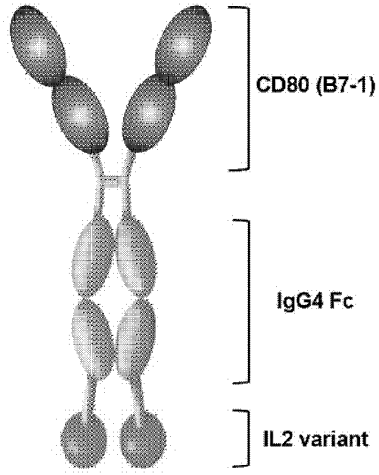
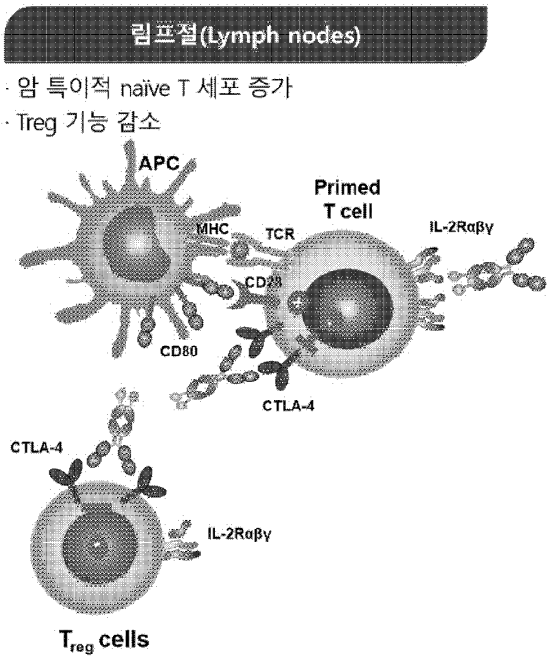
[도1]

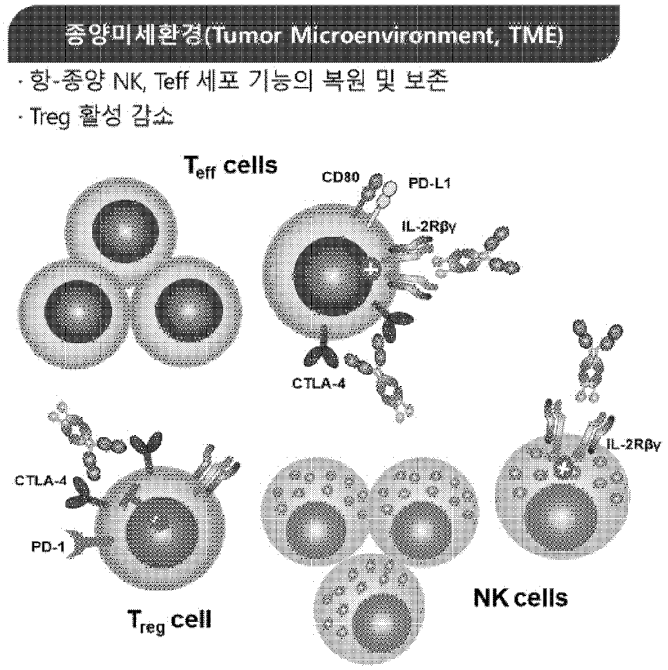


Size 190KDa

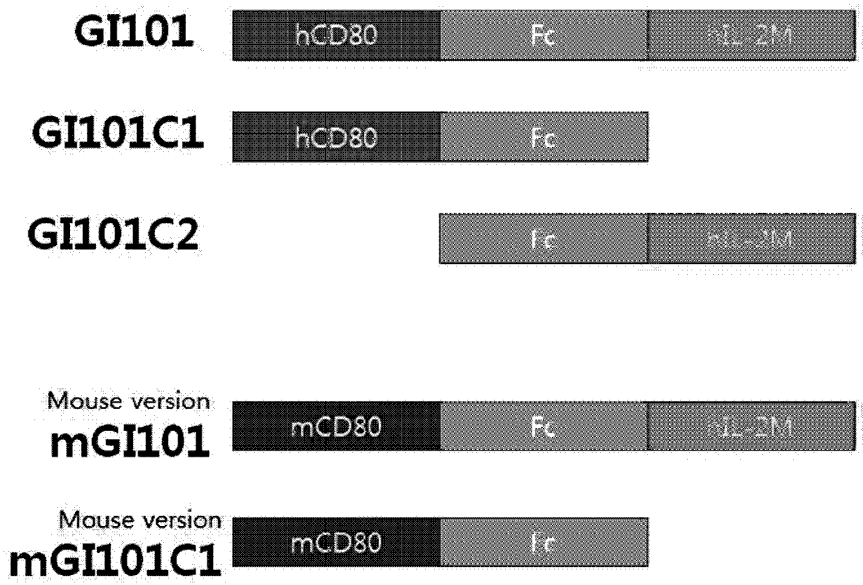
[도2]



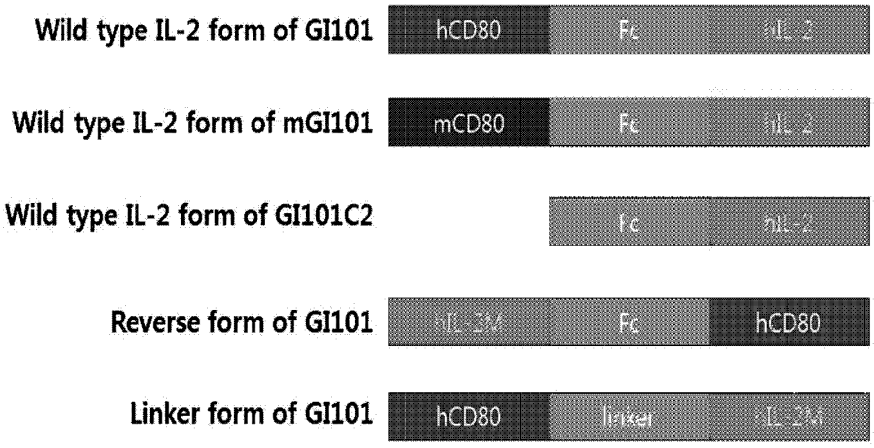
[도3]


종양미세환경(Tumor Microenvironment, TME)
· 항-종양 NK, Teff 세포 기능의 복원 및 보존
· Treg 활성 감소
Teff cells
CD80
PD-L1
IL-2Rβγ
CTLA-4
CTLA-4
PD-1
Treg cell
IL-2Rβγ
NK cells

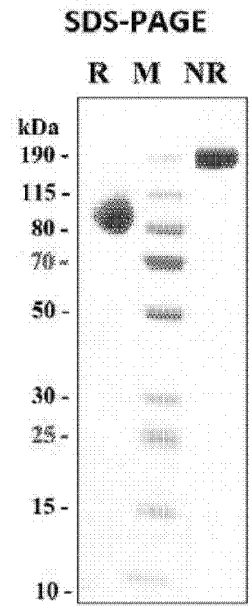
[도4]


GI101
hCD80
Fc
GI101C1
hCD80
Fc
GI101C2
Fc
Mouse version
mGI101
mCD80
Fc
Mouse version
mGI101C1
mCD80
Fc

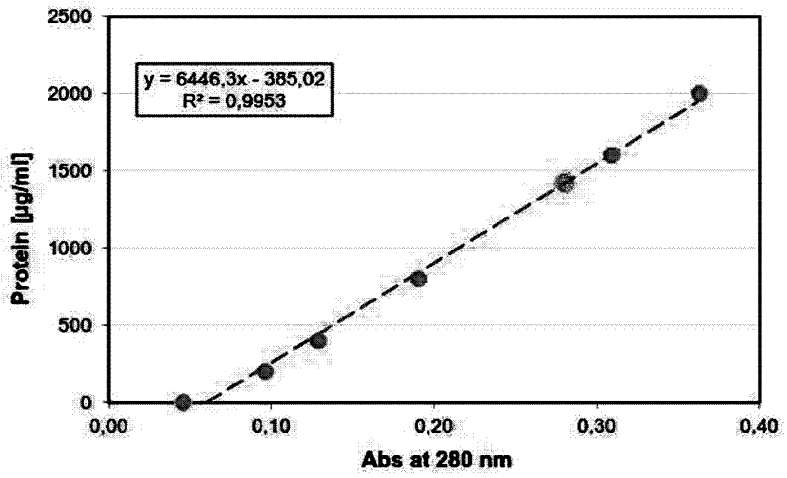
[도5]


Wild type IL-2 form of GI101
hCD80
Fc
Wild type IL-2 form of mGI101
mCD80
Fc
Wild type IL-2 form of GI101C2
Fc
Reverse form of GI101
Fc
hCD80
Linker form of GI101
hCD80
linker

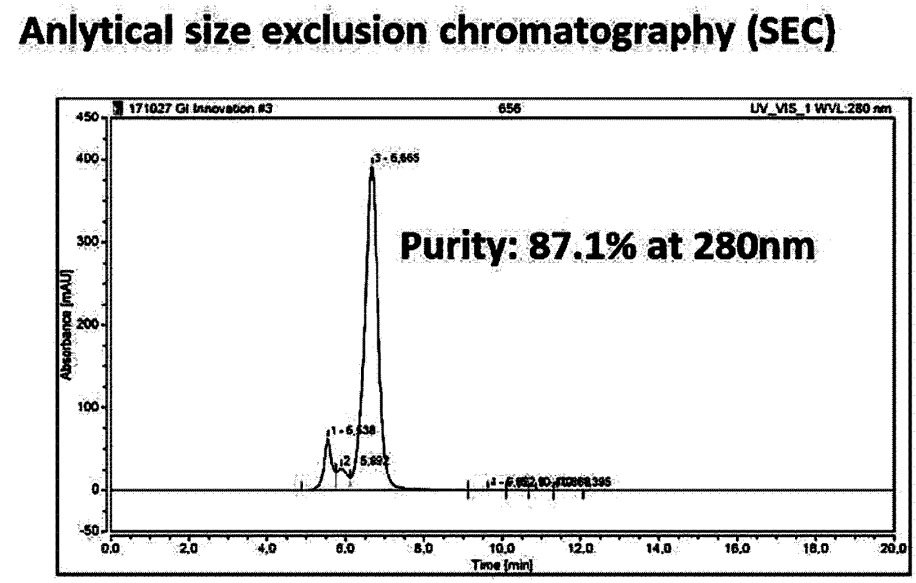
[도6]


SDS-PAGE
R M NR
kDa
190 -
115 -
80 -
70 -
50 -
30 -
25 -
15 -
10 -

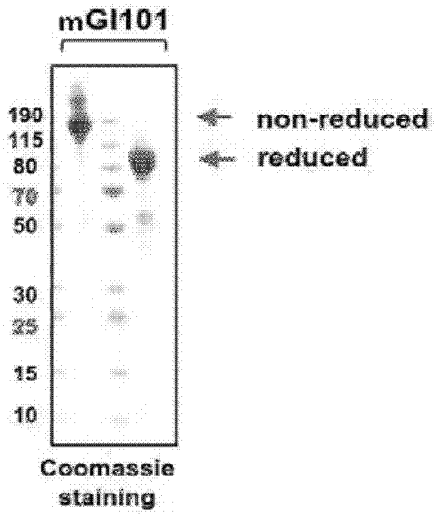
[도7]


y = 6446,3x - 385,02
R² = 0,9953
Protein [µg/ml]
2500
2000
1500
1000
500
0
0,00
0,10
0,20
0,30
0,40
Abs at 280 nm

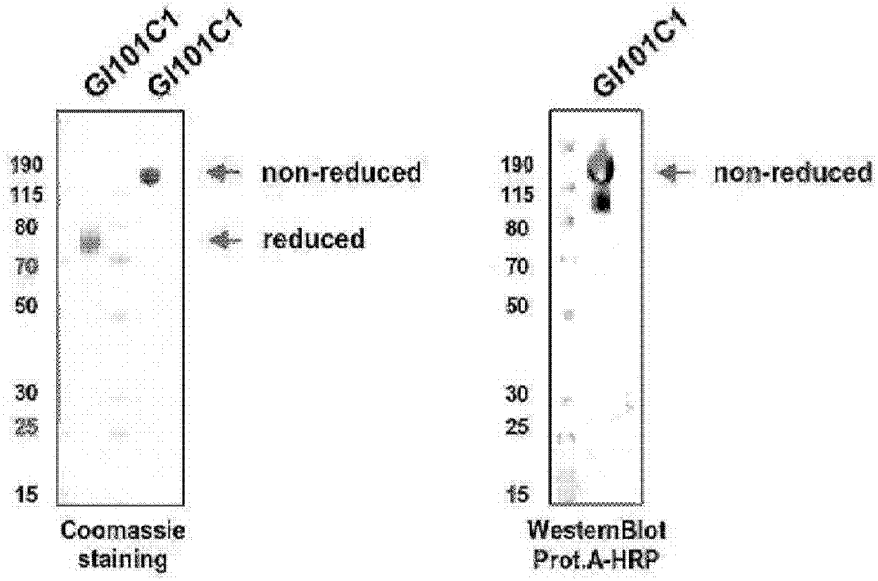
[도8]


Anlytical size exclusion chromatography (SEC)
171027 GI Innovation #3
656
UV_VIS_1 WVL:280 nm
Purity: 87.1% at 280nm
Absorbance [mAU]
Time [min]

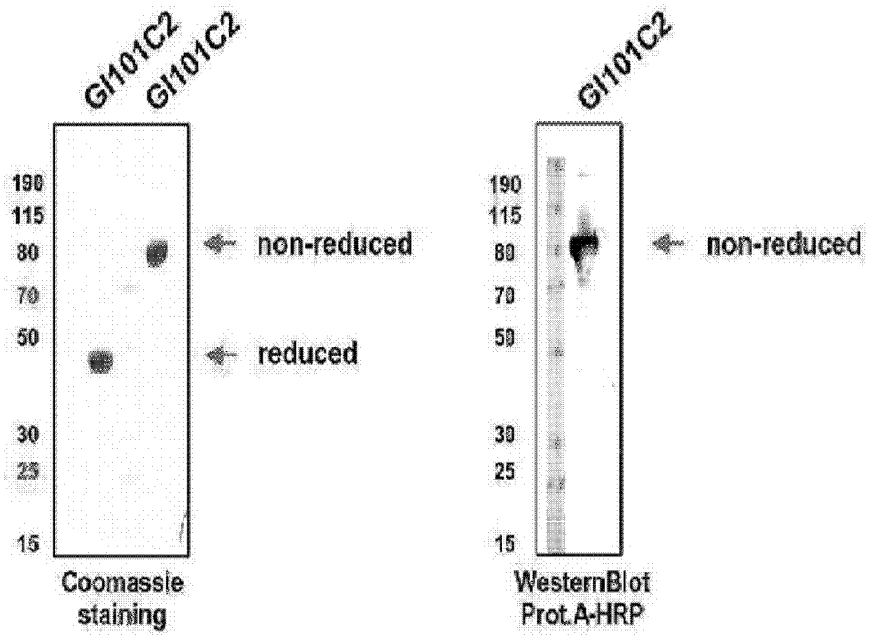
[도9]


mGI101
190
115
80
70
50
30
25
15
10
non-reduced
reduced
Coomassie staining

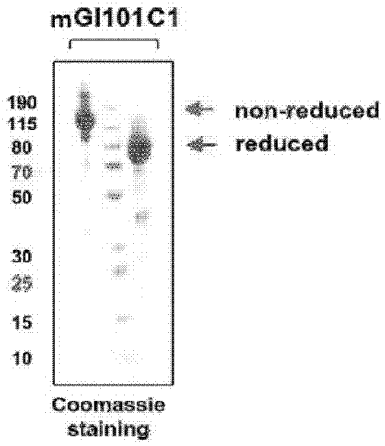
[도10]


GI101C1
GI101C1
190
115
80
70
50
30
25
15
non-reduced
reduced
Coomassie staining
GI101C1
190
115
80
70
50
30
25
15
non-reduced
WesternBlot Prot.A-HRP

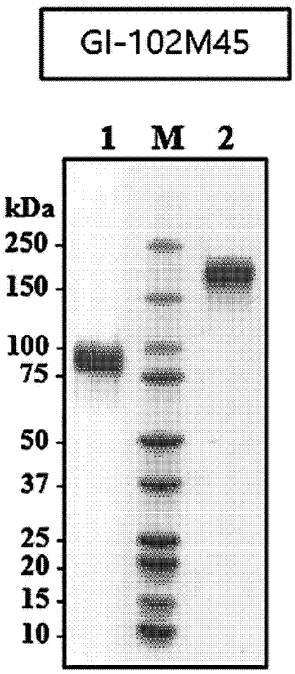
[도11]


GI101C2
GI101C2
190
115
80
70
50
30
25
15
non-reduced
reduced
Coomassie staining
GI101C2
190
115
80
70
50
30
25
15
non-reduced
WesternBlot Prot.A-HRP

[도12]



[도13]

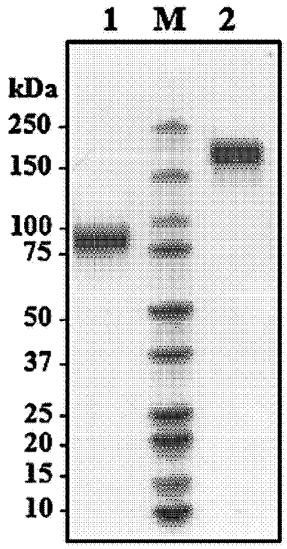


Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

[도14]

