYFAMDYWGQGTIVTVSS SEQ ID NO:10

LPH2

EVQLVQSGAEVKKPGASVKVSKASGNTFTSYVMHWVRQAPCQGLEWM
 GYINPYNDGTYNEKFKGRVTMTSDKSTSTAYMELSSLRSED TAVYYC
 ARSTARATPFYFAMDYWGQGTIVTVSS SEQ ID NO:11

LPH4

EVQLVQSGAEVKKPGASVKVSKASGNTFTSYVMHWVRQAPCQGLEWM
 GYINPYNDGTYNEKFKGRVTITSDKSASTAYMELSSLRSED TAVYYC
 ARSTARATPFYFAMDYWGQGTIVTVSS SEQ ID NO:12

Figure 1

Light chain framework variants

AL1

DIQMTQSPSSLSASVGDRVTITCKSSQSLLSVSNQNSYLSWYQQKPGK
 APKLLIYGASIRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQH
NHGSFLPYTFGQGTKVEIK SEQ ID NO:13

AL2

EIVLTQSPATLSLSPGERATLSCKSSQSLLSVSNQNSYLSWYQQKPGQ
 APRLLIYGASIRESGVPSRFSGSGSGTDFTLTISSLQPEDFAVYYCQH
NHGSFLPYTFGQGTKVEIK SEQ ID NO:14

AL3

DIVMTQSPDLSAVSLGERATINCKSSQSLLSVSNQNSYLSWYQQKPGQ
 PPKLLIYGASIRESGVPSRFSGSGSGTDFTLTISSLQAEADVAVYYCQH
NHGSFLPYTFGQGTKVEIK SEQ ID NO:15

LPL1

DIVMTQSPDLSAVSLGERATINCKSSQSLLSVSNQNSYLSWYQQKPGQ
 PPKLLIYGASIRESGVPSRFSGSGSGTDFTLTISSLQAEADVAVYYCQH
NHGSFLPYTFGQGTKLEIK SEQ ID NO:16

LPL2

DIVMTQSPDLSAVSLGERATINCKSSQSLLSVSNQNSYLSWYQLKPGQ
 PPKLLIYGASIRESGVPSRFSGSGSGTDFTLTISSLQAEADVAVYYCQH
NHGSFLPYTFGCGTKLEIK SEQ ID NO:17

LPL4

DIIMTQSPSSLSASVGDRVTITCKSSQSLLSVSNQNSYLSWYQLKPGQ
 PPKLLIYGASIRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQH
NHGSFLPYTFGCGTKLEIK SEQ ID NO:18

Figure 2

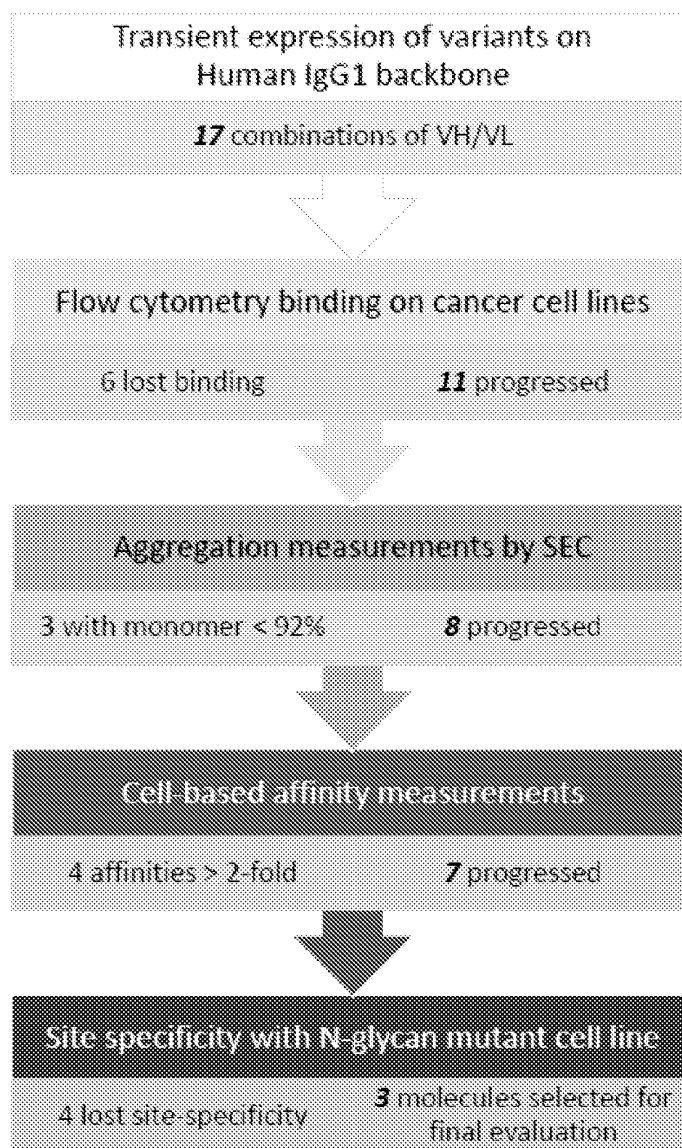


Figure 3A (continued)

VARIANTS: VH/VL	STEP 1: Flow Cytometry	STEP 2: Aggregation	STEP 3: Binding Affinity	STEP 4: Glycan Site Specificity
AH1/AL1	✓	✓	✓	✓
AH1/AL2	✗			
AH1/AL3	✓	✗		
AH2/AL1	✓	borderline	borderline	✗
AH2/AL2	✓	✗		
AH2/AL3	✓	✗		
AH3/AL1	✗			
AH3/AL2	✗			
AH3/AL3	✗			
LPH1/LPL2	✓	✓	✓	✓
LPH2/LPL1	✗			
LPH2/LPL2	✗			
LPH2/LPL4	✓	✓	✗	
LPH4/LPL2	✓	borderline	✓	✗
LPH4/LPL4	✓	✓	borderline	✗
AH1/LPL2	✓	✓	✓	✓
AH2/LPL2	✓	borderline	borderline	✗

✓: Clone meets passing criteria and progressed for next assay
✗: Clone does not meet passing criteria and is eliminated, not progressed for next assay
Borderline: Clone was at borderline of passing criteria, and kept in view for next assay

Figure 3B (end)

VH/VL	Flow cytometry binding			% Monomer	Affinity (nM)	Epitope site specificity	
	A549+	PC9	H1299			WT-CEACAM6	NtoA glycan mutant
Pass Criteria	++++/+++	++++/+++	-	> 92%	2-fold change	Binding	Reduced binding
mGR6A04	++++	++++	-		47 - 97	Bind	Reduced Binding
chGR6A04	++++	++++	-		75.7	Bind	Reduced Binding
AH1/AL1	++++	+++	-	96.57	139	Bind	Reduced Binding
AH1/AL2	++++	++	-				
AH1/AL3	++++	+++	-	92.05			
AH2/AL1	++++	+++	-	88.12 - 95.38	55 - 180	Bind	Bind
AH2/AL2	++++	+++	-	73.14			
AH2/AL3	++++	+++	-	83.52			
AH3/AL1	-	-	-				
AH3/AL2	-	-	-				
AH3/AL3	-	-	-				
LPH1/LPL2	++++	++++	-	99.12	109	Bind	Reduced Binding
LPH2/LPL1	++++	++	-				
LPH2/LPL2	++++	++++	+/-				
LPH2/LPL4	++++	++++	-	99.03	278		
LPH4/LPL2	++++	++++	-	94.51	126	Bind	Bind
LPH4/LPL4	++++	++++	-	99.32	215	Bind	Bind
AH1/LPL2	++++	+++	-	95.91	124	Bind	Reduced Binding
AH2/LPL2	++++	++++	-	92.78	218	Bind	Bind

Flow cytometry binding	
-	< 8%
+/-	8 - 15%
+	15 - 25%
++	25 - 50%
+++	50 - 75%
++++	75 - 100%

Red: Clone did not meet passing criteria and not progressed for next assay

Yellow: Clone was at borderline of passing criteria, and kept in view for next criteria

Note: Clone AH2/AL1 had significant batch-to-batch variation. Range of monomer % and affinity indicated.

