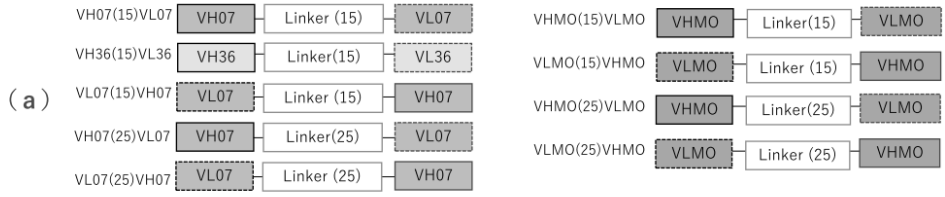
[Fig.1]



(b)

Heavy chain variable region (VH)

VH07	SEQ ID NO: 1	QVQLQQSGPGLVTPSQTLSLTCAISGDSVSSNSATWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSV KSRMSINPDTSKNQFSLQLNSVTPEDTAVYYCARGMMTYYYGMDVWGQGTTVTVSSGILGS
VH36	SEQ ID NO:3	QVQLQQSGPGLVTPSQTLSLTCAISGDSVSSNSATWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSV KSRMSINPDTSKNQFSLQLNSVTPEDTAVYYCARGMMTYYYGMDVWGQGTTVTVSSGILLS
VHMO	SEQ ID NO: 5	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGNYINWVRQAPGQGLEWMGIINPTKGWTLYAQKFQGR VTMTRDTSISTAYMELSSLRSEDTAVYYCARWHHGTWIFDYWGQGTLVTVSS

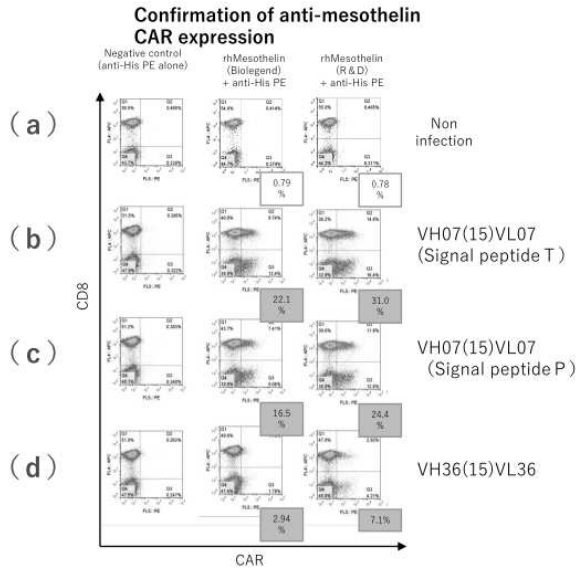
Light chain variable region (VL)

VL07	SEQ ID NO: 2	QPVLTQSSSLSASPGASASLTCTLRSGINVGPYRIYWYQQKPGSPPQYLLNYKSDSDKQQGSGVPSR FSGSKDASANAGVLLISGLRSEDEADYYCMIWHSSAAVFGGGTQLTVLS
VL36	SEQ ID NO: 4	QPVLTQSSSLSASPGASASLTCTLRSGINVGPRIYWYQQKPGSPPQYLLNYKSDSDKQQGSGVPSRF SGSKDASANAGVLLISGLRSEDEADYYCMIWHSSAAVFGGGTQLTVLS
VLMO	SEQ ID NO: 6	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLIYNDNQRPSGVPDRFSGSKS GTSASLAITGLQSEDEADYYCSTYDRRTFSVFGGGTKLTVL

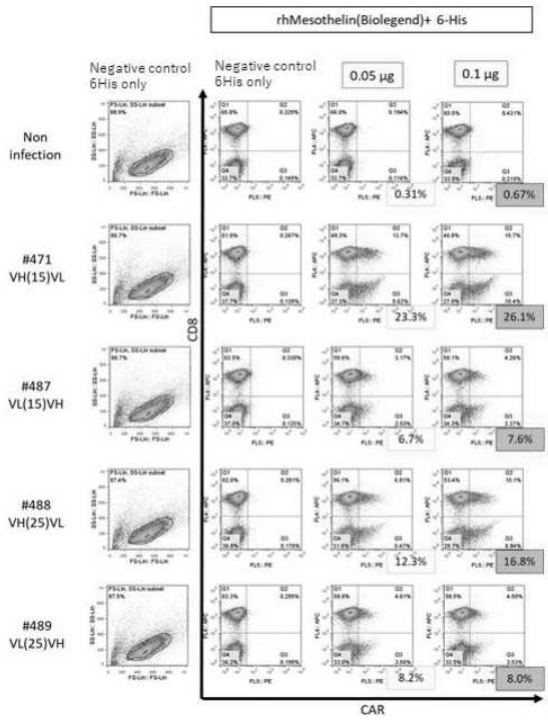
Linker

(15)	SEQ ID NO: 26	GGGGSGGGGSGGGGS
(25)	SEQ ID NO: 27	SSADDAKKDAAKKDDAKKDDAKKDG

[Fig.2]