GI-102M61

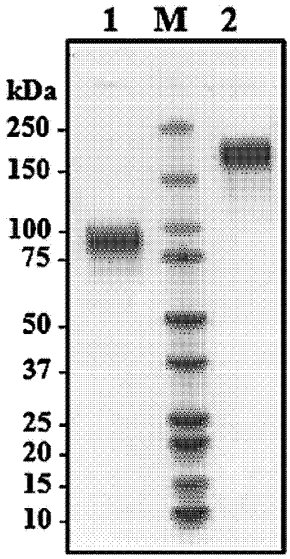


Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

[도15]

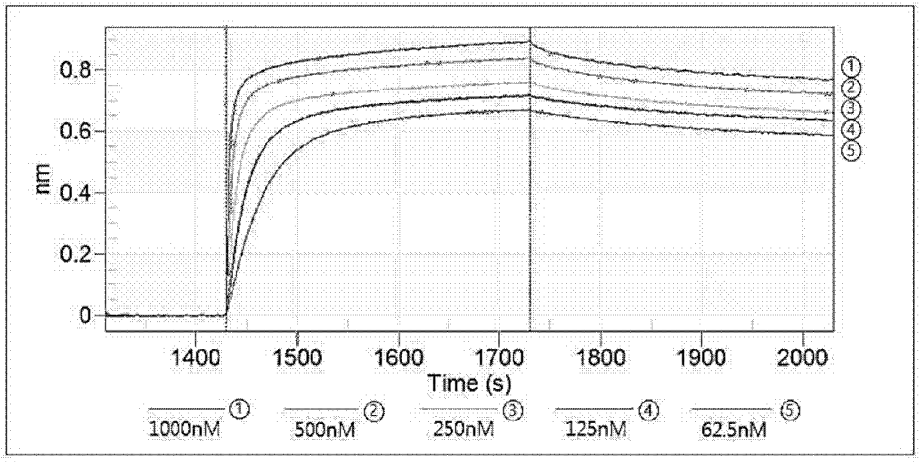
GI-102M72



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

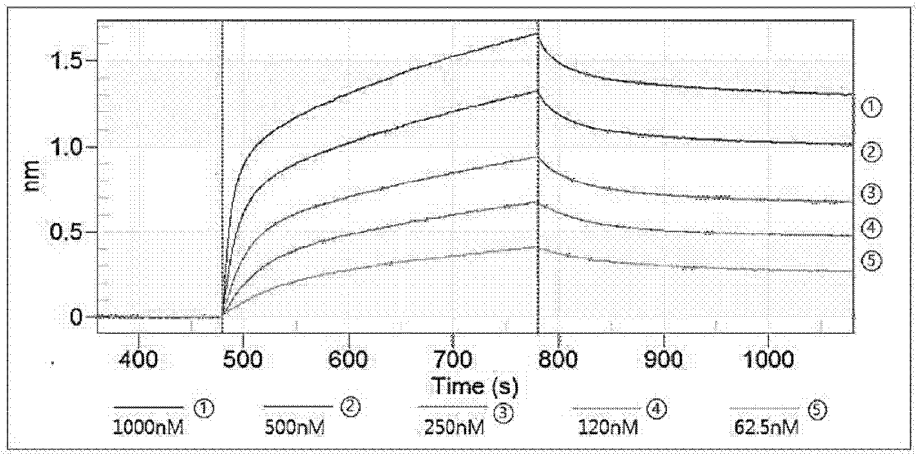
BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

[도16]



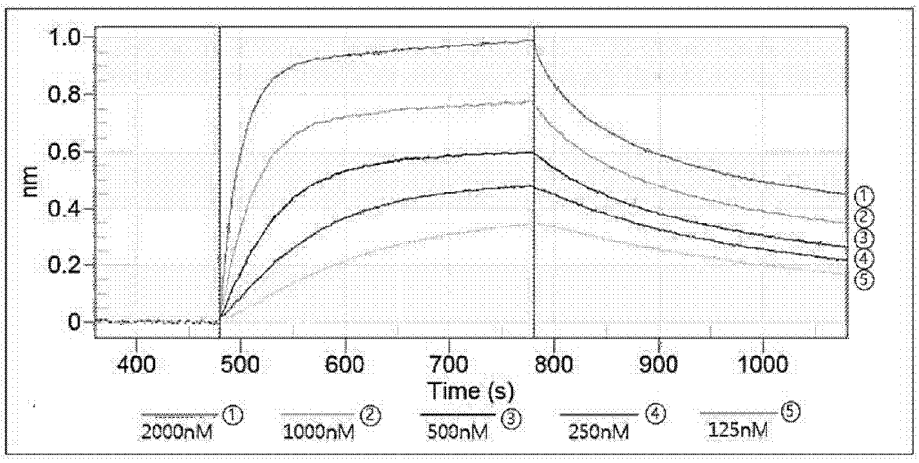
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
GI101	1.07E-09	3.86E+05	4.14E-04

[도17]



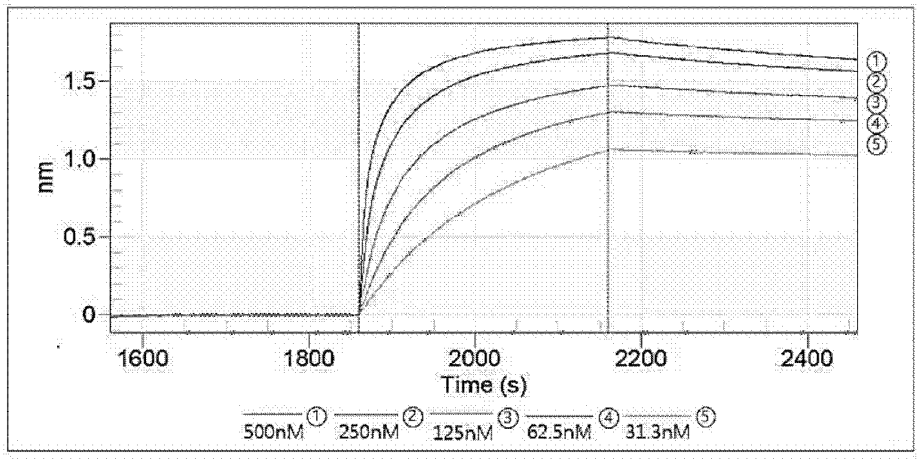
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
GI101	3.46E-08	7.0E+04	2.42E-03

[도18]



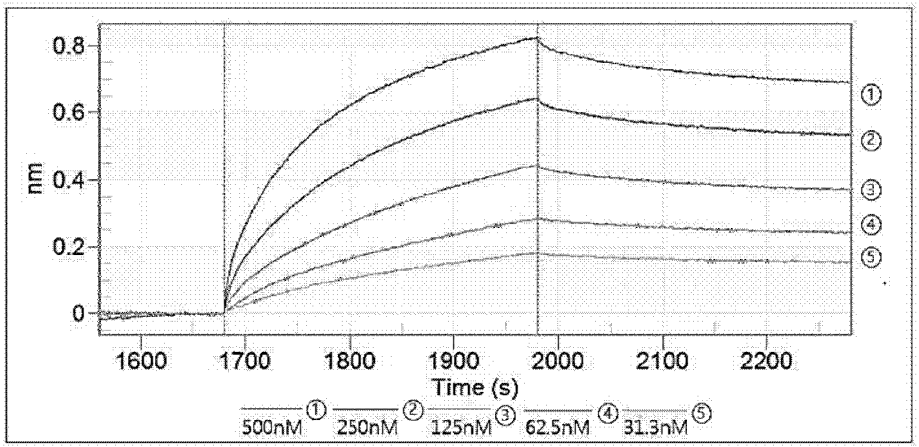
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
PD-1	1.13E-07	2.72E+04	3.07E-03

[도19]



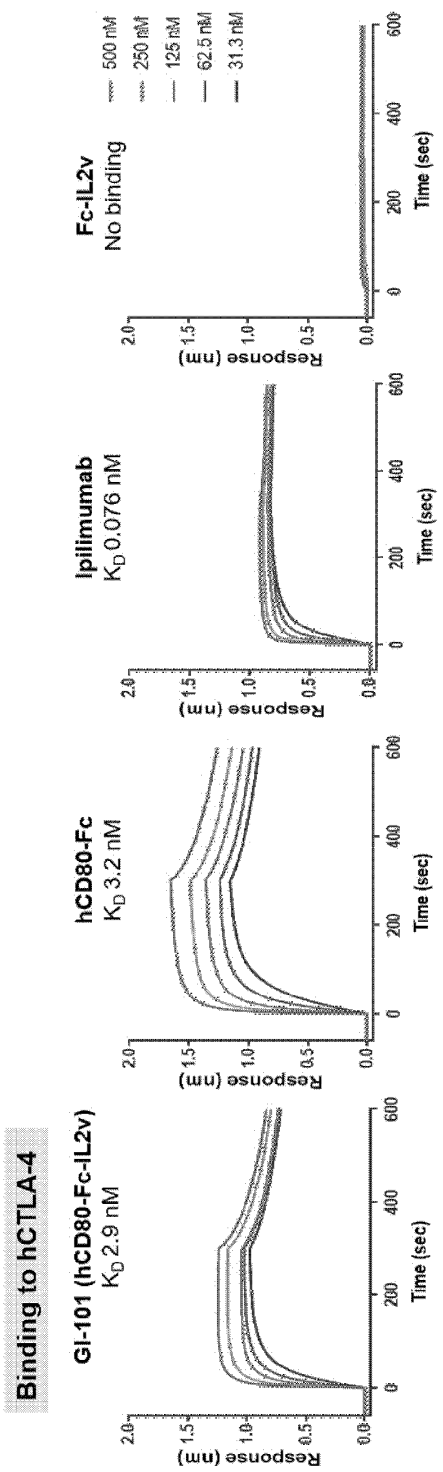
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
mGI101	2.58E-08	3.05E+05	7.88E-04

[도20]

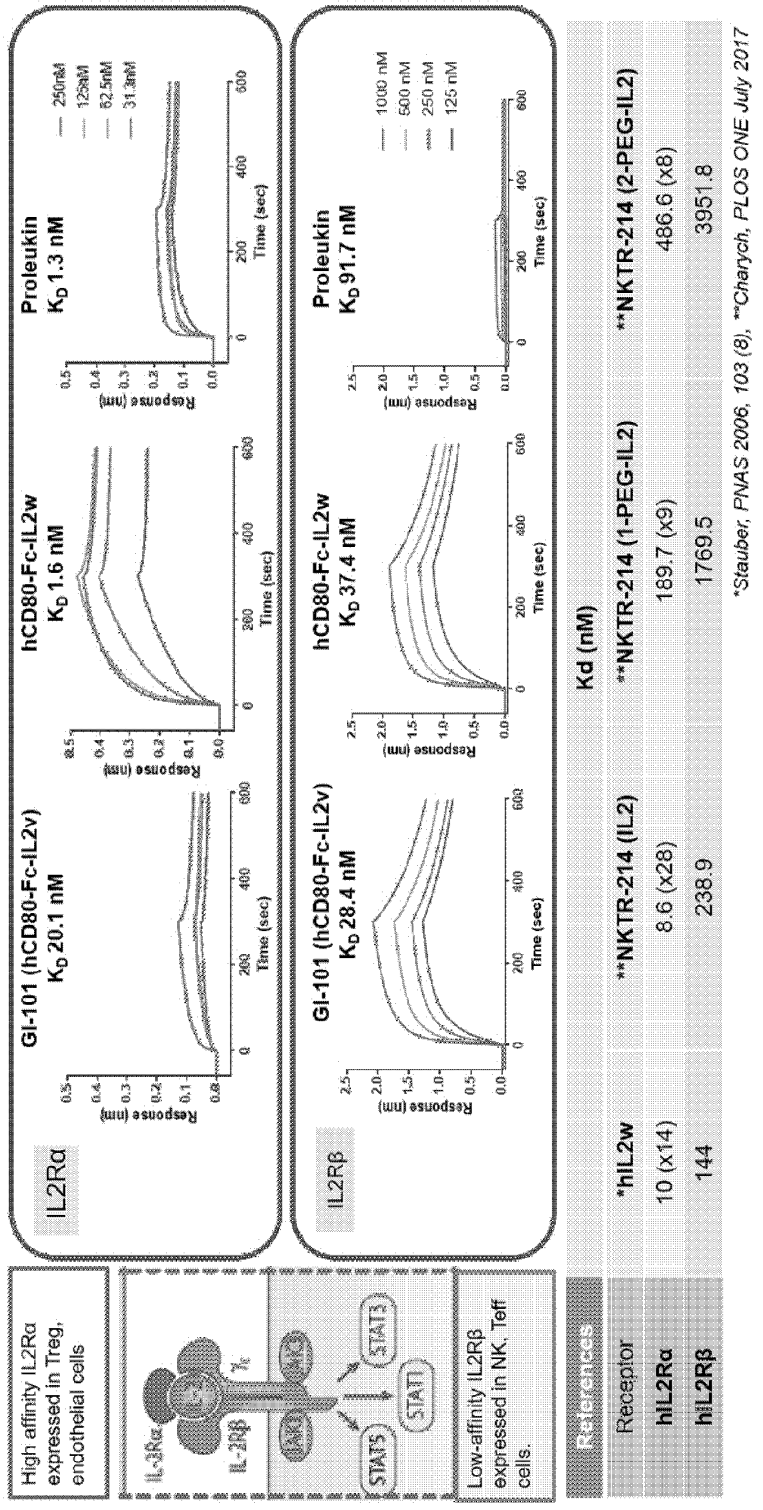


Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
mGI101	9.91E-09	8.12E+05	8.04E-03

[도21]


Binding to hCTLA-4
GI-101 (hCD80-Fc-IL2v)
K_D 2.9 nM
hCD80-Fc
K_D 3.2 nM
Ipilimumab
K_D 0.076 nM
Fc-IL2v
No binding
500 nM
250 nM
125 nM
62.5 nM
31.3 nM
Response (nm)
Time (sec)

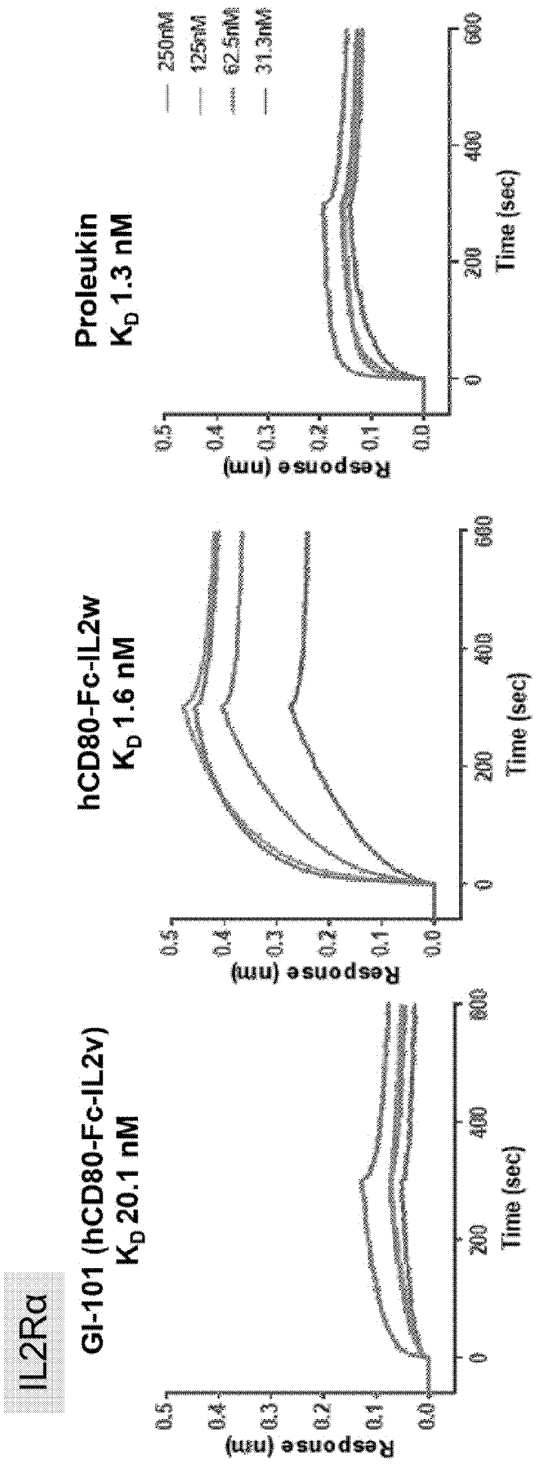
[도22]



