Human TMEFF2#19 (Pr1) anti-TENB2 Ab heavy chain amino acid sequence (signal peptide is underlined):

MAVLGLLLCLVTFPSCVLSDVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWSWIRQPPGKGLEWMGFIS
YDGSNKYNPSLKNRITISRDTSKNQFSLKLSSVTAADTAVYYCARGLRRGDYSMDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO:1

Human TMEFF2#19 (Pr1) anti-TENB2 Ab light chain amino acid sequence (signal peptide is underlined):

MDFQVQIFSFLLISASVIMSRGDIQMTQSPSSLSASVGDRVTITCKASQNVVTAVAWYQQKPGKAPKLLIYSASNRHT
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSYPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC SEQ ID NO:2

Figure 1

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A121C thio hu TMEFF2#19 (Pr1) heavy chain amino acid sequence (signal peptide is underlined):

MAVLGLLLCLVTFPSCVLSDVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWSWIRQPPGKGLEWMGFI
SYDGSNKYNPSLKNRITISRDTSKNQFSLKLSSVTAADTAVYYCARGLRRGDYSMDYWGQGTLVTVSSCST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO:3

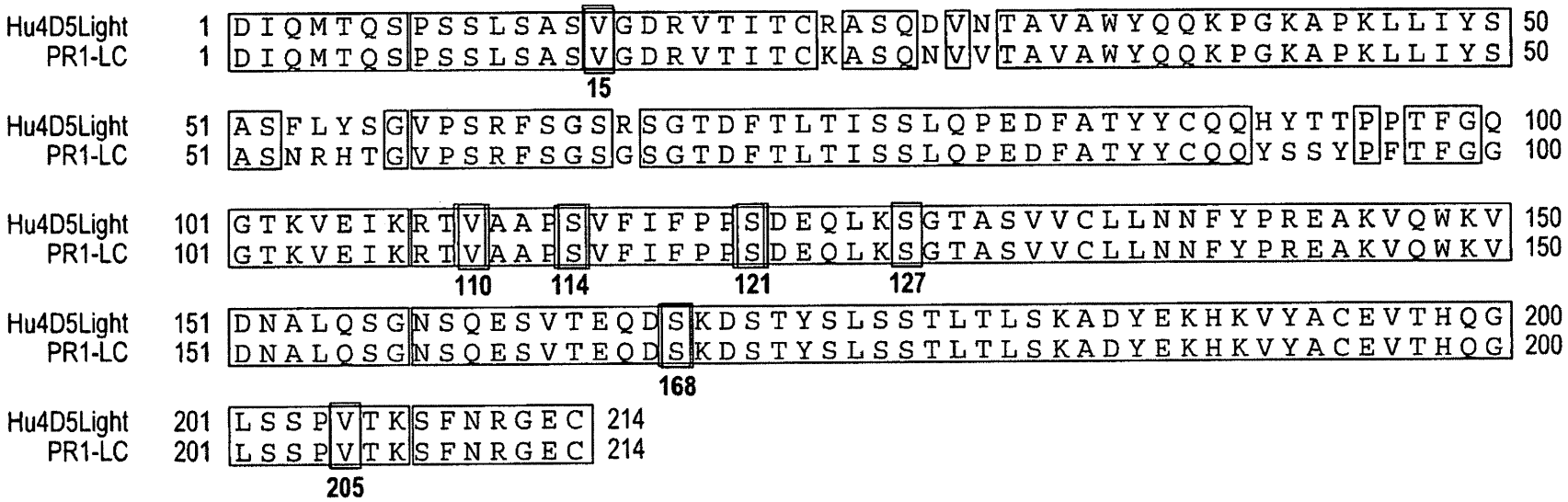
A121C thio hu TMEFF2#19 (Pr1) light chain amino acid sequence (signal peptide is underlined):

MDFQVQIFSFLLISASVIMSRGDIQMTQSPSSLSASVGDRVTITCKASQNVVTAVAWYQQKPGKAPKLLIYSASNRH
TGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSYPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC SEQ ID NO:2

Thio-anti-TENB2 heavy chain amino acid sequence (signal sequence is underlined). Cysteine substitution is at Ala-121 residue in the heavy chain

Figure 2

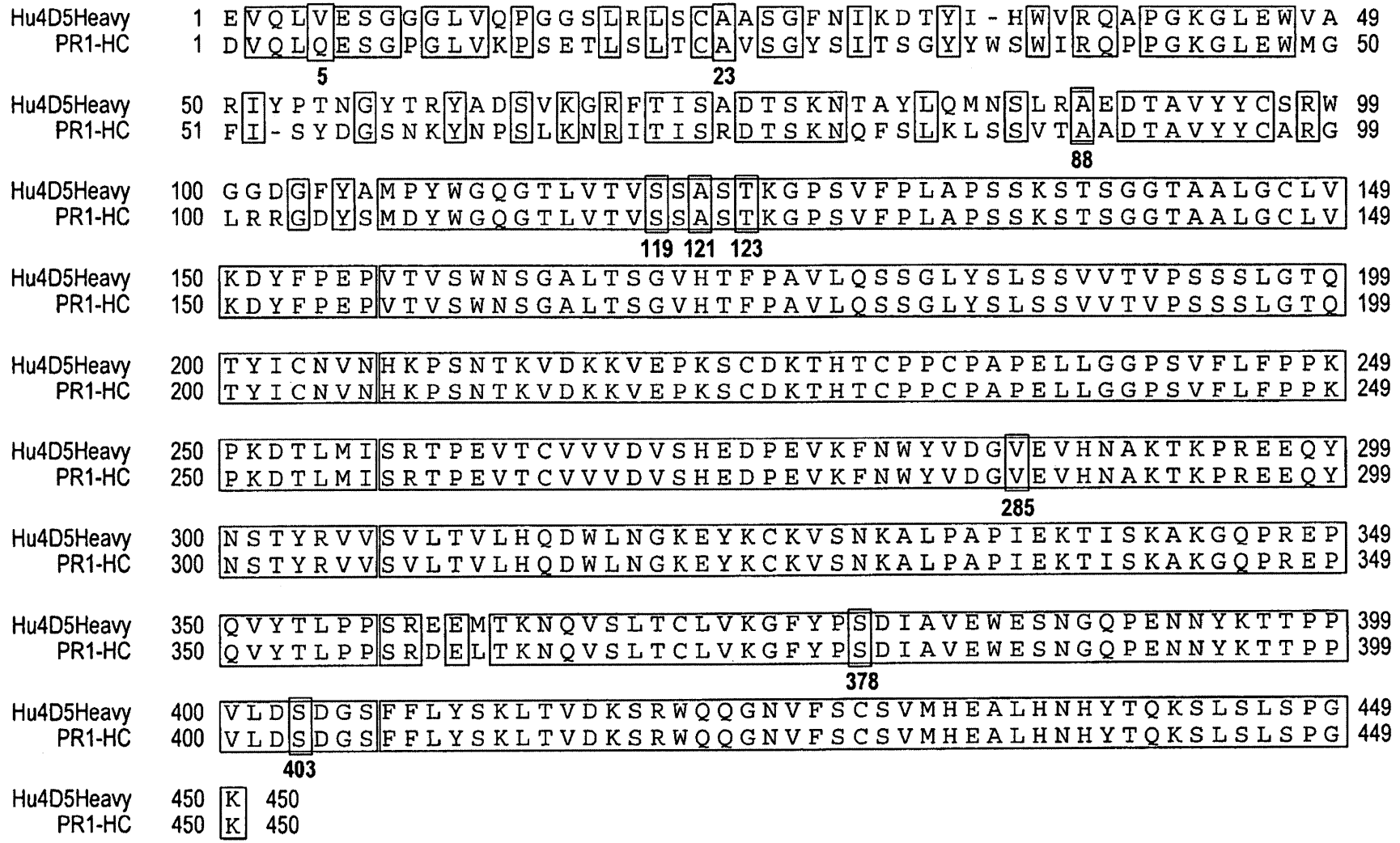
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The numbering denotes for PR1 (anti-TENB2) light chain according to sequential numbering