[Fig. 3] Confirmation of anti-mesothelin CAR expression



(a) Non infection

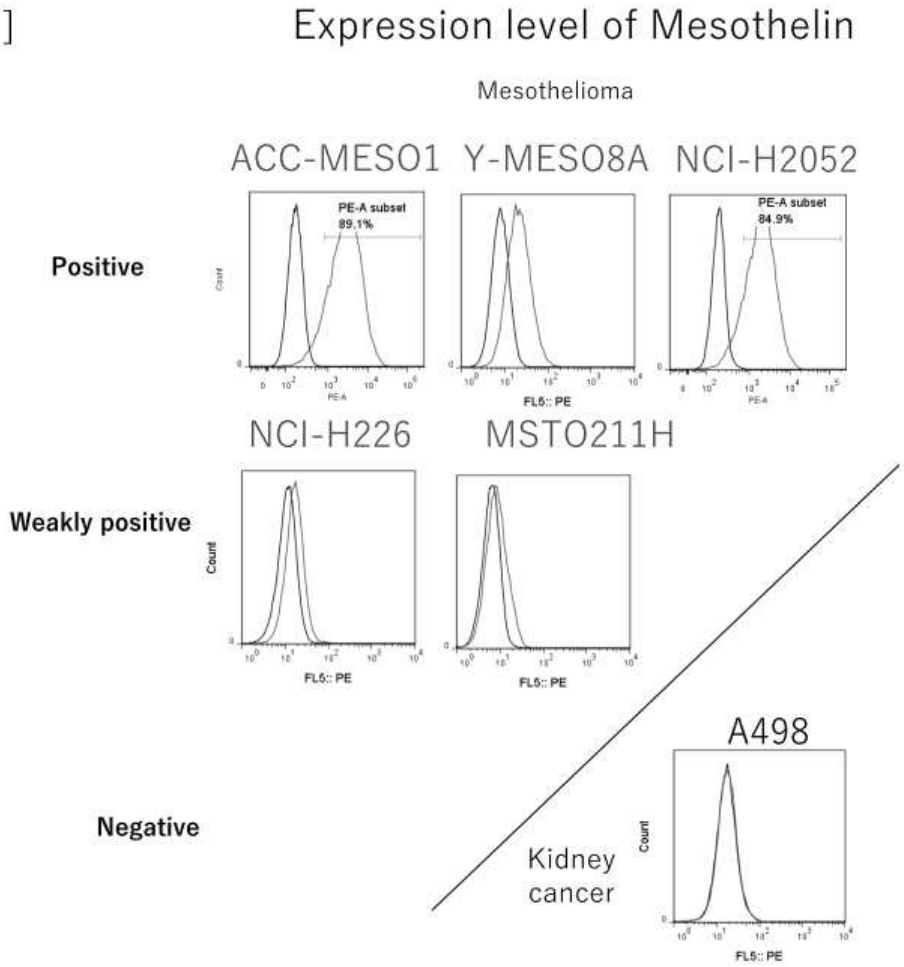
(b) VH07(15)VL07

(c) VL07(15)VH07

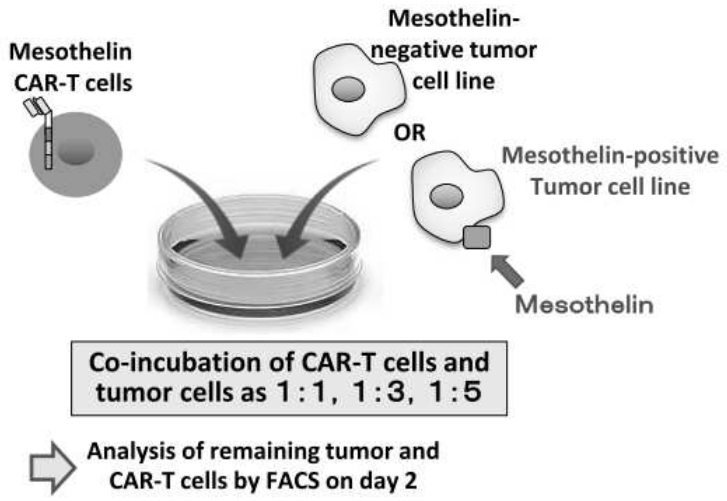
(d) VH07(25)VL07

(e) VL07(25)VH07

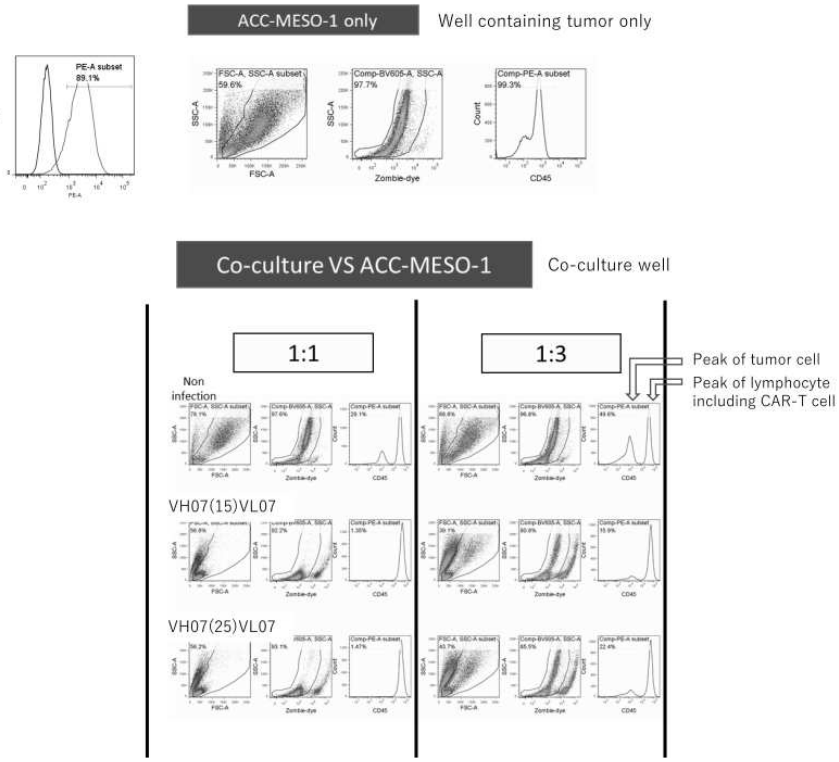
[Fig. 4]