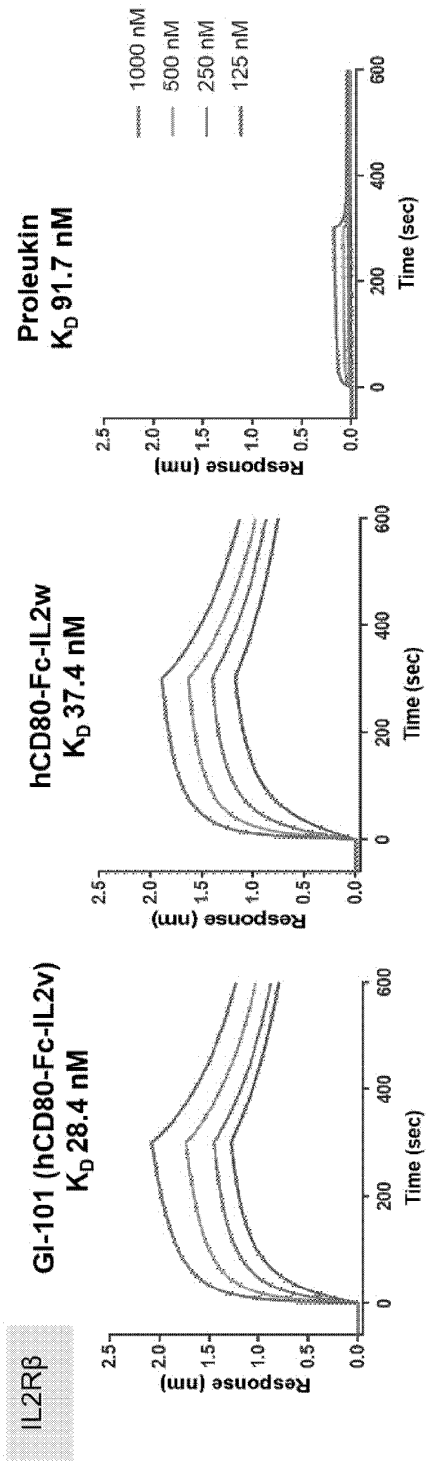
References	Kd (nM)			
Receptor	*hIL2w	**NKTR-214 (IL2)	**NKTR-214 (1-PEG-IL2)	**NKTR-214 (2-PEG-IL2)
hIL2Rα	10 (x14)	8.6 (x28)	189.7 (x9)	486.6 (x8)
hIL2Rβ	144	238.9	1769.5	3951.8

*Stauber, PNAS 2006, 103 (8), **Charych, PLOS ONE July 2017

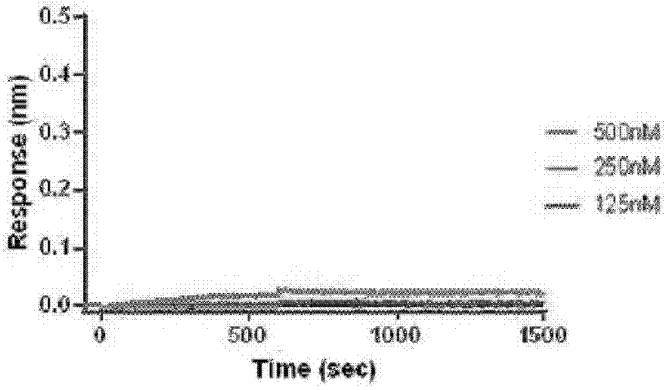
[도23]


IL2Rα
GI-101 (hCD80-Fc-IL2v)
K_D 20.1 nM
hCD80-Fc-IL2w
K_D 1.6 nM
Proleukin
K_D 1.3 nM
250nM
125nM
62.5nM
31.3nM
Response (nm)
Time (sec)
0.0
0.1
0.2
0.3
0.4
0.5
0
200
400
600
800

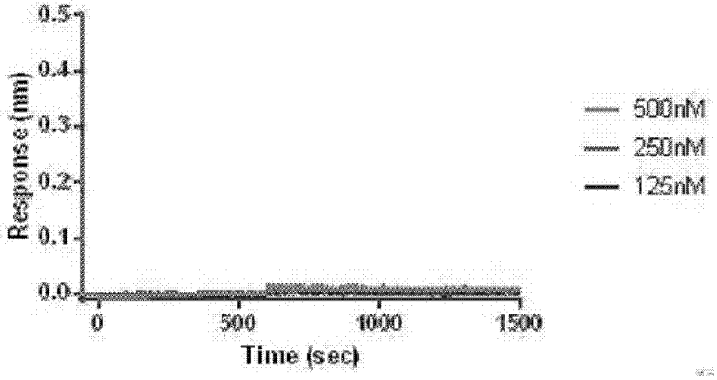
[도24]


IL2Rβ
GI-101 (hCD80-Fc-IL2v)
K_D 28.4 nM
hCD80-Fc-IL2w
K_D 37.4 nM
Proleukin
K_D 91.7 nM
1000 nM
500 nM
250 nM
125 nM
Response (nm)
Time (sec)
0.0
0.5
1.0
1.5
2.0
2.5
0
200
400
600

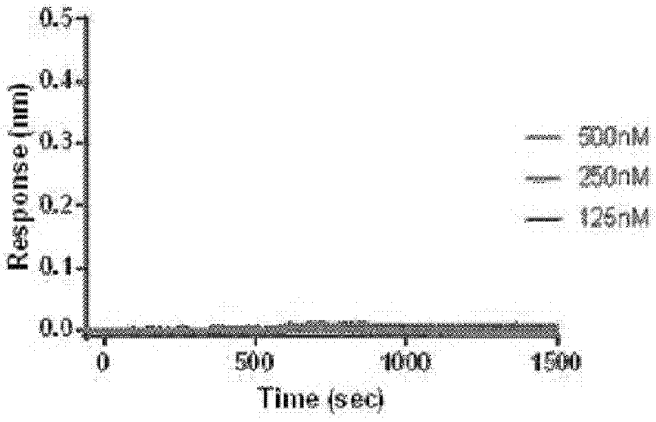
[도25]


Response (nm)
0.5
0.4
0.3
0.2
0.1
0.0
0
500
1000
1500
Time (sec)
500nM
250nM
125nM

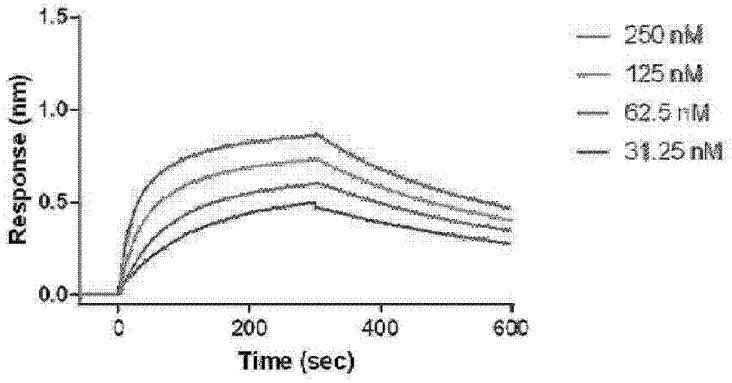
[도26]


Response (nm)
0.5
0.4
0.3
0.2
0.1
0.0
0
500
1000
1500
Time (sec)
500nM
250nM
125nM

[도27]

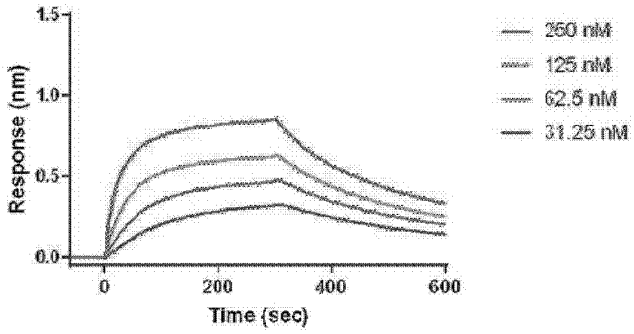

Response (nm)
0.5
0.4
0.3
0.2
0.1
0.0
0
500
1000
1500
Time (sec)
500nM
250nM
125nM

[도28]



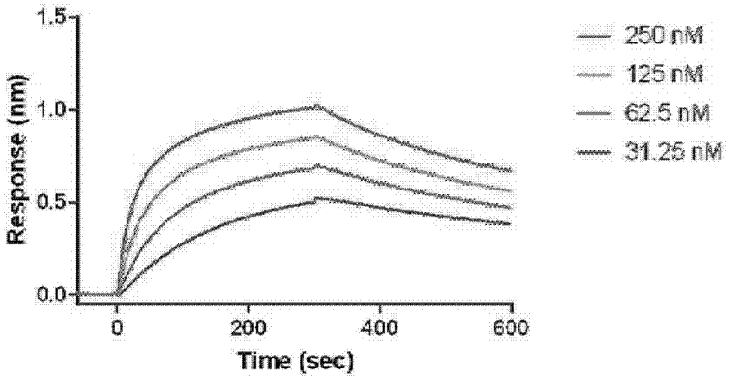
Kon	Koff	Kd
1.30X105	2.01X10-3	1.55X10-8

[도29]



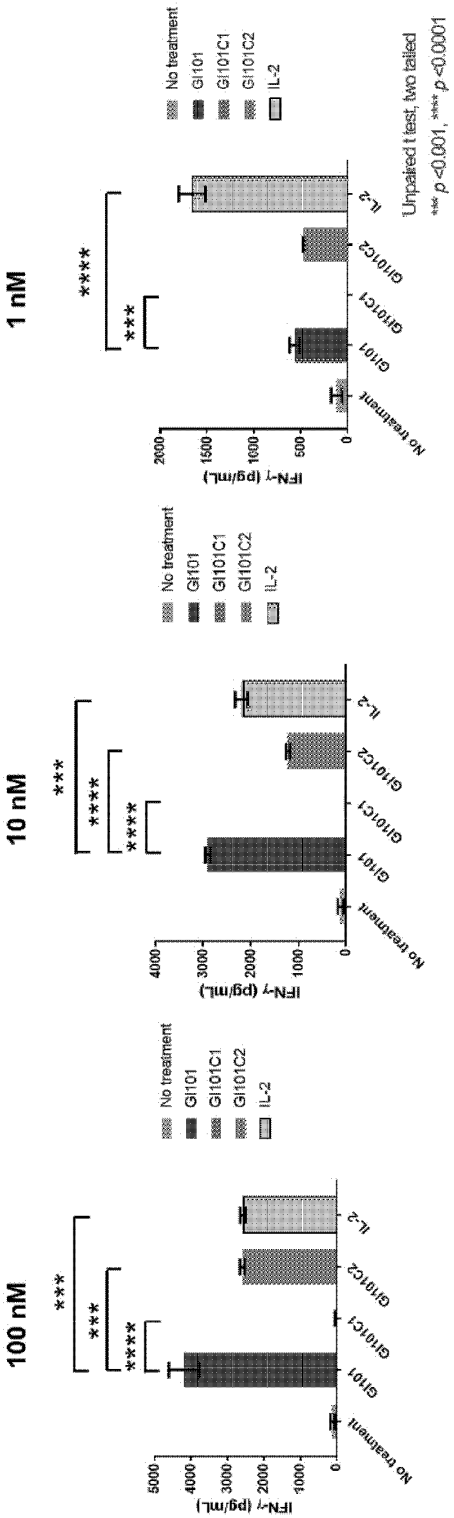
Kon	Koff	Kd
1.32X105	3.11X10-3	2.36X10-8

[도30]

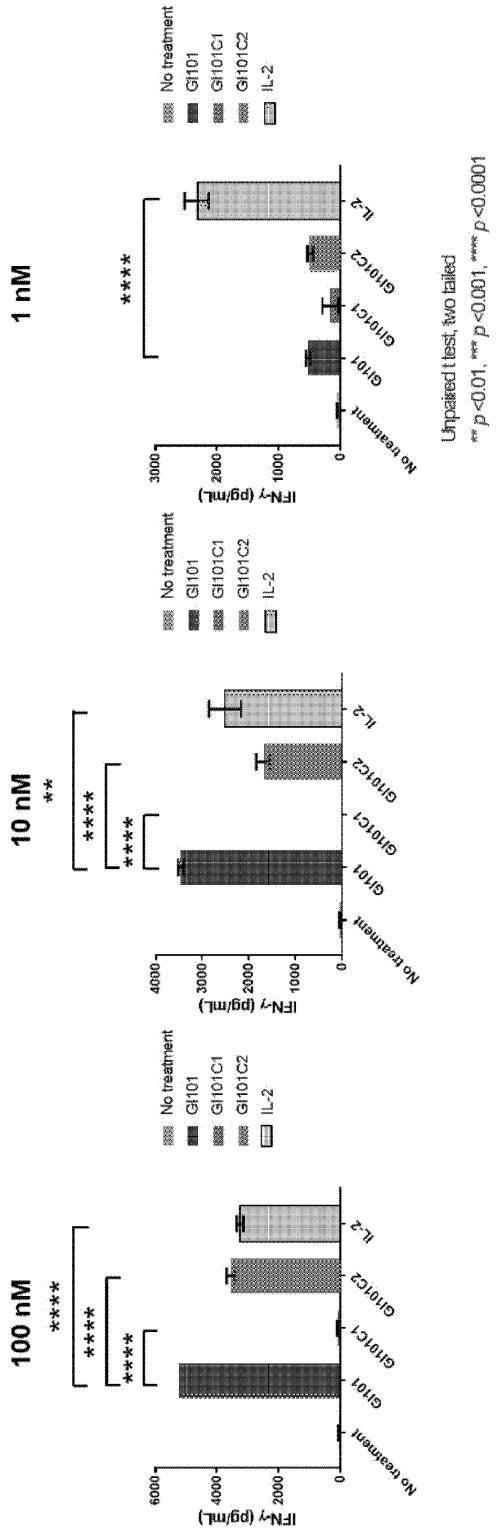


Kon	Koff	Kd
1.10X105	1.27X10-3	1.15X10-8

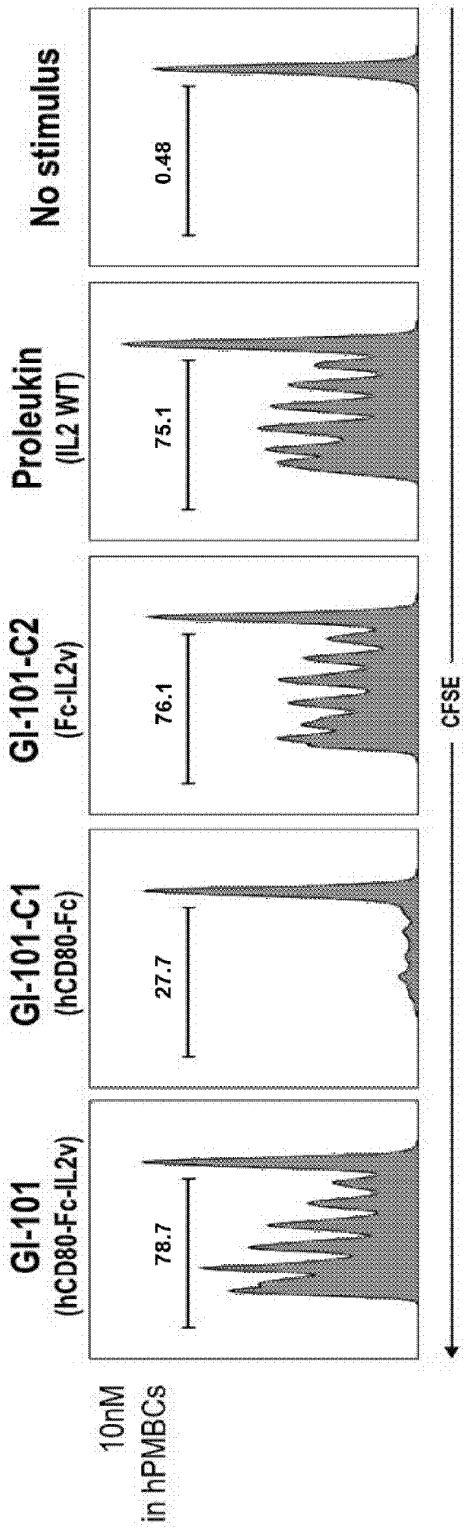
[도31]


1 nM
10 nM
100 nM
No treatment
GI101
GI101C1
GI101C2
IL-2
IFN-γ (pg/mL)
Unpaired t test, two tailed
*** p <0.001, **** p <0.0001

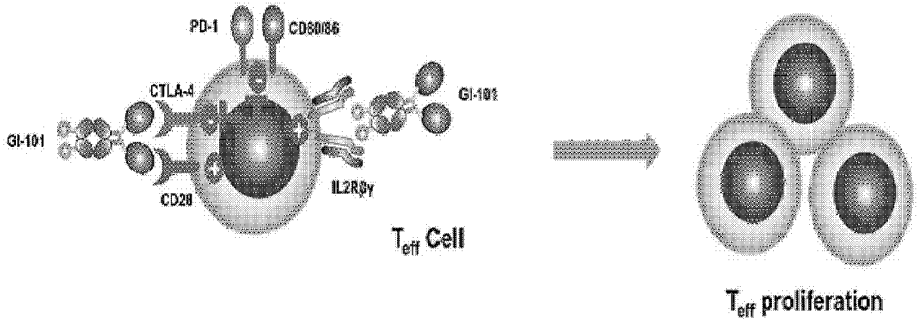
[도32]


100 nM
10 nM
1 nM
IFN-γ (pg/mL)
No treatment
GI101
GI101C1
GI101C2
IL-2
Unpaired t test, two tailed
** p <0.01, *** p <0.001, **** p <0.0001

