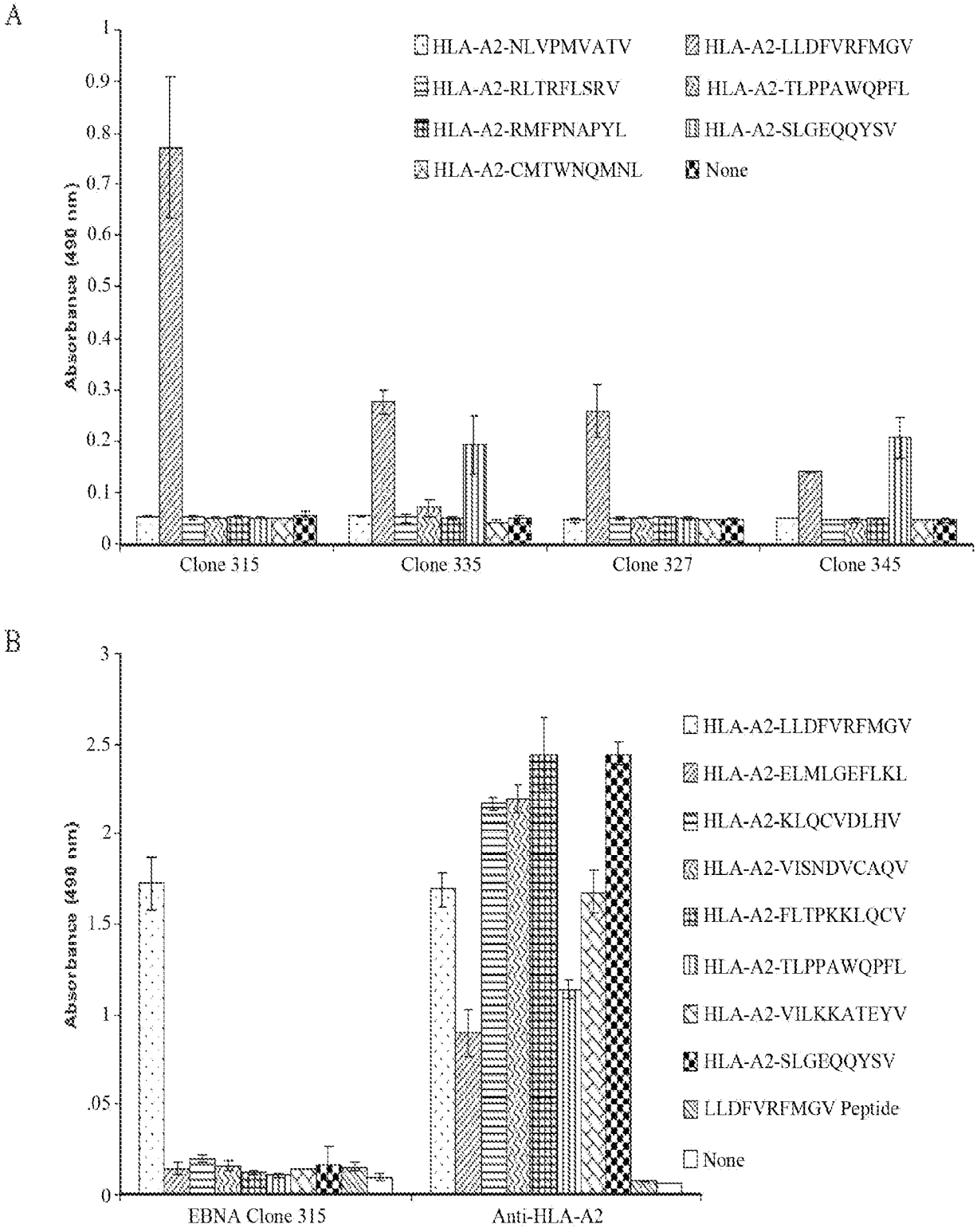

A
Absorbance (490 nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
HLA-A2-NLVPMVATV
HLA-A2-RLTRFLSRV
HLA-A2-RMFPNAPYL
HLA-A2-CMTWNQMNL
HLA-A2-LLDFVRFMGV
HLA-A2-TLPPAWQPFL
HLA-A2-SLGEQQYSV
None
Clone 315
Clone 335
Clone 327
Clone 345
B
Absorbance (490 nm)
3
2.5
2
1.5
1
.05
0
HLA-A2-LLDFVRFMGV
HLA-A2-ELMLGEFLKL
HLA-A2-KLQCVDLHV
HLA-A2-VISNDVCAQV
HLA-A2-FLTPKKLQCV
HLA-A2-TLPPAWQPFL
HLA-A2-VILKKATEYV
HLA-A2-SLGEQQYSV
LLDFVRFMGV Peptide
None
EBNA Clone 315
Anti-HLA-A2

Figure 1

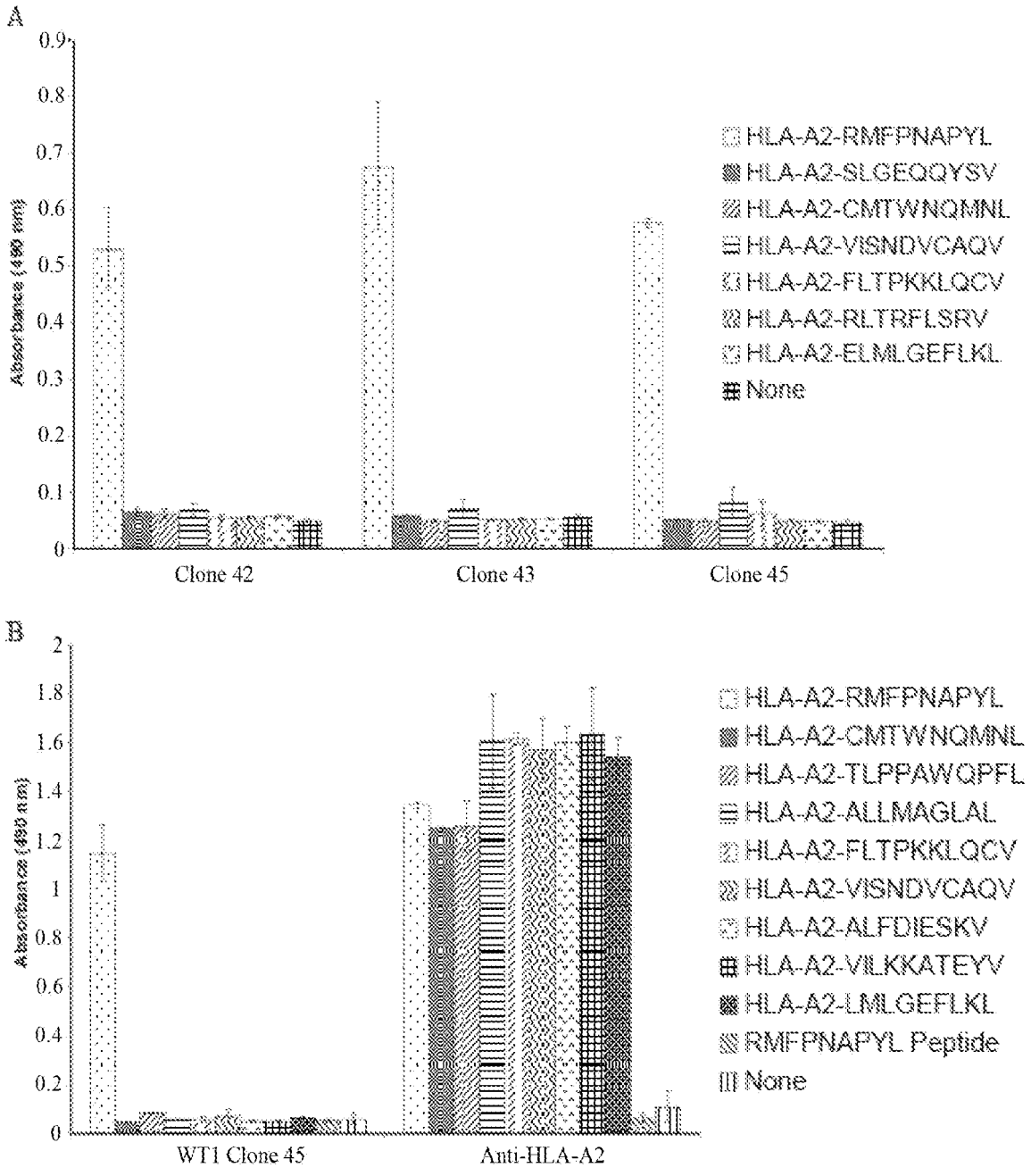

A
Absorbance (490 nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Clone 42
Clone 43
Clone 45
HLA-A2-RMFPNAPYL
HLA-A2-SLGEQQYSV
HLA-A2-CMTWNQMNL
HLA-A2-VISNDVCAQV
HLA-A2-FLTPKKLQCV
HLA-A2-RLTRFLSRV
HLA-A2-ELMLGEFLKL
None
B
Absorbance (490 nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT1 Clone 45
Anti-HLA-A2
HLA-A2-RMFPNAPYL
HLA-A2-CMTWNQMNL
HLA-A2-TLPPAWQPFL
HLA-A2-ALLMAGLAL
HLA-A2-FLTPKKLQCV
HLA-A2-VISNDVCAQV
HLA-A2-ALFDIESKV
HLA-A2-VILKKATEYV
HLA-A2-LMLGEFLKL
RMFPNAPYL Peptide
None

Figure 2

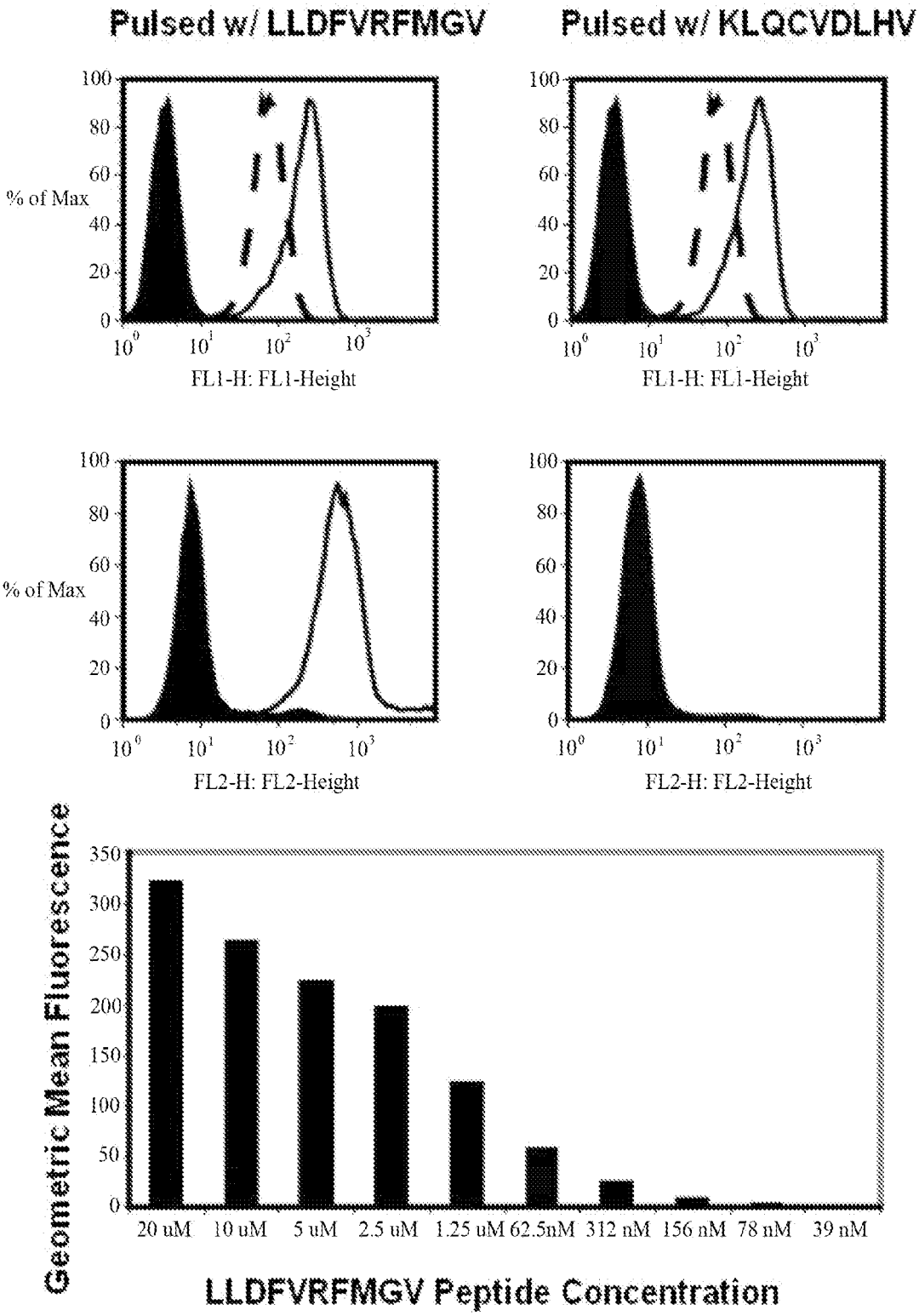

Pulsed w/ LLDFVRFMGV
Pulsed w/ KLQCVDLHV
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
Geometric Mean Fluorescence
20 uM
10 uM
5 uM
2.5 uM
1.25 uM
62.5nM
312 nM
156 nM
78 nM
39 nM
LLDFVRFMGV Peptide Concentration

