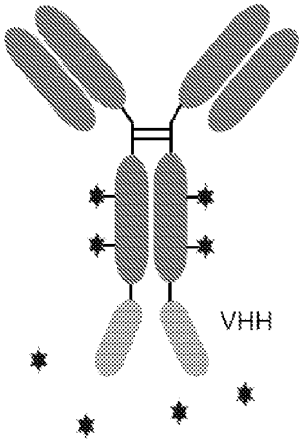


图1

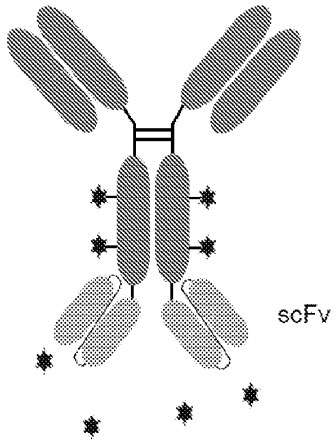


图2

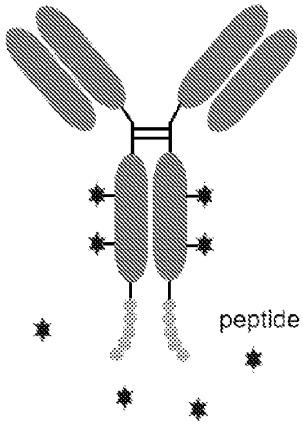


图3

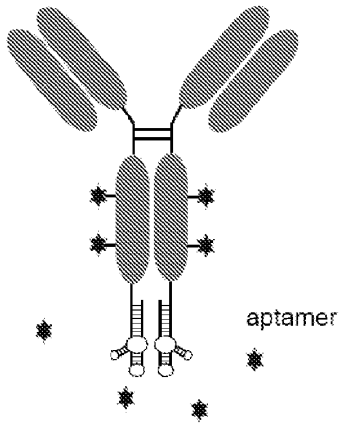


图4

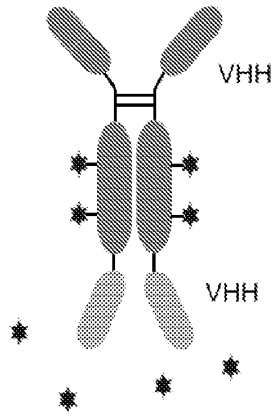


图5

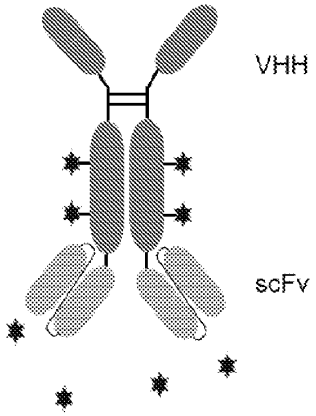


图6

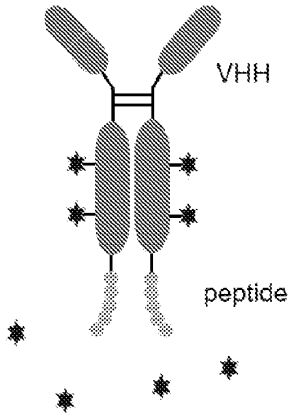


图7

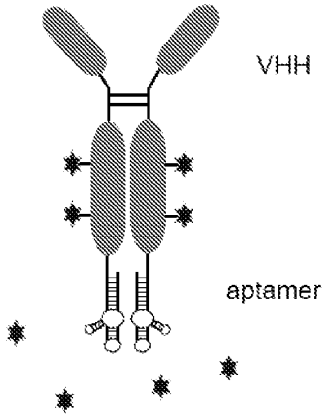


图8

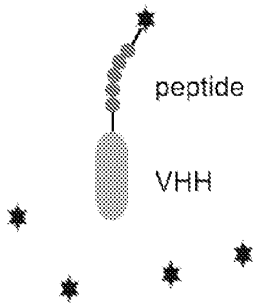


图9

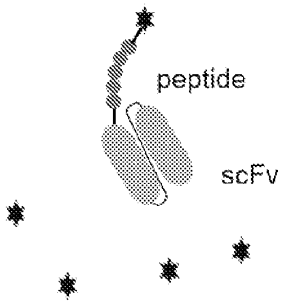


图10

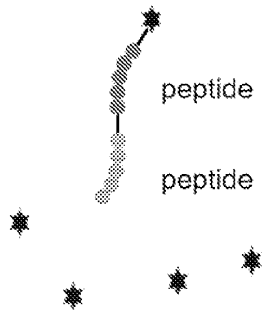


图11

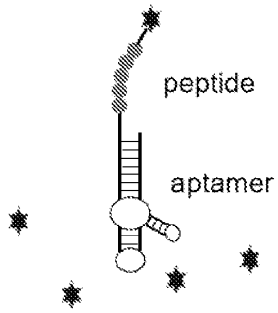


图12

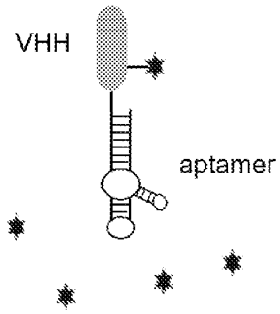


图13

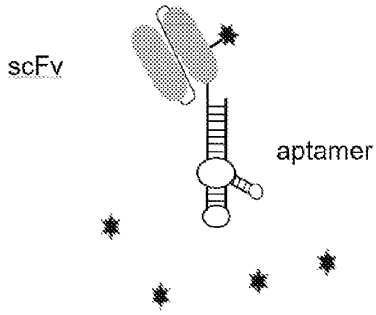


图14

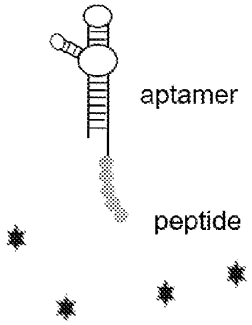


图15

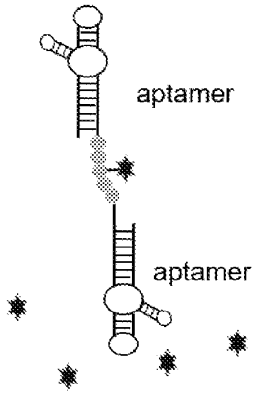


图16

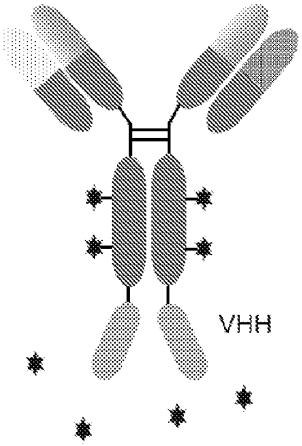


图17

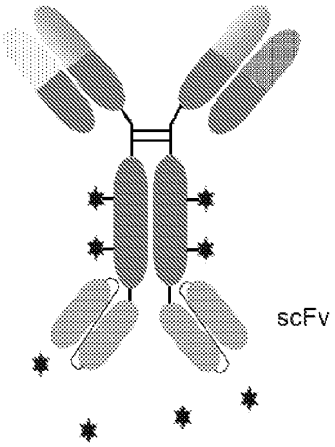


图18

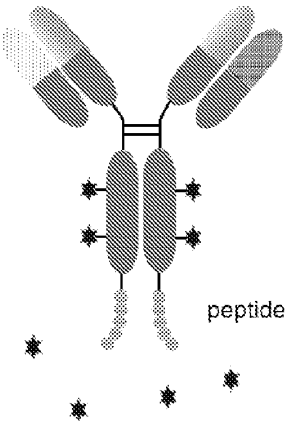


图19

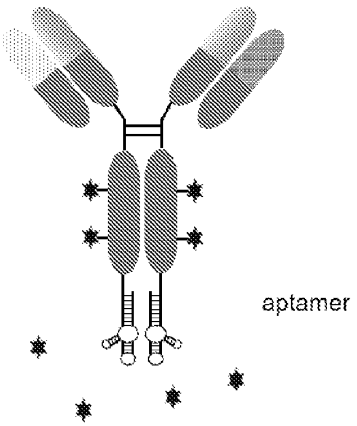


图20

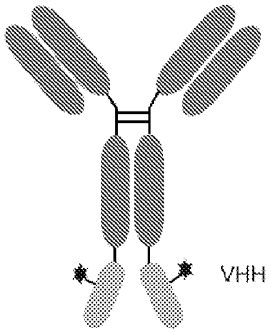


图21