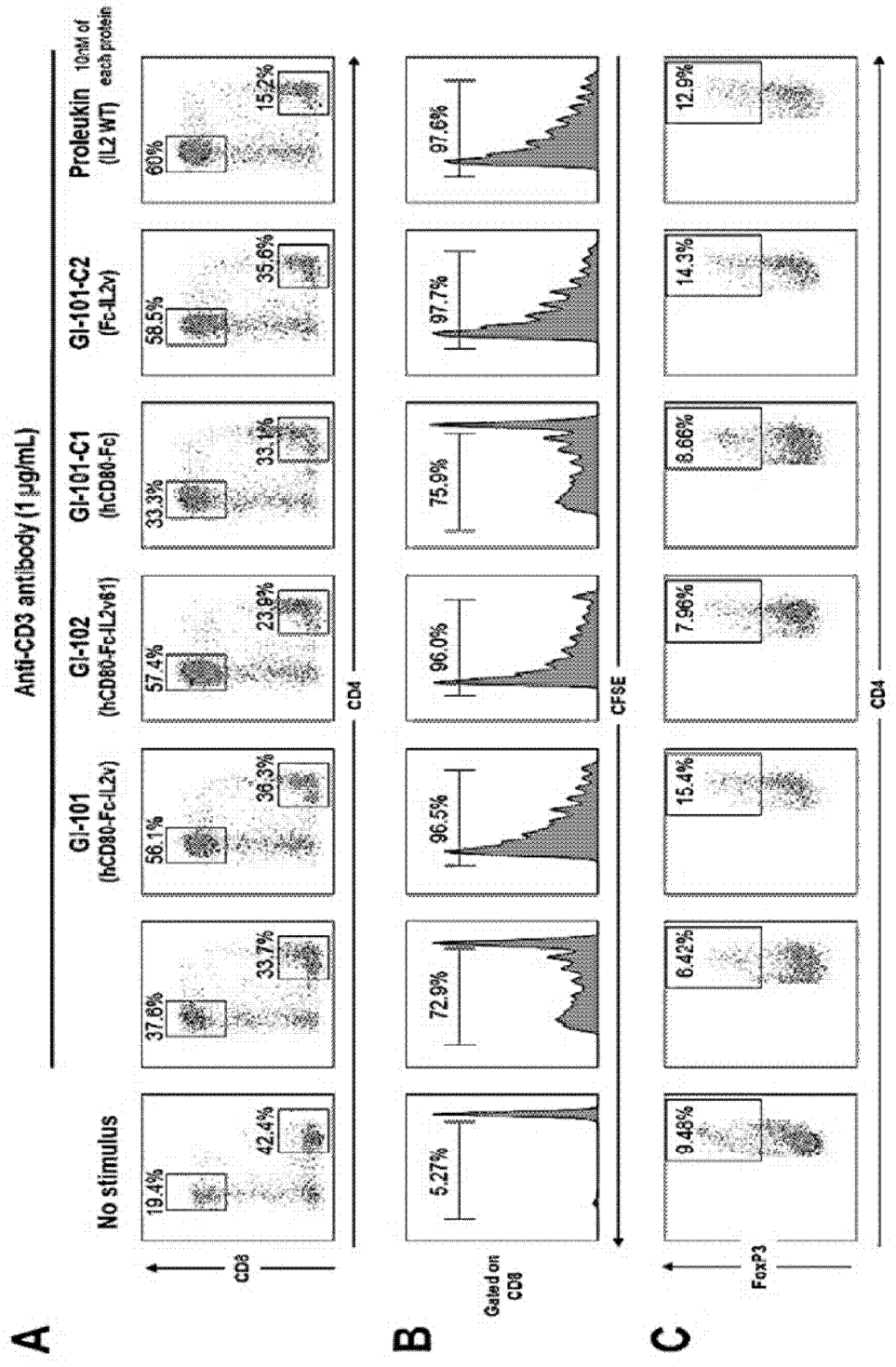
[도33]


No stimulus
0.48
Proleukin
(IL2 WT)
75.1
GI-101-C2
(Fc-IL2v)
76.1
GI-101-C1
(hCD80-Fc)
27.7
GI-101
(hCD80-Fc-IL2v)
78.7
CFSE
10nM
in hPMBCs

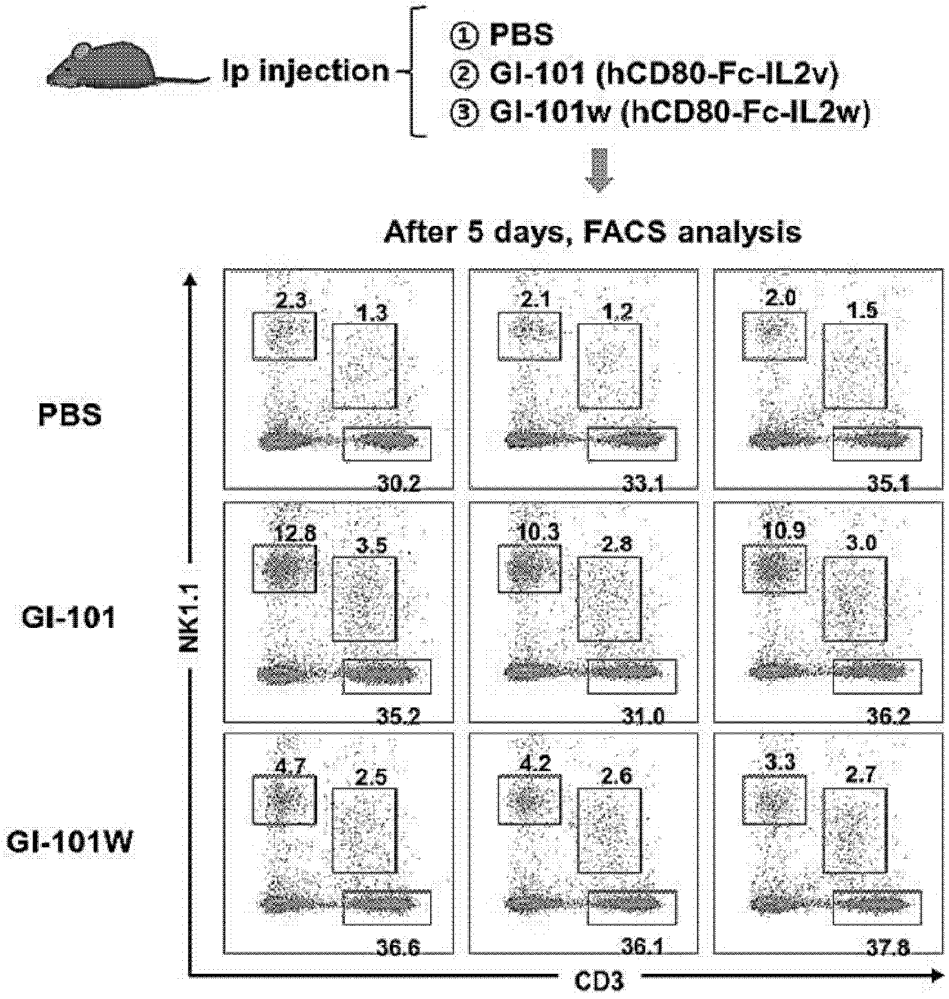
[도34]


PD-1
CD80/86
CTLA-4
GI-101
GI-101
CD28
IL2Rβγ
T_{eff} Cell
T_{eff} proliferation

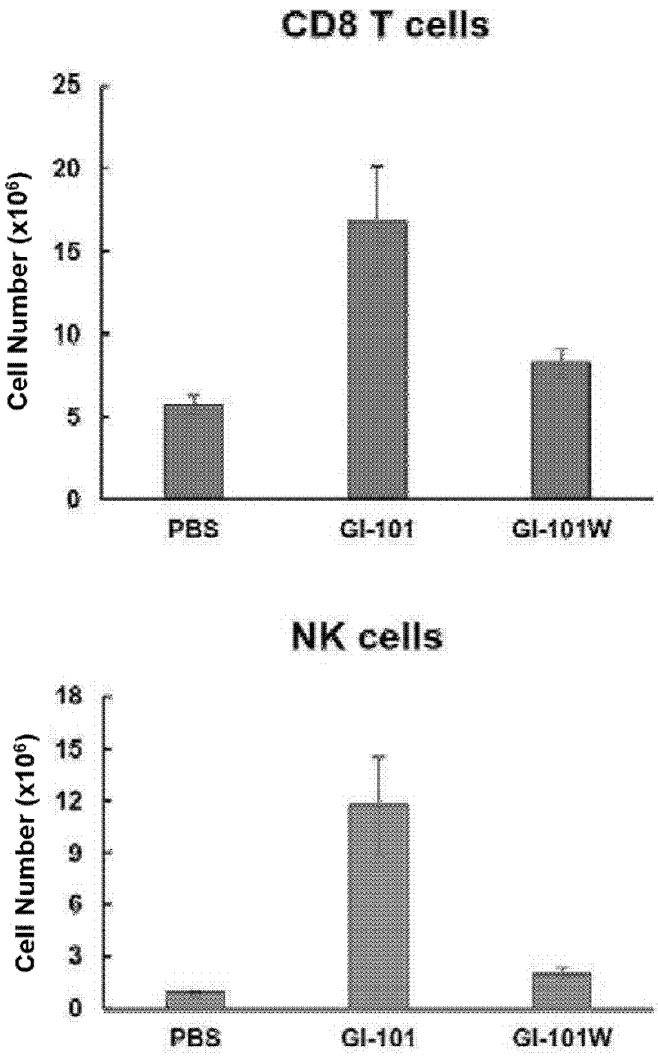
[도35]


A
B
C
Anti-CD3 antibody (1 μg/mL)
No stimulus
GI-101 (hCD80-Fc-IL2v)
GI-102 (hCD80-Fc-IL2v01)
GI-101-C1 (hCD80-Fc)
GI-101-C2 (Fc-IL2v)
Proleukin (IL2 WT) 10nM of each protein
CD8
CD4
Gated on CD8
CFSE
FoxP3
19.4%
42.4%
37.6%
33.7%
56.1%
38.3%
57.4%
23.8%
33.3%
33.1%
58.5%
35.6%
60%
15.2%
5.27%
72.9%
96.5%
96.0%
75.9%
97.7%
97.6%
9.48%
6.42%
15.4%
7.96%
8.66%
14.3%
12.9%

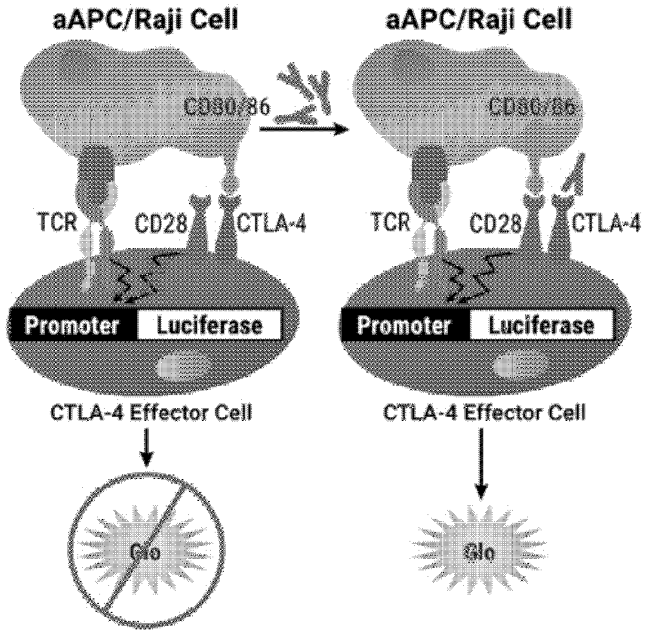
[도36]


Ip injection
① PBS
② GI-101 (hCD80-Fc-IL2v)
③ GI-101w (hCD80-Fc-IL2w)
After 5 days, FACS analysis
PBS
GI-101
GI-101W
NK1.1
CD3
2.3
1.3
30.2
2.1
1.2
33.1
2.0
1.5
35.1
12.8
3.5
35.2
10.3
2.8
31.0
10.9
3.0
36.2
4.7
2.5
36.6
4.2
2.6
36.1
3.3
2.7
37.8

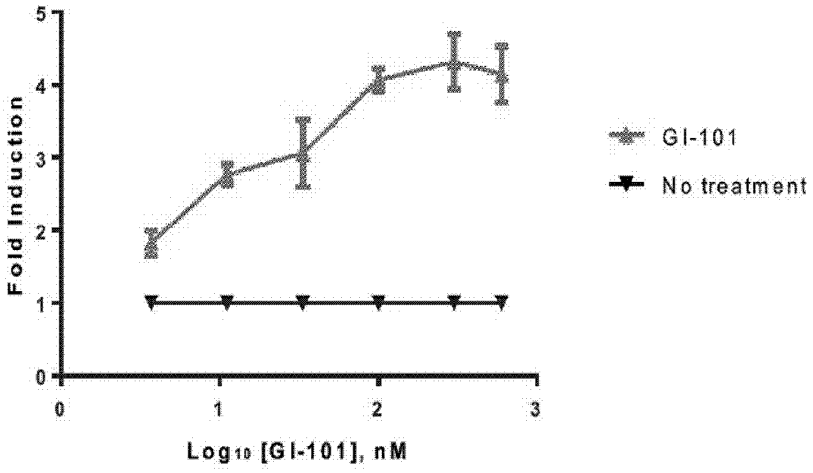
[도37]


CD8 T cells
Cell Number (x10⁶)
25
20
15
10
5
0
PBS
GI-101
GI-101W
NK cells
Cell Number (x10⁶)
18
15
12
9
6
3
0
PBS
GI-101
GI-101W

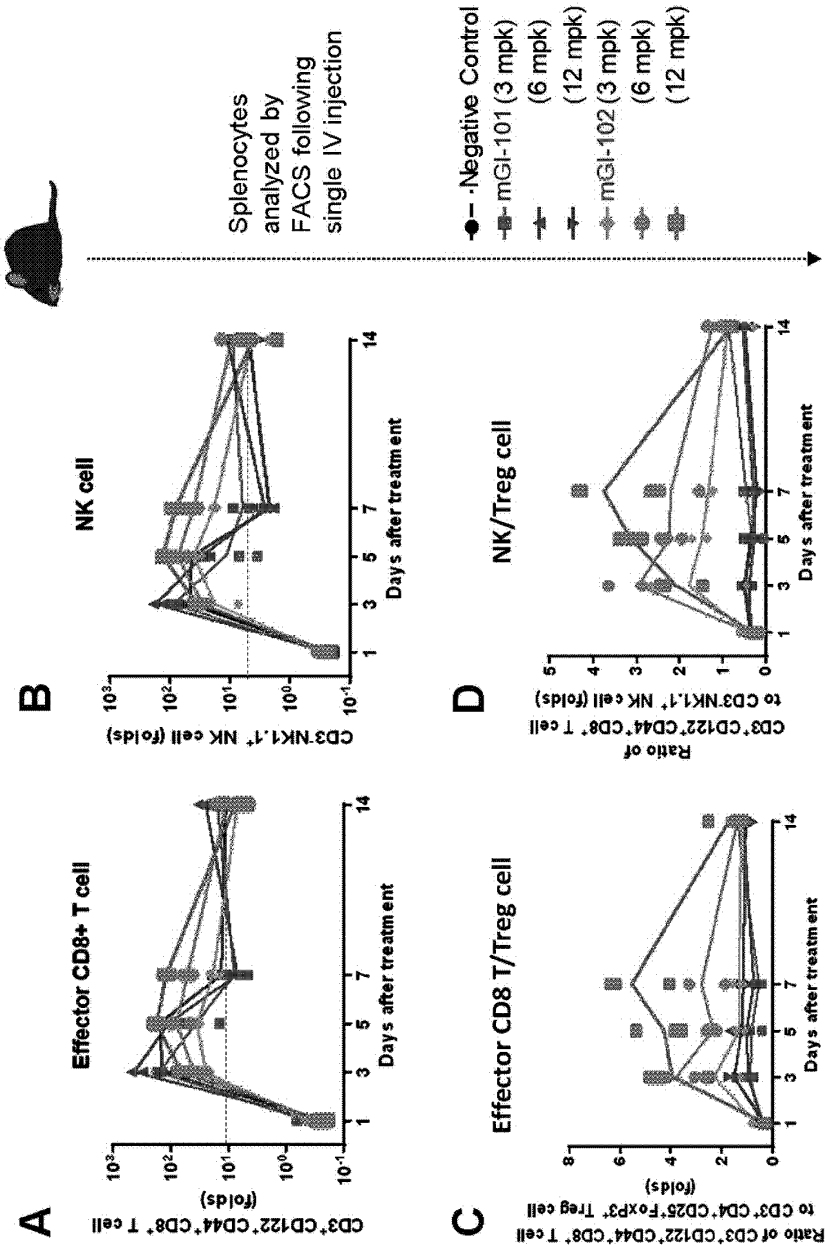
[도38]


aAPC/Raji Cell
aAPC/Raji Cell
CD80/86
CD80/86
TCR
CD28
CTLA-4
TCR
CD28
CTLA-4
Promoter
Luciferase
Promoter
Luciferase
CTLA-4 Effector Cell
CTLA-4 Effector Cell
Glo
Glo

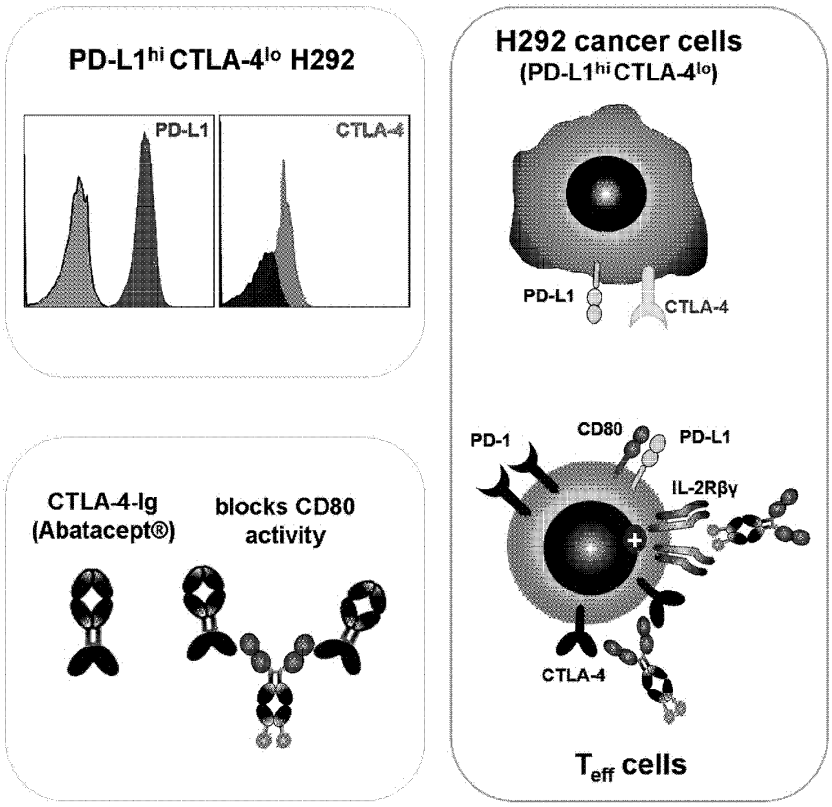
[도39]