Figure 3

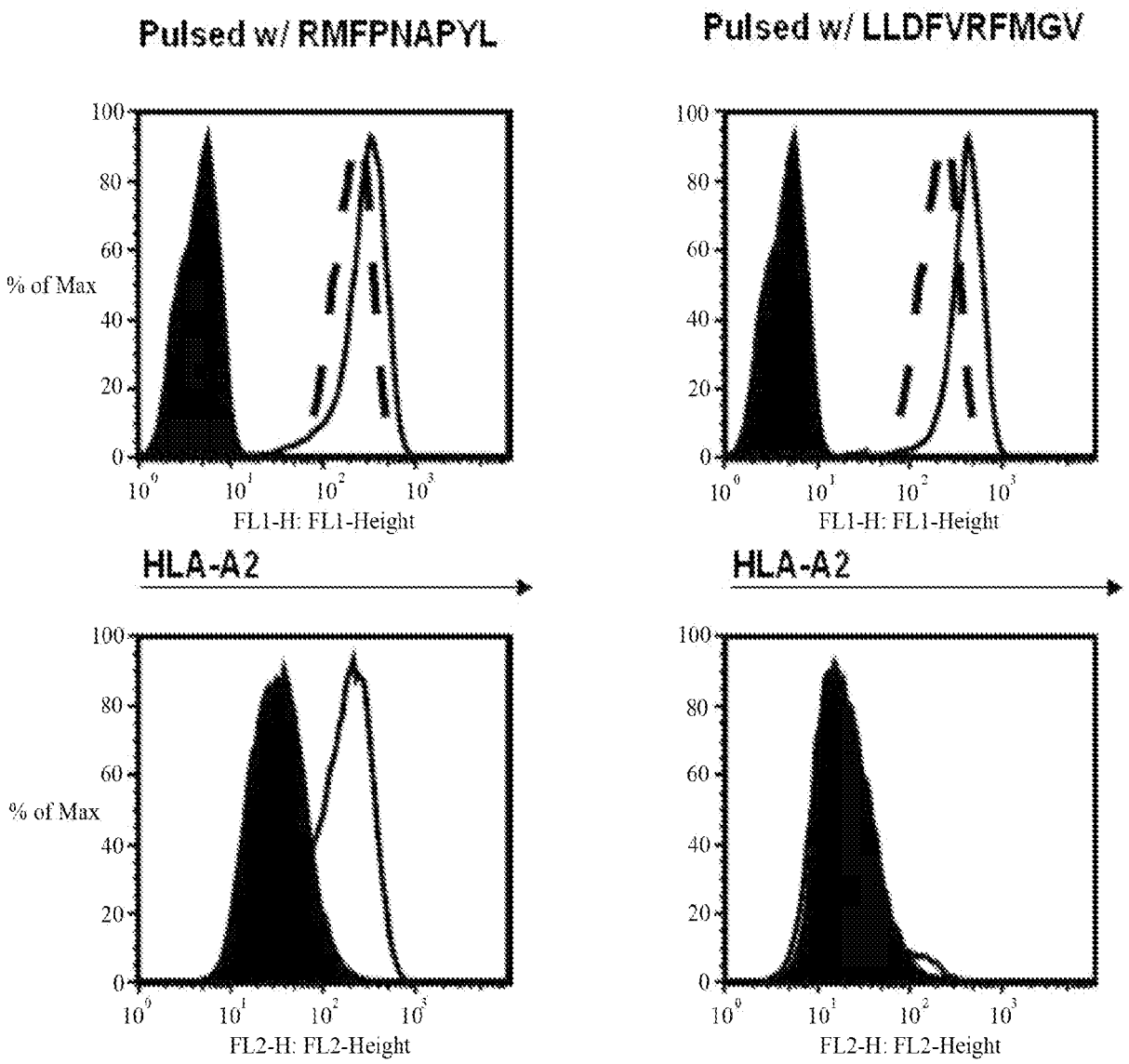

Pulsed w/ RMFPNAPYL
Pulsed w/ LLDFVRFMGV
% of Max
FL1-H: FL1-Height
HLA-A2
% of Max
FL2-H: FL2-Height

Figure 4

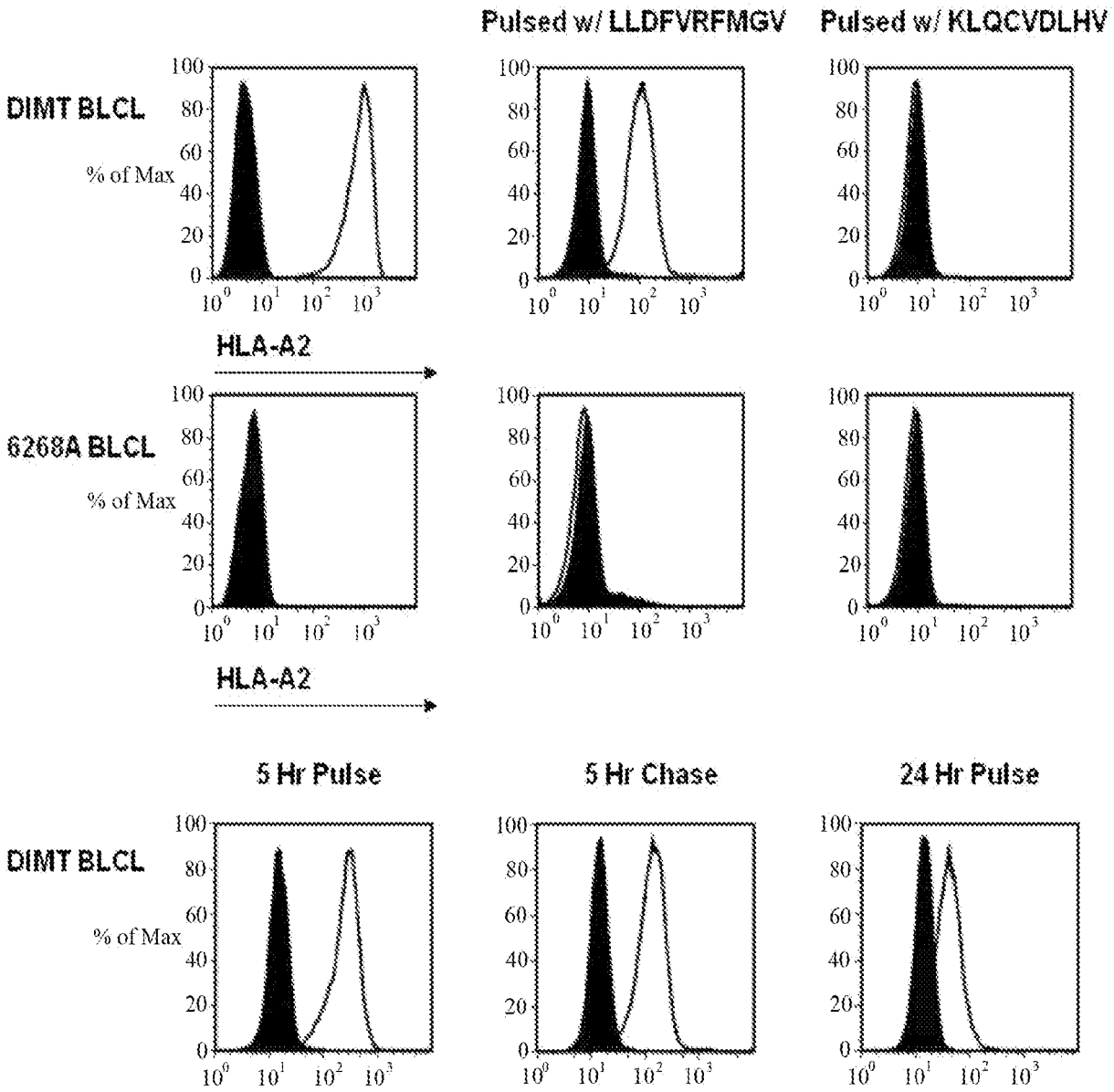
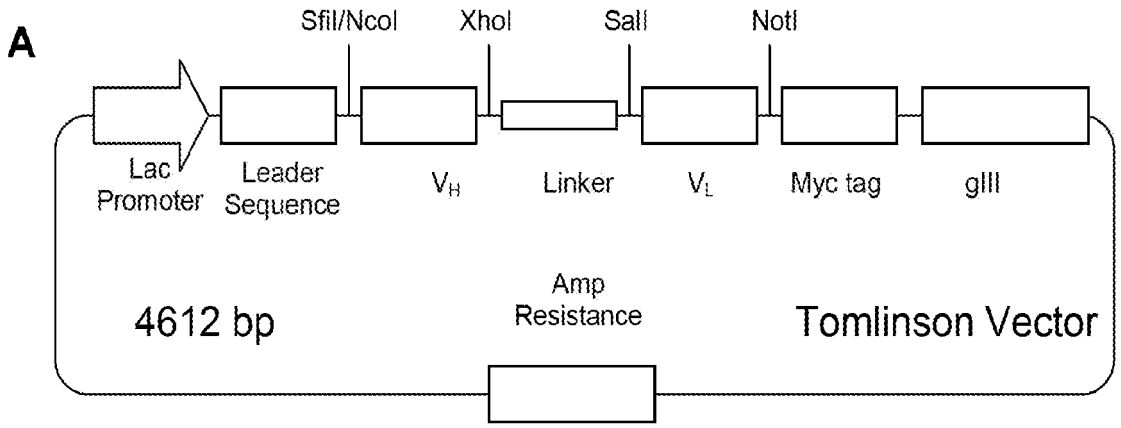

Pulsed w/ LLDFVRFMGV
Pulsed w/ KLQCVDLHV
DIMT BLCL
% of Max
HLA-A2
6268A BLCL
% of Max
HLA-A2
5 Hr Pulse
5 Hr Chase
24 Hr Pulse
DIMT BLCL
% of Max

Figure 5


A
SfiI/NcoI
XhoI
SalI
NotI
Lac Promoter
Leader Sequence
V_H
Linker
V_L
Myc tag
gIII
Amp Resistance
4612 bp
Tomlinson Vector