Figure 3

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The numbering denotes for PR1 (anti-TENB2) heavy chain according to sequential numbering

Figure 4

SUBSTITUTE SHEET (RULE 26)

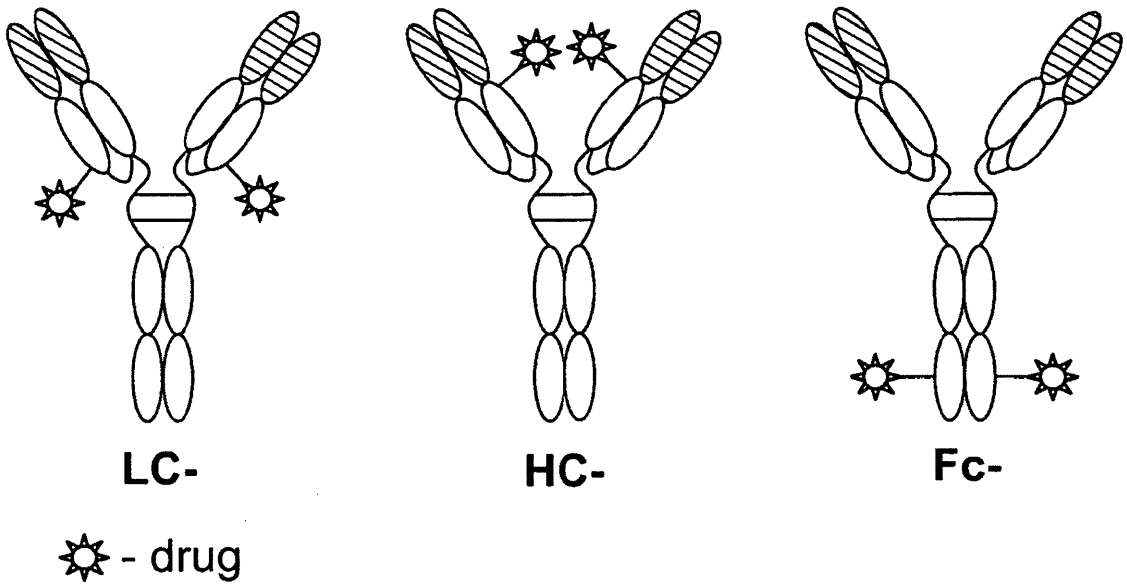


Figure 5

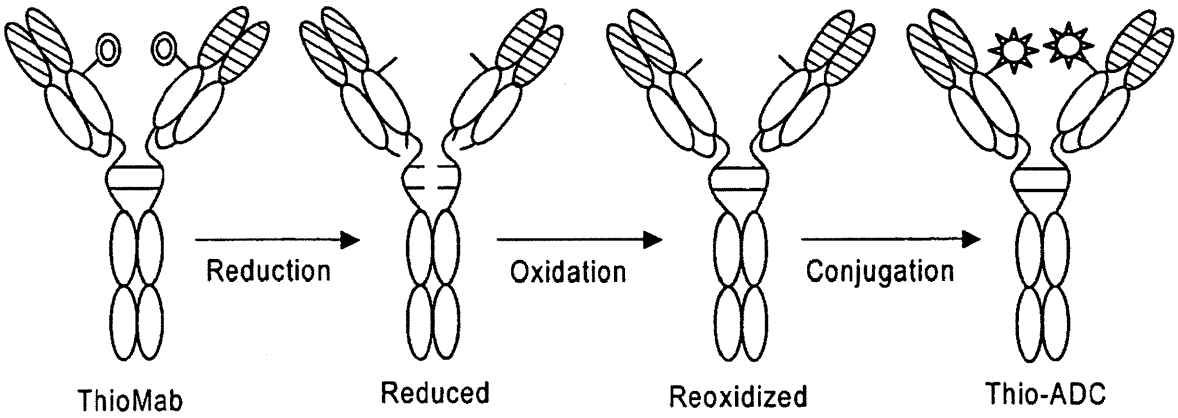

Reduction
Oxidation
Conjugation
ThioMab
Reduced
Reoxidized
Thio-ADC

Figure 6

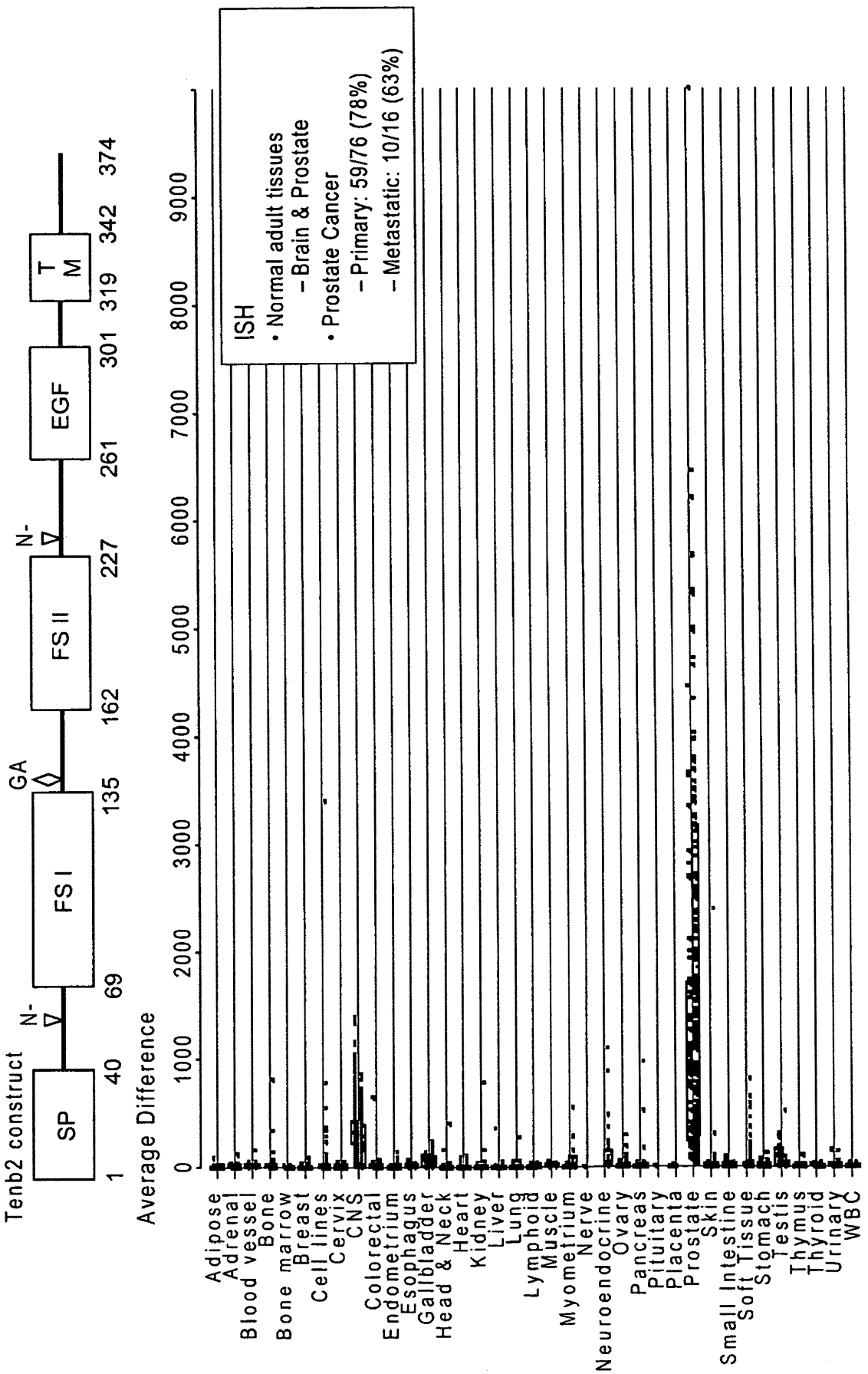
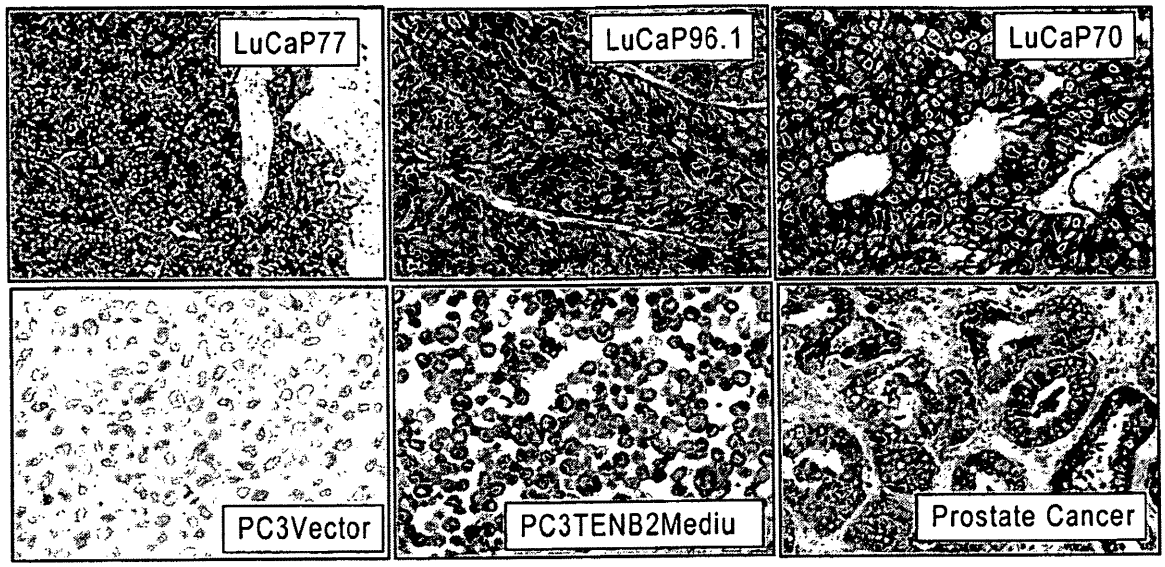

Tenb2 construct
SP
FS I
FS II
EGF
T
M
N-
GA
N-
1
40
69
135
162
227
261
301
319
342
374
Average Difference
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
ISH
• Normal adult tissues
– Brain & Prostate
• Prostate Cancer
– Primary: 59/76 (78%)
– Metastatic: 10/16 (63%)
Adipose
Adrenal
Blood vessel