Expression level of Mesothelin
Mesothelioma
ACC-MESO1
Y-MESO8A
NCI-H2052
Positive
PE-A subset
89.1%
PE-A subset
84.9%
Count
FL5:: PE
NCI-H226
MSTO211H
Weakly positive
Count
FL5:: PE
FL5:: PE
A498
Negative
Kidney cancer
Count
FL5:: PE

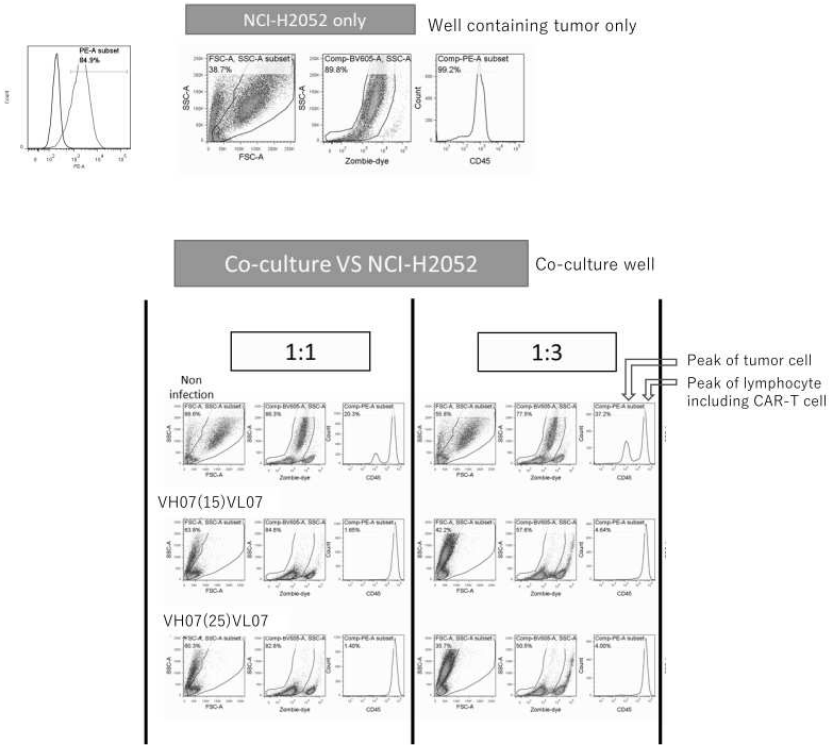
[Fig. 5]