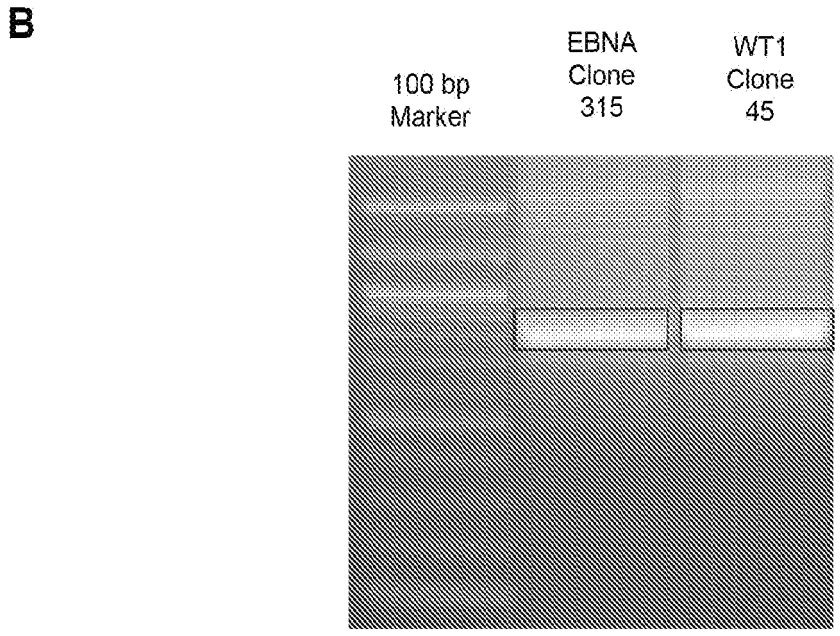

B
100 bp Marker
EBNA Clone 315
WT1 Clone 45

Figure 6

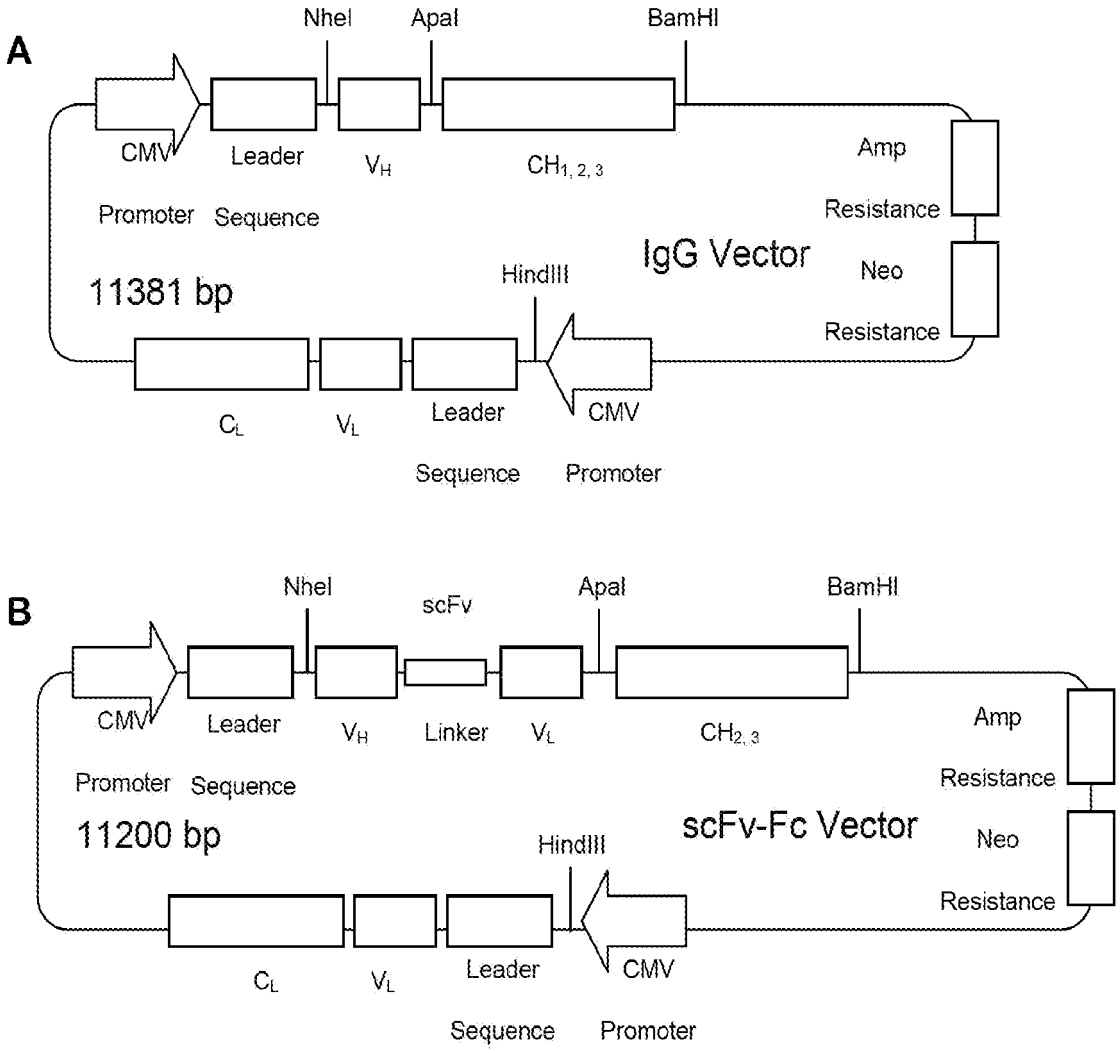

A
NheI
ApaI
BamHI
CMV
Promoter
Leader
Sequence
V_H
$CH_{1,2,3}$
Amp
Resistance
IgG Vector
Neo
Resistance
11381 bp
HindIII
C_L
V_L
Leader
Sequence
CMV
Promoter
B
NheI
scFv
ApaI
BamHI
CMV
Promoter
Leader
Sequence
V_H
Linker
V_L
$CH_{2,3}$
Amp
Resistance
11200 bp
scFv-Fc Vector
Neo
Resistance
HindIII
C_L
V_L
Leader
Sequence
CMV
Promoter

Figure 7

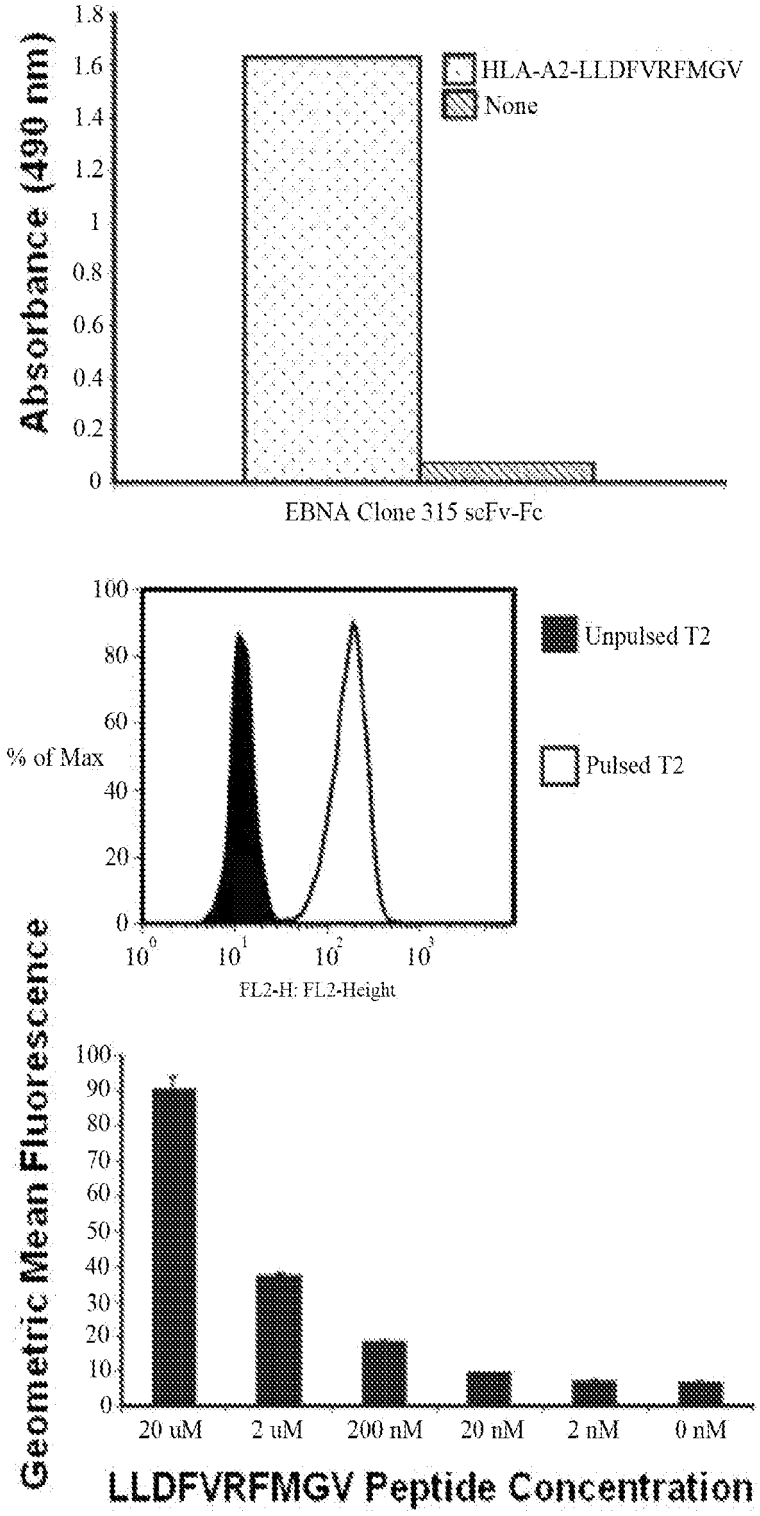
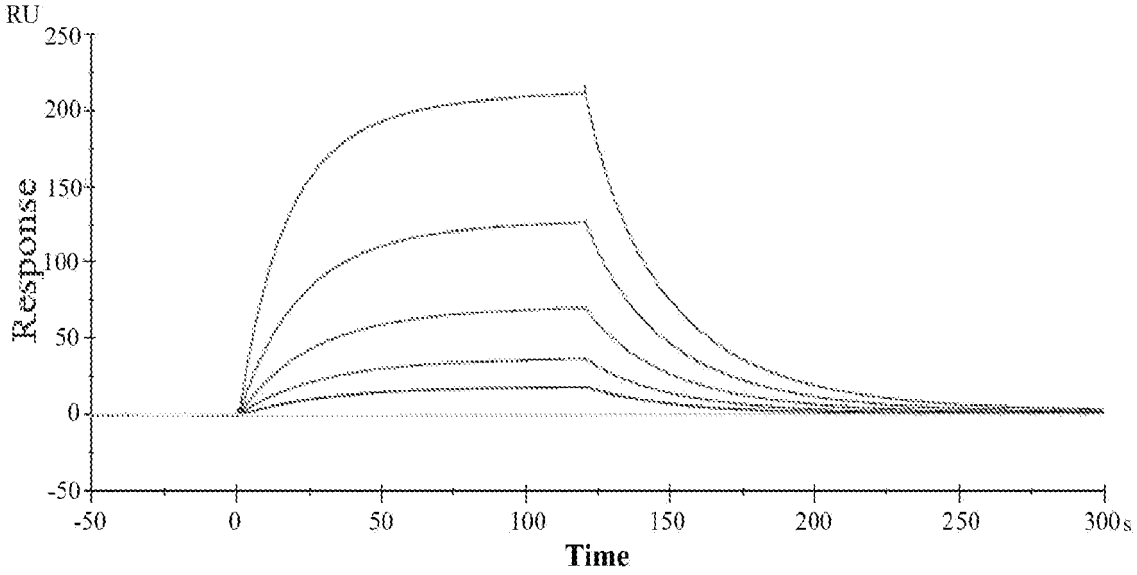

Absorbance (490 nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HLA-A2-LLDFVRFMGV
None
EBNA Clone 315 scFv-Fc
% of Max
100
80
60
40
20
0
Unpulsed T2
Pulsed T2
10^0
10^1
10^2
10^3
FL2-H: FL2-Height
Geometric Mean Fluorescence
100
90
80
70
60
50
40
30
20
10
0
20 uM
2 uM
200 nM
20 nM
2 nM
0 nM
LLDFVRFMGV Peptide Concentration

Figure 8



k_{on} (M^{-1} s^{-1}) = 2.361 x 10^5 k_{off} (s^{-1}) = 6.891 x 10^{-2} K_D (nM) = 291