Bone
Bone marrow
Breast
Cell lines
Cervix
CNS
Colorectal
Endometrium
Esophagus
Gallbladder
Head & Neck
Heart
Kidney
Liver
Lung
Lymphoid
Muscle
Myometrium
Nerve
Neuroendocrine
Ovary
Pancreas
Pituitary
Placenta
Prostate
Skin
Small Intestine
Soft Tissue
Stomach
Testis
Thymus
Thyroid
Urinary
WBC

Figure 7

IHC for TENB2 with anti-TENB2



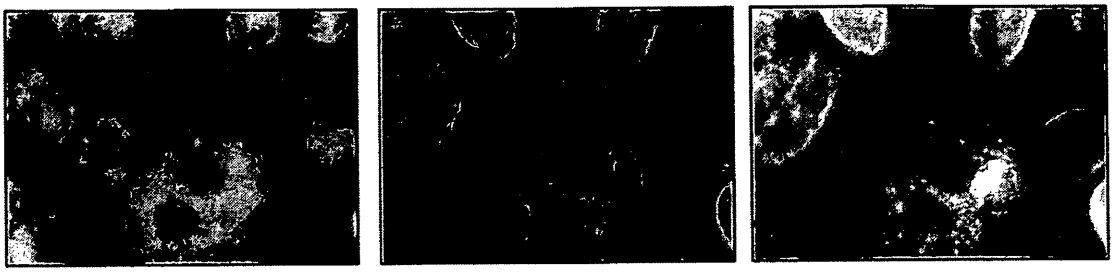
Immunohistochemistry: TENB2 expression in human prostate tumors

Top and bottom panels are from human prostate explant models, PC3TENB2 medium stable cell line with vector control and prostate tumor, respectively

Figure 8

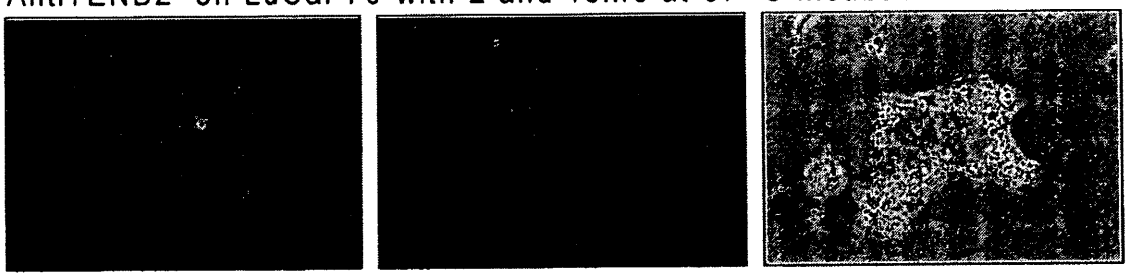
Internalization of TENB2 monoclonal antibody (Mab) on PC3TENB2Medium cell line and Lucap 70 tumor