Mesothelin
CAR-T cells
Mesothelin-
negative tumor
cell line
OR
Mesothelin-positive
Tumor cell line
Mesothelin
Co-incubation of CAR-T cells and
tumor cells as 1:1, 1:3, 1:5
Analysis of remaining tumor and
CAR-T cells by FACS on day 2

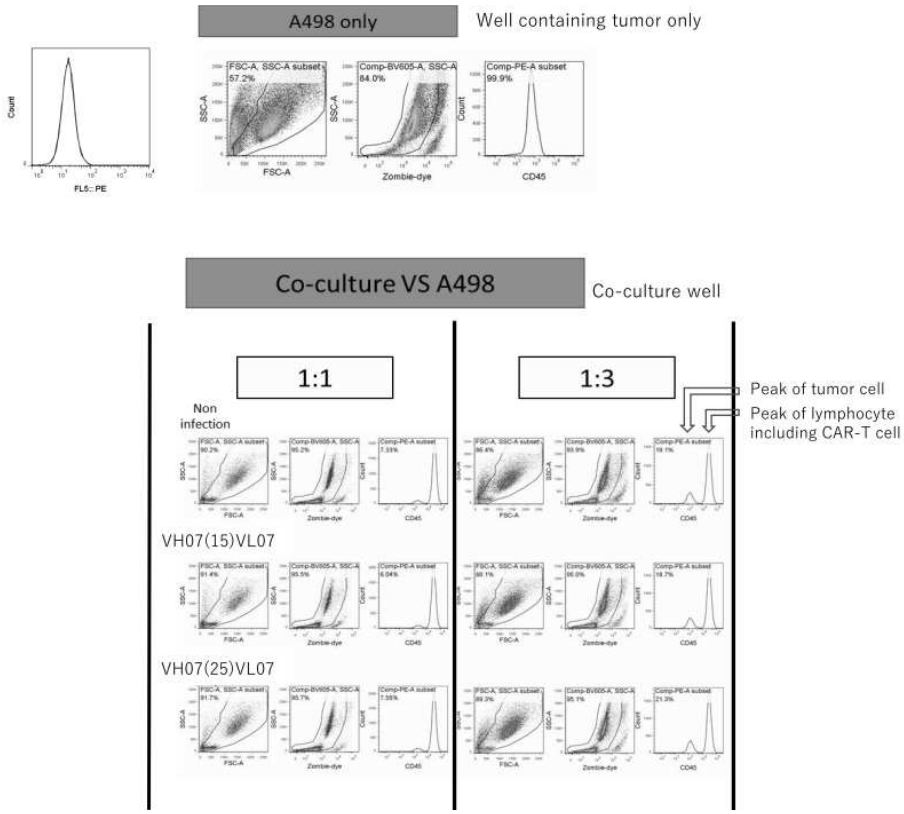
[Fig. 6 A]


ACC-MESO-1 only
Well containing tumor only
PE-A subset
89.1%
FSC-A, SSC-A subset
59.6%
Comp-BV605-A, SSC-A
97.7%
Comp-PE-A subset
99.3%
SSC-A
FSC-A
Zombie-dye
CD45
Count
Co-culture VS ACC-MESO-1
Co-culture well
1:1
1:3
Peak of tumor cell
Peak of lymphocyte
including CAR-T cell
Non
infection
VH07(15)VL07
VH07(25)VL07

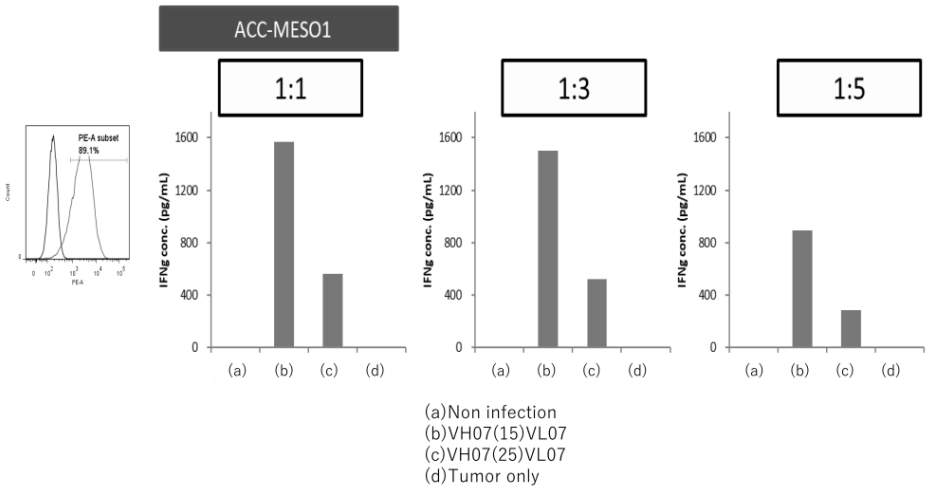
[Fig. 6B]