Figure 9

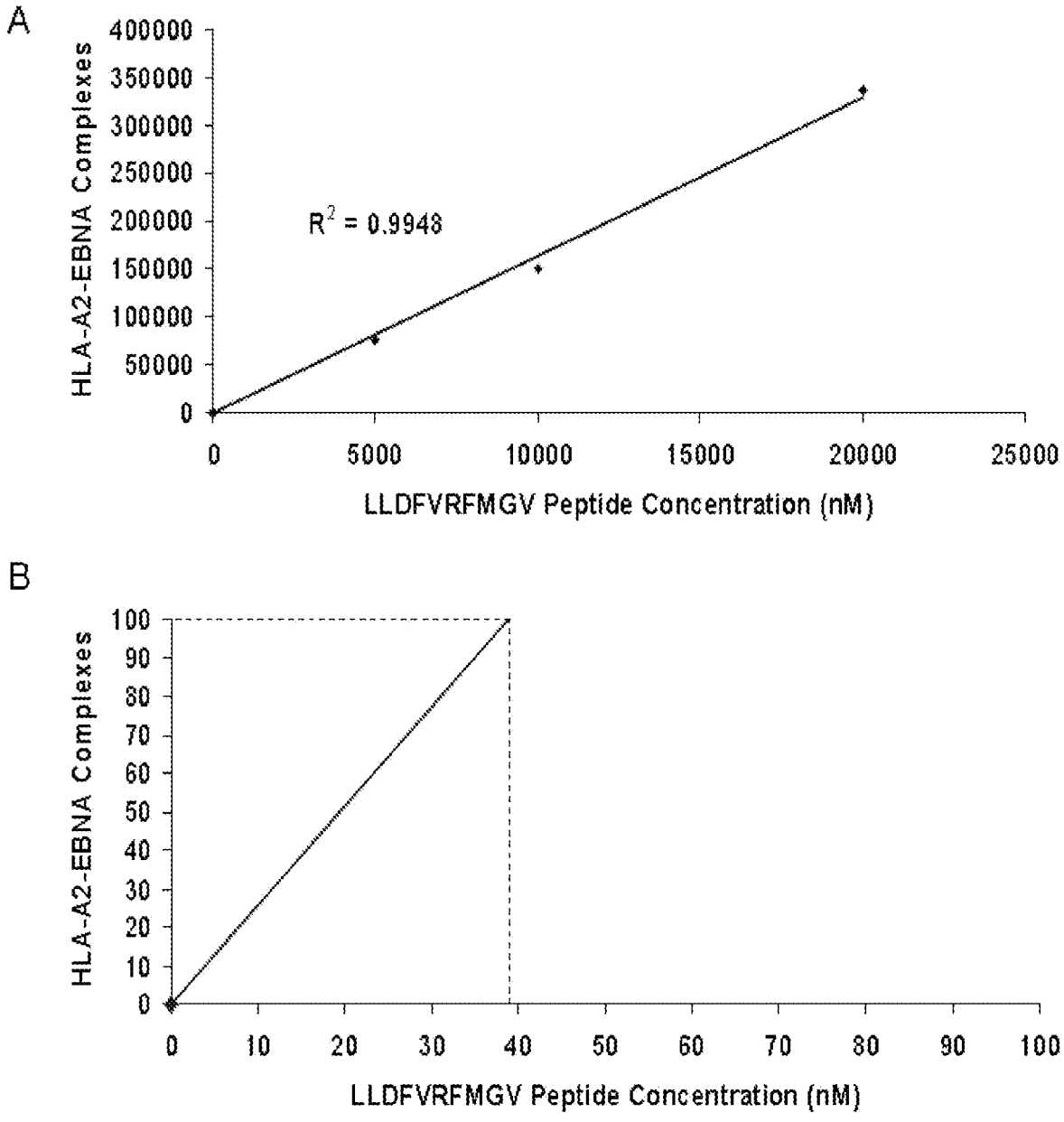
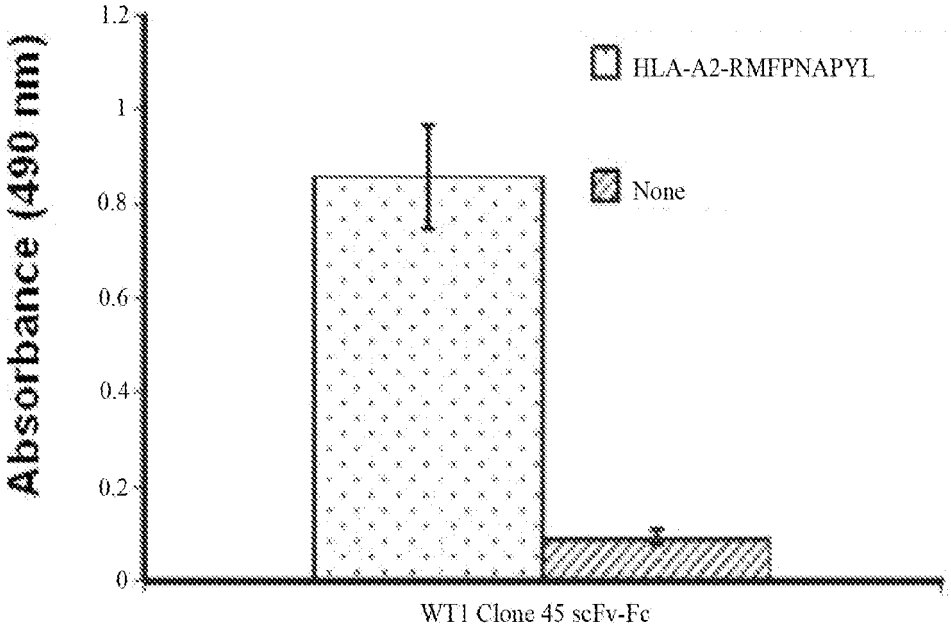

A
HLA-A2-EBNA Complexes
400000
350000
300000
250000
200000
150000
100000
50000
0
R^2 = 0.9948
0
5000
10000
15000
20000
25000
LLDFVRFMGV Peptide Concentration (nM)
B
HLA-A2-EBNA Complexes
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
LLDFVRFMGV Peptide Concentration (nM)

Figure 10


Absorbance (490 nm)
1.2
1
0.8
0.6
0.4
0.2
0
HLA-A2-RMFPNAPYL
None
WT1 Clone 45 scFv-Fc

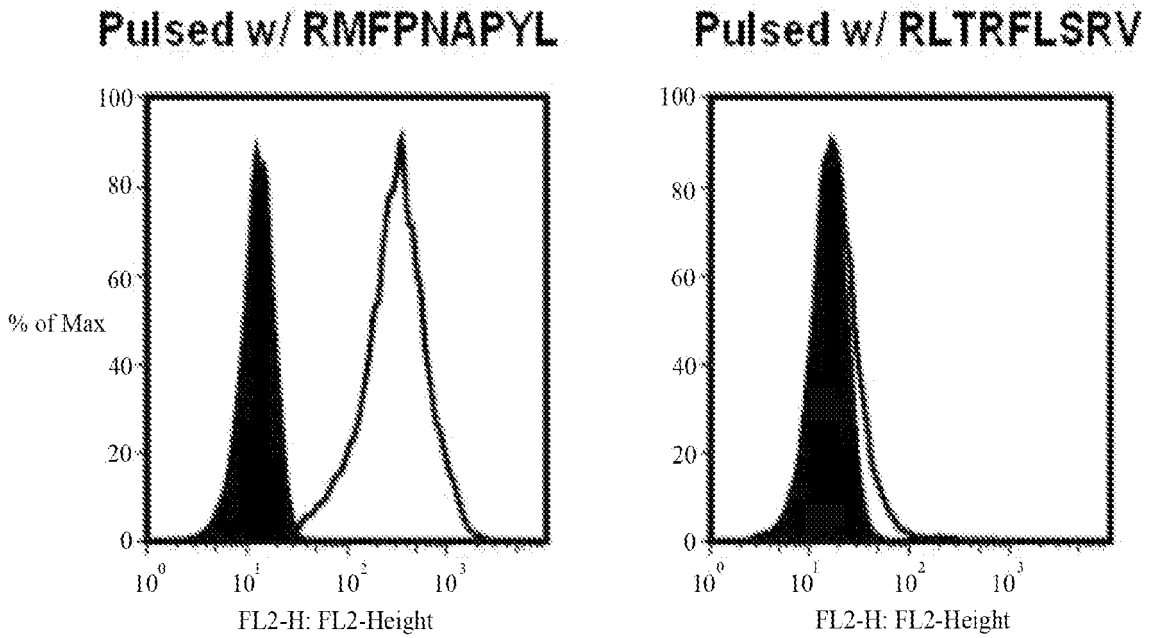

Pulsed w/ RMFPNAPYL
Pulsed w/ RLTRFLSRV
% of Max
100
80
60
40
20
0
10^0
10^1
10^2
10^3
FL2-H: FL2-Height
FL2-H: FL2-Height

Figure 11

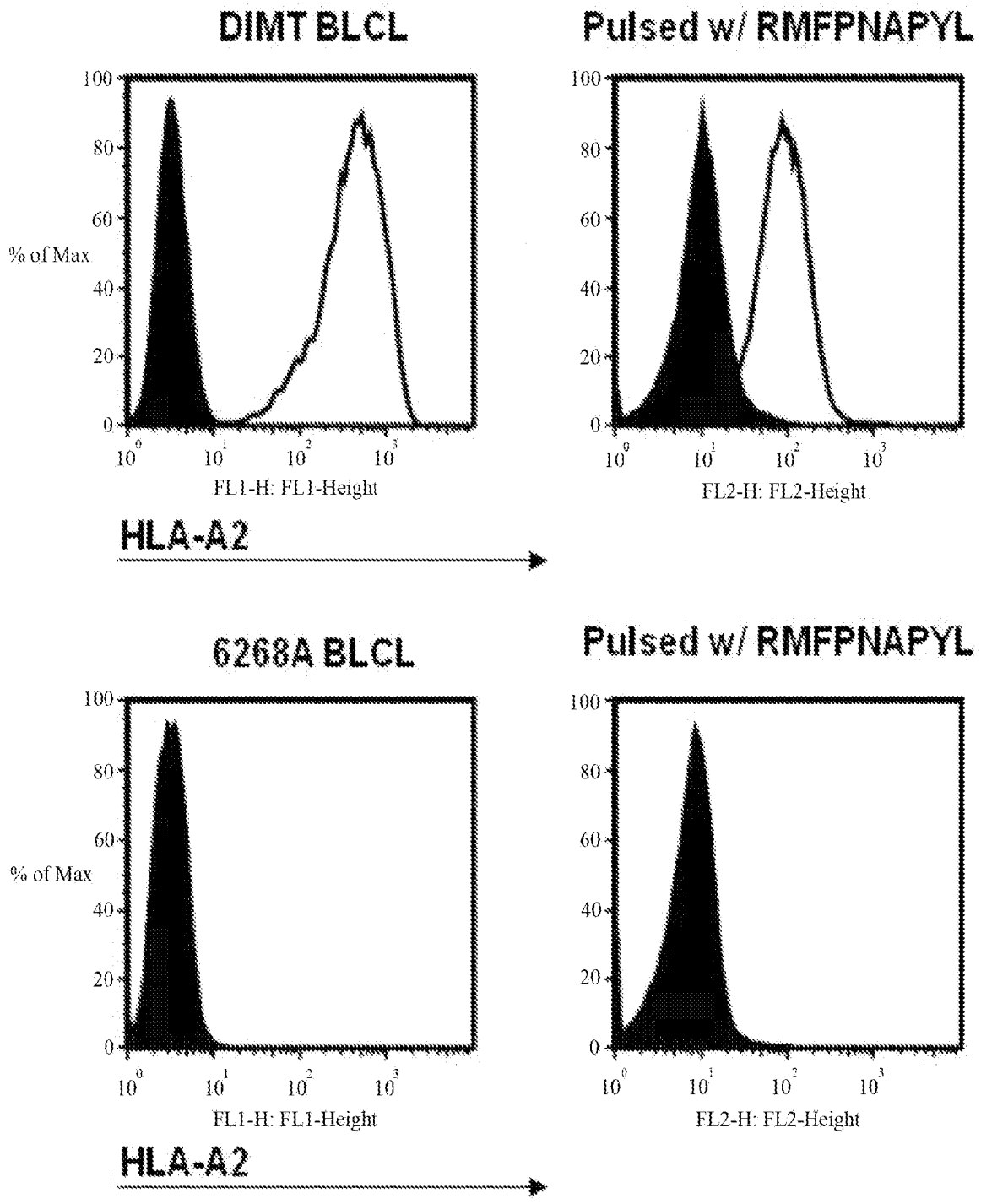

DIMT BLCL
Pulsed w/ RMFPNAPYL
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
HLA-A2
6268A BLCL
Pulsed w/ RMFPNAPYL
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
HLA-A2