Figure 4A (continued)

FR1 VHS		
AH1	QVQLVQSGGVEVKKRFGASVKVSCKAS	SEQ ID NO:35
AH2	EVQLVESGGGLVQPGGSLRLSCAAS	SEQ ID NO:37
AH3	QVQLKQSGPGGLVQPSQSLSITCTVQ	SEQ ID NO:38
LPH1	QVQLVQSGGVEVKKRFGASVKVSCKAS	SEQ ID NO:39
LPH2	EVQLVQSGGVEVKKRFGASVKVSCKAS	SEQ ID NO:40
LPH4	EVQLVQSGGVEVKKRFGASVKVSCKAS	SEQ ID NO:41

FR2 VHS		
AH1	WVRQAPFGGGLFWMA	SEQ ID NO:42
AH2	WVRQAPFGGLFWVA	SEQ ID NO:43
AH3	WVRQAPFGGLFWIA	SEQ ID NO:44
LPH1	WVRQAPFGGGLFWMA	SEQ ID NO:45
LPH2	WVRQAPFGGGLFWMA	SEQ ID NO:46
LPH4	WVRQAPFGGGLFWMA	SEQ ID NO:47

FR3 VHS		
AH1	RVTLTFFDSSTTTAYMELASLOFDDTAVVYCAR	SEQ ID NO:48
AH2	RFTLSVDRSENTELYLQMNSLRAEDTAVVYCAR	SEQ ID NO:49
AH3	RVTMLVDTSKNQPSLRSLSSVTAAATAVVYCAR	SEQ ID NO:50
LPH1	RVTMTTRDTSTSTVYMELESLRSED TAVVYCAR	SEQ ID NO:51
LPH2	RVTMTSDKSTSTAYMELESLRSED TAVVYCAR	SEQ ID NO:52
LPH4	RVTITSDKSASTAYMELESLRSEDTAVVYCAR	SEQ ID NO:53

FR4 VHS		
AH1	WGQGTLLVTVSS	SEQ ID NO:54
AH2	WGQGTLLVTVSS	SEQ ID NO:55
AH3	WGQGTLLVTVSS	SEQ ID NO:56
LPH1	WGQGTLLVTVSS	SEQ ID NO:57
LPH2	WGQGTLLVTVSS	SEQ ID NO:58
LPH4	WGQGTLLVTVSS	SEQ ID NO:59

Figure 4B (continued)

FR1 VLs		
AL1	DIQMTQSPSSLSASVGDRVITTC	SEQ ID NO: 60
AL2	EIVLTQSPATLSLSPGERATLSC	SEQ ID NO: 61
AL3	DIIVMTQSPDSSLAVSLGERATINC	SEQ ID NO: 62
LPL1	DIIVMTQSPDSSLAVSLGERATINC	SEQ ID NO: 63
LPL2	DIIVMTQSPDSSLAVSLGERATINC	SEQ ID NO: 64
LPL4	DILEMTQSPSSLSASVGDRVITTC	SEQ ID NO: 65

FR2 VLs		
AL1	WYQQKRPGRAPRIILYY	SEQ ID NO: 66
AL2	WYQQKPGQAPRLLIYY	SEQ ID NO: 67
AL3	WYQQKPGQPPKLLIYY	SEQ ID NO: 68
LPL1	WYQQKPGQPPKLLIYY	SEQ ID NO: 66
LPL2	WYQQKPGQPPKLLIYY	SEQ ID NO: 70
LPL4	WYQLKPGQPPKLLIYY	SEQ ID NO: 71

FR3 VLs		
AL1	GVPSSRFSGSGSGTDFTLTISSSLQPEDFATYYC	SEQ ID NO: 72
AL2	GVPARFSGSGSGTDFTLTISSLQPEDFAVYYC	SEQ ID NO: 73
AL3	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	SEQ ID NO: 74
LPL1	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	SEQ ID NO: 75
LPL2	GVPDRFSGSGSGTDFTLTISSLQAEDFAVYYC	SEQ ID NO: 76
LPL4	GVPSSRFSGSGSGTDFTLTISSLQPEDFATYYC	SEQ ID NO: 77

FR4 VLs		
AL1	FGGGTKVEIK	SEQ ID NO: 78
AL2	FGGGTKVEIK	SEQ ID NO: 79
AL3	FGGGTKVEIK	SEQ ID NO: 80
LPL1	FGGGTKVEIK	SEQ ID NO: 81
LPL2	FGGGTKVEIK	SEQ ID NO: 82
LPL4	FGGGYKVEIK	SEQ ID NO: 83

Figure 4C (end)

Light Chain Sequence

DIVMTQSPDSLAVSLGERATINCKSSQSLLWSVNQNSYLSWYQLKPGQPPKLLYGASIRESGVPDRFS
GSGSGTDFTLTISSLQAEDVAVYYCQHNGSFLPYTFGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC* (SEQ ID NO: 84)

Heavy Chain Sequence

QVQLVQSGAEVKKPGASVKVSCKASGNTFTSYVMHWVRQAPGQGLEWMGYINPYNDGTKYNEKFK
GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSTARATPYFYAMDYWGQGTLVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC
NVNHHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG* (SEQ ID NO: 85)