AntiTENB2 on PC3TENB2 medium with 2hrs treatment at 37°C



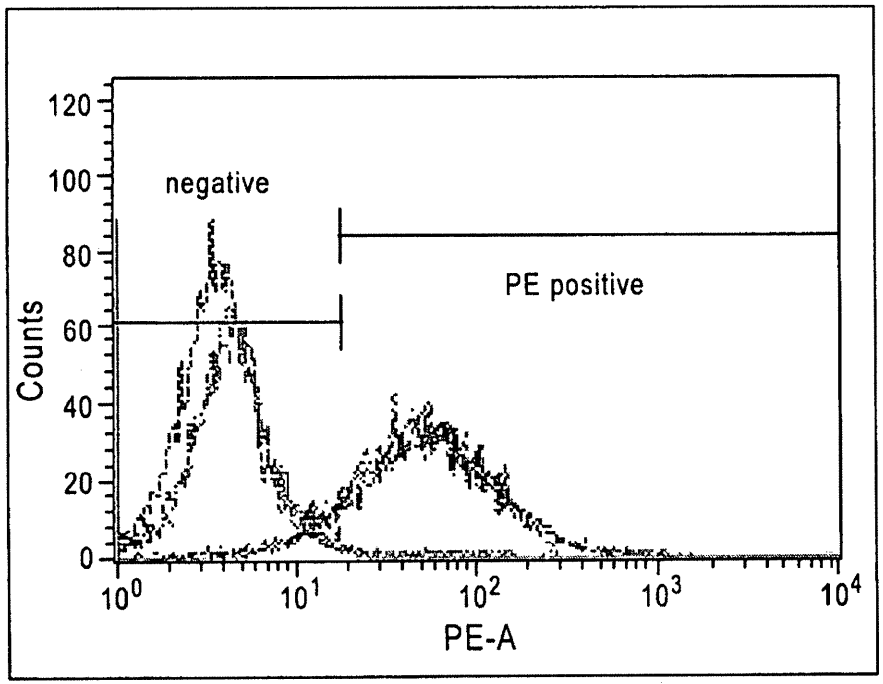
Anti-LAMP1 (lysosomal marker) antiTENB2 Combining

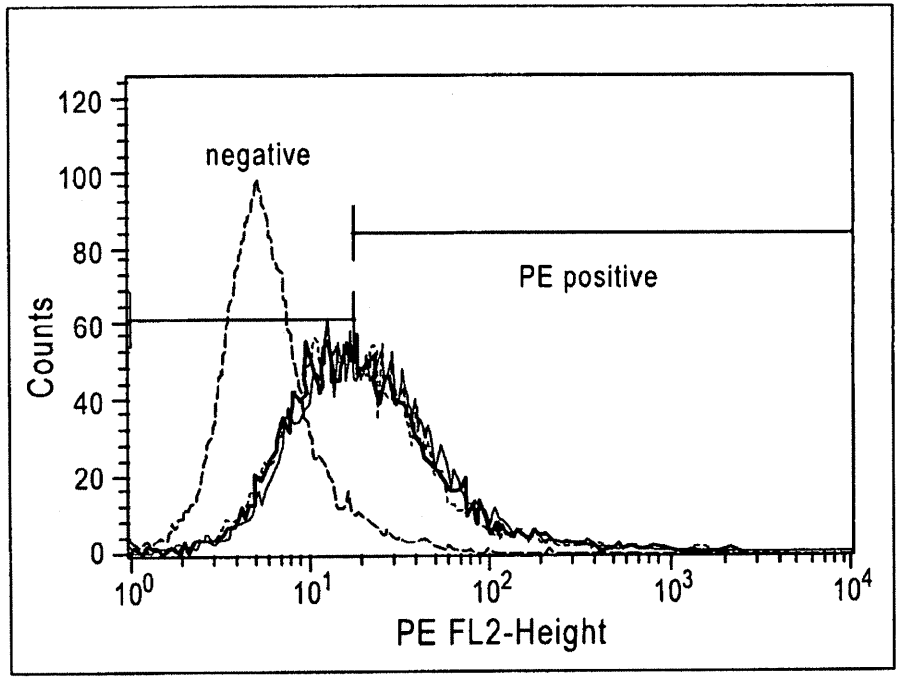
AntiTENB2 on LuCaP70 with 2 and 18hrs at 37° C incubation



AntiTENB2 AB internalized in PC3TENB2Medium cells faster than LuCaP70 tumor
AntiTENB2 AB was internalized more than 60% on LuCap70 cells at 18 hours

Figure 9





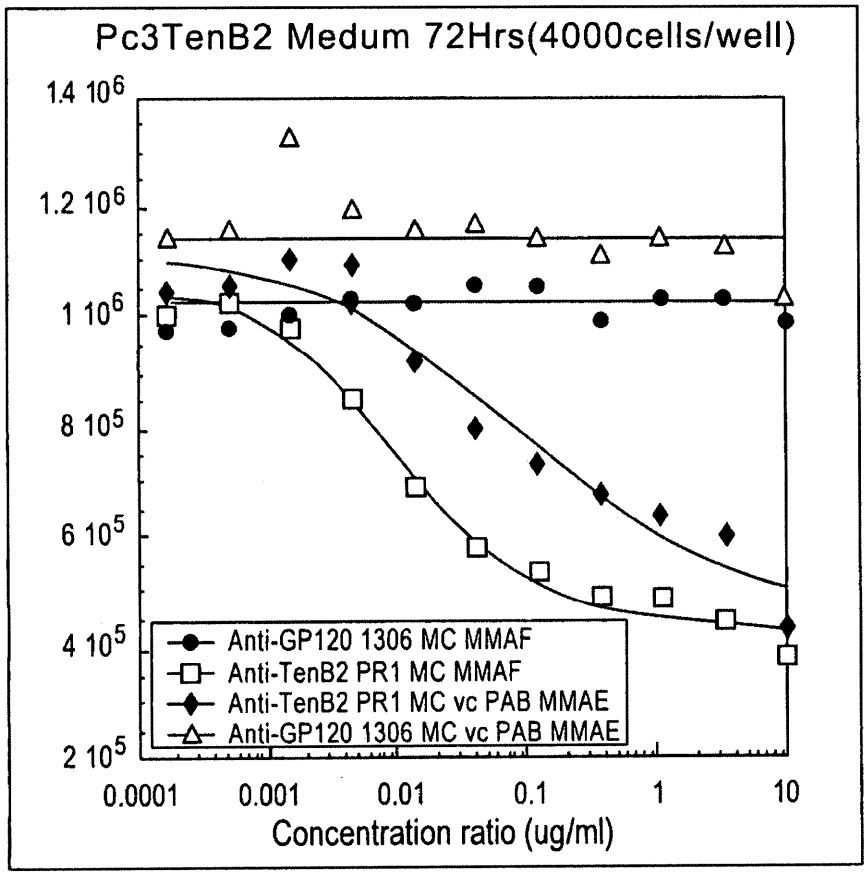
Name	Parameter	Gat	
WM_060506_LSR_Tube_001.fcs	PE-A	G1	Second AB
WM_060506_LSR_Tube_002.fcs	PE-A	G1	PR1(Pur12076)
WM_060506_LSR_Tube_003.fcs	PE-A	G1	Anti-GP120 1306 MC MMAF
WM_060506_LSR_Tube_004.fcs	PE-A	G1	Anti-TenB2 PR1 MC MMAF
WM_060506_LSR_Tube_005.fcs	PE-A	G1	Anti-TenB2 PR1 MC vc PAB MMAE
WM_060506_LSR_Tube_006.fcs	PE-A	G1	Anti-GP120 1306 MC vc PAB MMAE