Figure 12

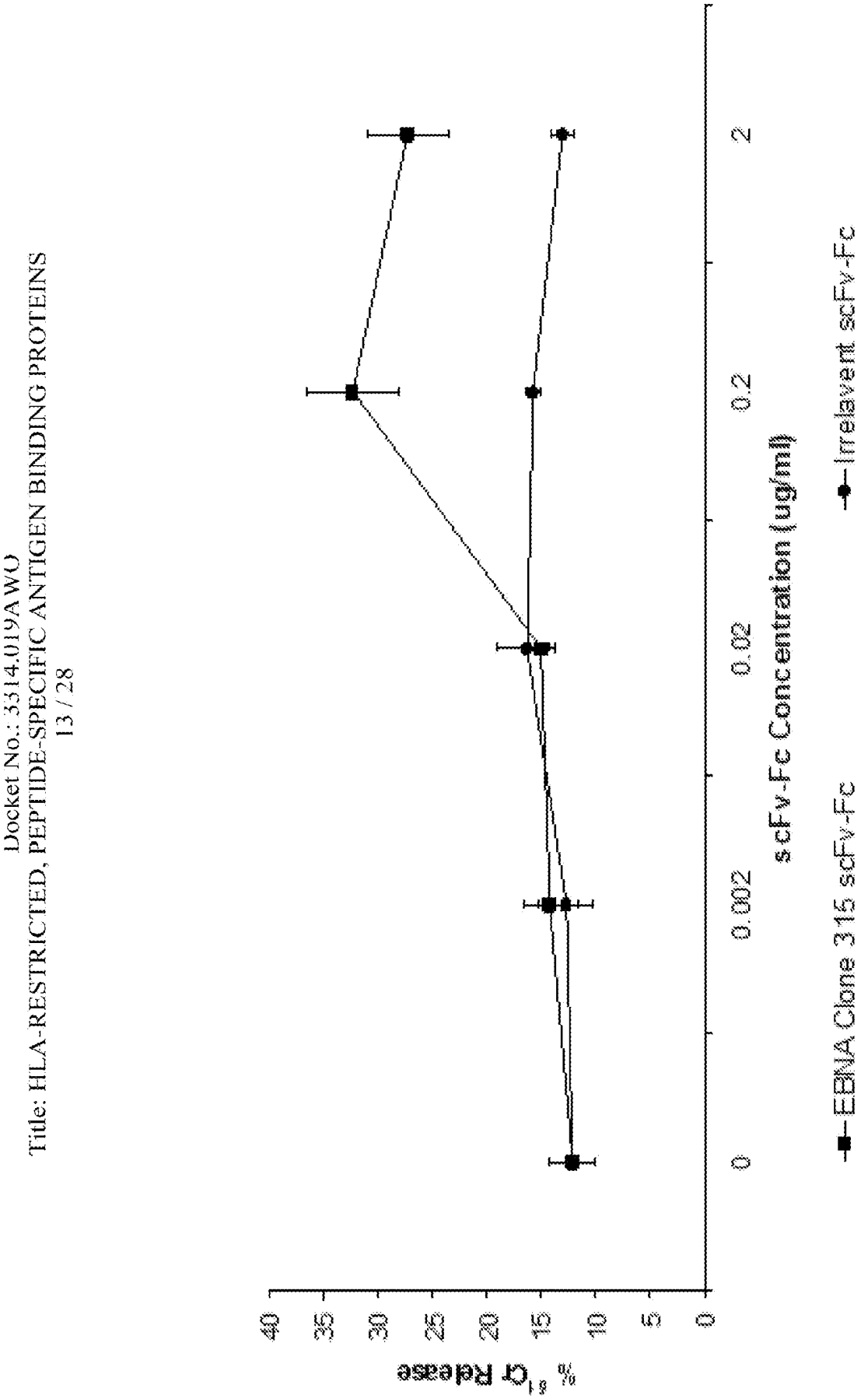

Docket No.: 3514.019AWO
Title: HLA-RESTRICTED, PEPTIDE-SPECIFIC ANTIGEN BINDING PROTEINS
13 / 28
% 51Cr Release
0
5
10
15
20
25
30
35
40
0
0.002
0.02
0.2
2
scFv-Fc Concentration (ug/ml)
EBNA Clone 315 scFv-Fc
Irrelavent scFv-Fc

Figure 13

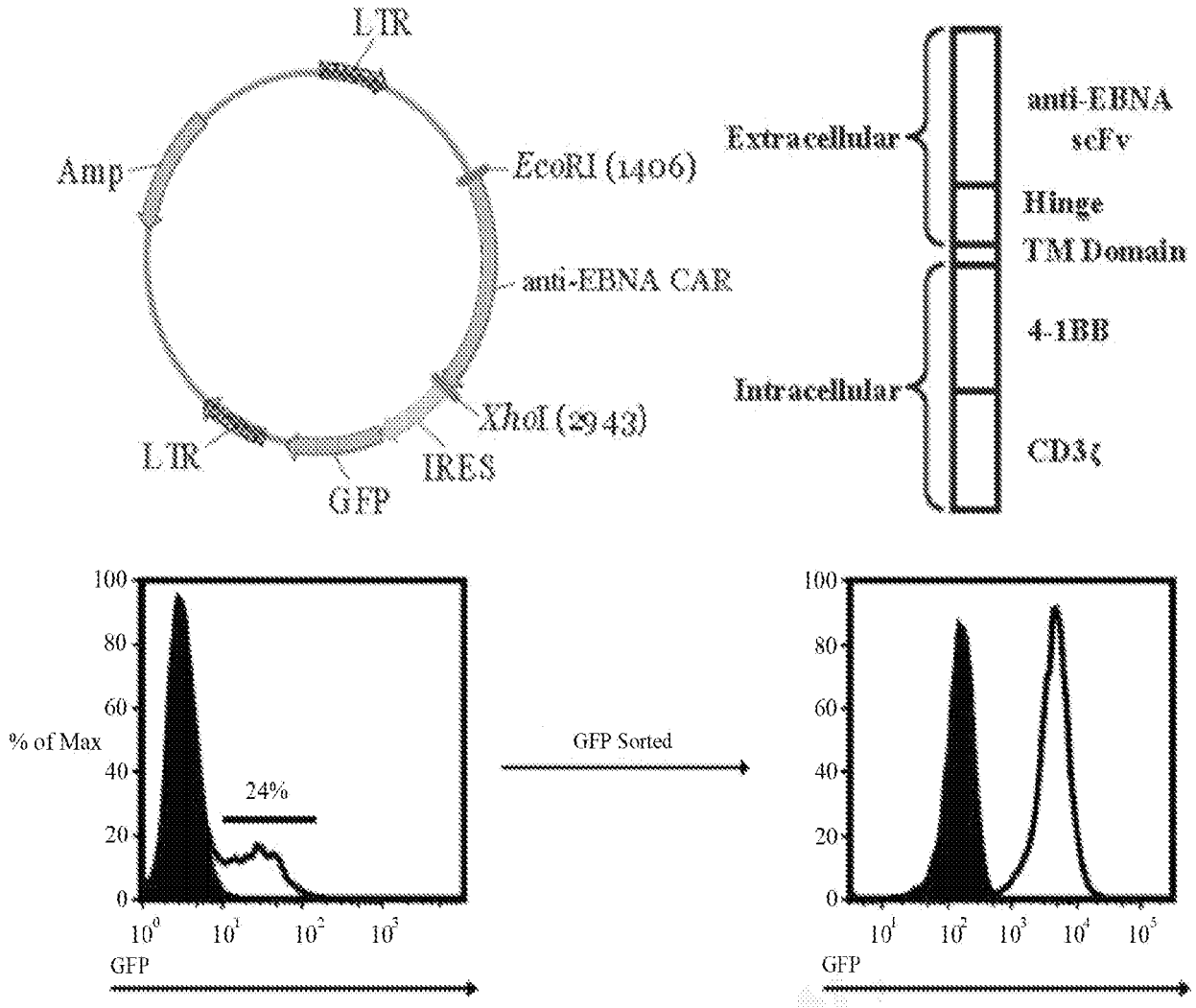

LTR
Amp
EcoRI (1406)
anti-EBNA CAR
XhoI (2943)
LTR
IRES
GFP
Extracellular
anti-EBNA scFv
Hinge
TM Domain
Intracellular
4-1BB
CD3ζ
% of Max
24%
GFP
GFP Sorted
GFP

Figure 14

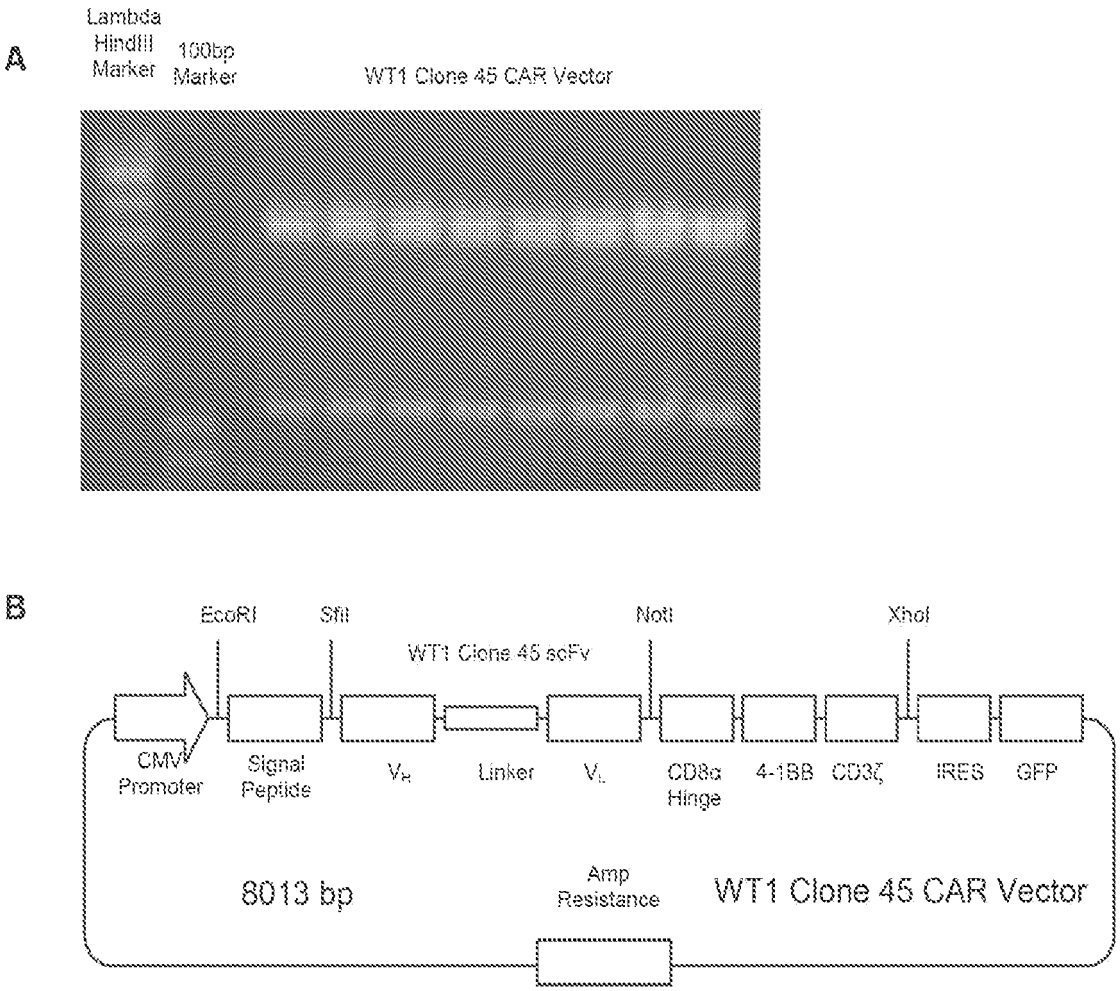

A
Lambda HindIII Marker
100bp Marker
WT1 Clone 45 CAR Vector
B
EcoRI
SfiI
NotI
XhoI
WT1 Clone 45 scFv
CMV Promoter
Signal Peptide
V_H
Linker
V_L
CD8α Hinge
4-1BB
CD3ζ
IRES
GFP
8013 bp
Amp Resistance
WT1 Clone 45 CAR Vector