Fold Induction
0
1
2
3
4
5
0
1
2
3
Log10 [GI-101], nM
GI-101
No treatment

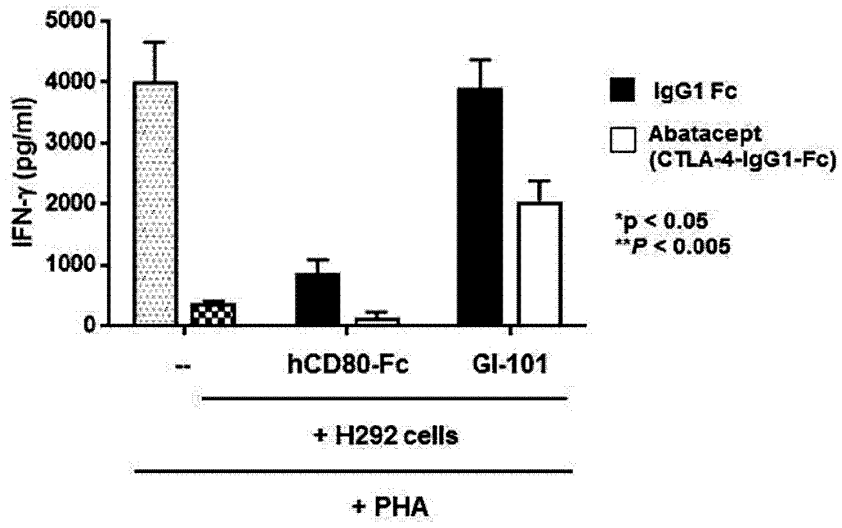
[도40]


A
Effector CD8+ T cell
CD3+CD122+CD44+CD8+ T cell (folds)
Days after treatment
B
NK cell
CD3-NK1.1+ NK cell (folds)
Days after treatment
C
Effector CD8 T/Treg cell
Ratio of CD3+CD122+CD44+CD8+ T cell to CD3+CD4+CD25+FoxP3+ Treg cell (folds)
Days after treatment
D
NK/Treg cell
Ratio of CD3+CD122+CD44+CD8+ T cell to CD3-NK1.1+ NK cell (folds)
Days after treatment
Splenocytes analyzed by FACS following single IV injection
Negative Control
mGI-101 (3 mpk)
(6 mpk)
(12 mpk)
mGI-102 (3 mpk)
(6 mpk)
(12 mpk)

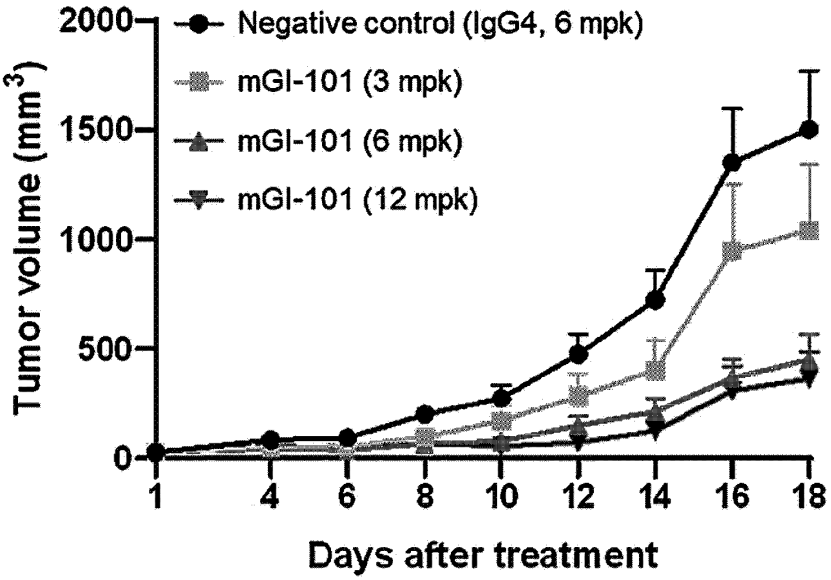
[도41]


PD-L1hi CTLA-4lo H292
PD-L1
CTLA-4
CTLA-4-Ig
(Abatacept®)
blocks CD80
activity
H292 cancer cells
(PD-L1hi CTLA-4lo)
PD-L1
CTLA-4
PD-1
CD80
PD-L1
IL-2Rβγ
CTLA-4
Teff cells

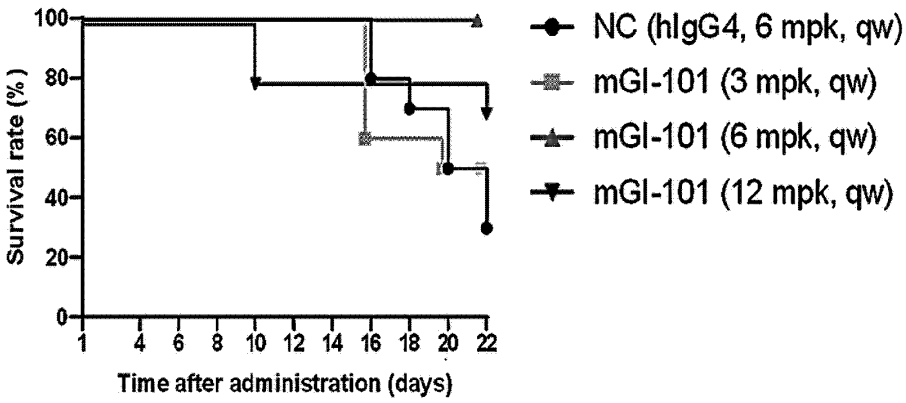
[도42]


IFN-γ (pg/ml)
5000
4000
3000
2000
1000
0
--
hCD80-Fc
GI-101
+ H292 cells
+ PHA
IgG1 Fc
Abatacept
(CTLA-4-IgG1-Fc)
*p < 0.05
**P < 0.005

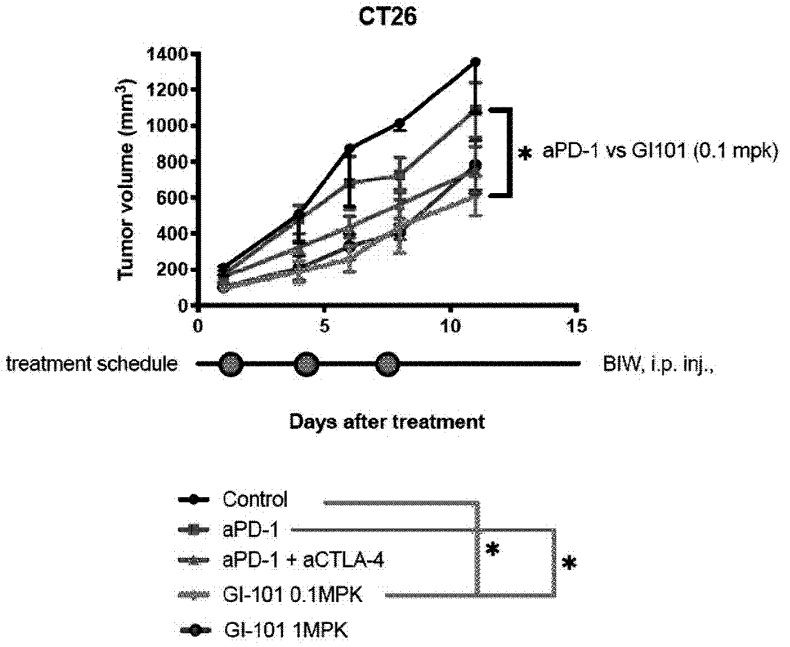
[도43]


Negative control (IgG4, 6 mpk)
mGI-101 (3 mpk)
mGI-101 (6 mpk)
mGI-101 (12 mpk)
Tumor volume (mm3)
2000
1500
1000
500
0
1
4
6
8
10
12
14
16
18
Days after treatment

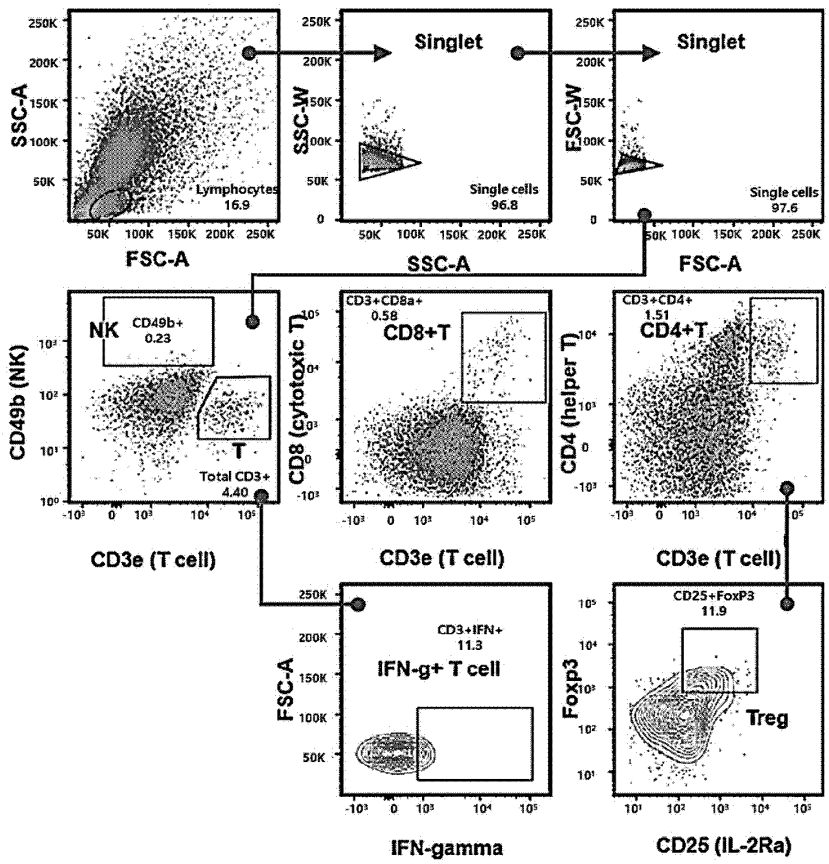
[도44]


NC (hIgG4, 6 mpk, qw)
mGI-101 (3 mpk, qw)
mGI-101 (6 mpk, qw)
mGI-101 (12 mpk, qw)
Survival rate (%)
100
80
60
40
20
0
1
4
6
8
10
12
14
16
18
20
22
Time after administration (days)

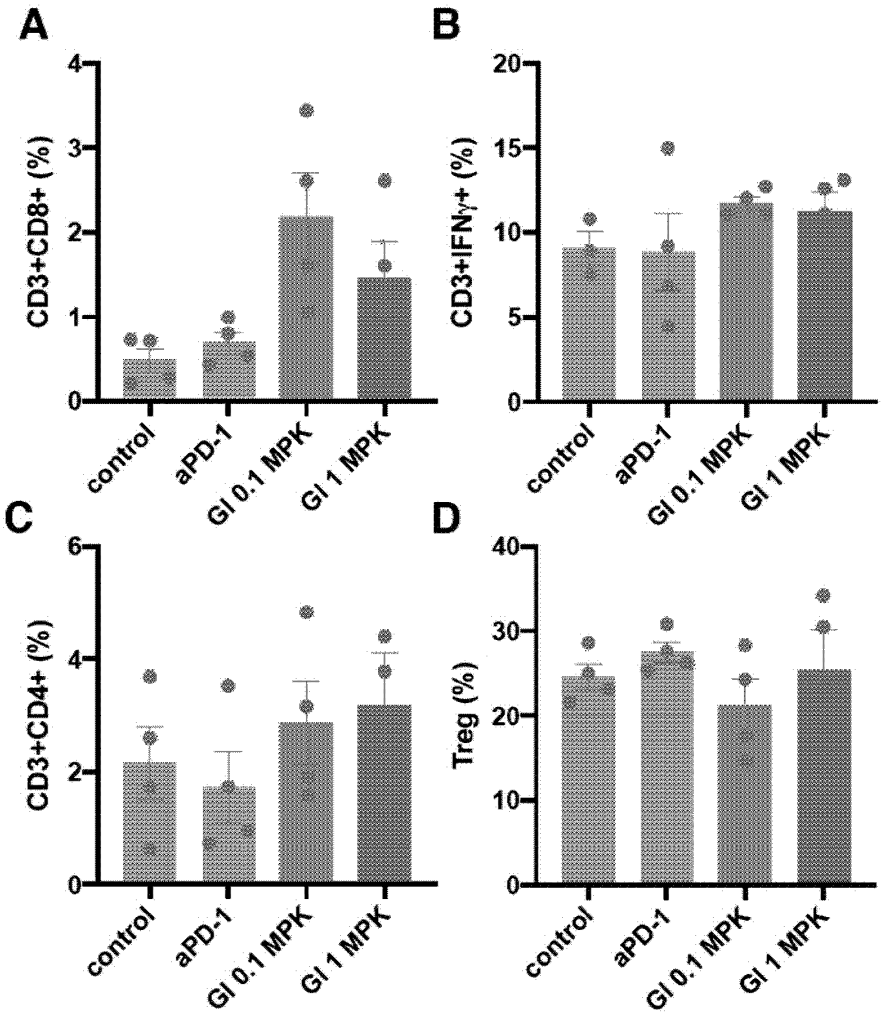
[도45]


CT26
Tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
5
10
15
* aPD-1 vs GI101 (0.1 mpk)
treatment schedule
BIW, i.p. inj.,
Days after treatment
Control
aPD-1
aPD-1 + aCTLA-4
GI-101 0.1MPK
GI-101 1MPK
*
*

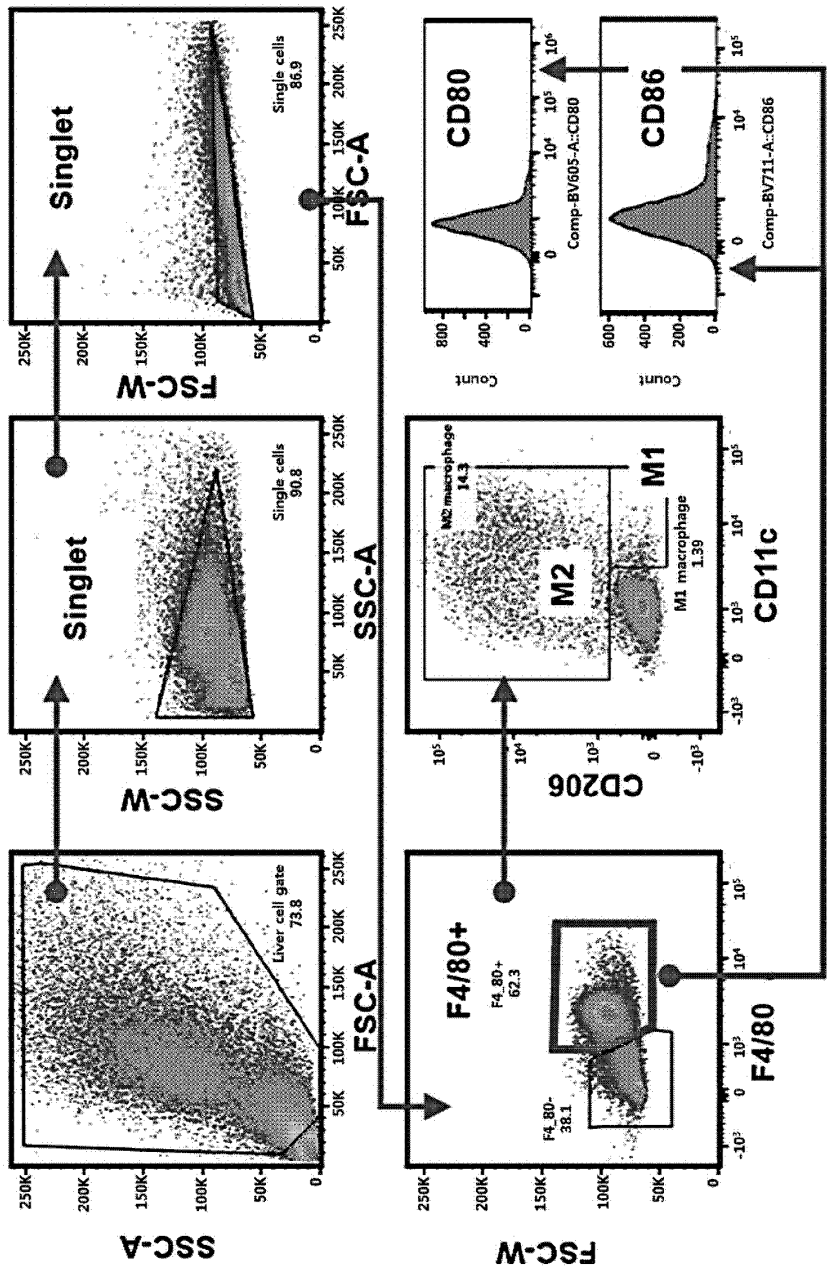
[도46]