	Name	Parameter	Gat	
- - -	WM _011007_S1.001	FL2-H	G1	1second AB
——	WM _011007_S1.002	FL2-H	G1	2PR1
-----	WM _011007_S1.003	FL2-H	G1	3CNJ1086 ThioPR1F
——	WM _011007_S1.004	FL2-H	G1	4CNJ1133 ThioPR1E

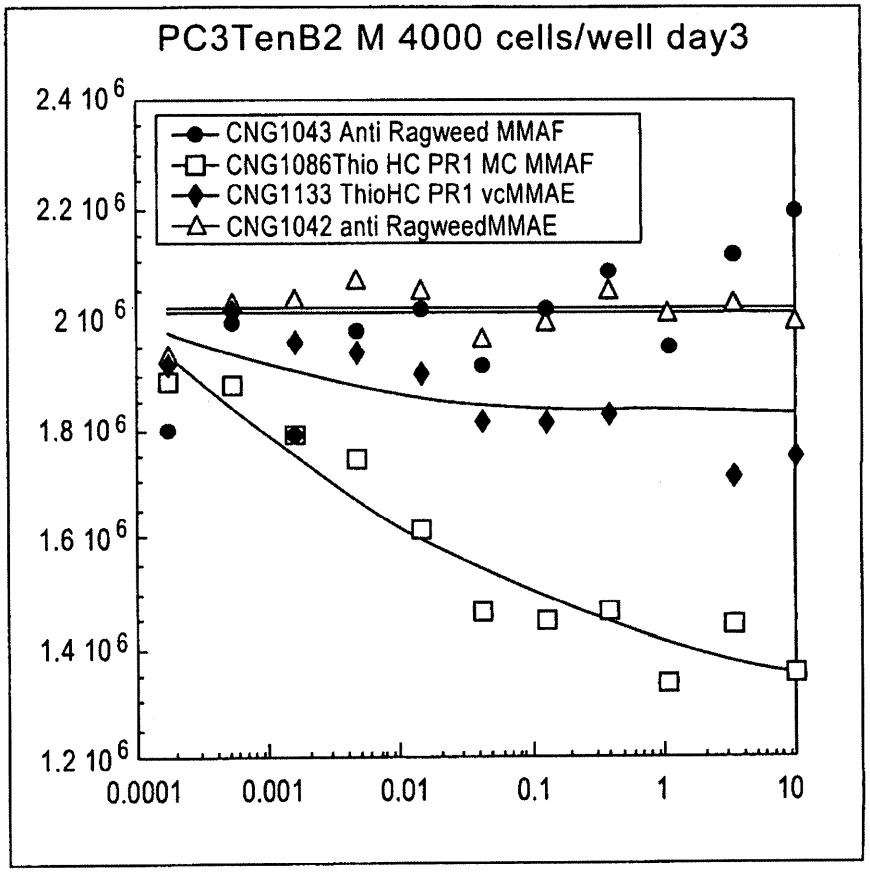
Figure 10

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Cell killing Assay on PC3TENB2 Medium cells with conventional anti-TENB2 and thio-anti-TENB2 ADCs



Conventional anti-TENB2-ADCs



thio-anti-TENB2-ADCs

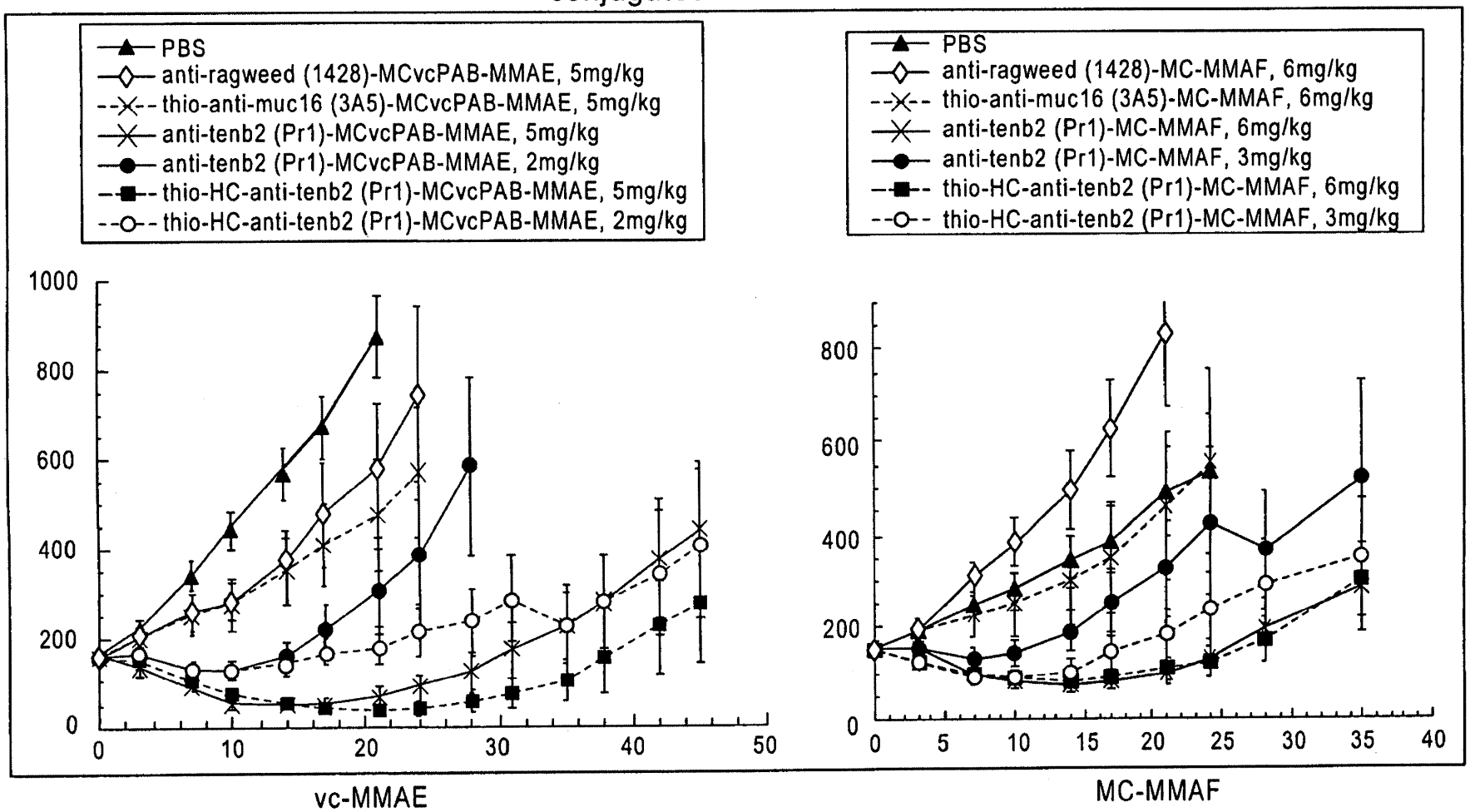
TENB2 -expressing PC3 cells was killed by conventional or thio-anti-TENB2 ADCs. The PC3 cell lines stably over expressing TENB2(PC3TENB2 Medium) was treated with increasing concentrations of conventional, Thio-anti-TENB2ADCs and control ADCs. Cell viability was measured after 3 days