Figure 15

Docket No.: 3514.019AWO
Title: HLA-RESTRICTED, PEPTIDE-SPECIFIC ANTIGEN BINDING PROTEINS
16 / 28

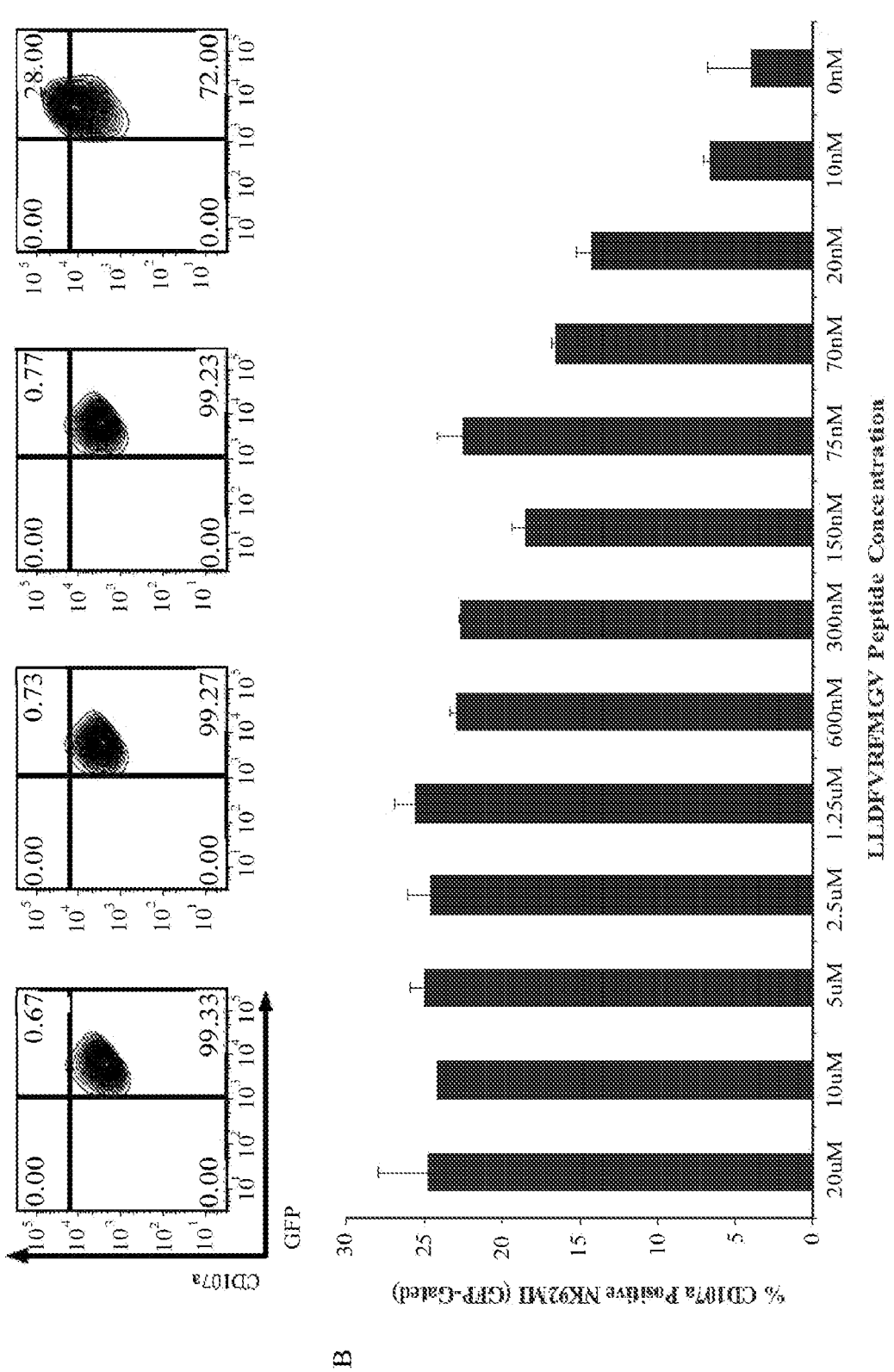


Figure 16

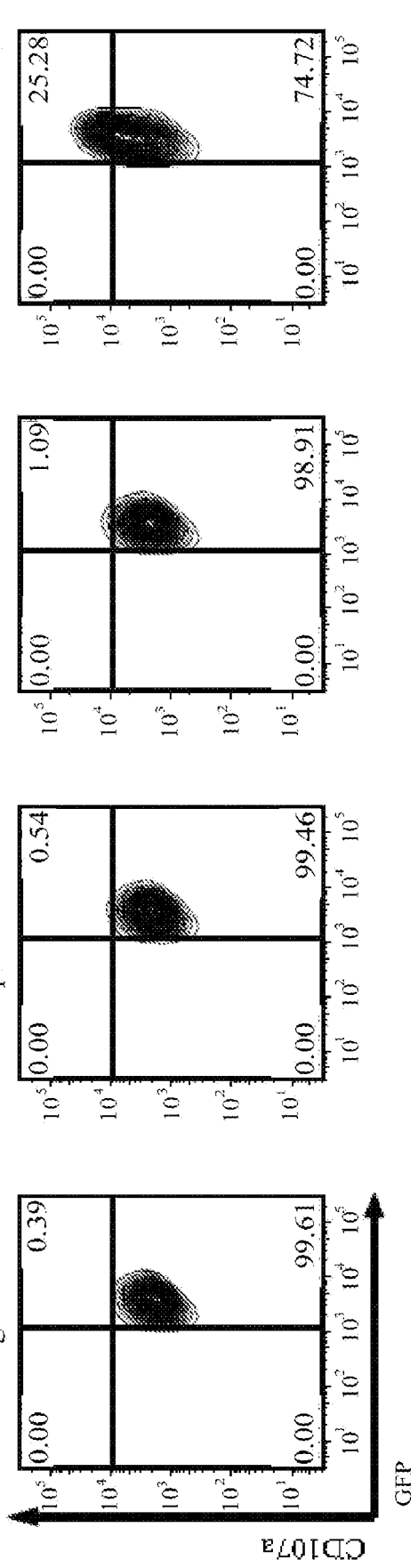

No Target Cells
Unpulsed T2
YMFPNAPYL-Pulsed T2
LLDFVRFMGV-Pulsed T2
0.00
0.39
99.61
0.00
0.54
99.46
0.00
1.09
98.91
0.00
25.28
74.72
CD107a
GFP

Figure 17

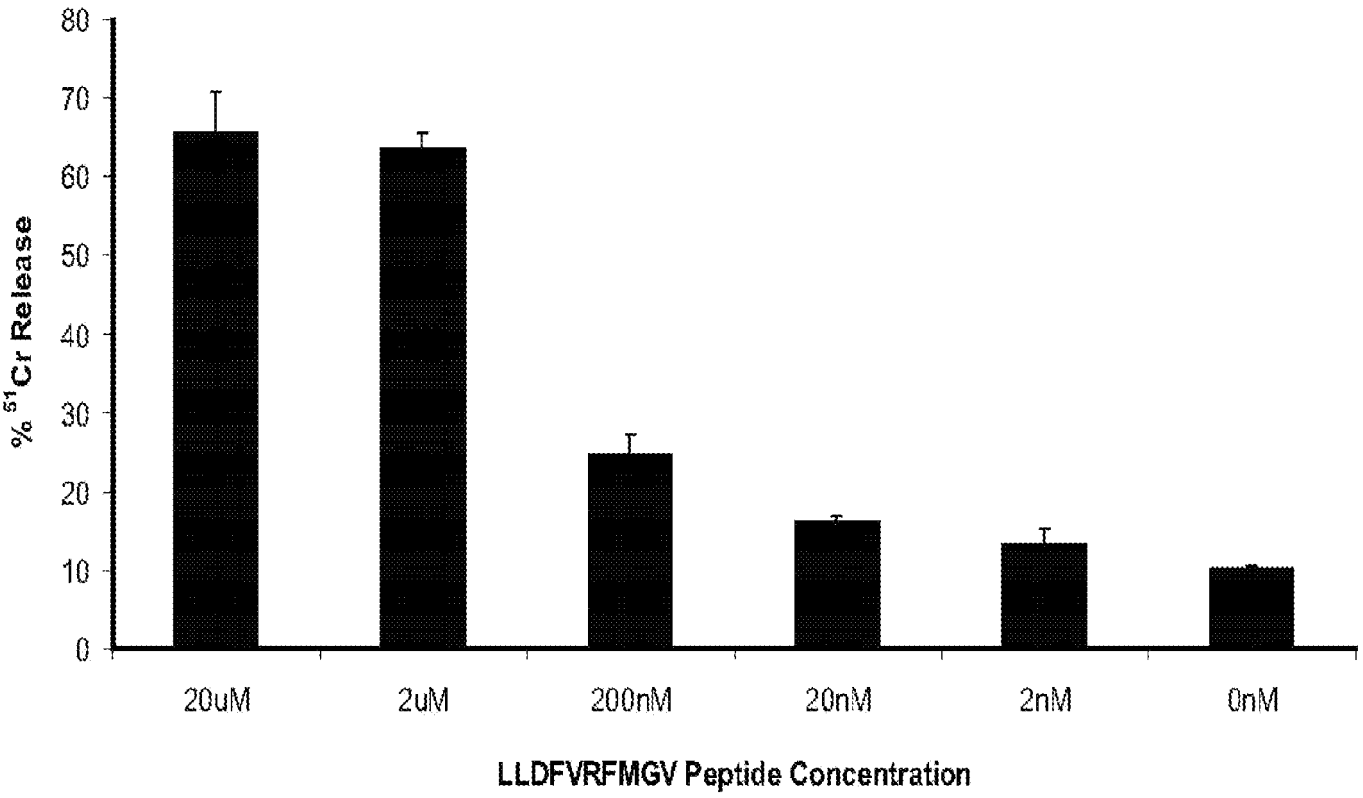

80
70
60
50
40
30
20
10
0
% 51Cr Release
20uM
2uM
200nM
20nM
2nM
0nM
LLDFVRFMGV Peptide Concentration

Figure 18

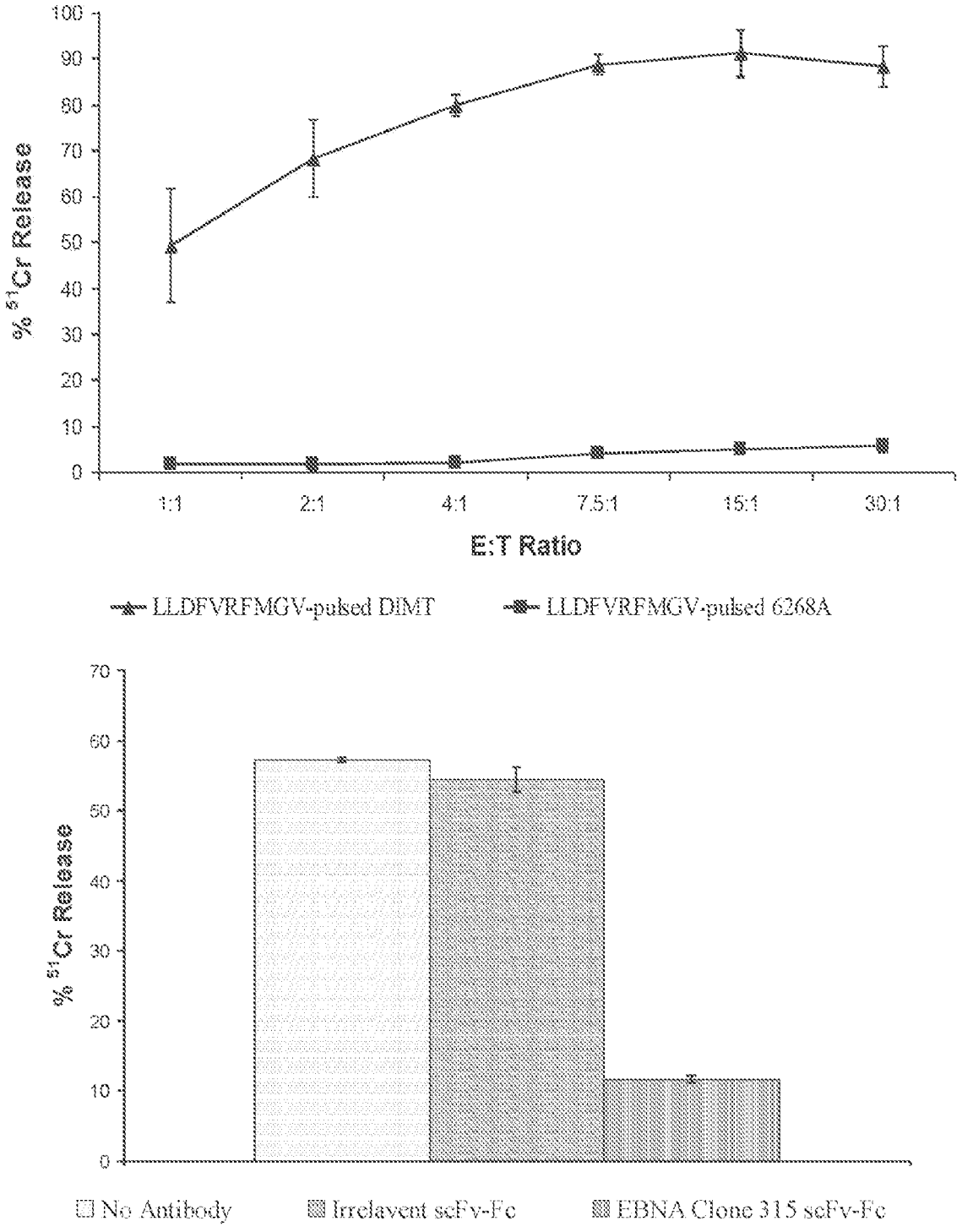
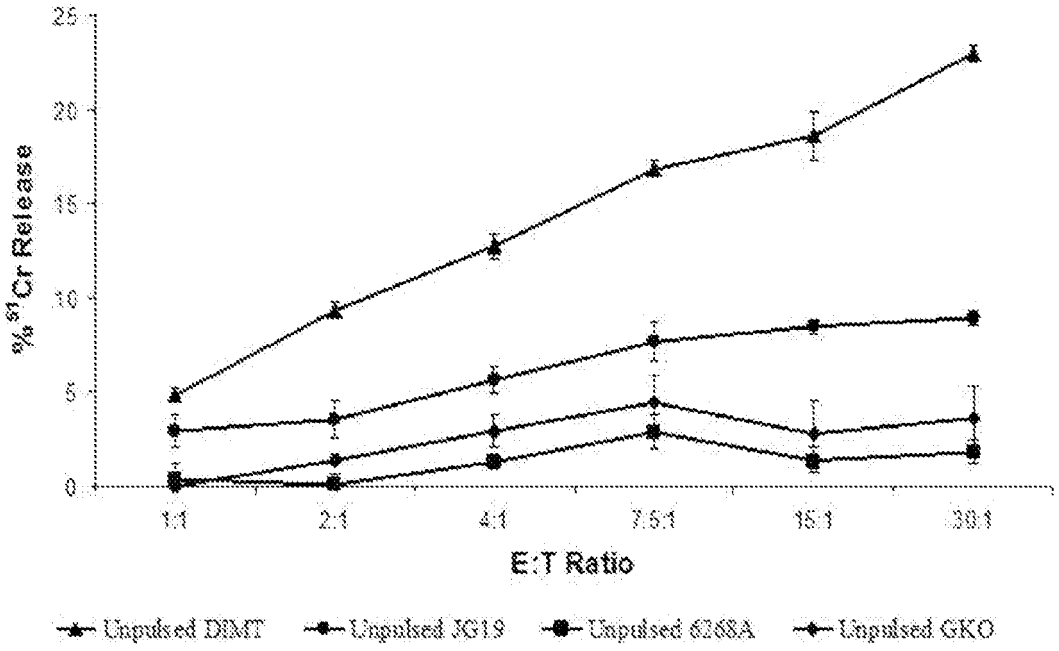