Figure 5

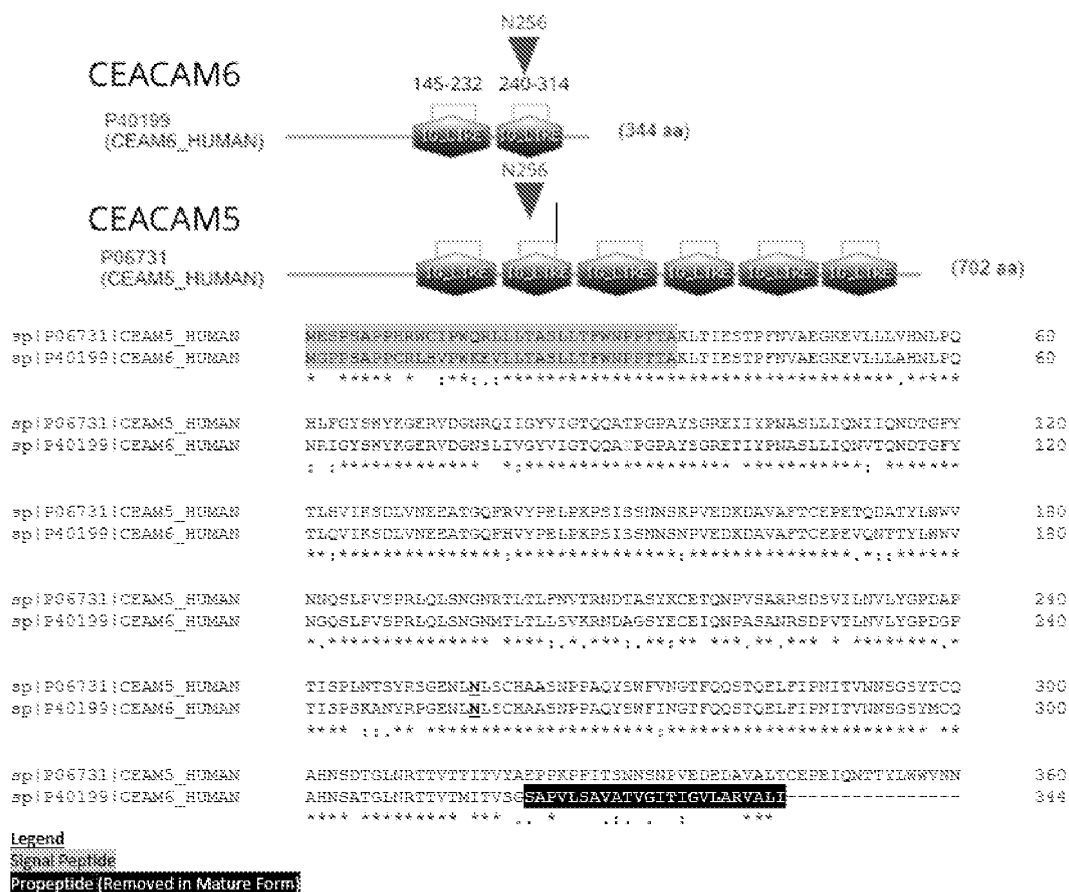


Figure 6

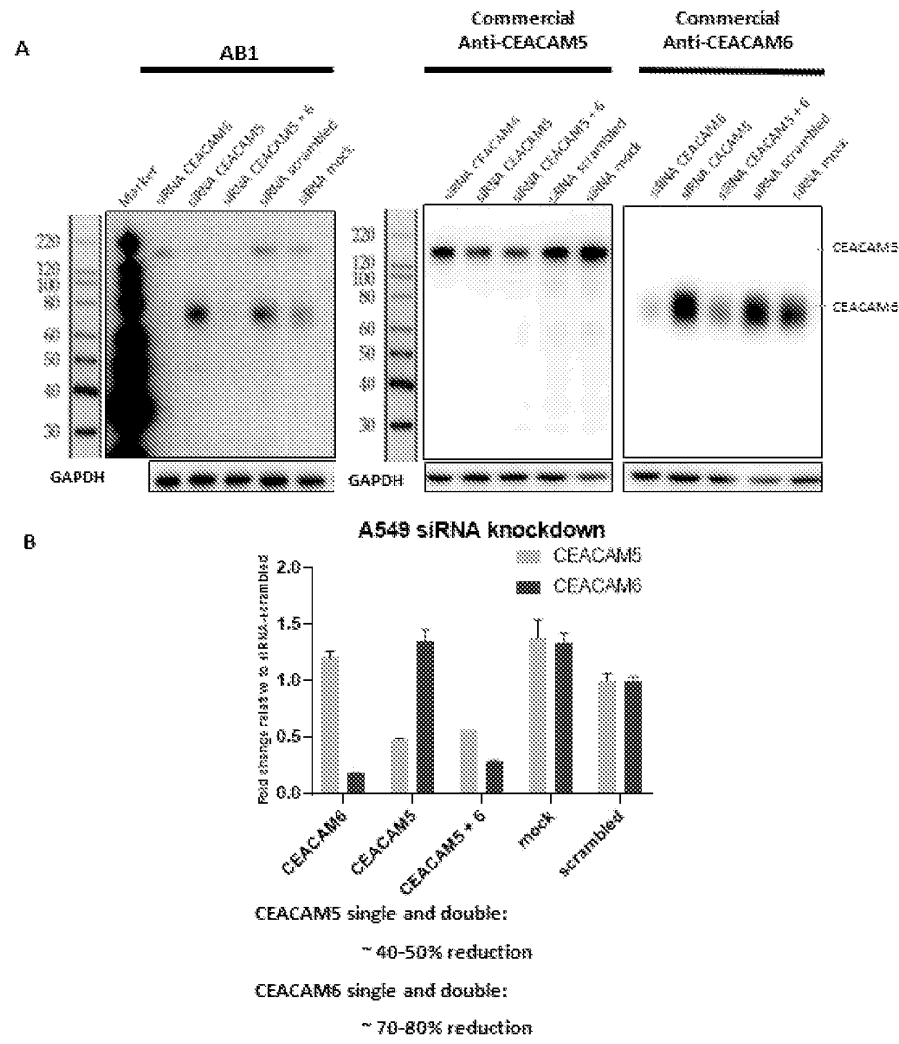


Figure 7

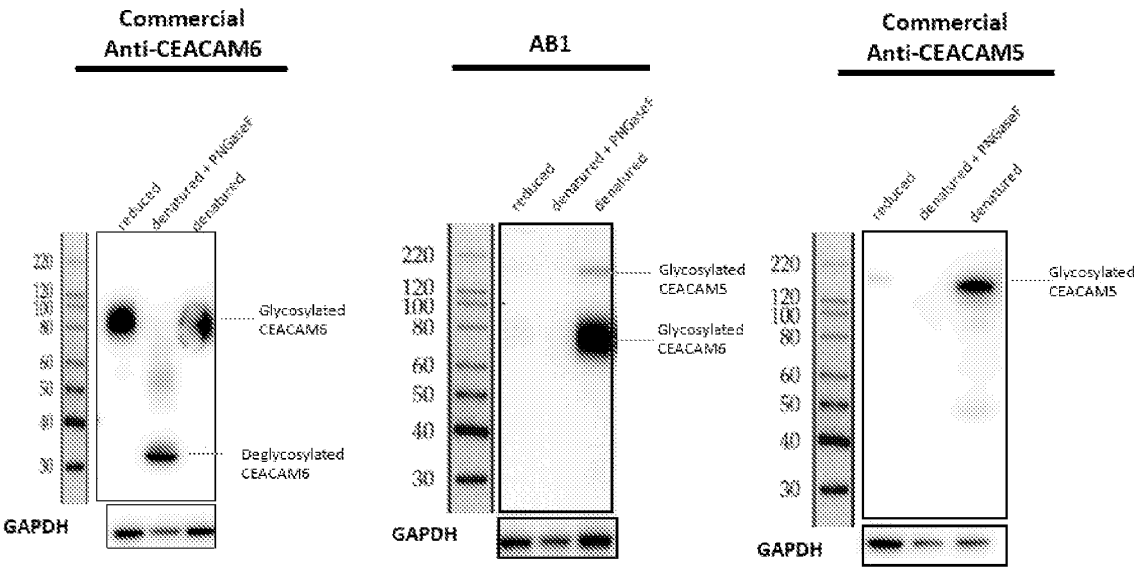
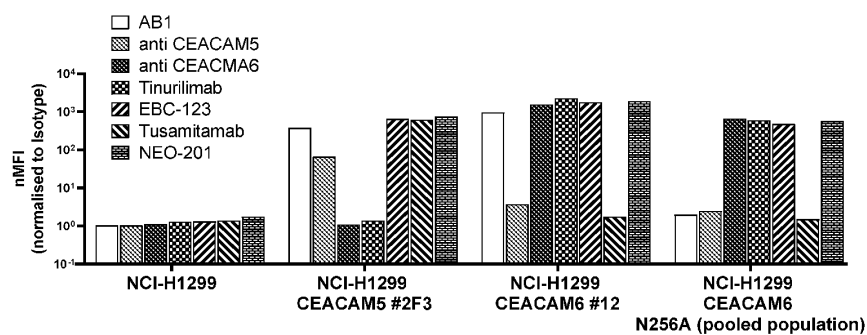
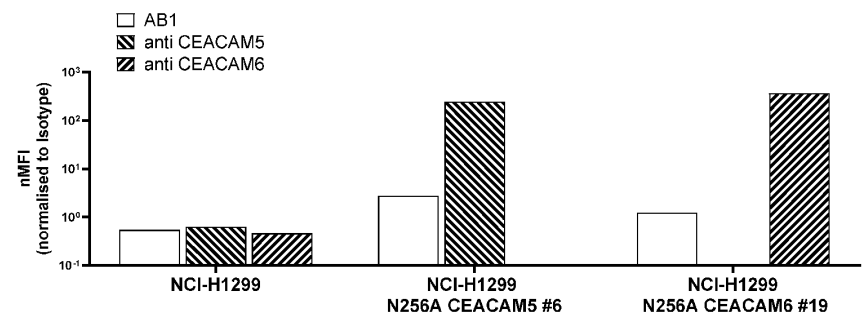