SSC-A
FSC-A
Lymphocytes
16.9
Singlet
SSC-W
SSC-A
Single cells
96.8
Singlet
FSC-W
FSC-A
Single cells
97.6
NK
CD49b+
0.23
T
Total CD3+
4.40
CD49b (NK)
CD3e (T cell)
CD3+CD8a+
0.58
CD8+T
CD8 (cytotoxic T)
CD3e (T cell)
CD3+CD4+
1.51
CD4+T
CD4 (helper T)
CD3e (T cell)
CD3+IFN+
11.3
IFN-g+ T cell
FSC-A
IFN-gamma
CD25+FoxP3
11.9
Treg
Foxp3
CD25 (IL-2Ra)

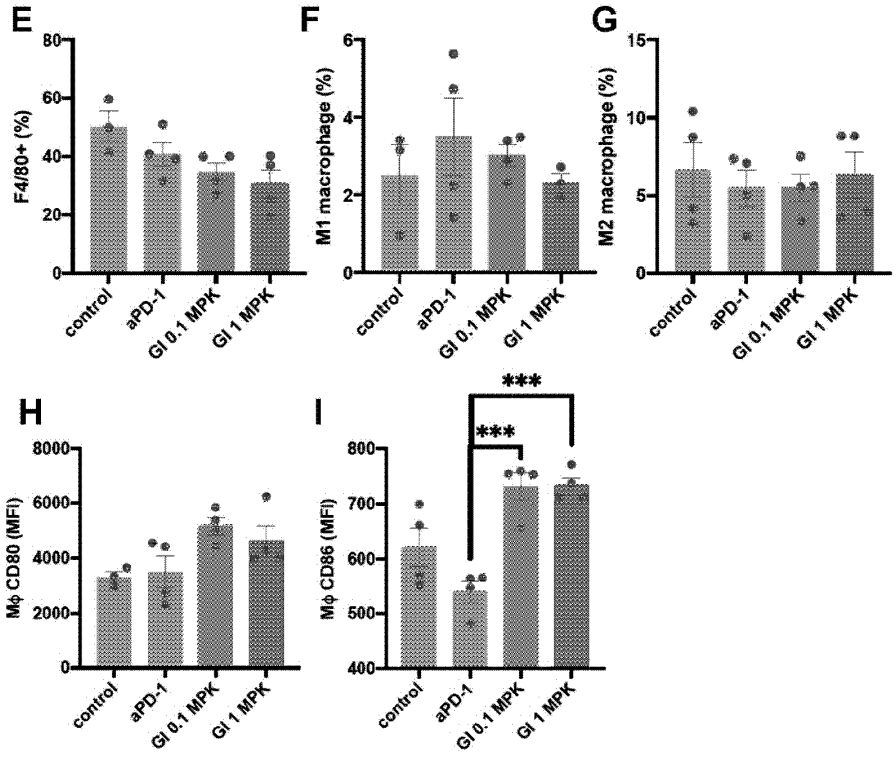
[도47]


A
CD3+CD8+ (%)
4
3
2
1
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
CD3+IFNγ+ (%)
20
15
10
5
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
CD3+CD4+ (%)
6
4
2
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
D
Treg (%)
40
30
20
10
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK

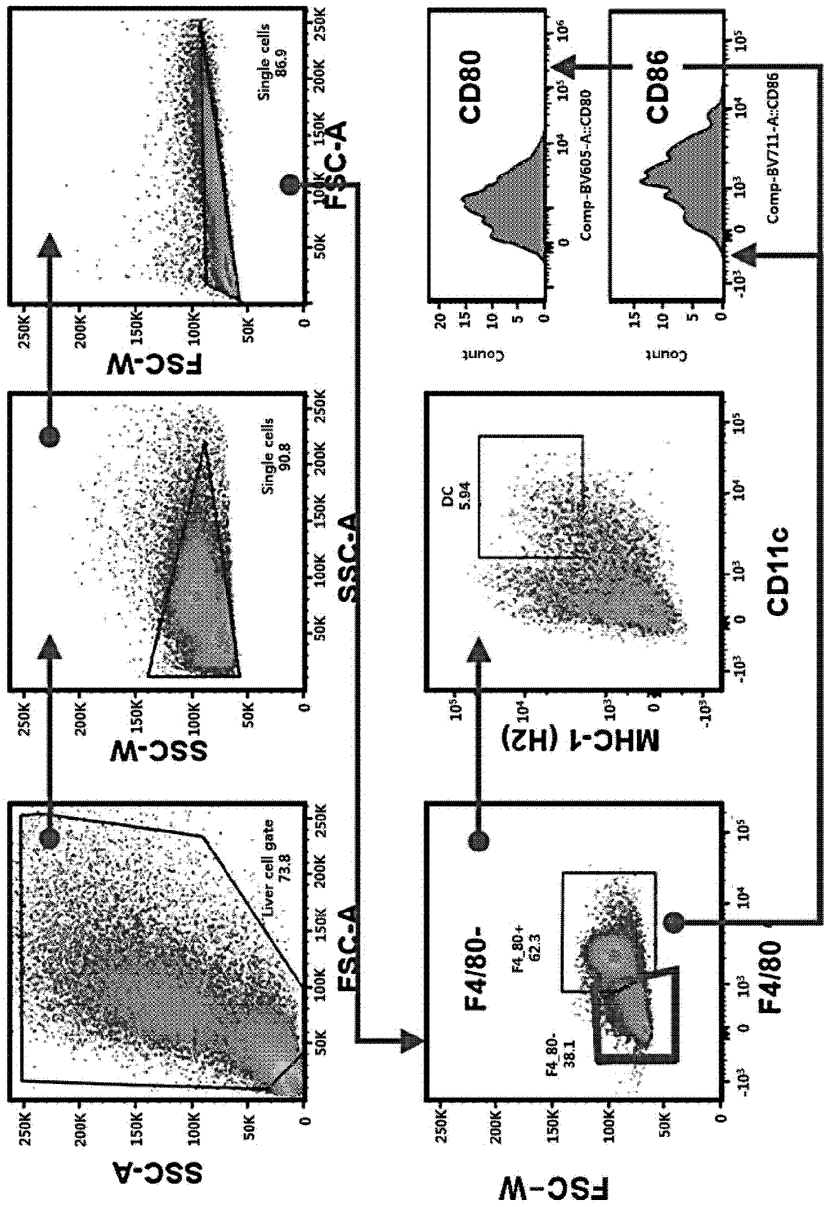
[도48]


Liver cell gate
73.8
SSC-A
FSC-W
Singlet
Single cells
86.9
FSC-W
FSC-A
Singlet
Single cells
90.8
SSC-W
SSC-A
F4/80+
F4_80+
62.3
F4_80-
38.1
F4/80
FSC-W
FSC-A
M2
M1
M2 macrophage
14.3
M1 macrophage
1.39
CD206
CD11c
CD80
Comp-BV605-A::CD80
Count
CD86
Comp-BV711-A::CD86
Count

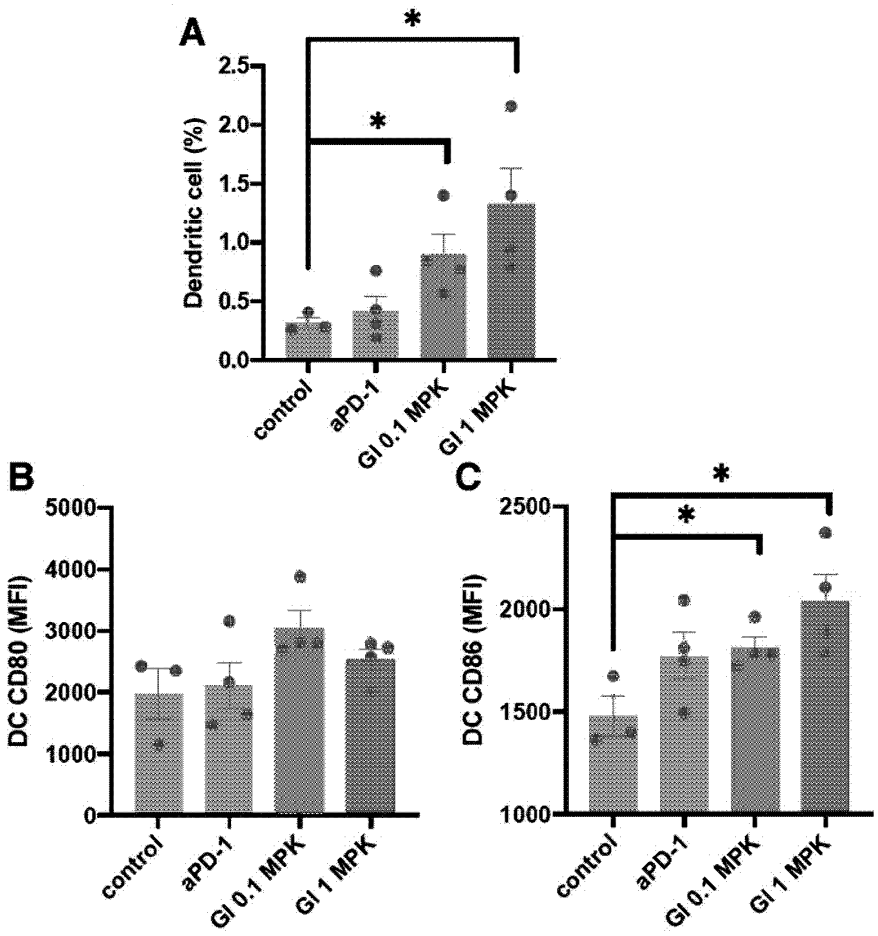
[도49]


E
F4/80+ (%)
F
M1 macrophage (%)
G
M2 macrophage (%)
H
Mϕ CD80 (MFI)
I
Mϕ CD86 (MFI)
***
***
control
aPD-1
GI 0.1 MPK
GI 1 MPK

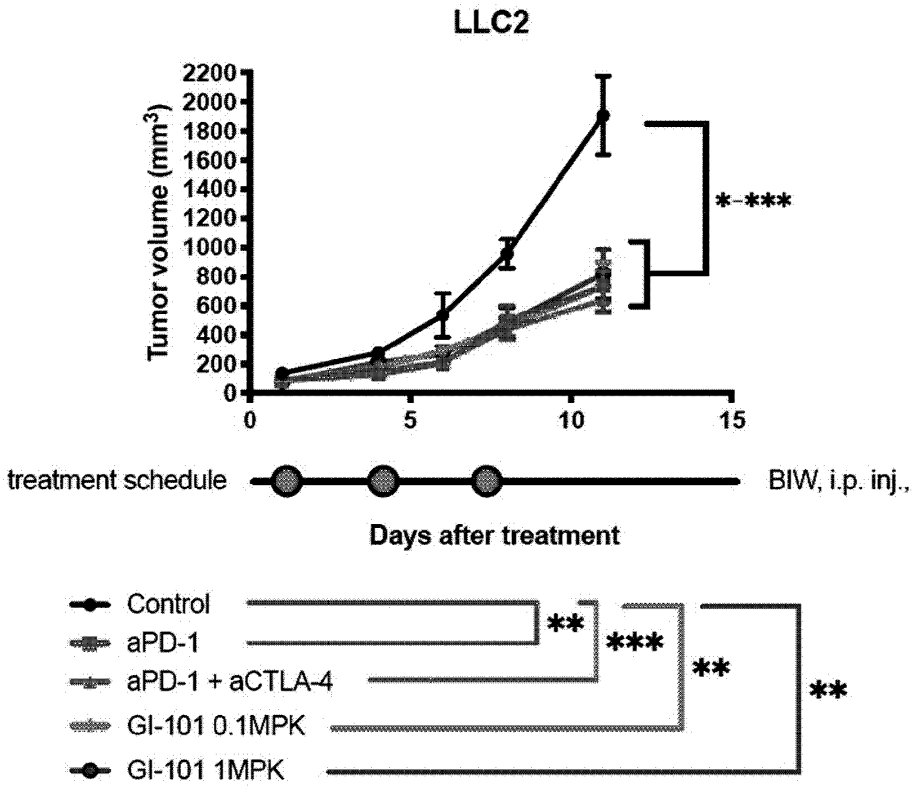
[도50]


SSC-A
FSC-W
Liver cell gate
73.8
FSC-A
SSC-W
Single cells
90.8
FSC-A
FSC-W
Single cells
86.9
FSC-A
SSC-A
F4/80-
F4_80+
62.3
F4_80-
38.1
F4/80
CD11c
DC
5.94
MHC-1 (H2)
CD80
Comp-BV605-A::CD80
Count
CD86
Comp-BV711-A::CD86
Count

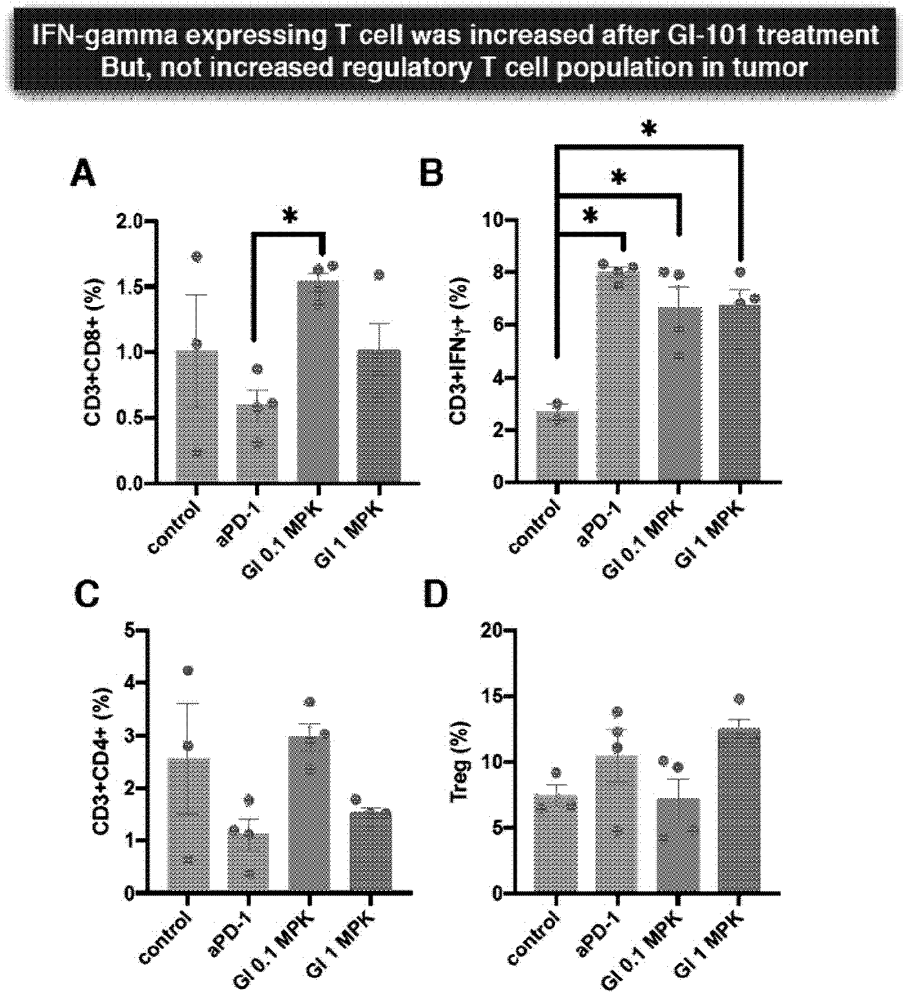
[도51]


A
Dendritic cell (%)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
DC CD80 (MFI)
5000
4000
3000
2000
1000
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
DC CD86 (MFI)
2500
2000
1500
1000
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK

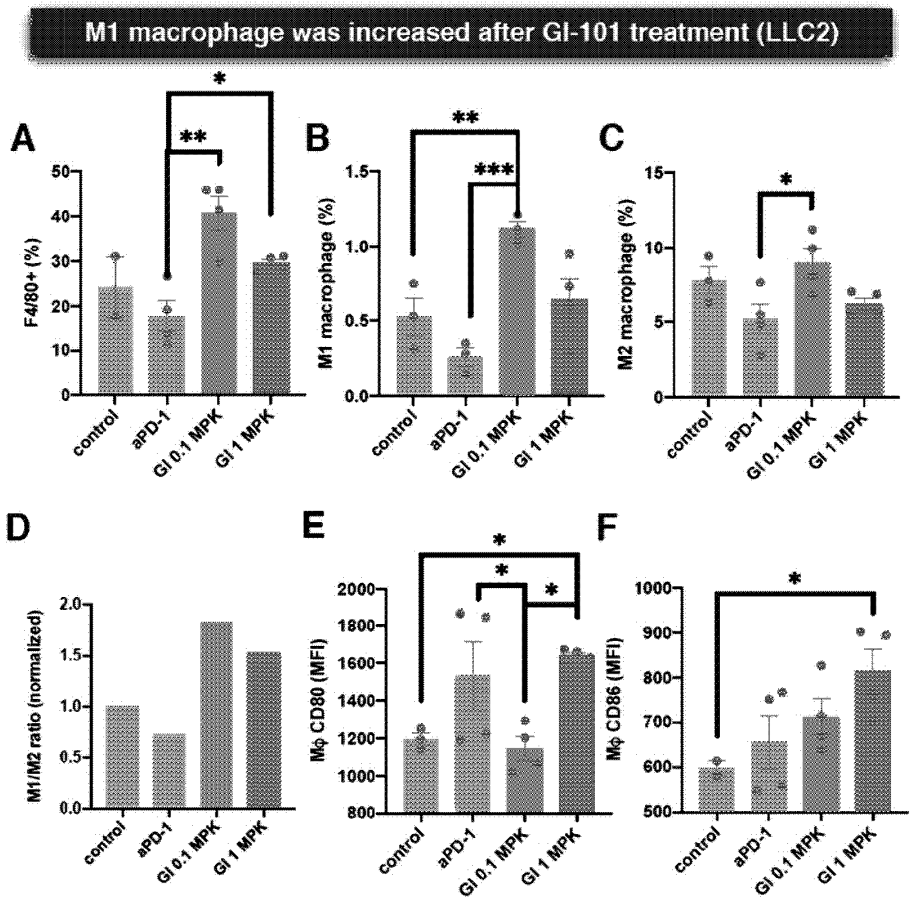
[도52]