100
90
80
70
60
50
40
30
20
10
0
% 51Cr Release
1:1
2:1
4:1
7.5:1
15:1
30:1
E:T Ratio
LLDFVRFMGV-pulsed DIMT
LLDFVRFMGV-pulsed 6268A
70
60
50
40
30
20
10
0
% 51Cr Release
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 19


% ^{51}Cr Release
25
20
15
10
5
0
1:1
2:1
4:1
7.5:1
15:1
30:1
E:T Ratio
Unpulsed DIMT
Unpulsed JG19
Unpulsed 6268A
Unpulsed GKO

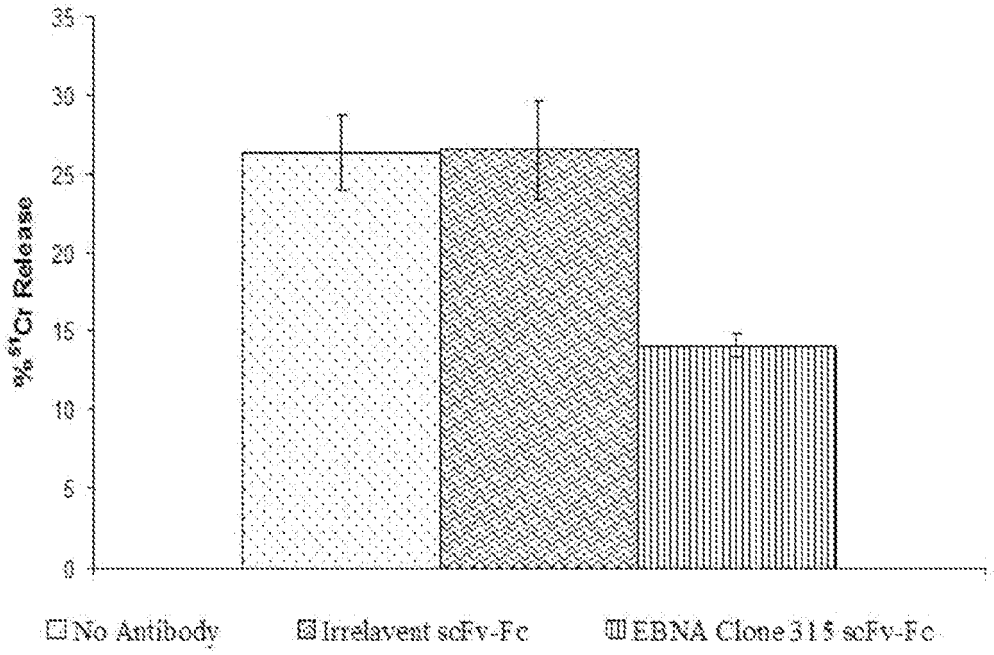

% ^{51}Cr Release
35
30
25
20
15
10
5
0
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 20

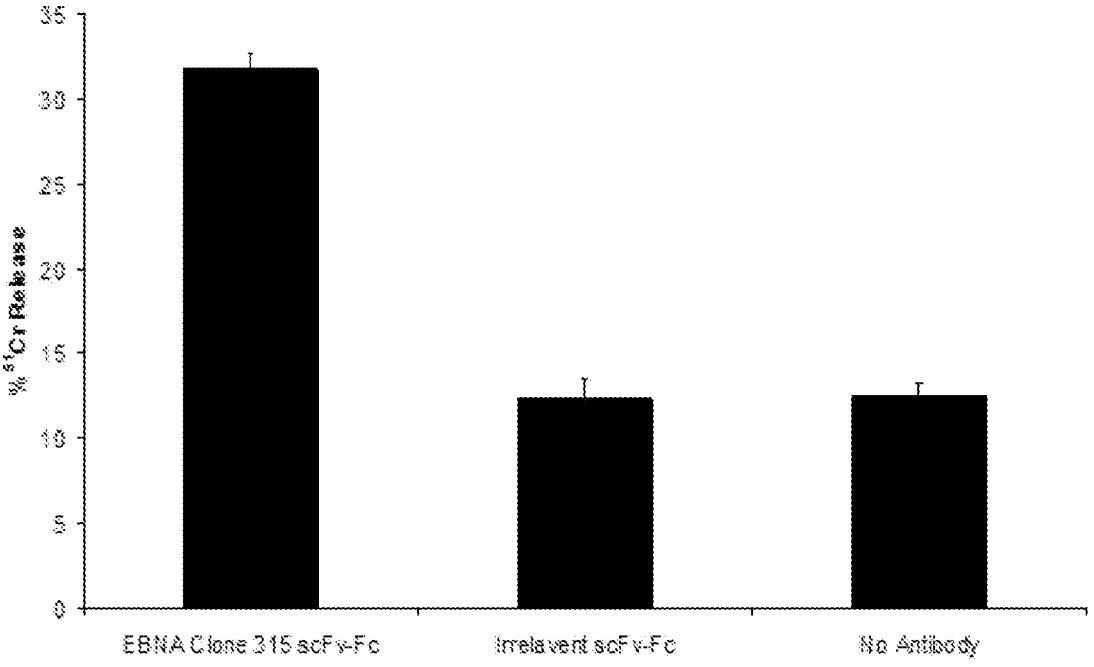

35
30
25
20
15
10
5
0
% ^{51}Cr Release
EBNA Clone 315 scFv-Fc
Irrelavent scFv-Fc
No Antibody

Figure 21

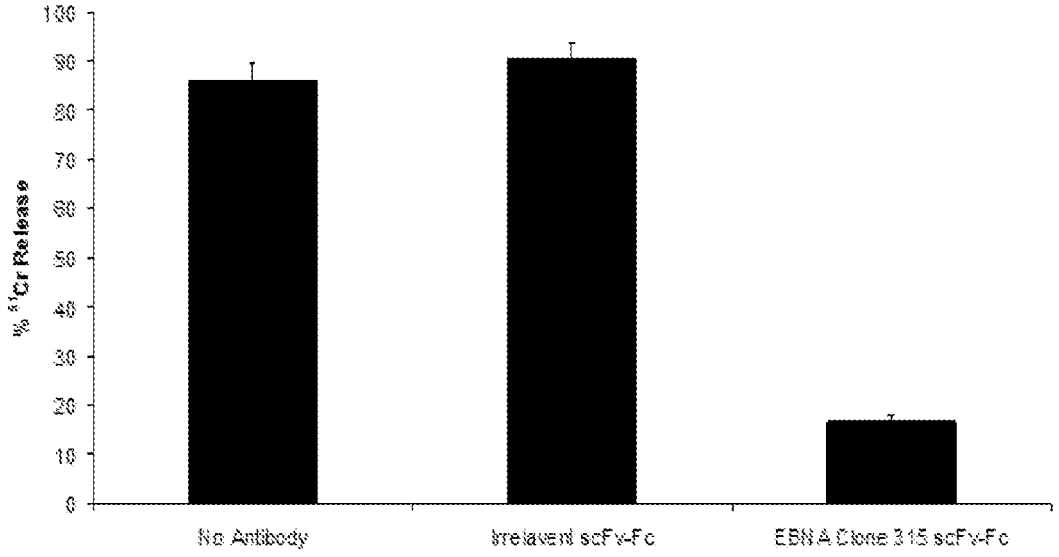

100
90
80
70
60
50
40
30
20
10
0
% 51Cr Release
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 22

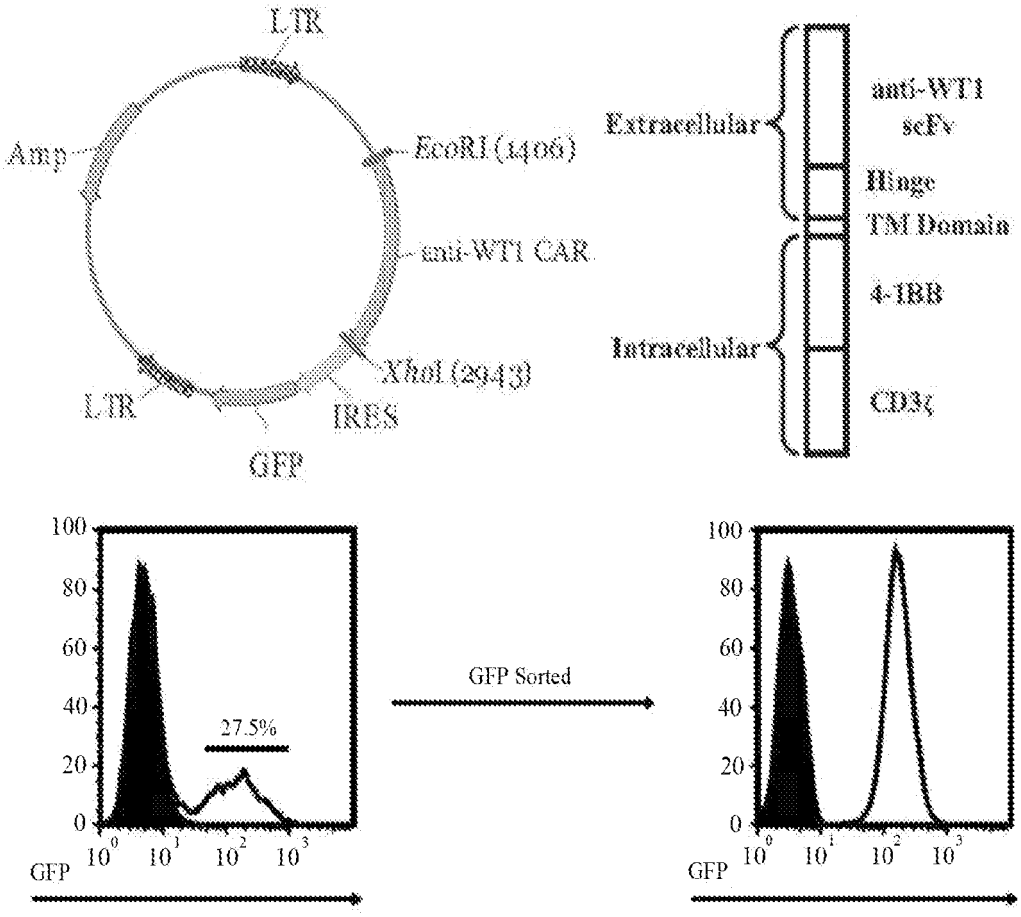

LTR
Amp
EcoRI (1406)
anti-WT1 CAR
XhoI (2943)
LTR
IRES
GFP
Extracellular
anti-WT1 scFv
Hinge
TM Domain
Intracellular
4-1BB
CD3ζ
100
80
60
40
20
0
27.5%
GFP
GFP Sorted