Figure 8



nMFI	NCI-H1299	NCI-H1299 CEACAM5 #2F3	NCI-H1299 CEACAM6 #12	NCI-H1299 N256A CEACAM6 (pooled population)
AB1	1.02	376	968	1.95
Anti CEACAM5	1.02	65.6	3.68	2.44
Anti CEACAM6	1.1	1.07	1520	654
Tinurilimab	1.27	1.38	2230	598
EBC-123	1.29	650	1750	473
Tusamitamab	1.36	614	1.75	1.49
NEO-201	1.74	742	1890	570

Figure 9A (continued)



nMFI	NCI-H1299	NCI-H1299 N256A CEACAM5 #6	NCI-H1299 N256A CEACAM6 #19
AB1	0.54	2.77	1.22
Anti CEACAM5	0.63	242	ND
Anti CEACAM6	0.46	ND	363

ND: Not Done

Figure 9B (end)

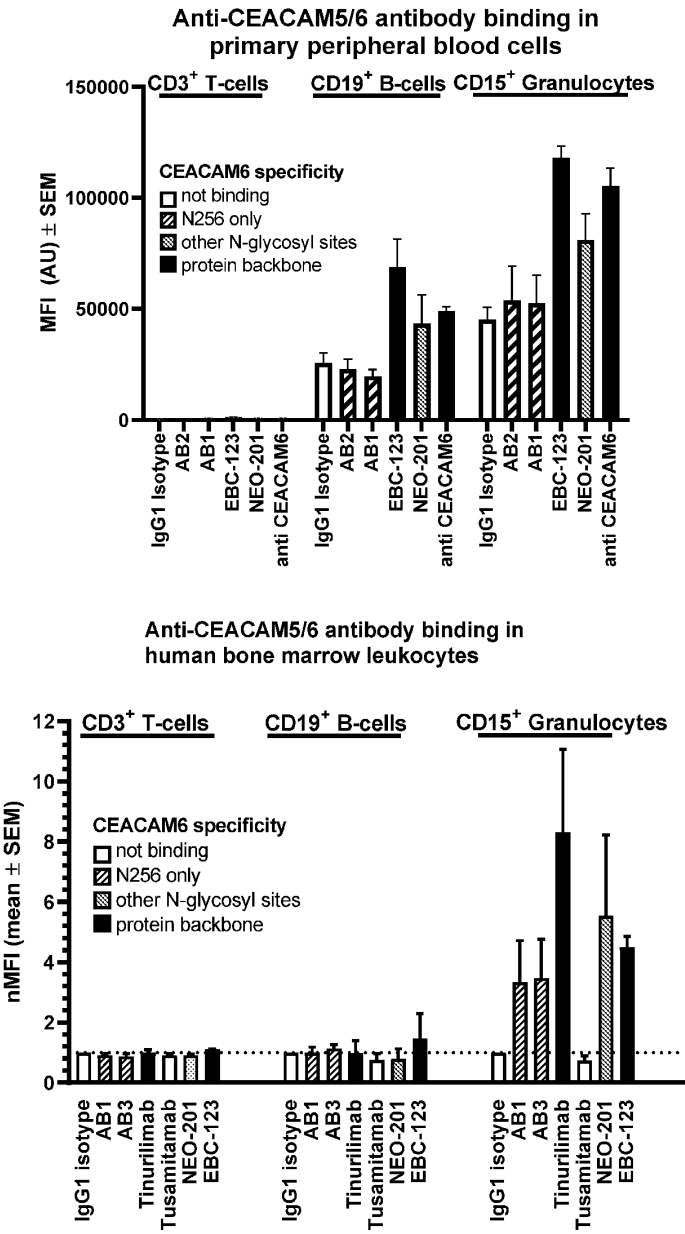


Figure 10

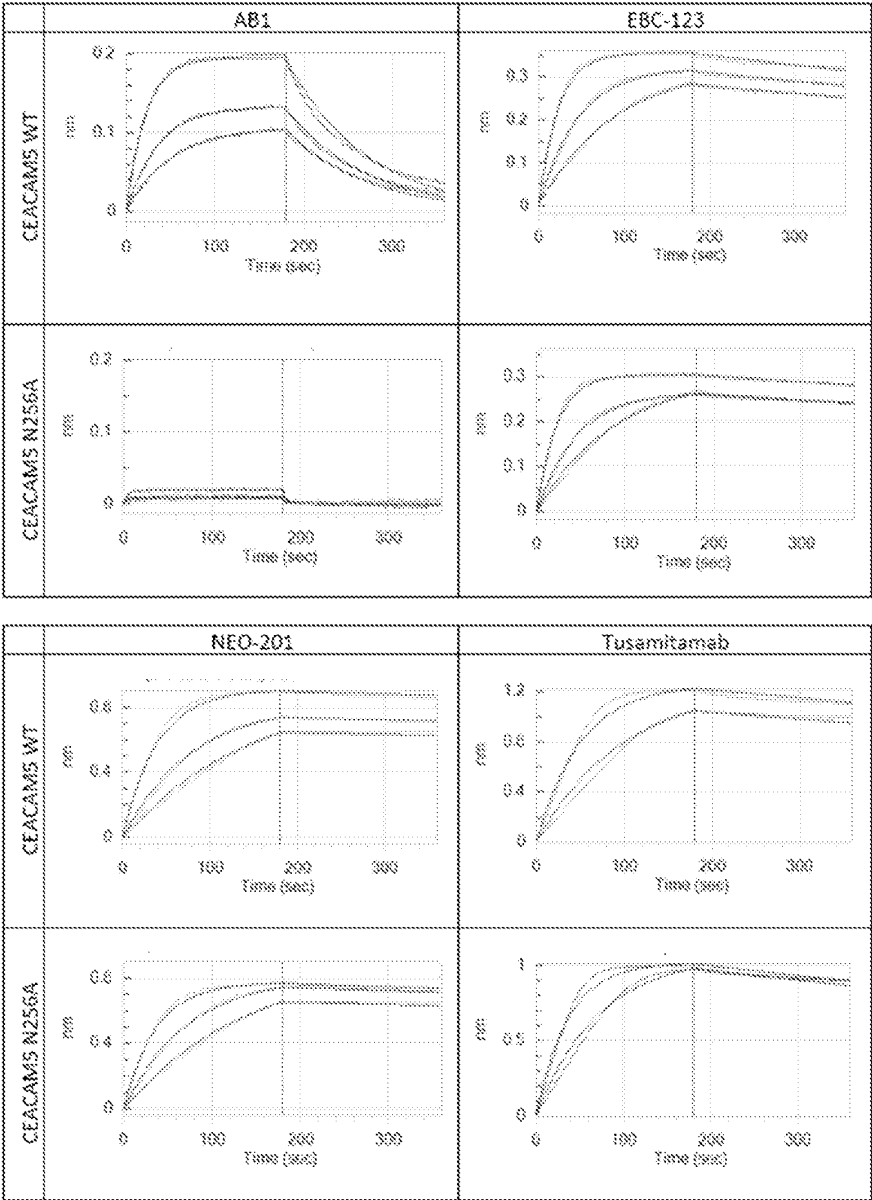


Figure 11A (continued)