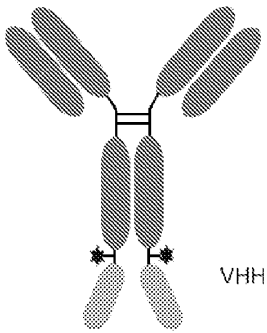


图22

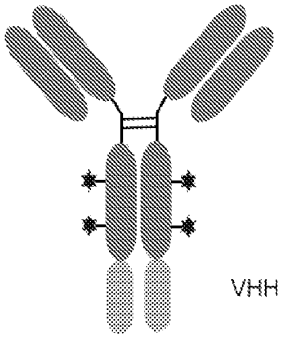

VHH

图23

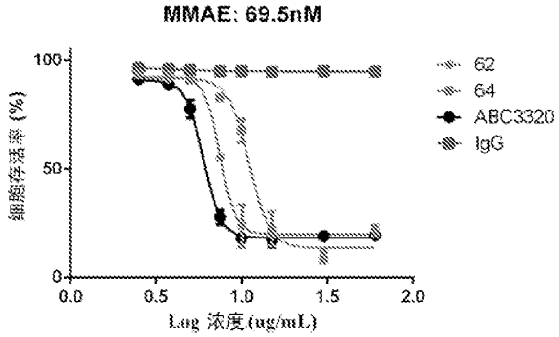

MMAE: 69.5nM
细胞存活率 (%)
100
50
0
0.0
0.5
1.0
1.5
2.0
Log 浓度(ug/mL)
62
64
ABC3320
IgG

图 24

5

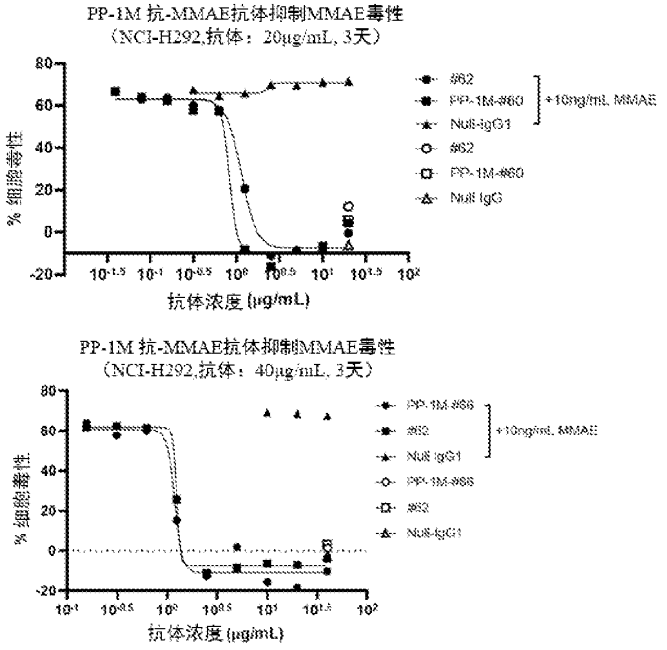

PP-1M 抗-MMAE抗体抑制MMAE毒性
（NCI-H292,抗体：20μg/mL, 3天）
% 细胞毒性
抗体浓度 (μg/mL)
#62
PP-1M-#60
Null-IgG1
#62
PP-1M-#60
Null IgG
+10ng/mL MMAE
PP-1M 抗-MMAE抗体抑制MMAE毒性
（NCI-H292,抗体：40μg/mL, 3天）
% 细胞毒性
抗体浓度 (μg/mL)
+10ng/mL MMAE

图 25

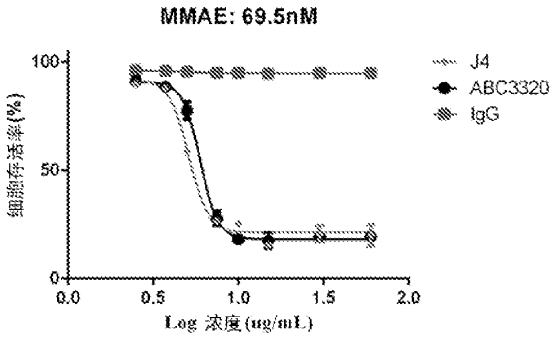

MMAE: 69.5nM
细胞存活率(%)
Log 浓度(ug/mL)
J4
ABC3320
IgG

图 26

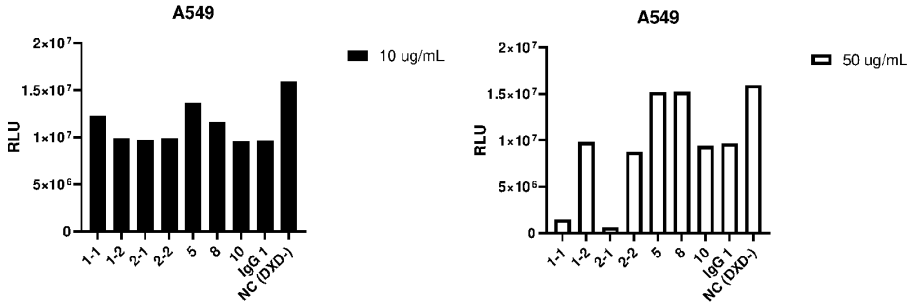

A549
10 ug/mL
RLU
2×10^7
1.5×10^7
1×10^7
5×10^6
0
1-1
1-2
2-1
2-2
5
8
10
IgG 1
NC (DXD-)
A549
50 ug/mL
RLU
2×10^7
1.5×10^7
1×10^7
5×10^6
0
1-1
1-2
2-1
2-2
5
8
10
IgG 1
NC (DXD-)

图 27

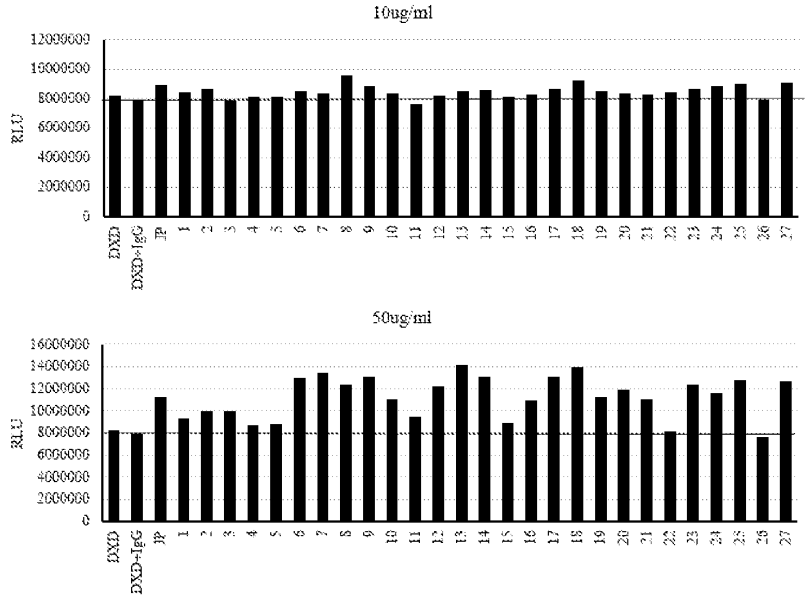

10ug/ml
RLU
12000000
10000000
8000000
6000000
4000000
2000000
0
DXD
DXD-IgG
IP
50ug/ml
RLU
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
DXD
DXD-IgG
IP