NCI-H2052 only
Well containing tumor only
PE-A subset
84.9%
FSC-A, SSC-A subset
38.7%
Comp-BV605-A, SSC-A
89.8%
Comp-PE-A subset
99.2%
SSC-A
FSC-A
Zombie-dye
Count
CD45
Co-culture VS NCI-H2052
Co-culture well
1:1
1:3
Peak of tumor cell
Peak of lymphocyte
including CAR-T cell
Non
infection
VH07(15)VL07
VH07(25)VL07

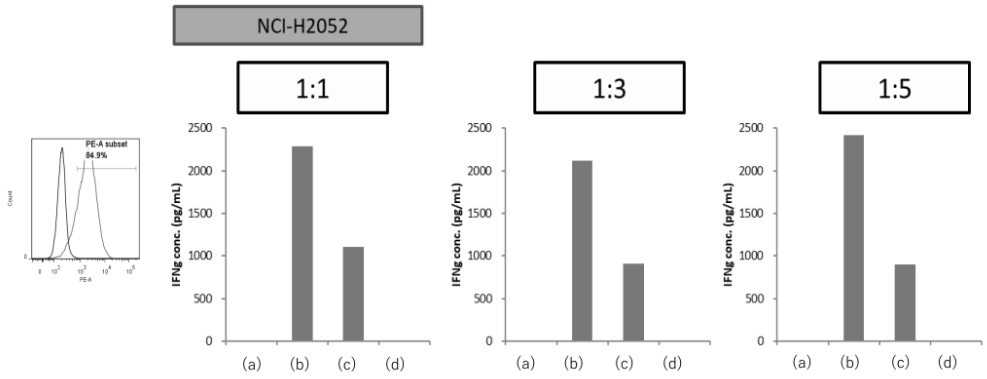
[Fig. 6 C]


A498 only
Well containing tumor only
Count
FL5::PE
FSC-A, SSC-A subset
57.2%
SSC-A
FSC-A
Comp-BV605-A, SSC-A
84.0%
Zombie-dye
Comp-PE-A subset
99.9%
CD45
Co-culture VS A498
Co-culture well
1:1
1:3
Peak of tumor cell
Peak of lymphocyte including CAR-T cell
Non infection
VH07(15)VL07
VH07(25)VL07

[Fig.7A]

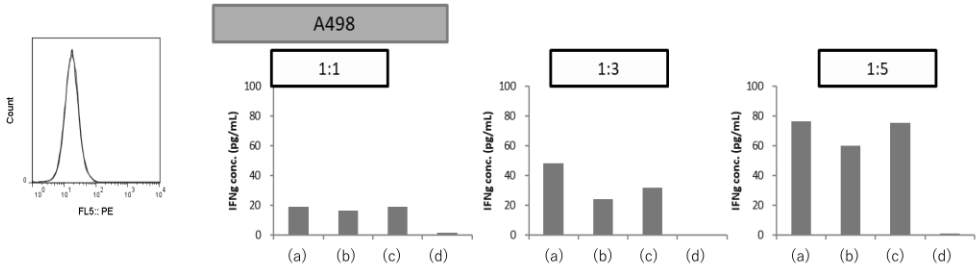

ACC-MESO1
1:1
1:3
1:5
PE-A subset
89.1%
IFNg conc. (pg/mL)
1600
1200
800
400
0
(a)
(b)
(c)
(d)
(a)Non infection
(b)VH07(15)VL07
(c)VH07(25)VL07
(d)Tumor only

[Fig.7B]



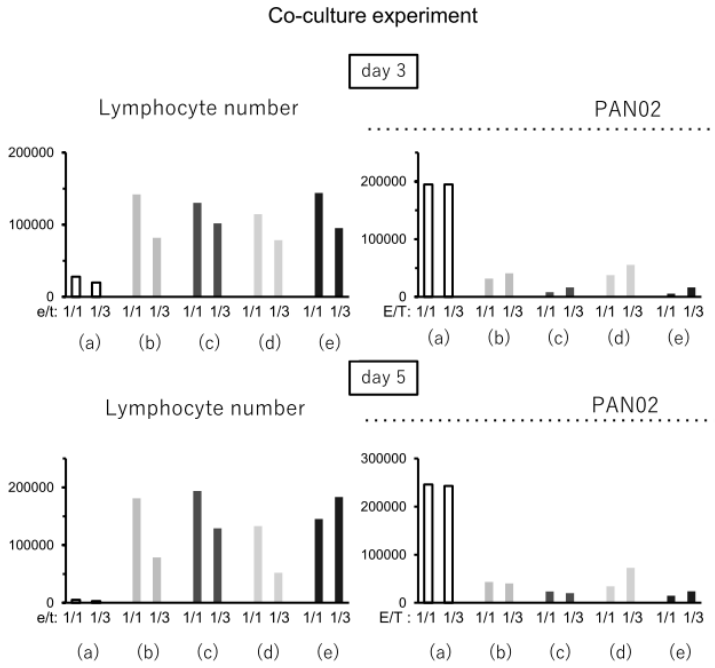
(a)Non infection
(b)VH07(15)VL07
(c)VH07(25)VL07
(d)Tumor only