Figure 11

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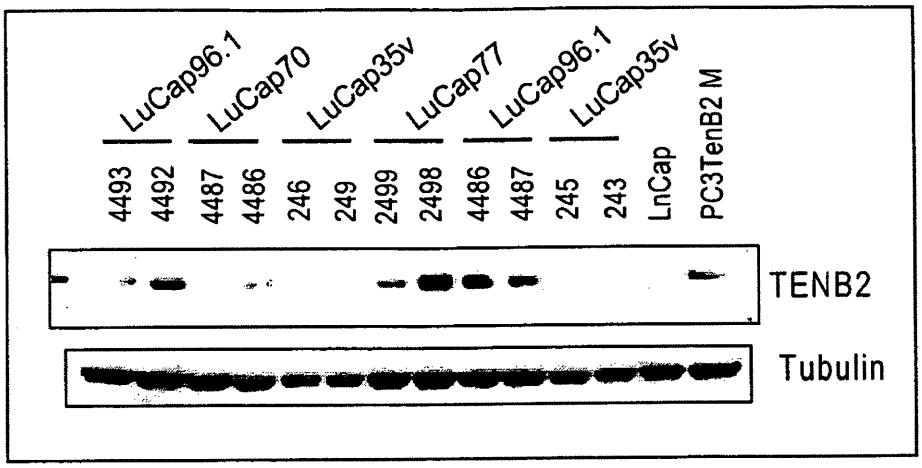
Efficacy study on PC3TENB2 Medium cells using anti-TENB2 and thio-anti-TENB2 conjugated with vc-MMAE or MC-MMAF



The efficacy of the thio-anti-TENB2 ADCs is equal or better than ADCs produced by the conventional method

Figure 12

Western Blot for TENB2 with different LuCaPtumors

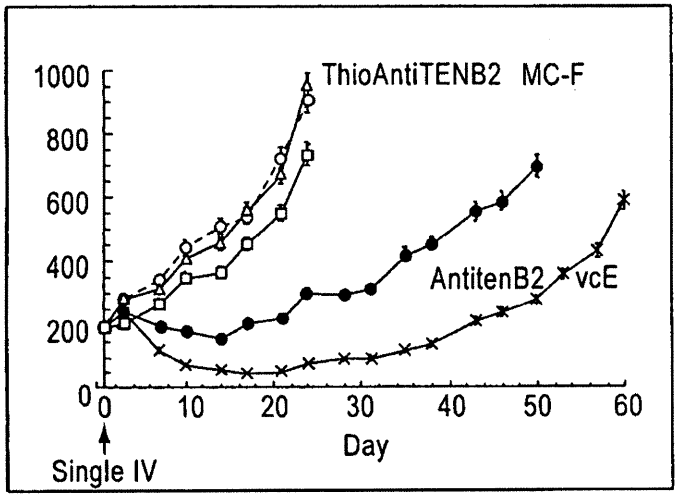


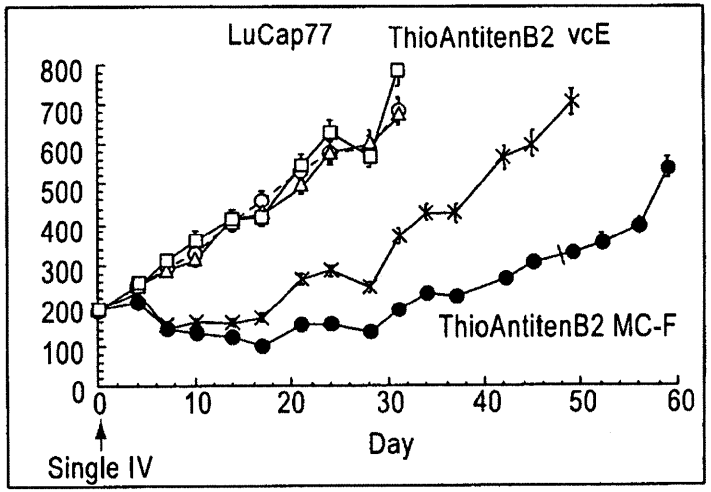
Different level TENB2 protein expression levels observed on LuCaP transplant tumors, in which LuCaP77 is highest and LuCaP35V is lowest This data is consistent to our Taqman data on those tumor lines (data not show in here)

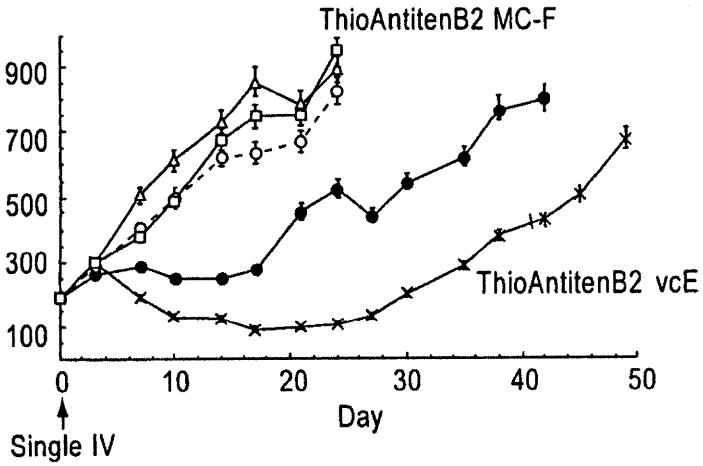
Figure 13

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Human Prostate Cancer LuCaP70, 77 and 96.1 xenograft
We found a good correlation between efficacy and expression level of TENB2 with those models