图 28

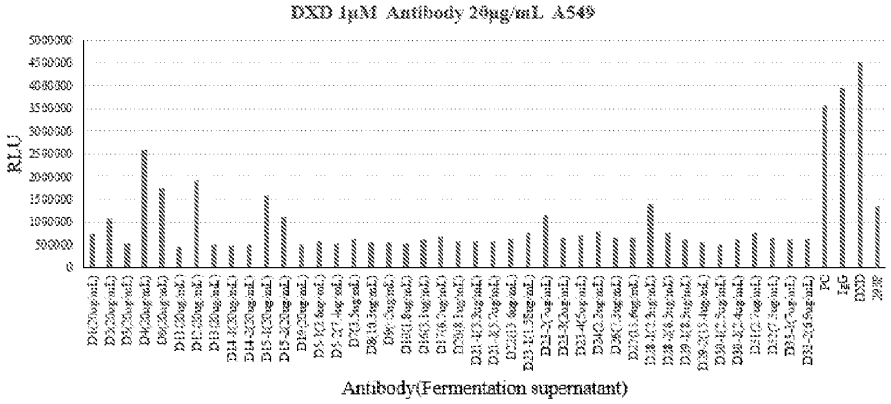

DXD 1μM Antibody 20μg/mL A549
RLU
Antibody(Fermentation supernatant)
PC
IgG
DXD

图 29

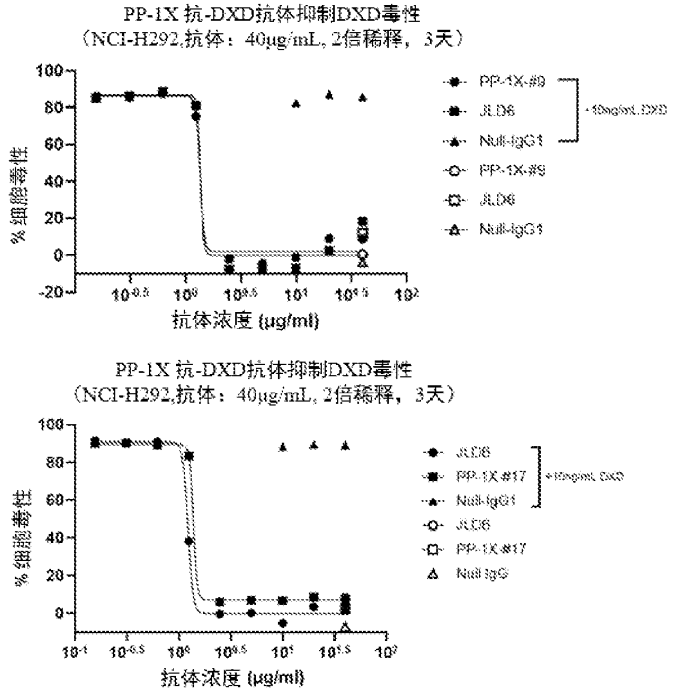

PP-1X 抗-DXD抗体抑制DXD毒性
（NCI-H292,抗体：40μg/mL, 2倍稀释，3天）
% 细胞毒性
抗体浓度 (μg/ml)
PP-1X-#9
JLD6
Null-IgG1
PP-1X-#9
JLD6
Null-IgG1
PP-1X 抗-DXD抗体抑制DXD毒性
（NCI-H292,抗体：40μg/mL, 2倍稀释，3天）
% 细胞毒性
抗体浓度 (μg/ml)
JLD6
PP-1X-#17
Null-IgG1
JLD6
PP-1X-#17
Null IgG

图 30

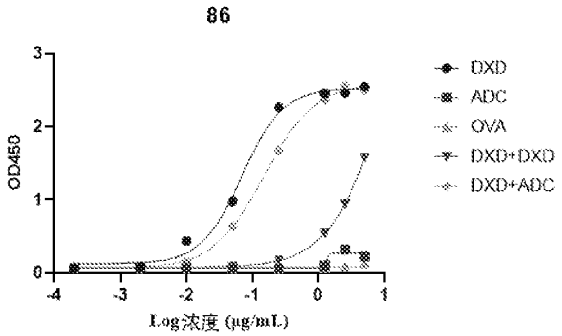

86
OD450
Log浓度 (μg/mL)
DXD
ADC
OVA
DXD+DXD
DXD+ADC

图 31

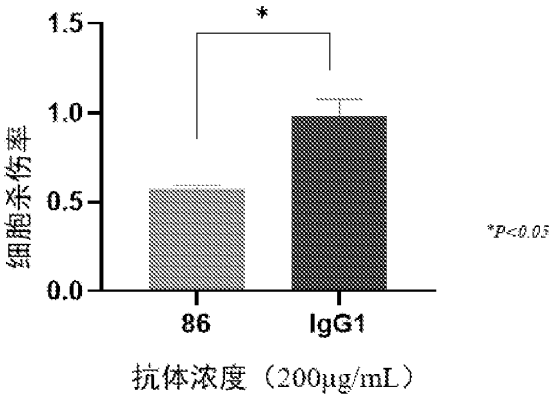

1.5
1.0
0.5
0.0
细胞杀伤率
*
*P<0.05
86
IgG1
抗体浓度（200μg/mL）

图 32

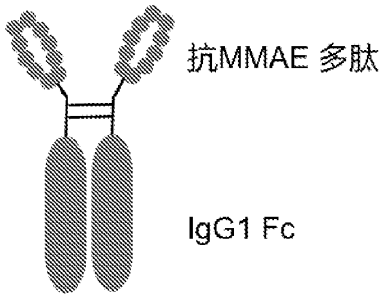
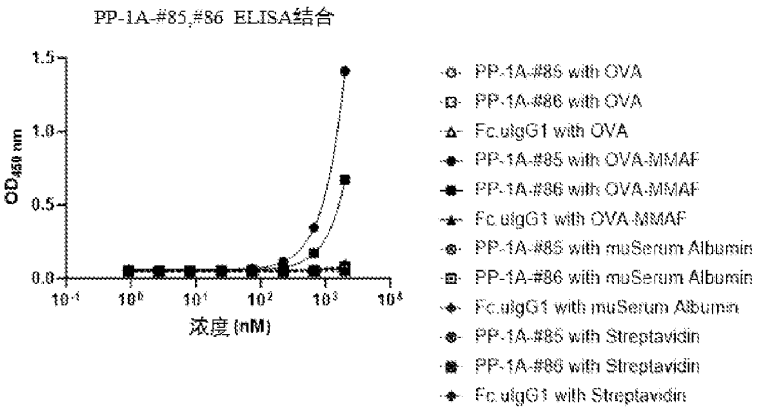
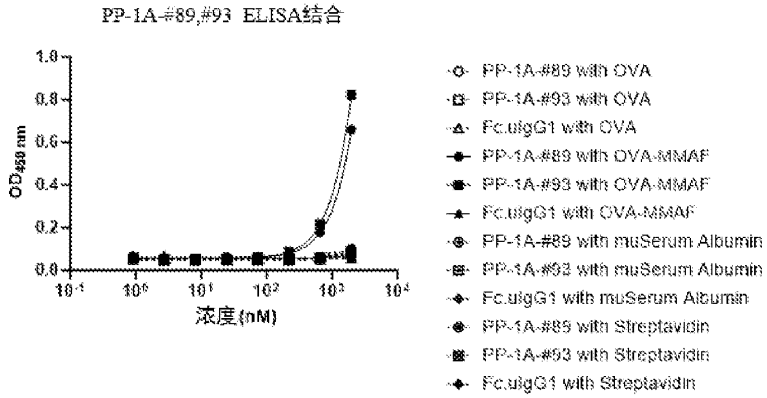