[Fig.7C]



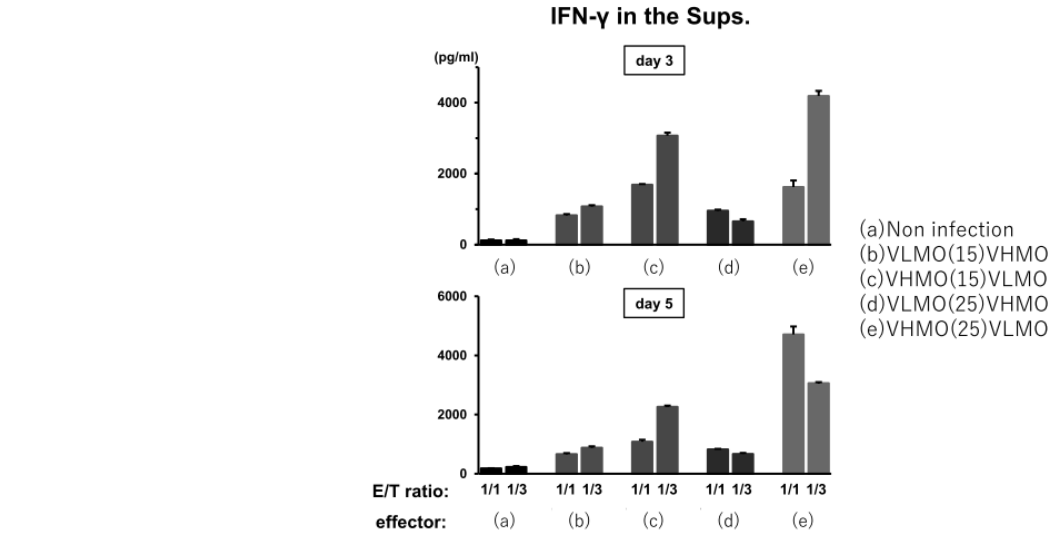
(a)Non infection
(b)VH07(15)VL07
(c)VH07(25)VL07
(d)Tumor only

[Fig.8]

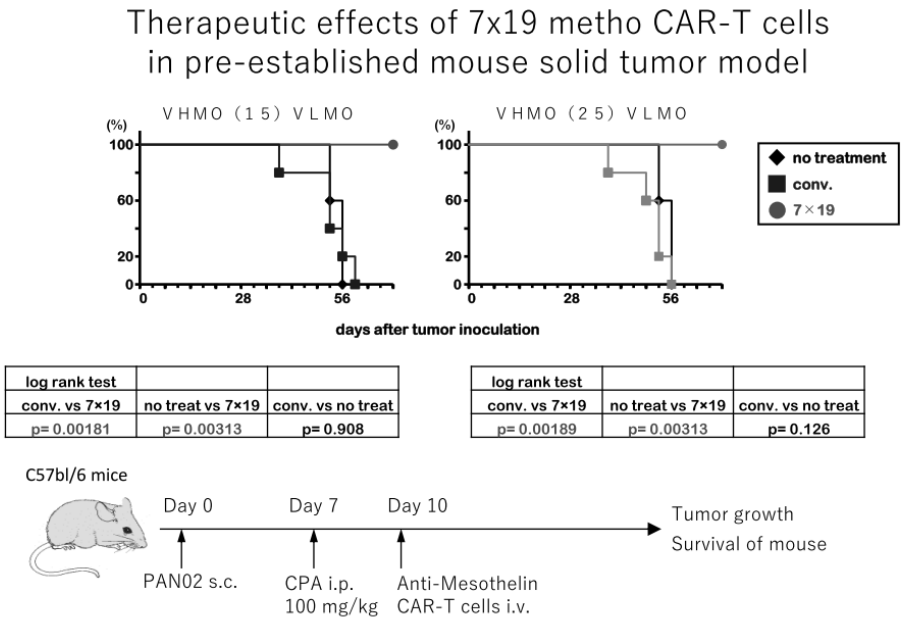


(a)Non infection
(b)VLMO(15)VHMO
(c)VHMO(15)VLMO
(d)VLMO(25)VHMO
(e)VHMO(25)VLMO

[Fig.9]