LLC2
Tumor volume (mm3)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
*-***
treatment schedule
BIW, i.p. inj.,
Days after treatment
Control
aPD-1
aPD-1 + aCTLA-4
GI-101 0.1MPK
GI-101 1MPK
**
***
**
**

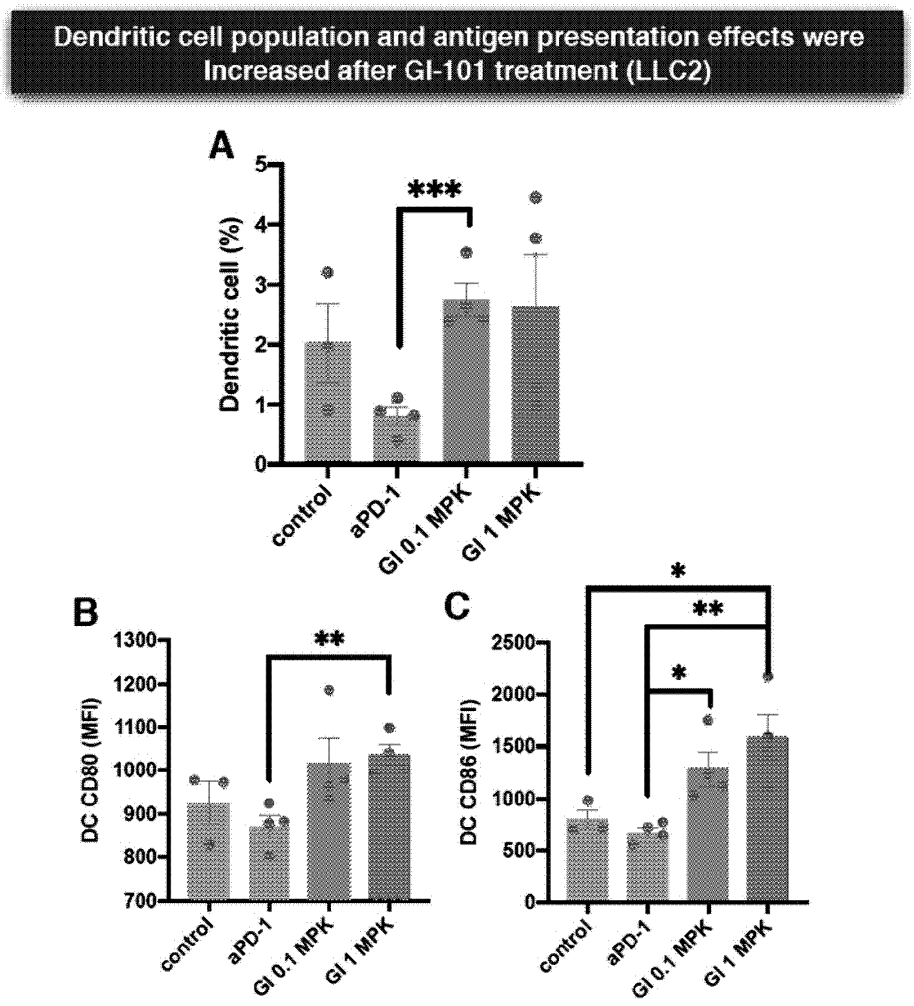
[도53]


IFN-gamma expressing T cell was increased after GI-101 treatment
But, not increased regulatory T cell population in tumor
A
CD3+CD8+ (%)
B
CD3+IFNγ+ (%)
C
CD3+CD4+ (%)
D
Treg (%)
control
aPD-1
GI 0.1 MPK
GI 1 MPK

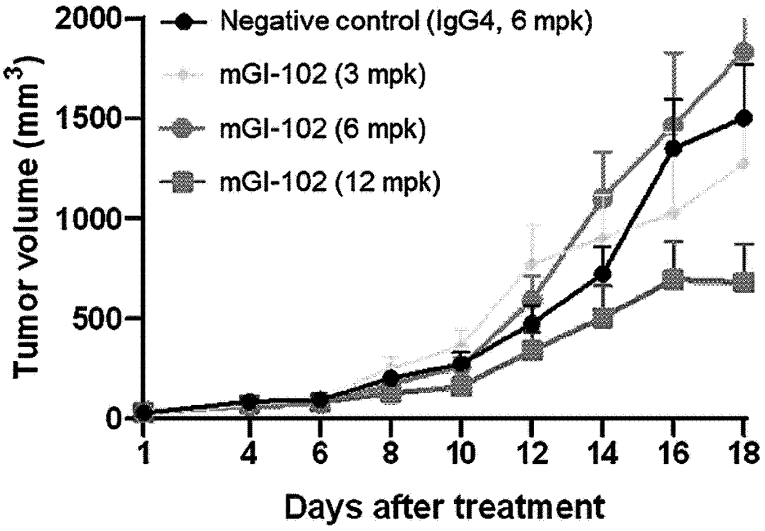
[도54]


M1 macrophage was increased after GI-101 treatment (LLC2)
A
F4/80+ (%)
B
M1 macrophage (%)
C
M2 macrophage (%)
D
M1/M2 ratio (normalized)
E
Mφ CD80 (MFI)
F
Mφ CD86 (MFI)
control
aPD-1
GI 0.1 MPK
GI 1 MPK

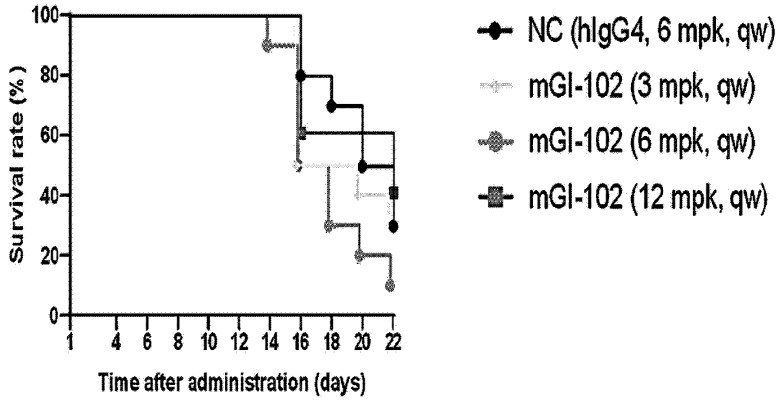
[도55]


Dendritic cell population and antigen presentation effects were Increased after GI-101 treatment (LLC2)
A
Dendritic cell (%)
***
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
DC CD80 (MFI)
**
C
DC CD86 (MFI)
*
**
*

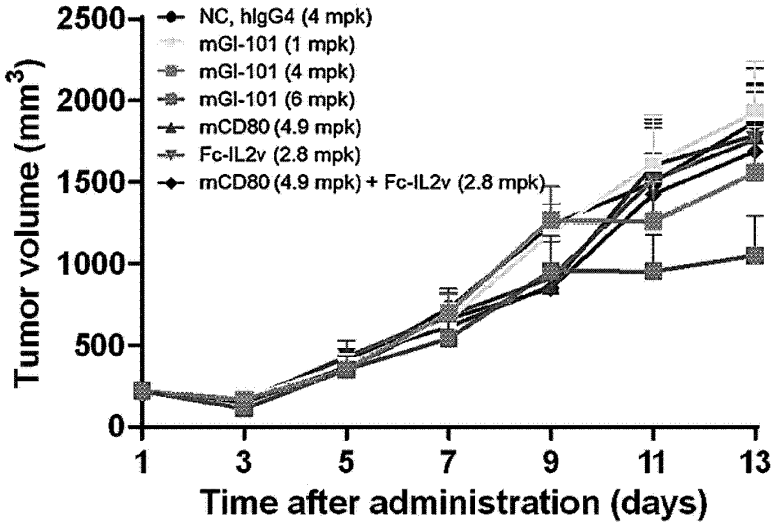
[도56]


Negative control (IgG4, 6 mpk)
mGI-102 (3 mpk)
mGI-102 (6 mpk)
mGI-102 (12 mpk)
Tumor volume (mm3)
Days after treatment

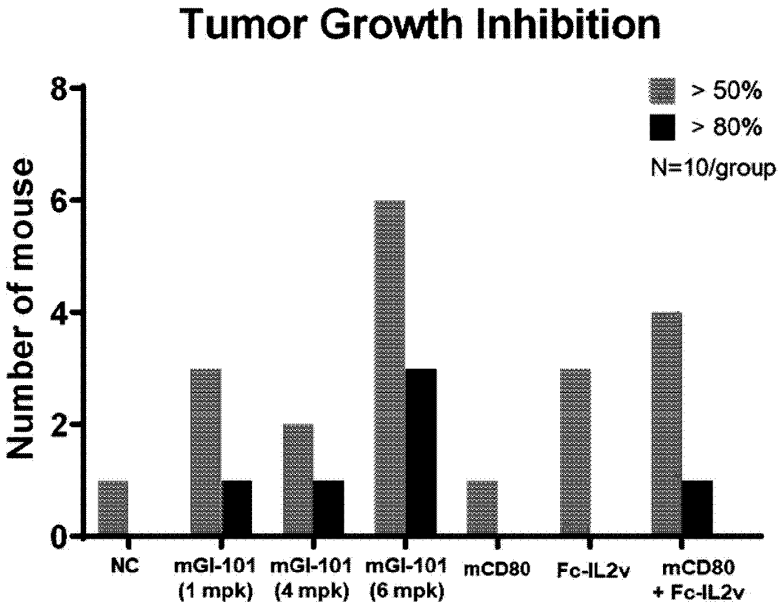
[도57]


Survival rate (%)
100
80
60
40
20
0
1 4 6 8 10 12 14 16 18 20 22
Time after administration (days)
NC (hIgG4, 6 mpk, qw)
mGl-102 (3 mpk, qw)
mGl-102 (6 mpk, qw)
mGl-102 (12 mpk, qw)

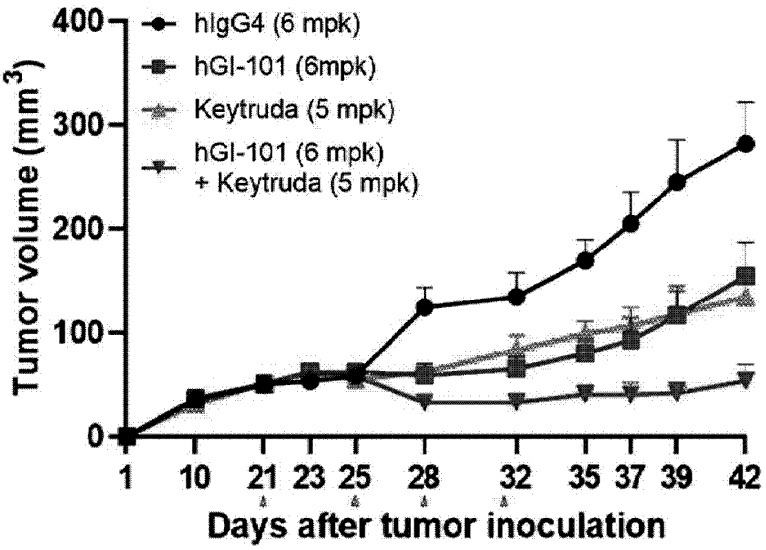
[도58]


Tumor volume (mm3)
2500
2000
1500
1000
500
0
1 3 5 7 9 11 13
Time after administration (days)
NC, hIgG4 (4 mpk)
mGl-101 (1 mpk)
mGl-101 (4 mpk)
mGl-101 (6 mpk)
mCD80 (4.9 mpk)
Fc-IL2v (2.8 mpk)
mCD80 (4.9 mpk) + Fc-IL2v (2.8 mpk)

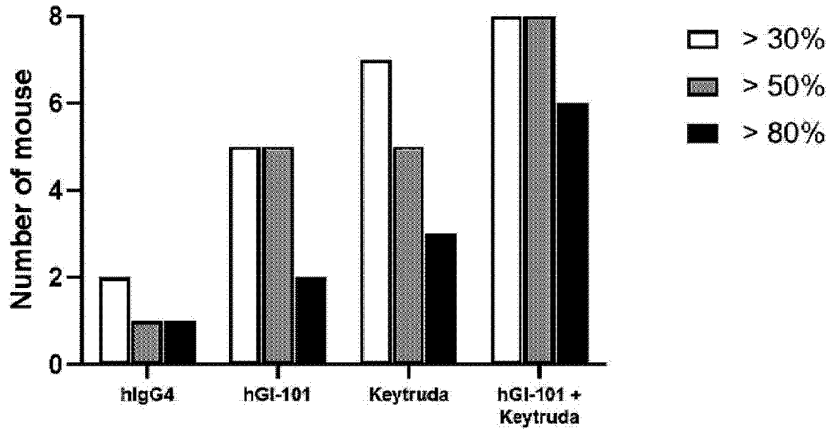
[도59]


Tumor Growth Inhibition
Number of mouse
8
6
4
2
0
> 50%
> 80%
N=10/group
NC
mGl-101 (1 mpk)
mGl-101 (4 mpk)
mGl-101 (6 mpk)
mCD80
Fc-IL2v
mCD80 + Fc-IL2v

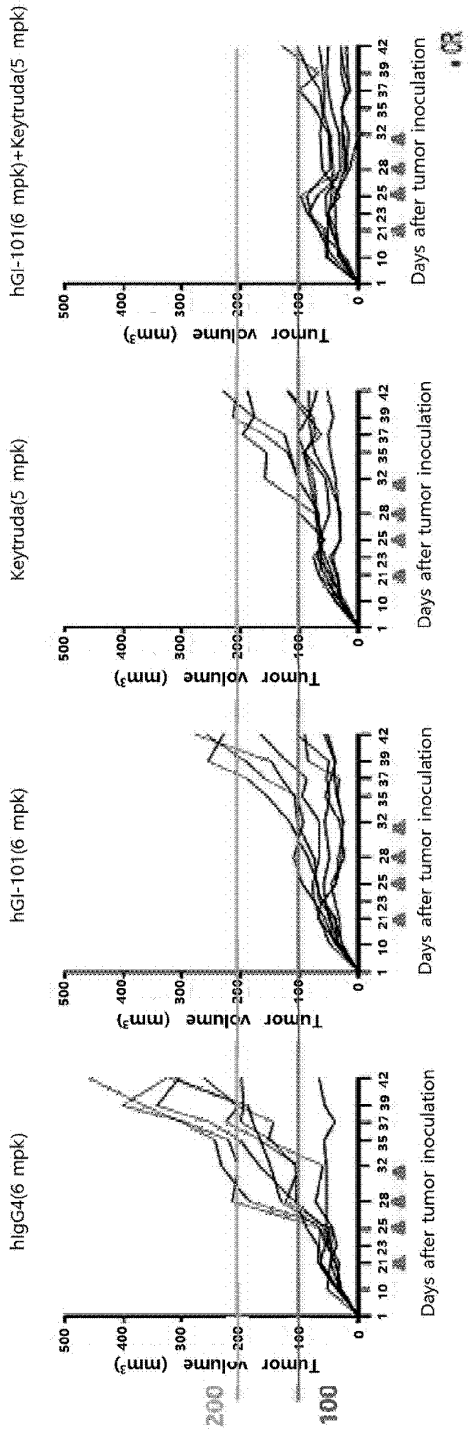
[도60]


hIgG4 (6 mpk)
hGI-101 (6mpk)
Keytruda (5 mpk)
hGI-101 (6 mpk)
+ Keytruda (5 mpk)
Tumor volume (mm3)
400
300
200
100
0
1
10
21
23
25
28
32
35
37
39
42
Days after tumor inoculation

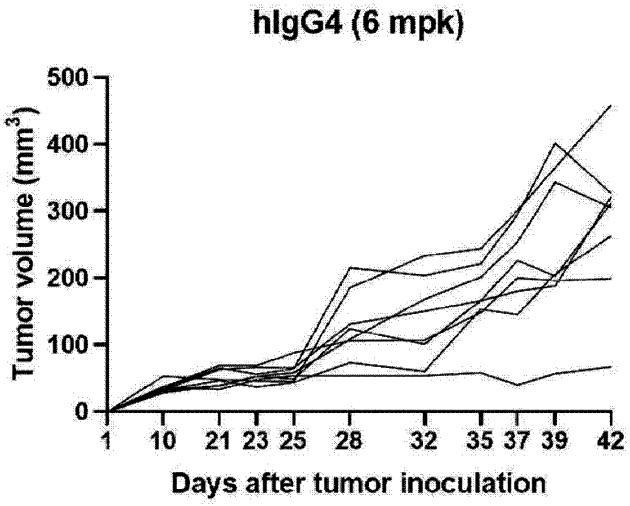
[도61]


Number of mouse
8
6
4
2
0
hIgG4
hGI-101
Keytruda
hGI-101 +
Keytruda
> 30%
> 50%
> 80%