抗MMAE 多肽
IgG1 Fc

图 33


PP-1A-#85,#86 ELISA结合
OD450 nm
1.5
1.0
0.5
0.0
10-1
100
101
102
103
104
浓度 (nM)
PP-1A-#85 with OVA
PP-1A-#86 with OVA
Fc.ulgG1 with OVA
PP-1A-#85 with OVA-MMAF
PP-1A-#86 with OVA-MMAF
Fc.ulgG1 with OVA-MMAF
PP-1A-#85 with muSerum Albumin
PP-1A-#86 with muSerum Albumin
Fc.ulgG1 with muSerum Albumin
PP-1A-#85 with Streptavidin
PP-1A-#86 with Streptavidin
Fc.ulgG1 with Streptavidin


PP-1A-#89,#93 ELISA结合
OD450 nm
浓度(nM)
PP-1A-#89 with OVA
PP-1A-#93 with OVA
Fc.uIgG1 with OVA
PP-1A-#89 with OVA-MMAF
PP-1A-#93 with OVA-MMAF
Fc.uIgG1 with OVA-MMAF
PP-1A-#89 with muSerum Albumin
PP-1A-#93 with muSerum Albumin
Fc.uIgG1 with muSerum Albumin
PP-1A-#89 with Streptavidin
PP-1A-#93 with Streptavidin
Fc.uIgG1 with Streptavidin

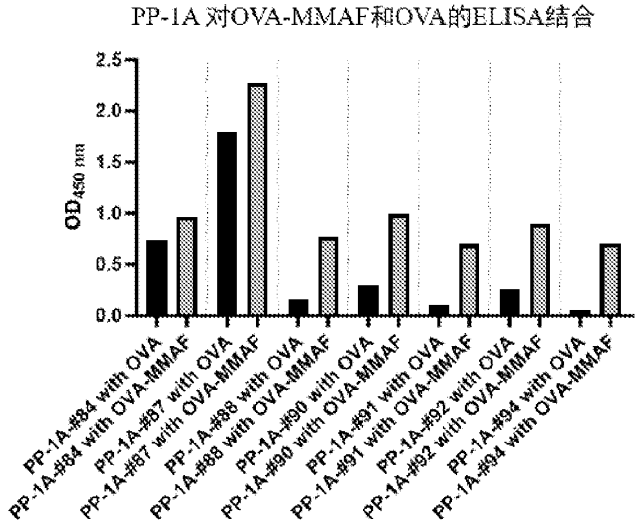

PP-1A 对OVA-MMAF和OVA的ELISA结合
OD450 nm
PP-1A-#84 with OVA
PP-1A-#84 with OVA-MMAF
PP-1A-#87 with OVA
PP-1A-#87 with OVA-MMAF
PP-1A-#88 with OVA
PP-1A-#88 with OVA-MMAF
PP-1A-#90 with OVA
PP-1A-#90 with OVA-MMAF
PP-1A-#91 with OVA
PP-1A-#91 with OVA-MMAF
PP-1A-#92 with OVA
PP-1A-#92 with OVA-MMAF
PP-1A-#94 with OVA
PP-1A-#94 with OVA-MMAF

图 34

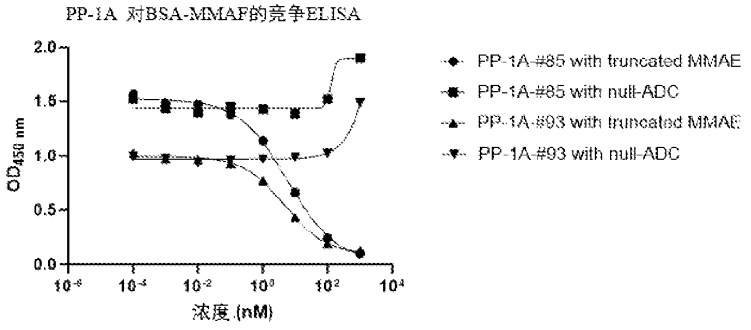

PP-1A 对BSA-MMAF的竞争ELISA
OD450 nm
浓度 (nM)
PP-1A-#85 with truncated MMAE
PP-1A-#85 with null-ADC
PP-1A-#93 with truncated MMAE
PP-1A-#93 with null-ADC

图 35

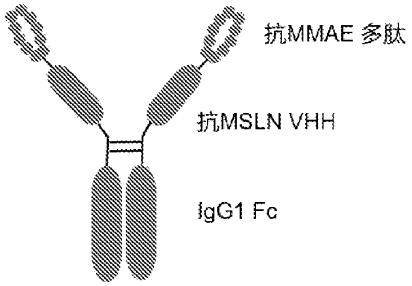

抗MMAE 多肽
抗MSLN VHH
IgG1 Fc

图 36

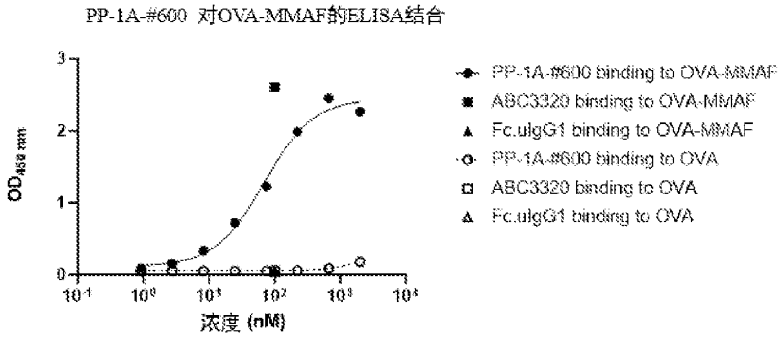

PP-1A-#600 对OVA-MMAF的ELISA结合
OD450 nm
浓度 (nM)
PP-1A-#600 binding to OVA-MMAF
ABC3320 binding to OVA-MMAF
Fc.uIgG1 binding to OVA-MMAF
PP-1A-#600 binding to OVA
ABC3320 binding to OVA
Fc.uIgG1 binding to OVA