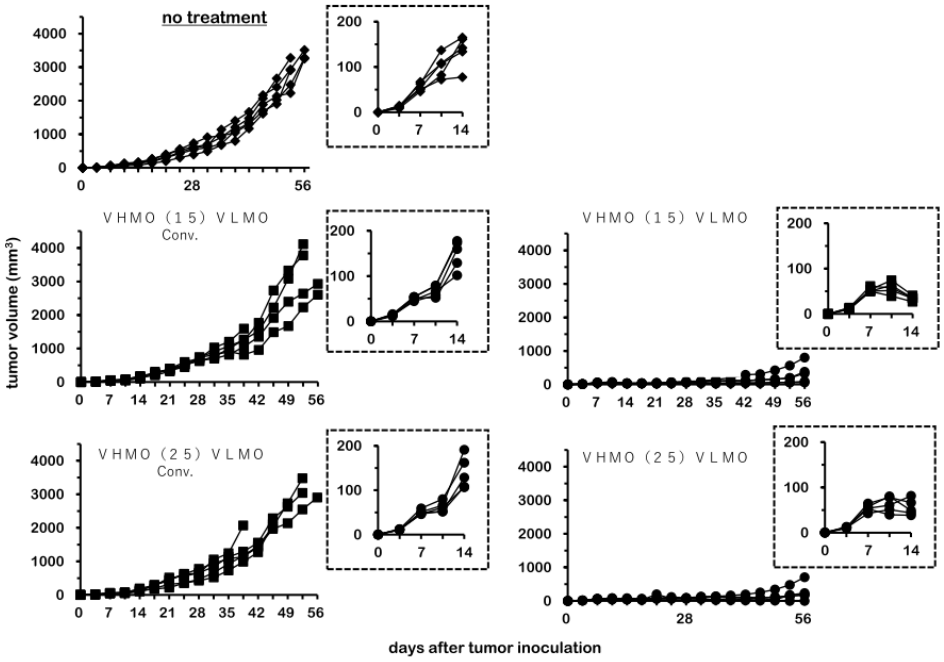
[Fig.10]



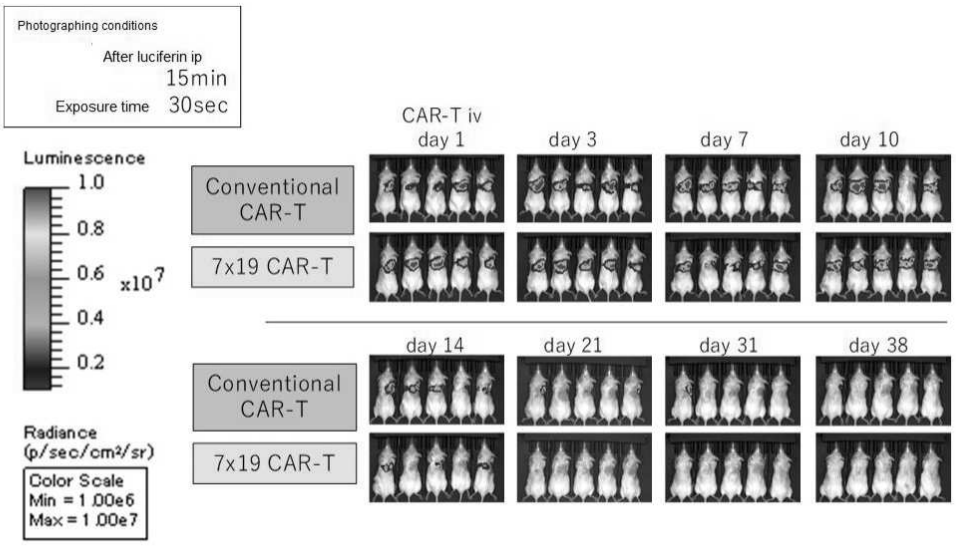
log rank test		
conv. vs 7×19	no treat vs 7×19	conv. vs no treat
p= 0.00181	p= 0.00313	**p= 0.908**

log rank test		
conv. vs 7×19	no treat vs 7×19	conv. vs no treat
p= 0.00189	p= 0.00313	**p= 0.126**