Analyte	Ligand	KD (M)	k _{on} (1/Ms)	k _{dis} (1/s)
AB1	CEACAM5	6.38E-08	1.74E+05	1.11E-02
	CEACAM5 N256A	> 2E-07	-	-
EBC-123	CEACAM5	7.75E-10	8.65E+05	6.71E-04
	CEACAM5 N256A	5.55E-10	8.64E+05	4.80E-04
NEO-201	CEACAM5	3.21E-10	5.12E+05	1.64E-04
	CEACAM5 N256A	4.01E-10	5.48E+05	2.20E-04
Tusamitamab	CEACAM5	1.37E-09	3.87E+05	5.30E-04
	CEACAM5 N256A	1.27E-09	5.34E+05	6.77E-04

Figure 11A (continued)

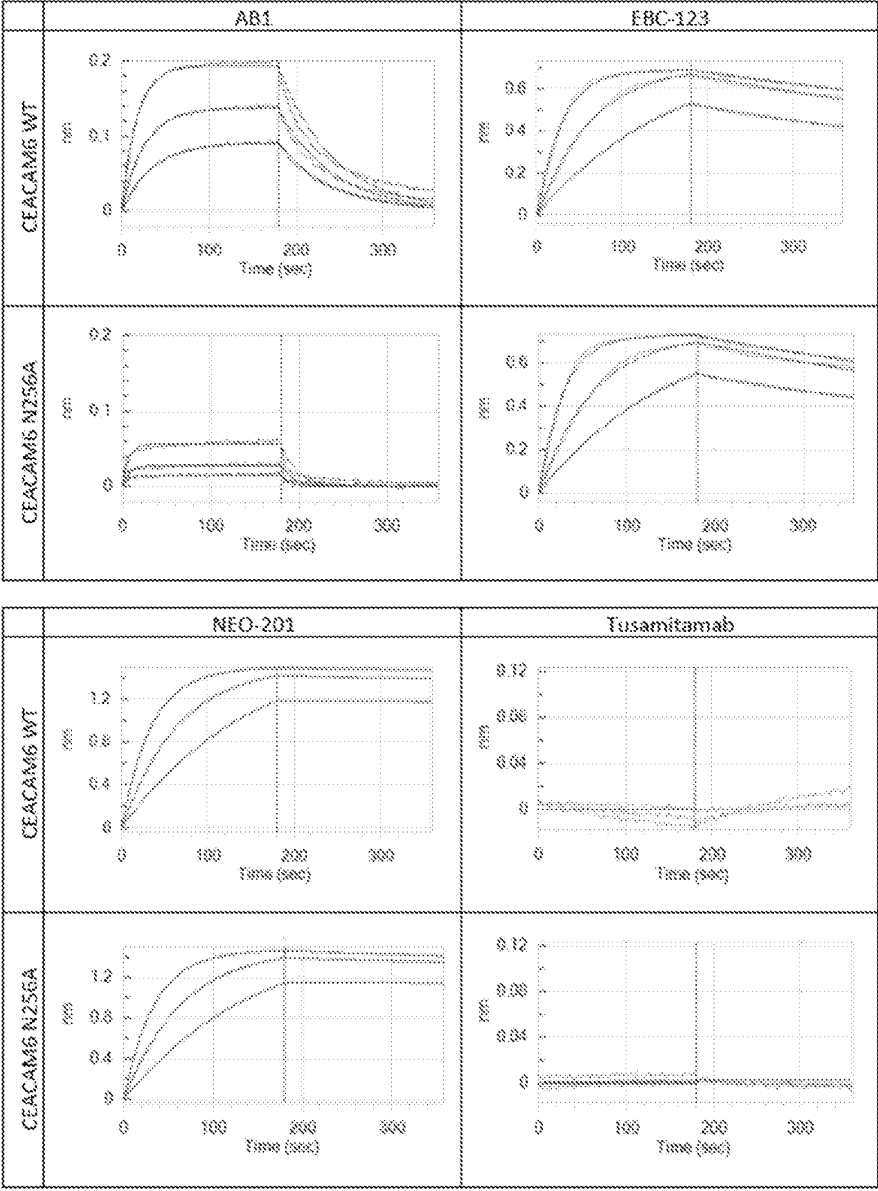


Figure 11B (continued)

Analyte	Ligand	KD (M)	k _{on} (1/Ms)	k _{dis} (1/s)
AB1	CEACAM6	7.15E-08	2.23E+05	1.60E-02
	CACAM6 N256A	3.18E-06	4.19E+04	1.33E-01
EBC-123	CEACAM6	1.57E-09	6.26E+05	9.85E-04
	CEACAM6 N256A	1.61E-09	6.60E+05	1.06E-03
NEO-201	CEACAM6	1.26E-10	5.80E+05	7.31E-05
	CEACAM6 N256A	2.17E-10	5.90E+05	1.28E-04
Tusamitamab	CEACAM6	NB	NB	NB
	CEACAM6 N256A	NB	NB	NB

Figure 11B (end)

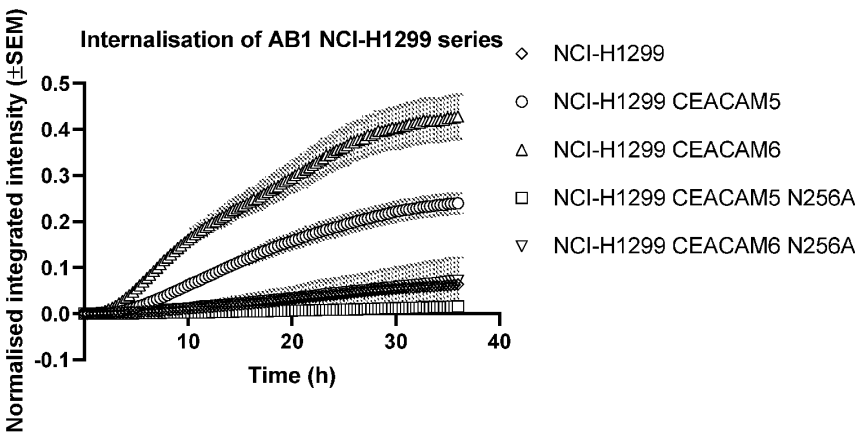


Figure 12(A) (continued)