图 37

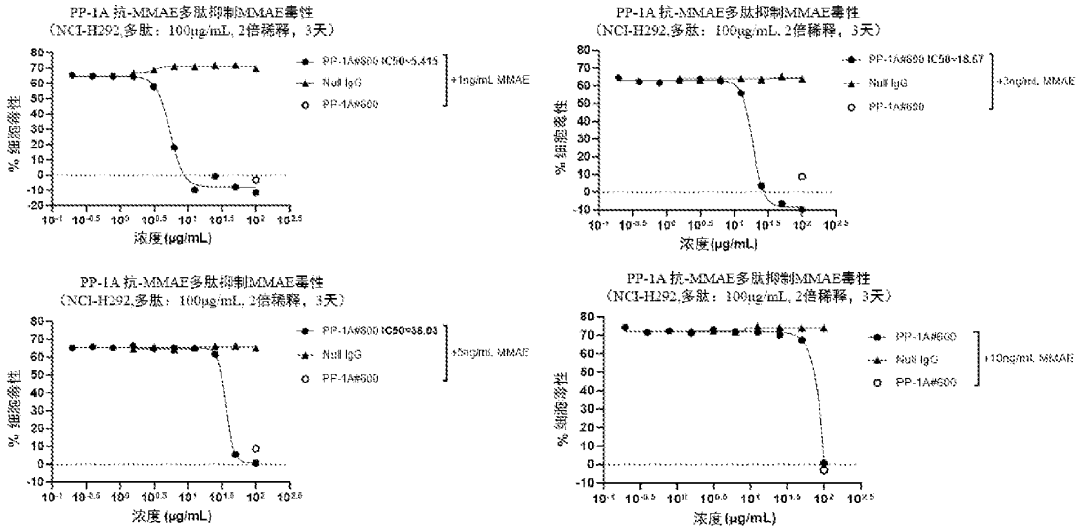

PP-1A 抗-MMAE多肽抑制MMAE毒性
（NCI-H292,多肽：100μg/mL, 2倍稀释，3天）
PP-1A6600 IC50=5.415
Null IgG
PP-1A6600
+1ng/mL MMAE
% 细胞毒性
浓度(μg/mL)
PP-1A 抗-MMAE多肽抑制MMAE毒性
（NCI-H292,多肽：100μg/mL, 2倍稀释，3天）
PP-1A6600 IC50=18.67
Null IgG
PP-1A6600
+3ng/mL MMAE
% 细胞毒性
浓度(μg/mL)
PP-1A 抗-MMAE多肽抑制MMAE毒性
（NCI-H292,多肽：100μg/mL, 2倍稀释，3天）
PP-1A6600 IC50=38.03
Null IgG
PP-1A6600
+5ng/mL MMAE
% 细胞毒性
浓度(μg/mL)
PP-1A 抗-MMAE多肽抑制MMAE毒性
（NCI-H292,多肽：100μg/mL, 2倍稀释，3天）
PP-1A6600
Null IgG
PP-1A6600
+10ng/mL MMAE
% 细胞毒性
浓度(μg/mL)

图 38

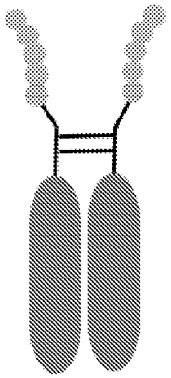


图 39

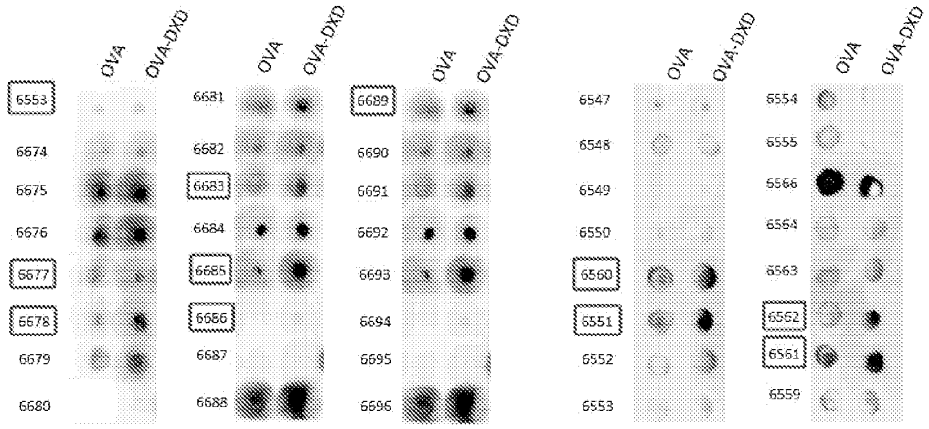
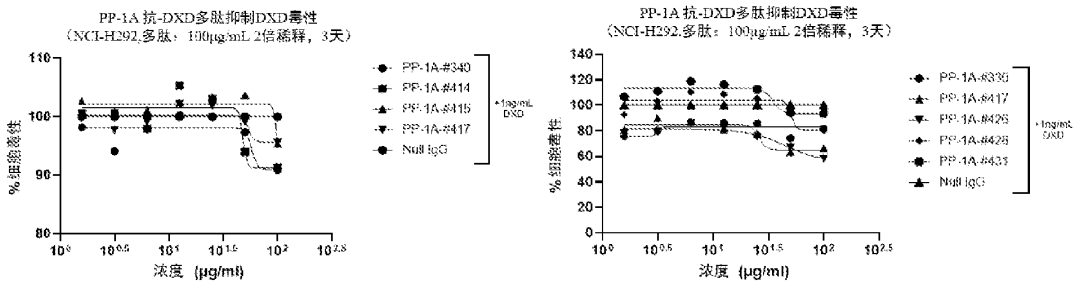


图 40



5 图 41

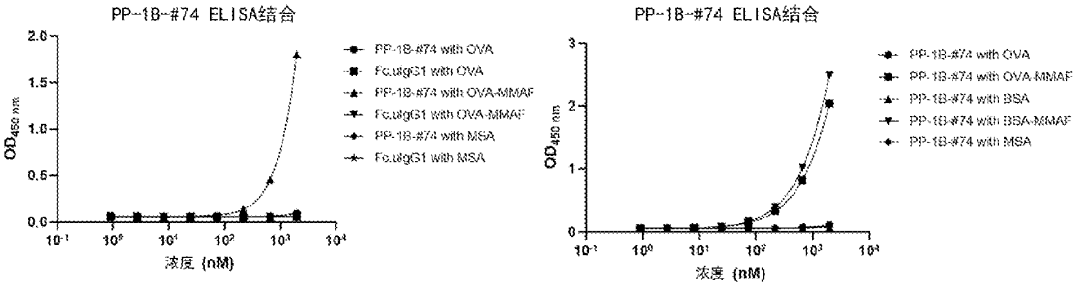


图 42

10

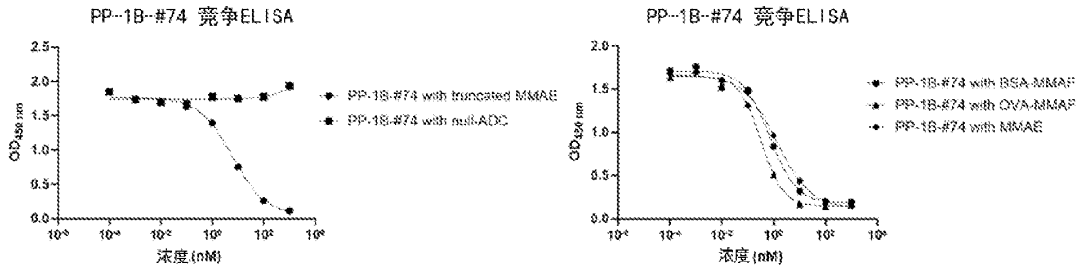

PP--1B--#74 竞争ELISA
OD450 nm
浓度(nM)
PP-1B-#74 with truncated MMAE
PP-1B-#74 with null-ADC
PP--1B--#74 竞争ELISA
OD450 nm
浓度(nM)
PP-1B-#74 with BSA-MMAF
PP-1B-#74 with OVA-MMAF
PP-1B-#74 with MMAE