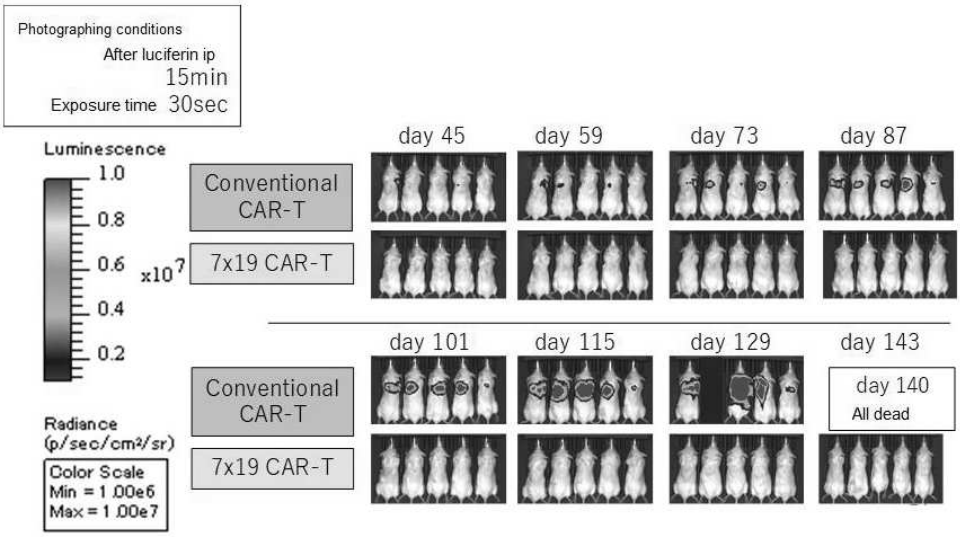
[Fig. 11]



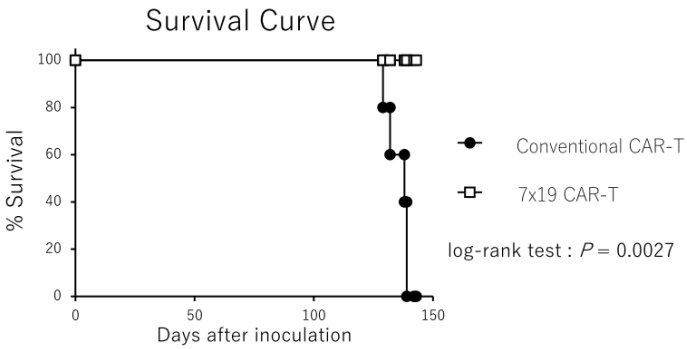
[Fig.12A]



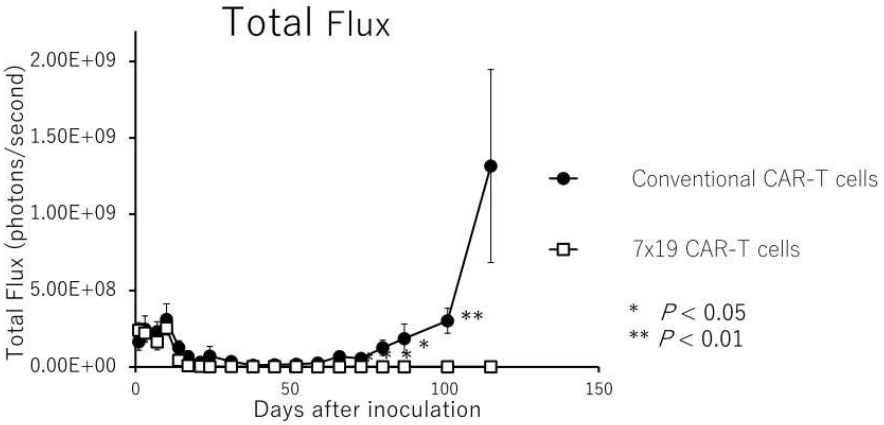
[Fig.12B]


Photographing conditions
After luciferin ip
15min
Exposure time 30sec
Luminescence
1.0
0.8
0.6
x10^7
0.4
0.2
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 1.00e6
Max = 1.00e7
Conventional CAR-T
7x19 CAR-T
day 45
day 59
day 73
day 87
Conventional CAR-T
7x19 CAR-T
day 101
day 115
day 129
day 143
day 140
All dead

[Fig. 13]


Survival Curve
% Survival
100
80
60
40
20
0
0
50
100
150
Days after inoculation
Conventional CAR-T
7x19 CAR-T
log-rank test : P = 0.0027

[Fig. 14]


Total Flux
Total Flux (photons/second)
2.00E+09
1.50E+09
1.00E+09
5.00E+08
0.00E+00
0
50
100
150
Days after inoculation
Conventional CAR-T cells
7x19 CAR-T cells
* P < 0.05
** P < 0.01