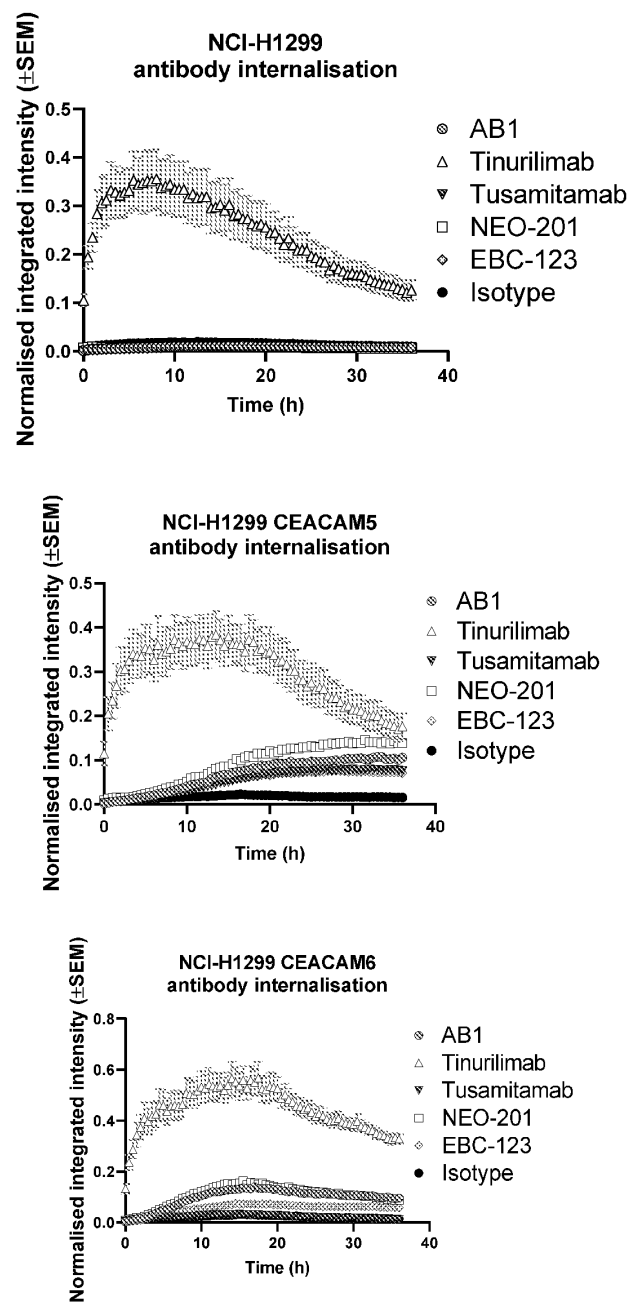


Figure 12(B) (end)

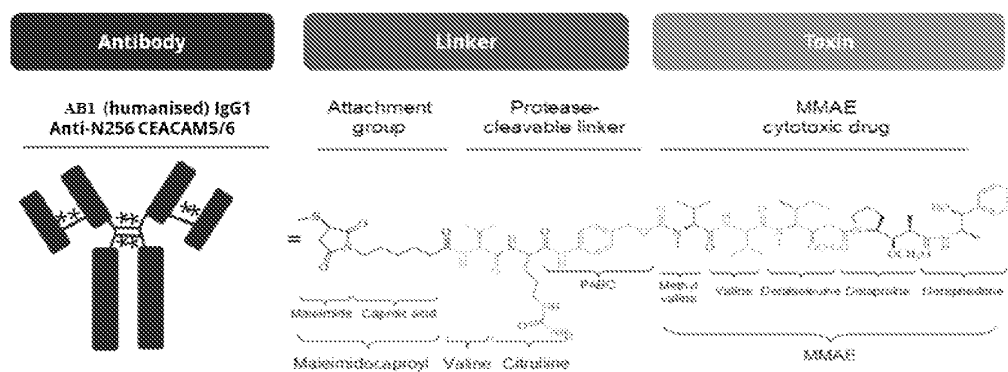


Figure 13

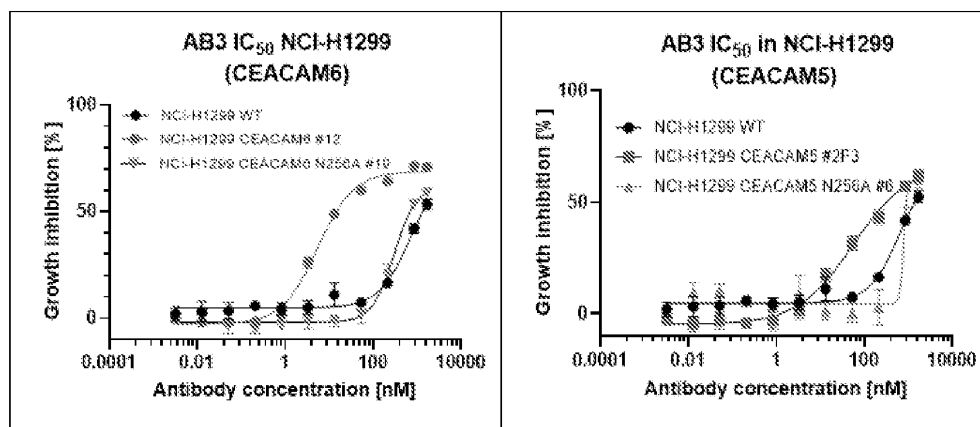


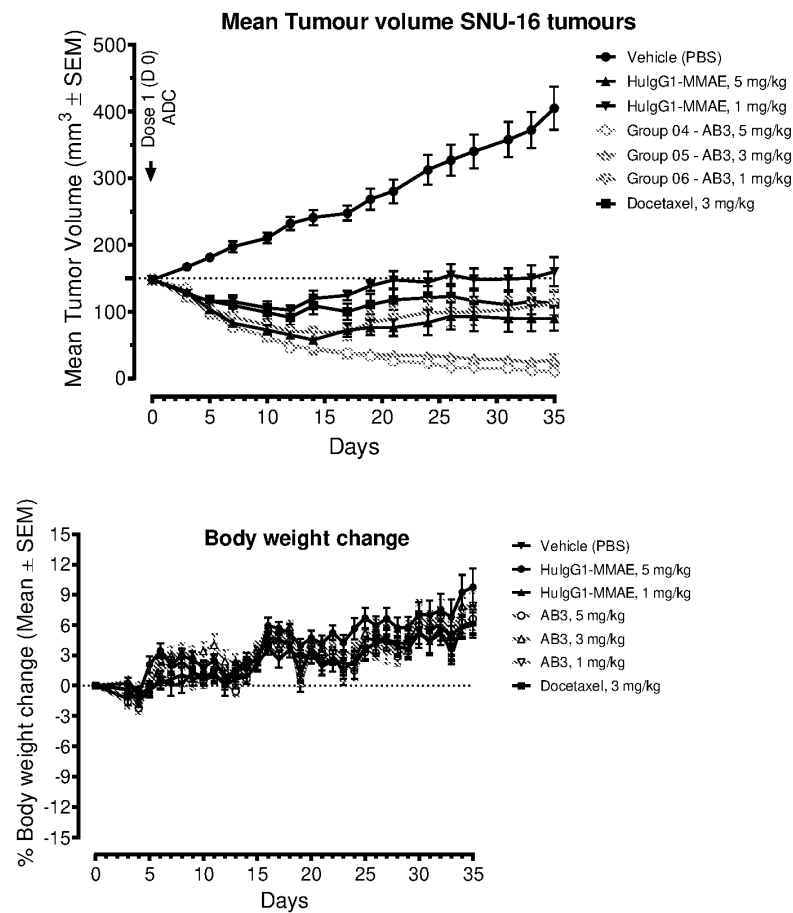
Figure 14A (continued)