图 43

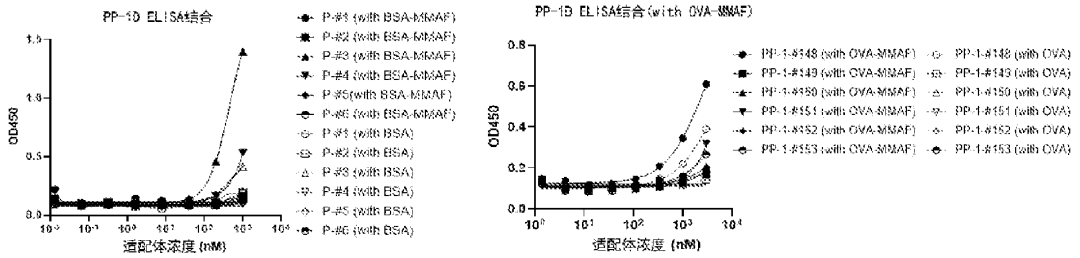

PP-1B ELISA结合
OD450
适配体浓度 (nM)
P-#1 (with BSA-MMAF)
P-#2 (with BSA-MMAF)
P-#3 (with BSA-MMAF)
P-#4 (with BSA-MMAF)
P-#5(with BSA-MMAF)
P-#6 (with BSA-MMAF)
P-#1 (with BSA)
P-#2 (with BSA)
P-#3 (with BSA)
P-#4 (with BSA)
P-#5 (with BSA)
P-#6 (with BSA)
PP-1B ELISA结合(with OVA-MMAF)
OD450
适配体浓度 (nM)
PP-1-#148 (with OVA-MMAF)
PP-1-#149 (with OVA-MMAF)
PP-1-#150 (with OVA-MMAF)
PP-1-#151 (with OVA-MMAF)
PP-1-#152 (with OVA-MMAF)
PP-1-#153 (with OVA-MMAF)
PP-1-#148 (with OVA)
PP-1-#149 (with OVA)
PP-1-#150 (with OVA)
PP-1-#151 (with OVA)
PP-1-#152 (with OVA)
PP-1-#153 (with OVA)

图 44

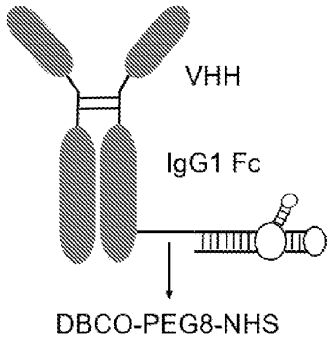

VHH
IgG1 Fc
DBCO-PEG8-NHS

图 45

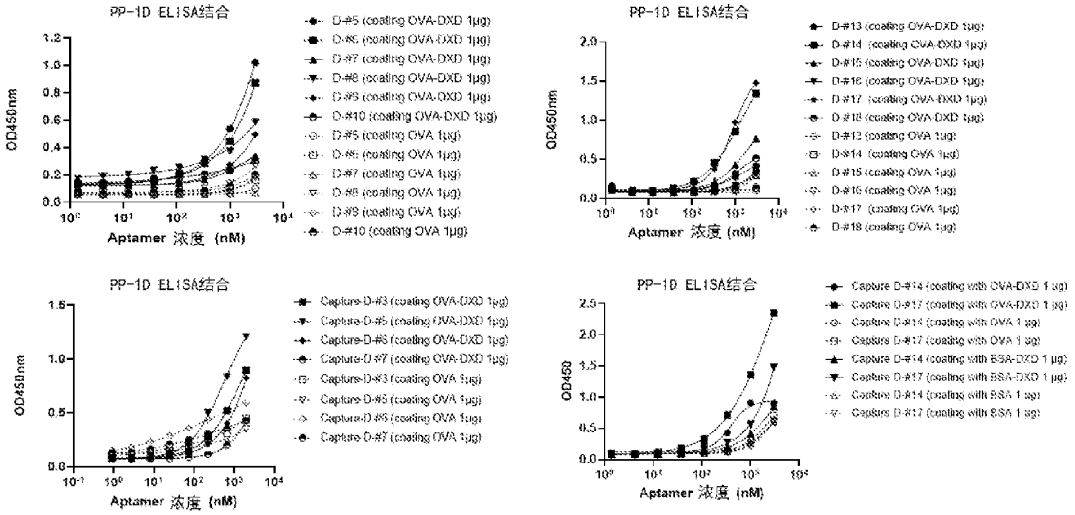

PP-1D ELISA结合
OD450nm
Aptamer 浓度 (nM)
D-#5 (coating OVA-DXD 1μg)
D-#6 (coating OVA-DXD 1μg)
D-#7 (coating OVA-DXD 1μg)
D-#8 (coating OVA-DXD 1μg)
D-#9 (coating OVA-DXD 1μg)
D-#10 (coating OVA-DXD 1μg)
D-#5 (coating OVA 1μg)
D-#6 (coating OVA 1μg)
D-#7 (coating OVA 1μg)
D-#8 (coating OVA 1μg)
D-#9 (coating OVA 1μg)
D-#10 (coating OVA 1μg)
PP-1D ELISA结合
OD450nm
Aptamer 浓度 (nM)
D-#13 (coating OVA-DXD 1μg)
D-#14 (coating OVA-DXD 1μg)
D-#15 (coating OVA-DXD 1μg)
D-#16 (coating OVA-DXD 1μg)
D-#17 (coating OVA-DXD 1μg)
D-#18 (coating OVA-DXD 1μg)
D-#13 (coating OVA 1μg)
D-#14 (coating OVA 1μg)
D-#15 (coating OVA 1μg)
D-#16 (coating OVA 1μg)
D-#17 (coating OVA 1μg)
D-#18 (coating OVA 1μg)
PP-1D ELISA结合
OD450nm
Aptamer 浓度 (nM)
Capture-D-#3 (coating OVA-DXD 1μg)
Capture-D-#5 (coating OVA-DXD 1μg)
Capture-D-#6 (coating OVA-DXD 1μg)
Capture-D-#7 (coating OVA-DXD 1μg)
Capture-D-#3 (coating OVA 1μg)
Capture-D-#5 (coating OVA 1μg)
Capture-D-#6 (coating OVA 1μg)
Capture-D-#7 (coating OVA 1μg)
PP-1D ELISA结合
OD450
Aptamer 浓度 (nM)
Capture D-#14 (coating with OVA-DXD 1 μg)
Capture D-#17 (coating with OVA-DXD 1 μg)
Capture D-#14 (coating with OVA 1 μg)
Capture D-#17 (coating with OVA 1 μg)
Capture D-#14 (coating with BSA-DXD 1 μg)
Capture D-#17 (coating with BSA-DXD 1 μg)
Capture D-#14 (coating with BSA 1 μg)
Capture D-#17 (coating with BSA 1 μg)