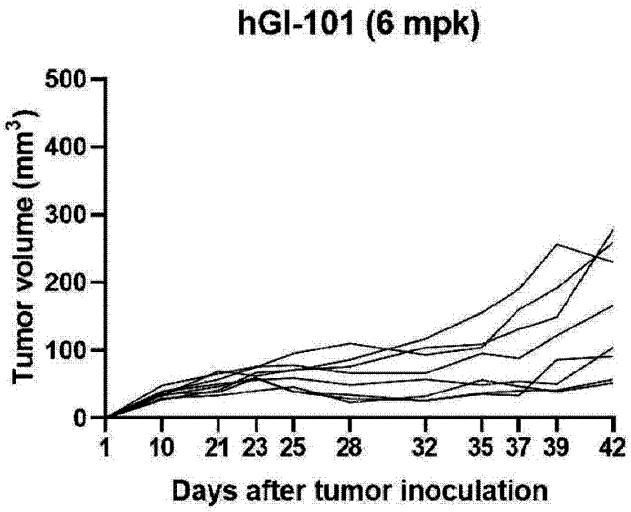
[도62]


hIgG4(6 mpk)
hGI-101(6 mpk)
Keytruda(5 mpk)
hGI-101(6 mpk)+Keytruda(5 mpk)
Tumor volume (mm³)
Days after tumor inoculation
200
100

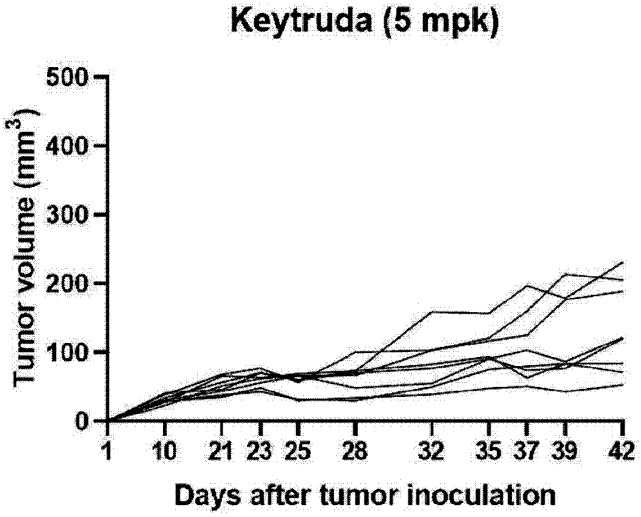
[도63]


hIgG4 (6 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1 10 21 23 25 28 32 35 37 39 42
Days after tumor inoculation

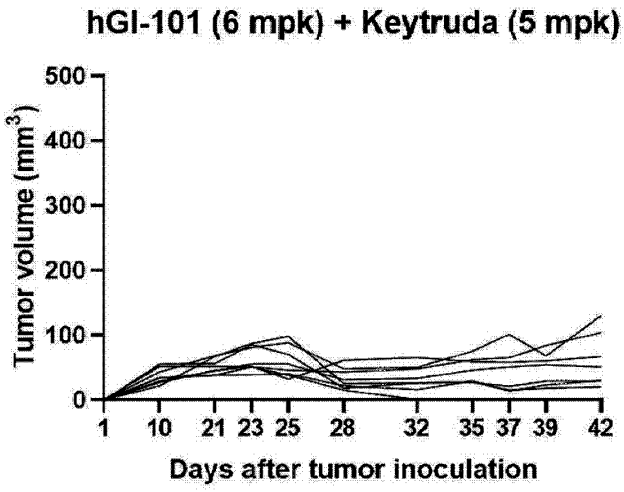
[도64]


hGI-101 (6 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1 10 21 23 25 28 32 35 37 39 42
Days after tumor inoculation

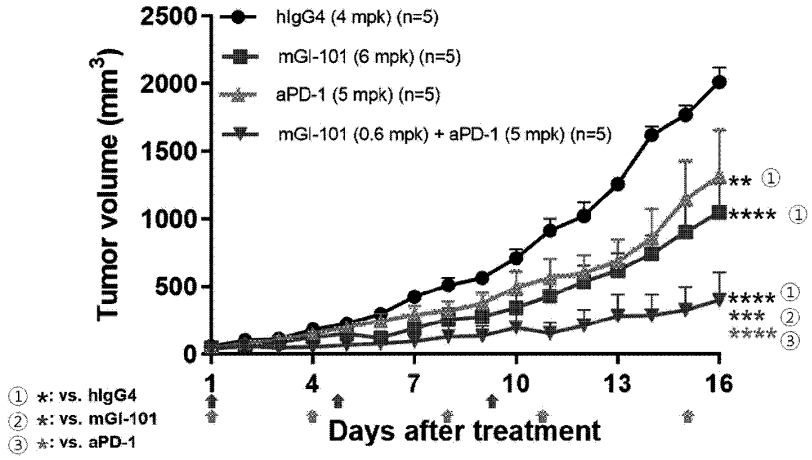
[도65]


Keytruda (5 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1 10 21 23 25 28 32 35 37 39 42
Days after tumor inoculation

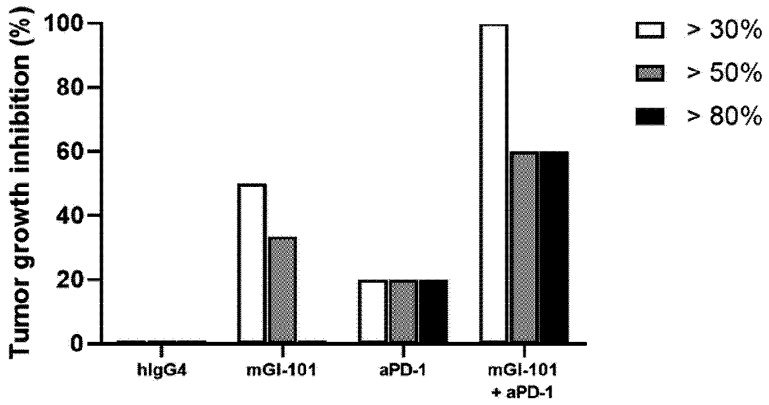
[도66]


hGI-101 (6 mpk) + Keytruda (5 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1 10 21 23 25 28 32 35 37 39 42
Days after tumor inoculation

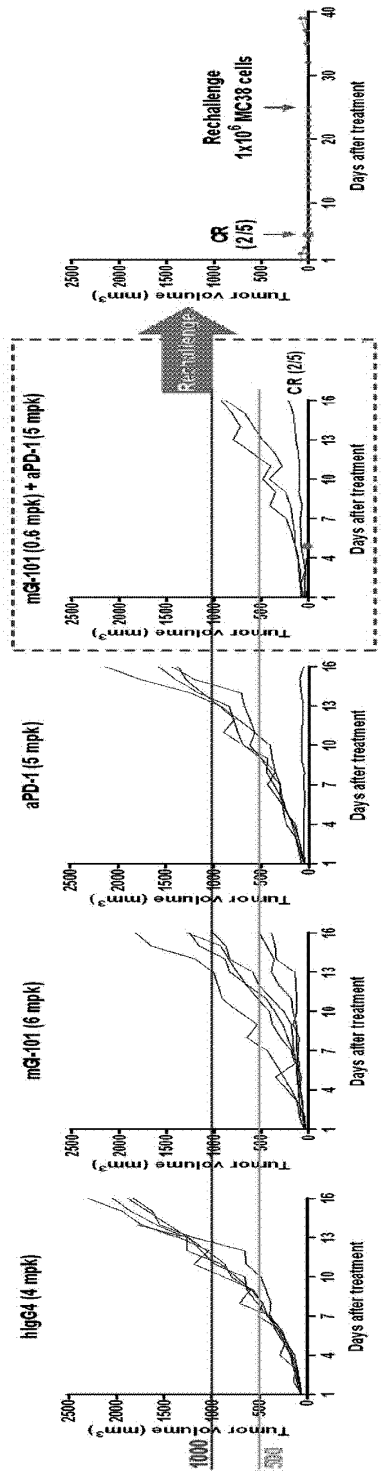
[도67]


hIgG4 (4 mpk) (n=5)
mGI-101 (6 mpk) (n=5)
aPD-1 (5 mpk) (n=5)
mGI-101 (0.6 mpk) + aPD-1 (5 mpk) (n=5)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
** ①
**** ①
**** ①
*** ②
**** ③
1 4 7 10 13 16
Days after treatment
① *: vs. hIgG4
② *: vs. mGI-101
③ *: vs. aPD-1

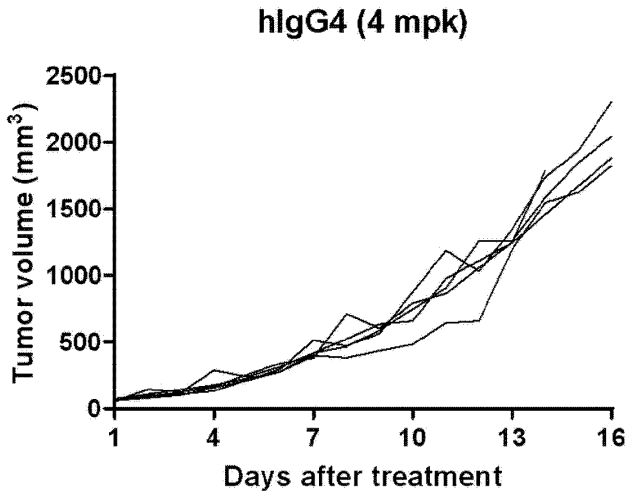
[도68]


Tumor growth inhibition (%)
100
80
60
40
20
0
hIgG4
mGI-101
aPD-1
mGI-101 + aPD-1
> 30%
> 50%
> 80%

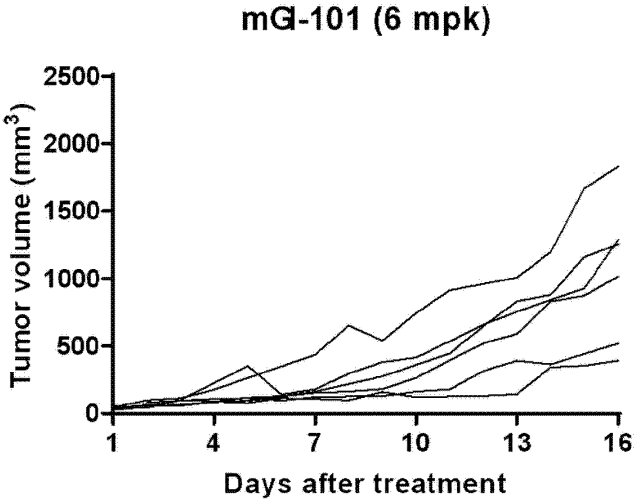
[도69]


hIgG4 (4 mpk)
mGA101 (6 mpk)
aPD-1 (5 mpk)
mGA101 (0.6 mpk) + aPD-1 (5 mpk)
Tumor volume (mm3)
Days after treatment
CR (2/5)
Rechallenge
1x10^5 MC38 cells
1000
351

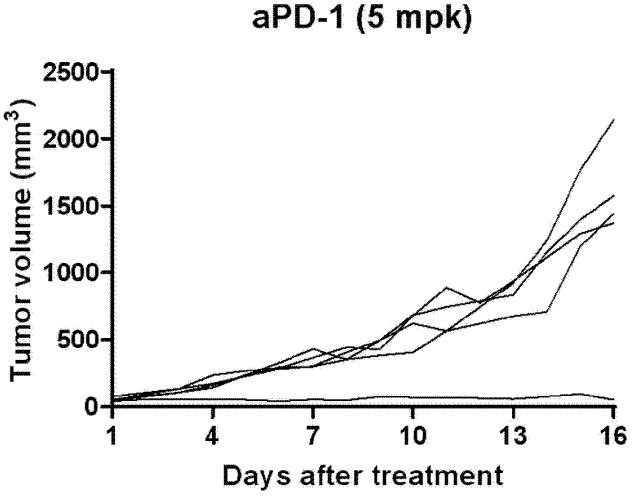
[도70]


hIgG4 (4 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment

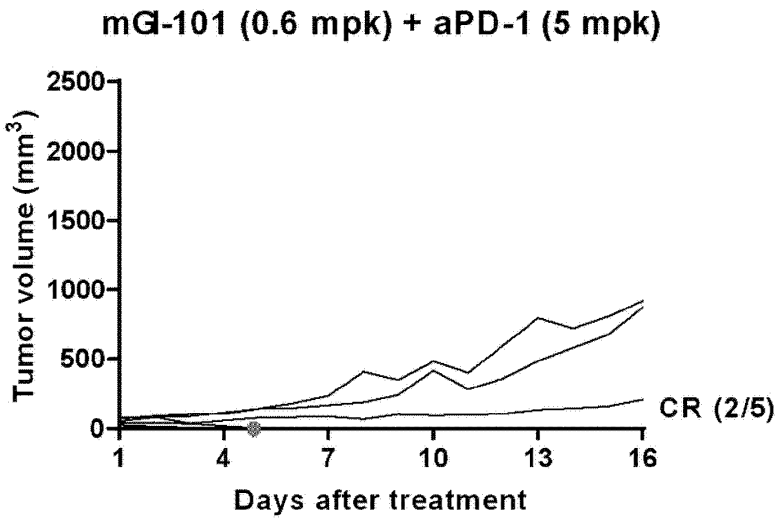
[도71]


mGI-101 (6 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment

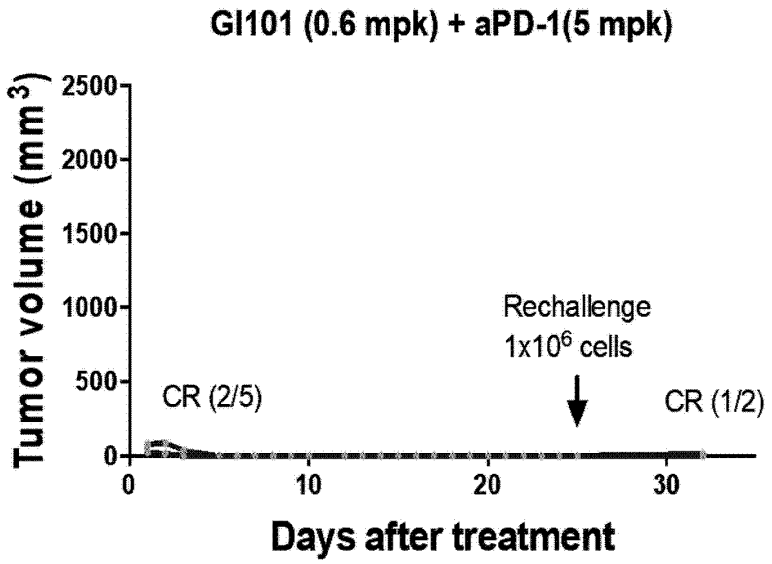
[도72]


aPD-1 (5 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment

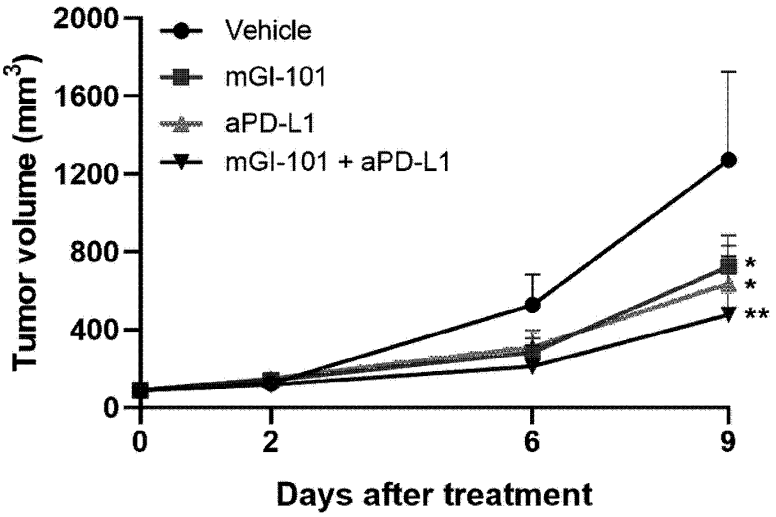
[도73]


mGI-101 (0.6 mpk) + aPD-1 (5 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment
CR (2/5)

[도74]


GI101 (0.6 mpk) + aPD-1(5 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
0
10
20
30
Days after treatment
CR (2/5)
Rechallenge
1x10^6 cells
CR (1/2)

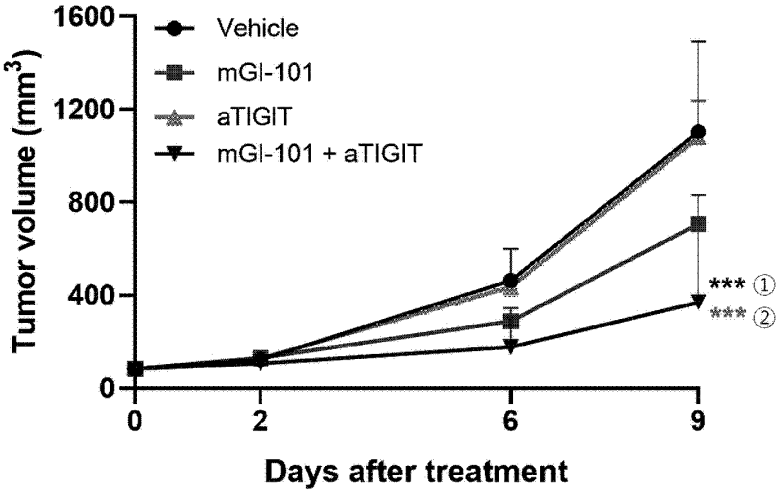
[도75]



*; $p < 0.05$ vs. Vehicle

**; $p < 0.01$ vs. Vehicle

[도76]



① ***; $p < 0.001$ vs. Vehicle

② ***; $p < 0.001$ vs. aTIGIT