Cell line	IC ₅₀ (nM)/Max. inhibition (%)		
	AB3	IgG1-MMAE	MMAE alone
NCI-H1299	>1700 / 54.85	580.7 / 59.84	2.17 / 84.5
NCI-H1299 CEACAM6 #12	15.19 / 67.5	358.4 / 67	1.14 / 77.68
NCI-H1299 CEACAM5 #2F3	54.26 / 60.58	568.3 / 52.69	2.83 / 73.7
NCI-H1299 CEACAM6 N256A #19	1374.55 / 56.39	536.8 / 59.41	2.99 / 82.95
NCI-H1299 CEACAM5 N256A #6	463.25 / 75.06	512.75 / 69.98	1.52 / 80.88

Figure 14B (continued)

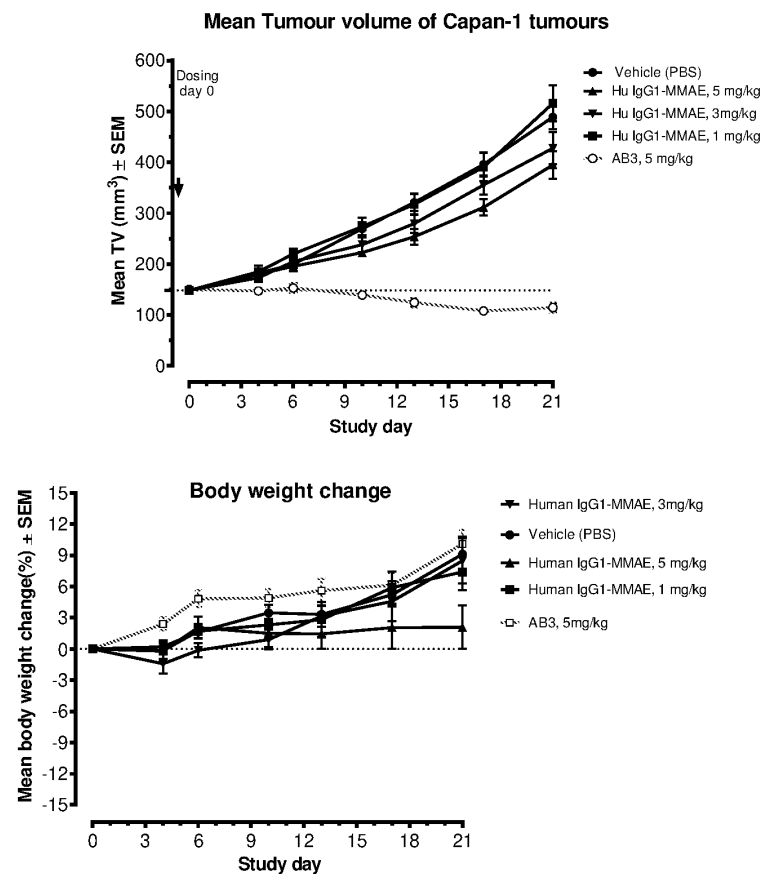
Cell line	IC ₅₀ (nM)/Max. inhibition (%)	
	AB3	Tusamitamab-MMAE
Capan-1 (Pancreas)	2.07 / 62.54	78.14 / 58.84
CFPAC-1 (Pancreas)	52.33 / 63.11	854.7 / 42.73
HCC4006 (Lung)	1.96 / 70.61	112.8 / 72.81
SNU-16 (Gastric)	5.29 / 96.68	0.28 / 93.65
HT-29 (Colon)	56.37 / 87.56	65.93 / 85

Figure 14C (end)



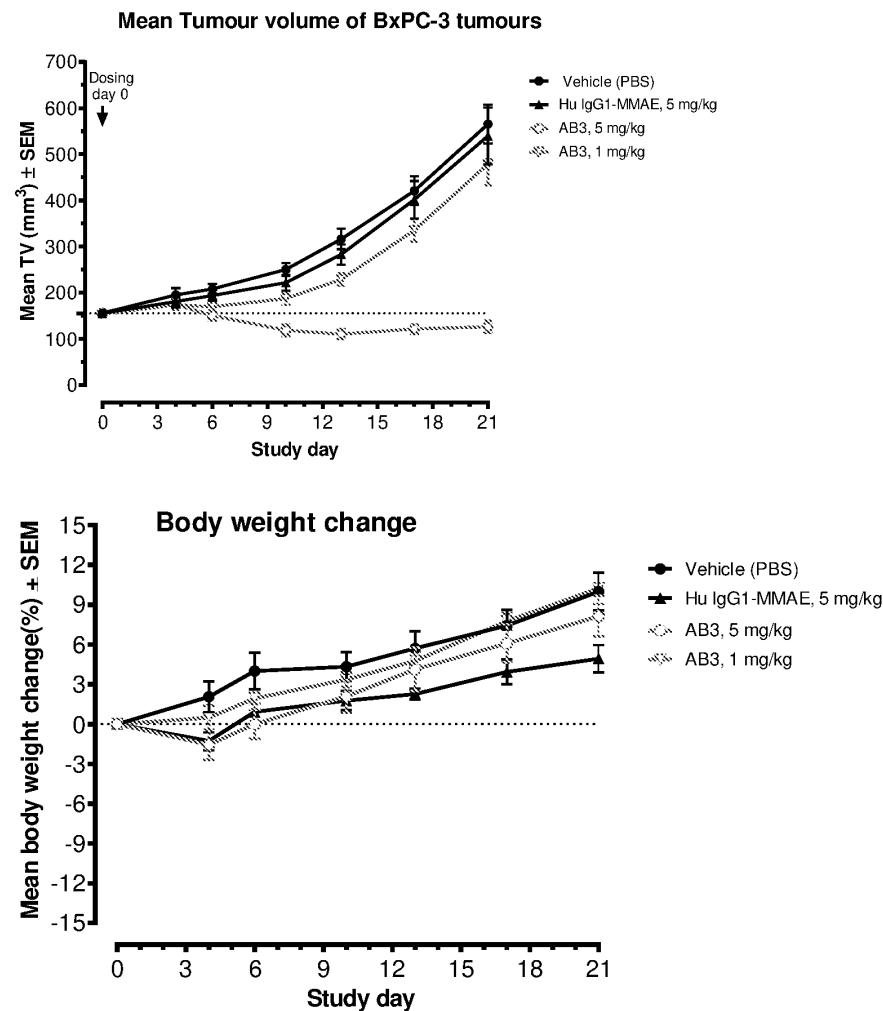
Day 35 Data Summary													
Treatment Regimen					Tumour Growth Analysis					Toxicity			
Group	n/Group	Compound	Dose	Route	Schedule	Mean TV (mm³)	TGI (%)	ANOVA (P Value)	PR (n/all)	CR (n/all)	Max. BW Loss (%)	TRD (n/all)	NTRD (n/all)
1	10	PBS, 10 ml/kg	-	IV	Once on day 0	404.85	NA	NA	0/10	0/10	-0.87 (day 4)	0/10	0/10
2	10	Hu IgG1-MMAE	5 mg/kg			88.89	123	***	6/10	0/10	-1.46 (day 4)	0/10	0/10
3	10	Hu IgG1-MMAE	1 mg/kg			160.17	95	***	0/10	0/10	-1.22 (day 3)	0/10	0/10
4	10	AB3	5 mg/kg			10.10	154	***	4/10	5/10	-2.20 (day 4)	0/10	0/10
5	10	AB3	3 mg/kg			27.67	147	***	7/10	3/10	-0.32 (day 4)	0/10	0/10
6	10	AB3	1 mg/kg			112.33	114	***	3/10	1/10	-1.88 (day 4)	0/10	0/10
7	10	Docetaxel	3 mg/kg			QW x 3		112.41	114	***	3/10	1/10	-0.61 (day 4)