图 46

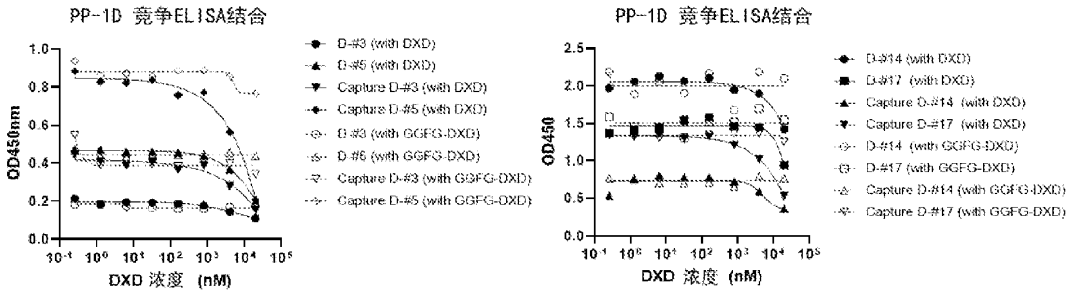

PP-1D 竞争ELISA结合
OD450nm
DXD 浓度 (nM)
D-#3 (with DXD)
D-#5 (with DXD)
Capture D-#3 (with DXD)
Capture D-#5 (with DXD)
D-#3 (with GGFG-DXD)
D-#5 (with GGFG-DXD)
Capture D-#3 (with GGFG-DXD)
Capture D-#5 (with GGFG-DXD)
PP-1D 竞争ELISA结合
OD450
DXD 浓度 (nM)
D-#14 (with DXD)
D-#17 (with DXD)
Capture D-#14 (with DXD)
Capture D-#17 (with DXD)
D-#14 (with GGFG-DXD)
D-#17 (with GGFG-DXD)
Capture D-#14 (with GGFG-DXD)
Capture D-#17 (with GGFG-DXD)

图 47

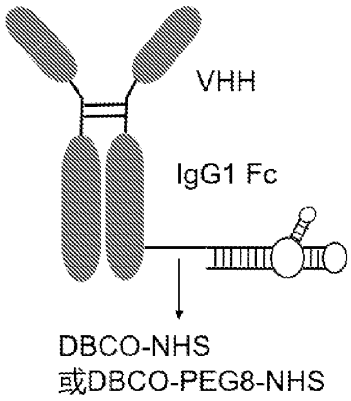

VHH
IgG1 Fc
DBCO-NHS
或DBCO-PEG8-NHS

图 48

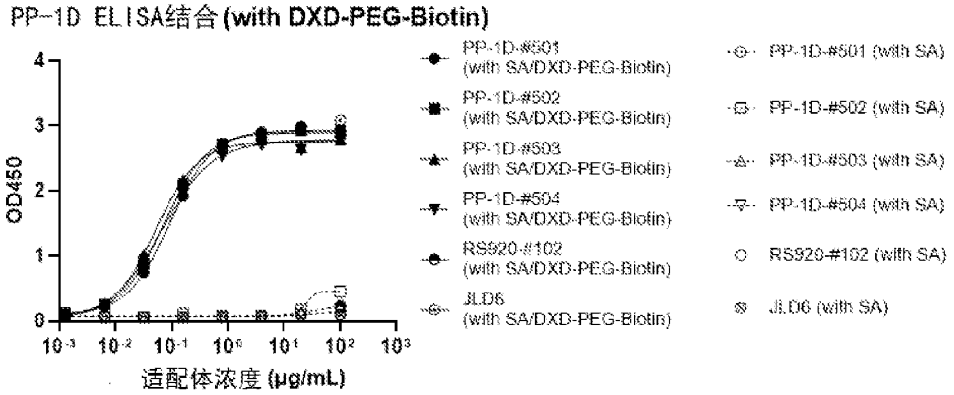

PP-1D ELISA结合(with DXD-PEG-Biotin)
OD450
适配体浓度(μg/mL)
PP-1D-#501 (with SA/DXD-PEG-Biotin)
PP-1D-#502 (with SA/DXD-PEG-Biotin)
PP-1D-#503 (with SA/DXD-PEG-Biotin)
PP-1D-#504 (with SA/DXD-PEG-Biotin)
RS920-#102 (with SA/DXD-PEG-Biotin)
JLD6 (with SA/DXD-PEG-Biotin)
PP-1D-#501 (with SA)
PP-1D-#502 (with SA)
PP-1D-#503 (with SA)
PP-1D-#504 (with SA)
RS920-#102 (with SA)
JLD6 (with SA)

图 49

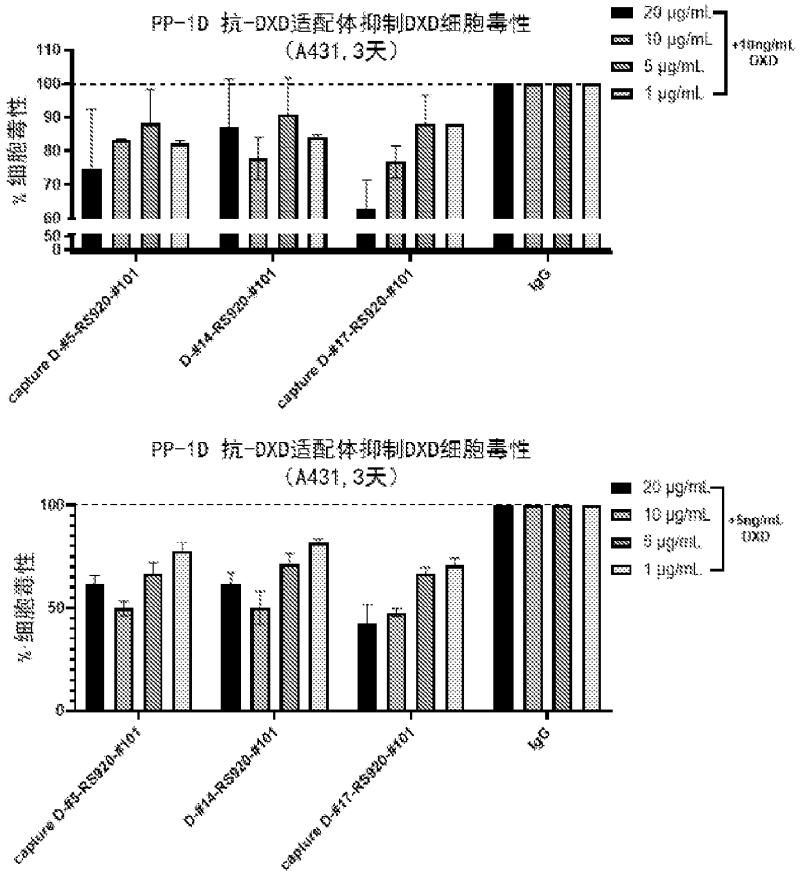

PP-1D 抗-DXD适配体抑制DXD细胞毒性
（A431, 3天）
20 μg/mL
10 μg/mL
5 μg/mL
1 μg/mL
+10ng/mL DXD
% 细胞毒性
110
100
90
80
70
60
50
0
capture D-#5-RS920-#101
D-#14-RS920-#101
capture D-#17-RS920-#101
IgG
PP-1D 抗-DXD适配体抑制DXD细胞毒性
（A431, 3天）
20 μg/mL
10 μg/mL
5 μg/mL
1 μg/mL
+5ng/mL DXD
% 细胞毒性
100
50
0
capture D-#5-RS920-#101
D-#14-RS920-#101
capture D-#17-RS920-#101
IgG

图 50

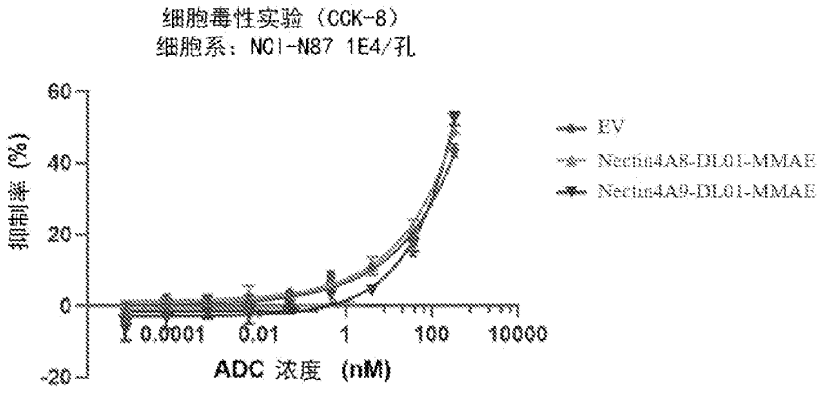

细胞毒性实验（CCK-8）
细胞系：NCI-N87 1E4/孔
抑制率（%）
60
40
20
0
-20
0.0001
0.01
1
100
10000
ADC 浓度（nM）
EV
Nectin4A8-DL01-MMAE
Nectin4A9-DL01-MMAE