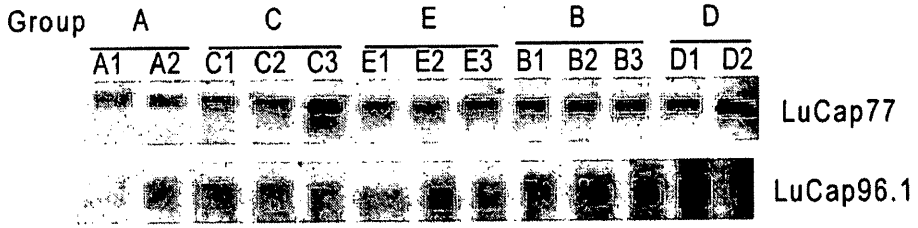






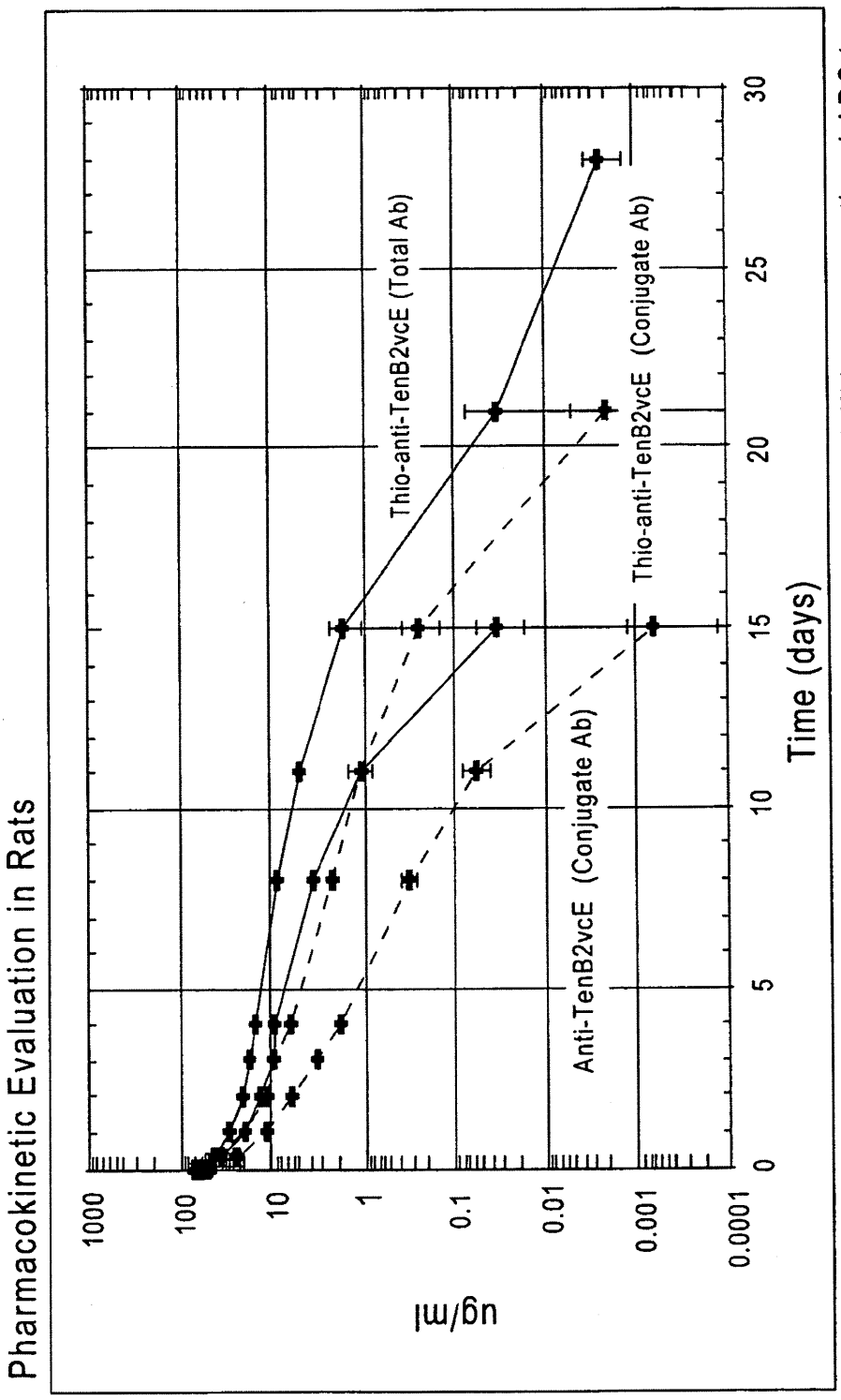
A-Vehicle
B-thio-HC-anti-muc16-MCvcPAB-MMAE, 5 mg/kg
C-thio-HC-anti-muc16-MC-MMAF, 5 mg/kg
D-thio-HC-ant-tenb2-MCvcPAB-MMAE, 5 mg/kg
E-thio-HC-anti-tenb2-MC-MMAF, 5 mg/kg

TenB2 Western blot on Lucap77 and 96.1 tumors with different treatment groups



Note: TenB2 expression level not different between groups

Figure 14



Serum TENB2 ADC concentrations following a 5mg/kg IV bolus dose of Thio or conventional ADC to rats

Thio-anti-TENB2 ADCs are more stable in the serum than conventional anti-TENB2 ADCs

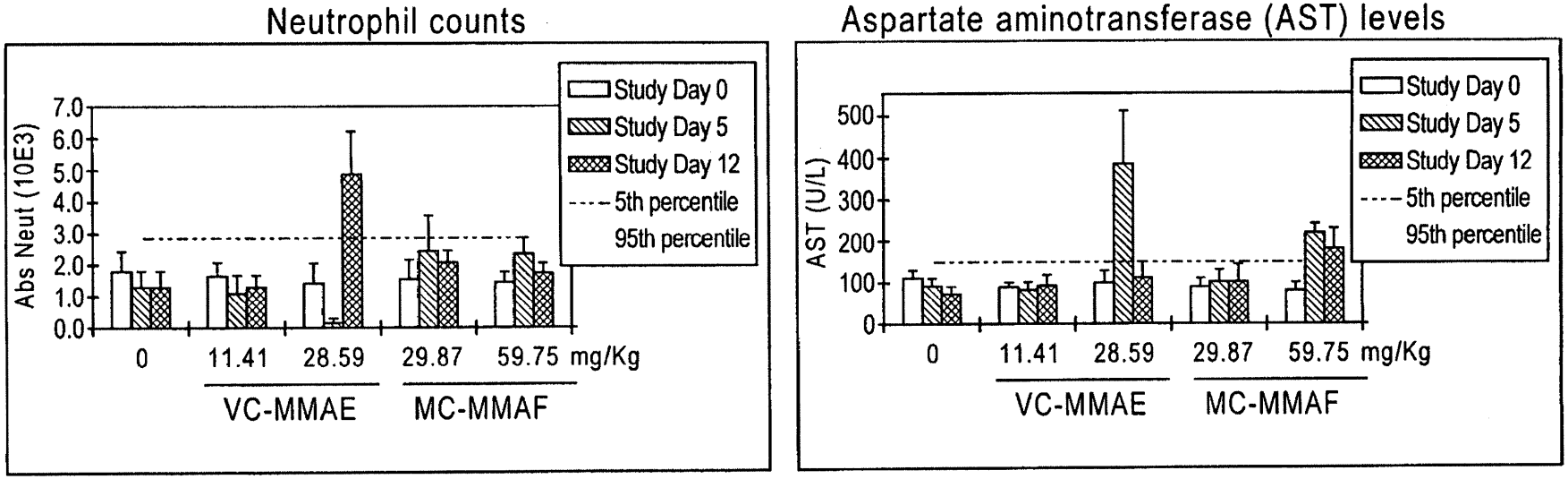
anti-TENB2vcE = anti-TENB2-vc-MMAE

thio-anti-TENB2vcE = thio-anti-TENB2-vc-MMAE

Figure 15

Safety Assessment

Anti-TENB2-vc-MMAE and –MC-MMAF in rats:

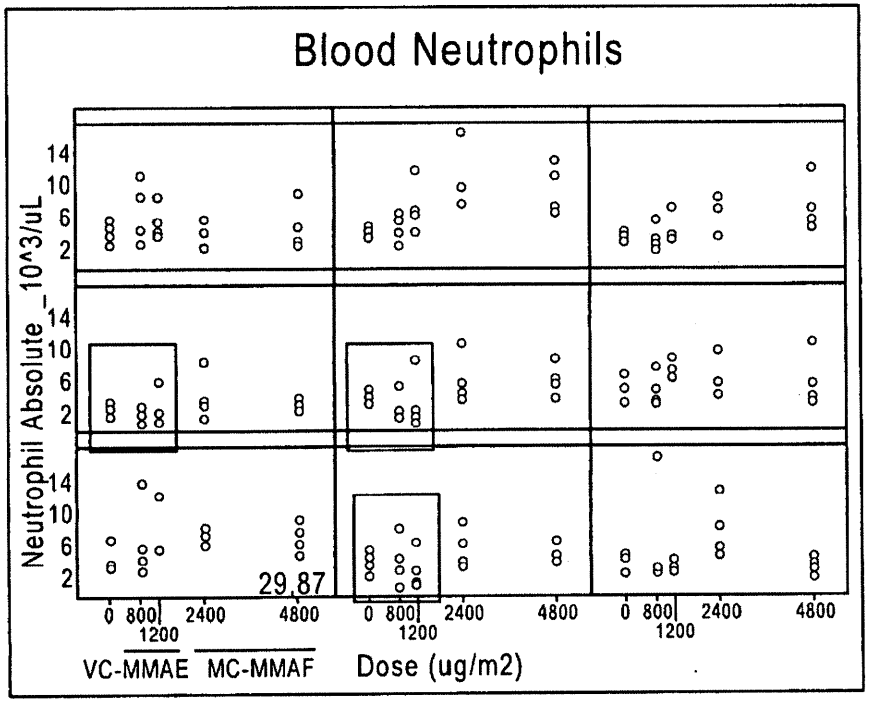


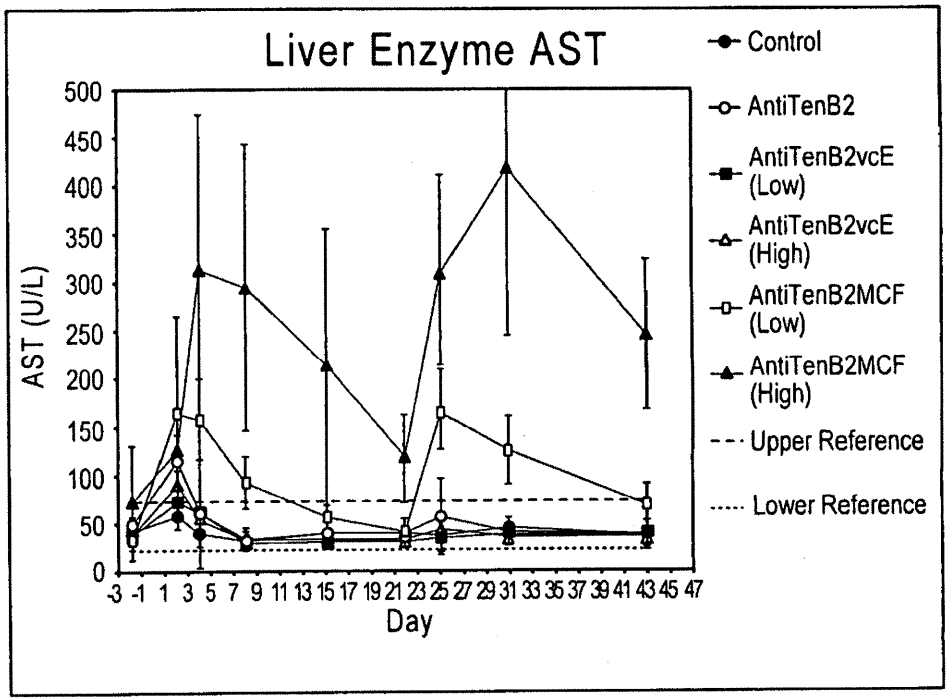
Toxicology studies with anti-TENB2 ADCs in rats and cynomolgus monkeys (both binding species) were performed and revealed no target-related toxicities in normal tissues and non-target-dependent toxicities at doses substantially higher than the anticipated efficacious dose

Figure 16

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Anti-TENB2-vc-MMAE and –MC-MMAF in cynomolgus monkeys:





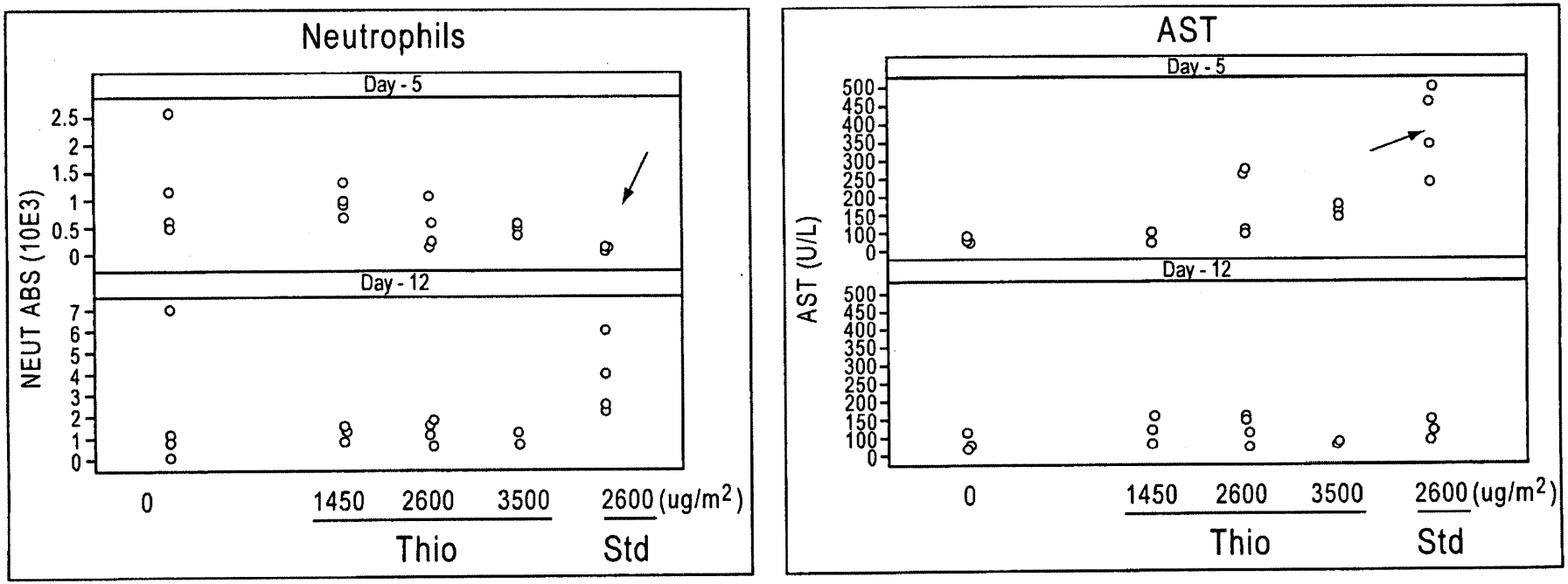
Groups: 1 Vehicle, 2 Naked PR1, 3 and 4 PR1vcE (800,1200 ug/m ^{2}), 5 and 6 PR1MC F (2400, 4800 ug/m ^{2}). Note: 800ug/m^2 equivalent to 8.7mg/kg.

Toxicology studies with anti-TENB2 ADCs in rats and cynomolgus monkeys (both binding species) were performed and revealed no target-related toxicities in normal tissues and non-target-dependent toxicities at doses substantially higher than the anticipated efficacious dose

Figure 17

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Thio-anti-TENB2-vc-MMAE compared with anti-TENB2-vc-MMAE in rats



Thio-anti-TENB2-vc-MMAE: improvement in neutrophil counts and AST levels

Figure 18

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