Figure 15



Day 21 Data Summary													
Treatment Regimen						Tumour Growth Analysis					Toxicity		
Group	n/Group	Test article	Dose	Route	Schedule	Mean TV (mm ³)	TVI (%)	ANOVA (P Value)	PR (n/all)	CR (n/all)	Max. BW Loss (%)	TRD (n/all)	NTRD (n/all)
1	10	PBS	-	iv	Once/study on day 0	488.73	-	-	0/10	0/10	No BW loss	0/10	0/10
2	10	IgG1-MMAE	5 mg/kg			384.61	28	ns	0/10	0/10	-0.20 (Day 4)	0/10	0/10
3	10	IgG1-MMAE	3 mg/kg			427.72	18	ns	0/10	0/10	-1.43 (Day 4)	0/10	0/10
4	10	IgG1-MMAE	1 mg/kg			515.88	-8	ns	0/10	0/10	-0.15 (Day 4)	0/10	0/10
5	10	AB3	5 mg/kg			114.426	110	****	10/10	0/10	No BW loss	0/10	0/10

Figure 16



Day 21 Data Summary													
Treatment Regimen						Tumour Growth Analysis					Toxicity		
Group	n/Group	Test article	Dose	Route	Schedule	Mean TV (mm ³)	TGI (%)	ANOVA (P Value)	PR (n/all)	CR (n/all)	Max. BW Loss (%)	TRD (n/all)	NTRD (n/all)
1	8	PBS	-	iv	Once/study on day 0	565.13	-	-	0/8	0/8	No BW loss	0/8	0/8
2	8	IgG1-MMAE	5 mg/kg			539.78	6	ns	0/8	0/8	-1.28 (Day 4)	0/8	0/8
3	8	AB3	5 mg/kg			125.29	197	****	6/8	0/8	-3.59 (Day 4)	0/8	0/8
4	8	AB3	1 mg/kg			478.73	21	ns	0/8	0/8	No BW loss	0/8	0/8

Figure 17

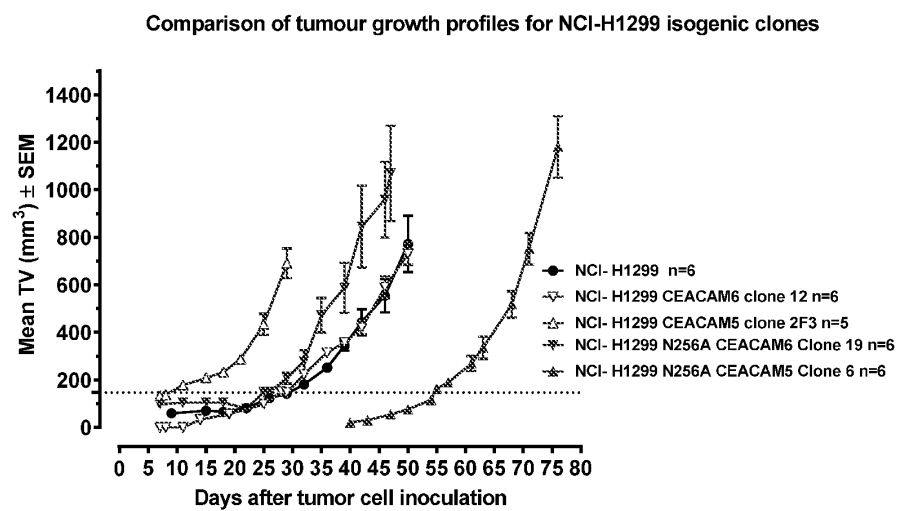
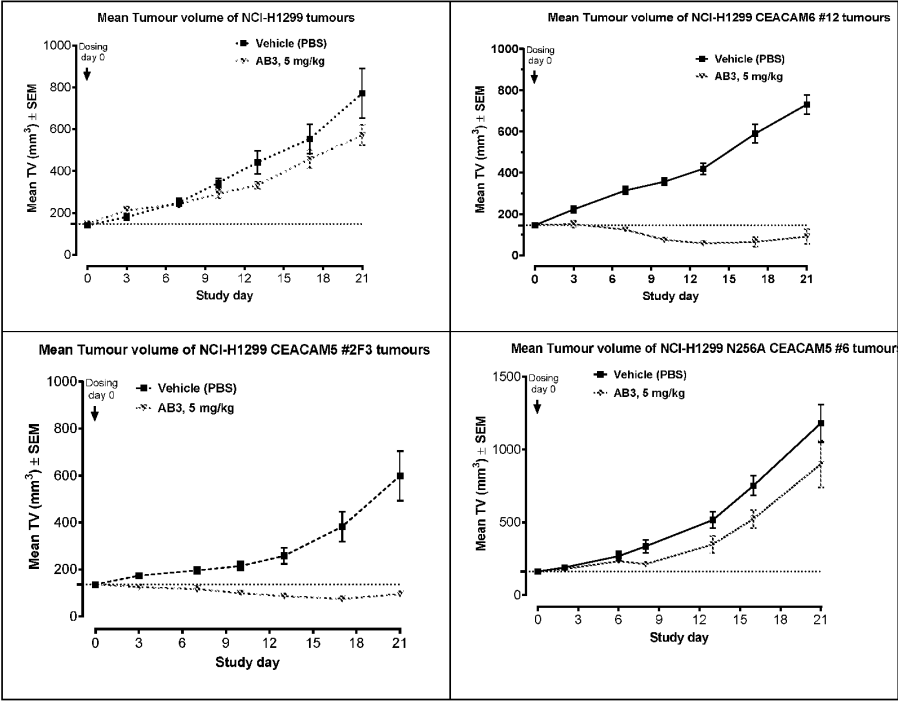


Figure 18



Day 21 Data Summary													
Treatment Regimen						Tumour Growth Analysis					Toxicity		
Cell line	n/Group	Test article	Dose	Route	Schedule	Mean TV (mm³)	TG1 (%)	ANOVA (n/all)	PR (n/all)	CR (n/all)	Max. BW Loss (%)	TRD (n/all)	NTRD (n/all)
NCI-H1299	6	PBS	-	IV	Dosing on Day 0	772.03	-	-	0/6	0/6	No BW loss	0/6	0/6
	6	AB3	5 mg/kg			571.52	32	ns	0/6	0/6	On day 3 (-1.24 %)	0/6	0/6
NCI-H1299 CEACAM6 #12	6	PBS	-	IV	Dosing on Day 0	730.51	-	-	0/6	0/6	On day 3 (-1.52 %)	0/6	0/6
	6	AB3	5 mg/kg			90.37	110	****	5/6	0/6	On day 3 (-0.14 %)	0/6	0/6
NCI-H1299 CEACAM5 #2F3	6	PBS	-	IV	Dosing on Day 0	598.44	-	-	0/6	0/6	No BW loss	0/6	0/6
	6	AB3	5 mg/kg			96.38	109	***	6/6	0/6	No BW loss	0/6	0/6
NCI-H1299 N256A CEACAM5 #6	6	PBS	-	IV	Dosing on Day 0	1180.72	NA	-	0/6	0/6	No BW loss	0/6	0/6
	6	AB3	5 mg/kg			899.74	28	ns	0/6	0/6	No BW loss	0/6	0/6

Figure 19