图 51

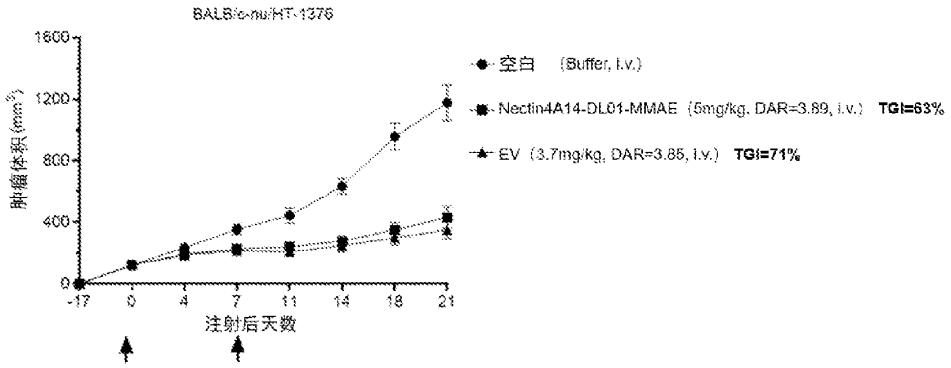

BALB/c-nu/HT-1376
肿瘤体积 (mm³)
1600
1200
800
400
0
-17
0
4
7
11
14
18
21
注射后天数
空白 (Buffer, i.v.)
Nectin4A14-DL01-MMAE (6mg/kg, DAR=3.89, i.v.) TGI=63%
EV (3.7mg/kg, DAR=3.85, i.v.) TGI=71%

图 52

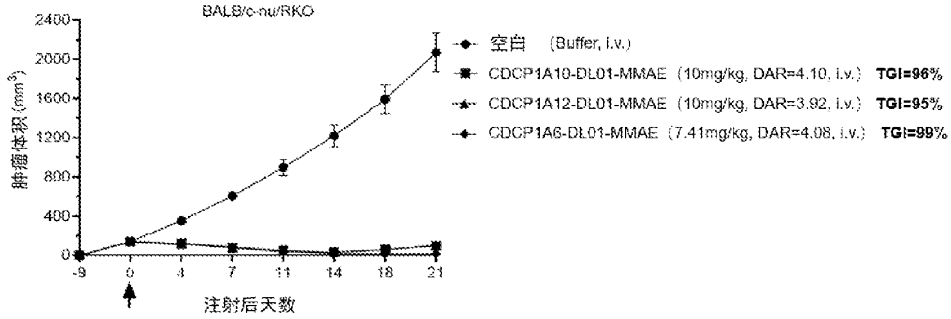

BALB/c-nu/RKO
肿瘤体积 (mm³)
2400
2000
1600
1200
800
400
0
-9
0
4
7
11
14
18
21
注射后天数
空白 (Buffer, i.v.)
CDCP1A10-DL01-MMAE (10mg/kg, DAR=4.10, i.v.) TGI=96%
CDCP1A12-DL01-MMAE (10mg/kg, DAR=3.92, i.v.) TGI=95%
CDCP1A6-DL01-MMAE (7.41mg/kg, DAR=4.08, i.v.) TGI=99%

图 53

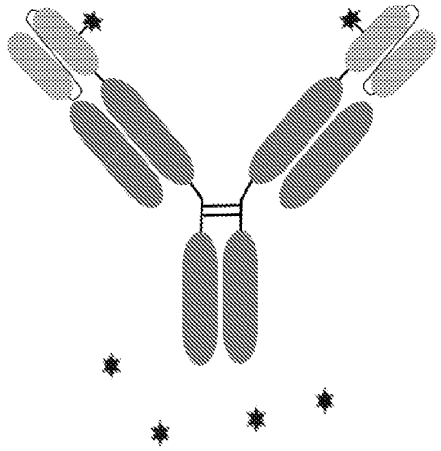


图 54

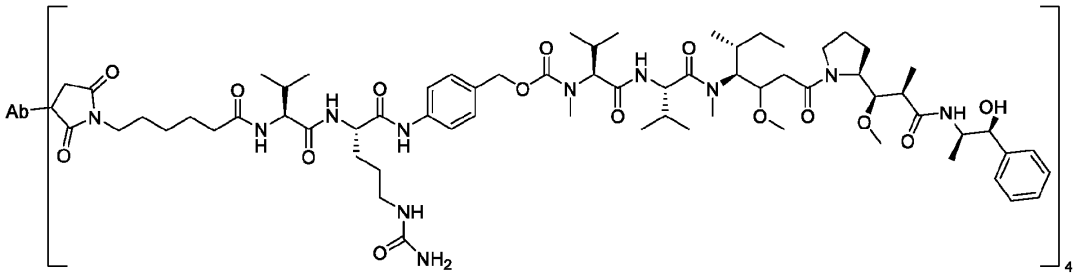


图 55

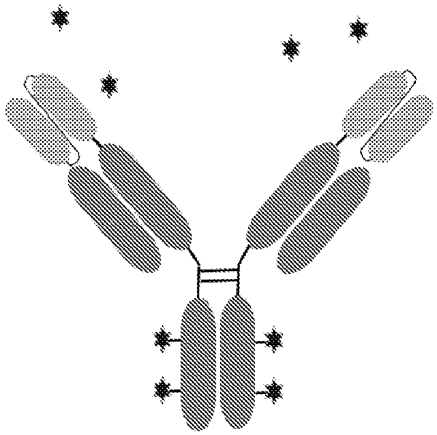


图 56

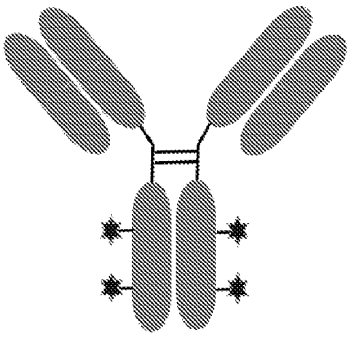


图 57

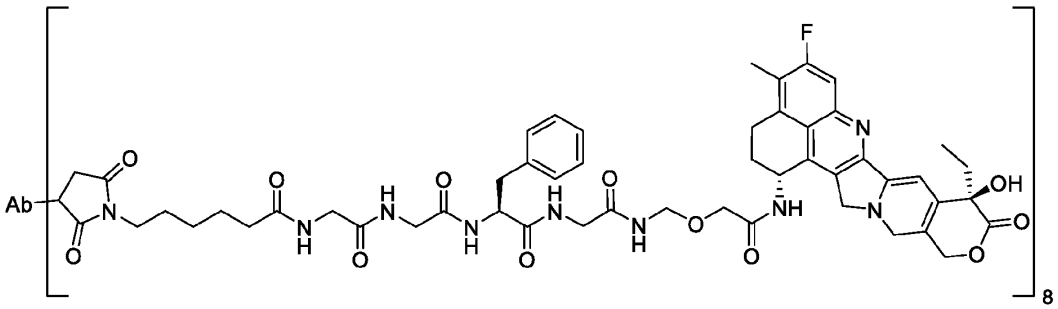


图 58