Figure 23

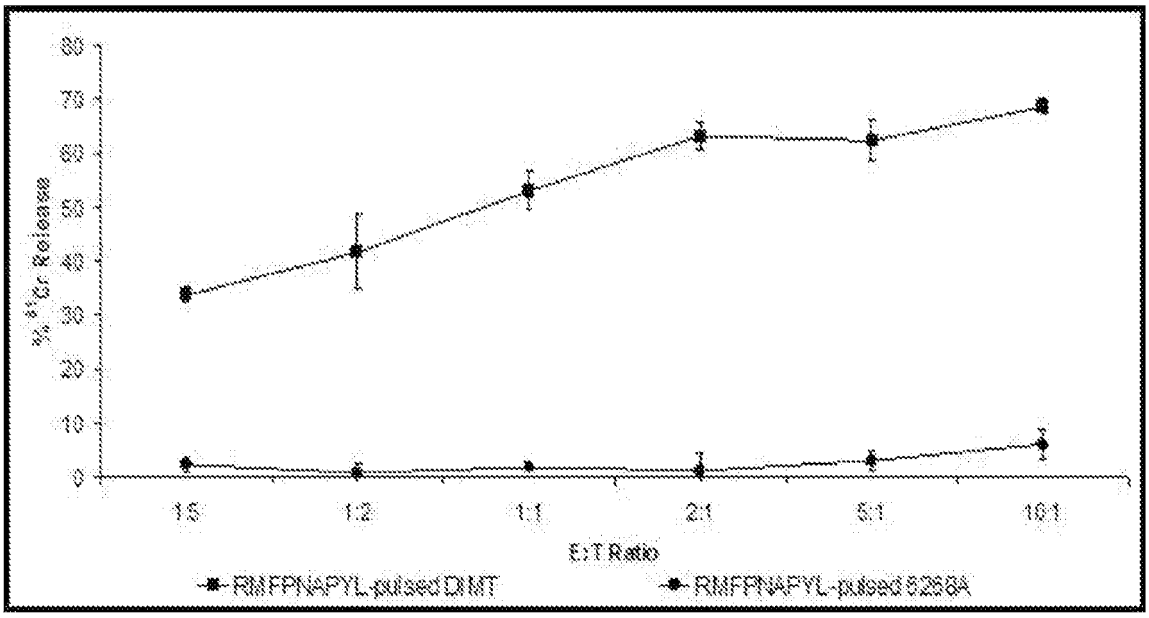

80
70
60
50
40
30
20
10
0
% ^{51}Cr Release
1:5
1:2
1:1
2:1
5:1
10:1
E:T Ratio
RMFPNAPYL-pulsed DMT
RMFPNAPYL-pulsed 526A

Figure 24

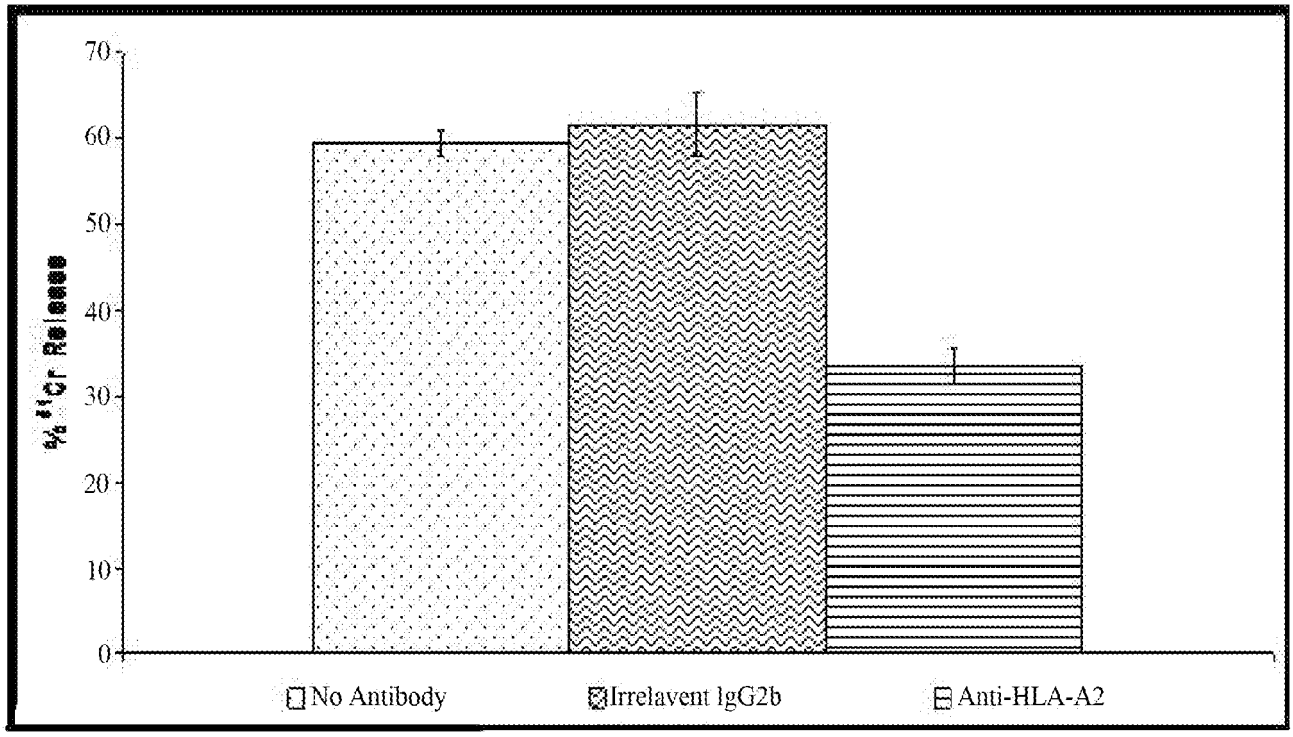


Figure 25

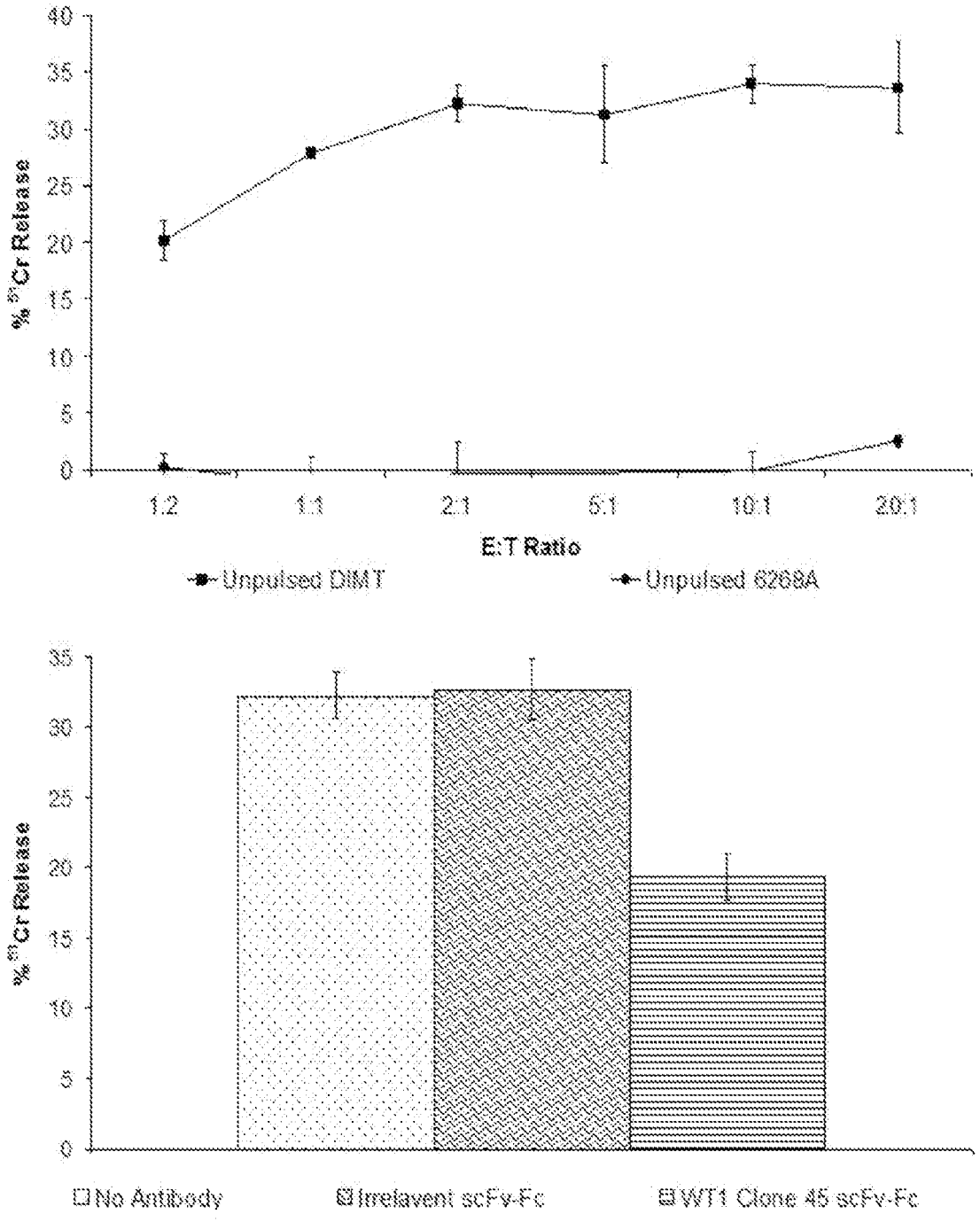

40
35
30
25
20
15
10
5
0
% 51Cr Release
1:2
1:1
2:1
5:1
10:1
20:1
E:T Ratio
Unpulsed DIMT
Unpulsed 6268A
35
30
25
20
15
10
5
0
% 51Cr Release
No Antibody
Irrelavent scFv-Fc
WT1 Clone 45 scFv-Fc

Figure 26

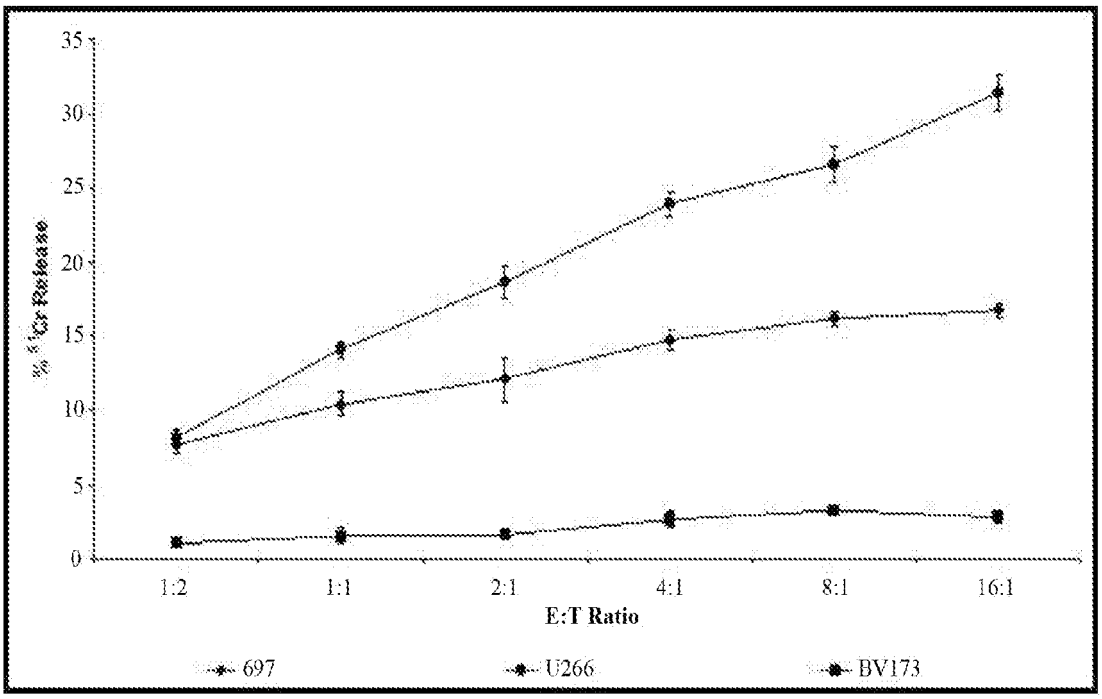

35
30
25
20
15
10
5
0
% ^{51}Cr Release
1:2
1:1
2:1
4:1
8:1
16:1
E:T Ratio
697
U266
BV173

Figure 27

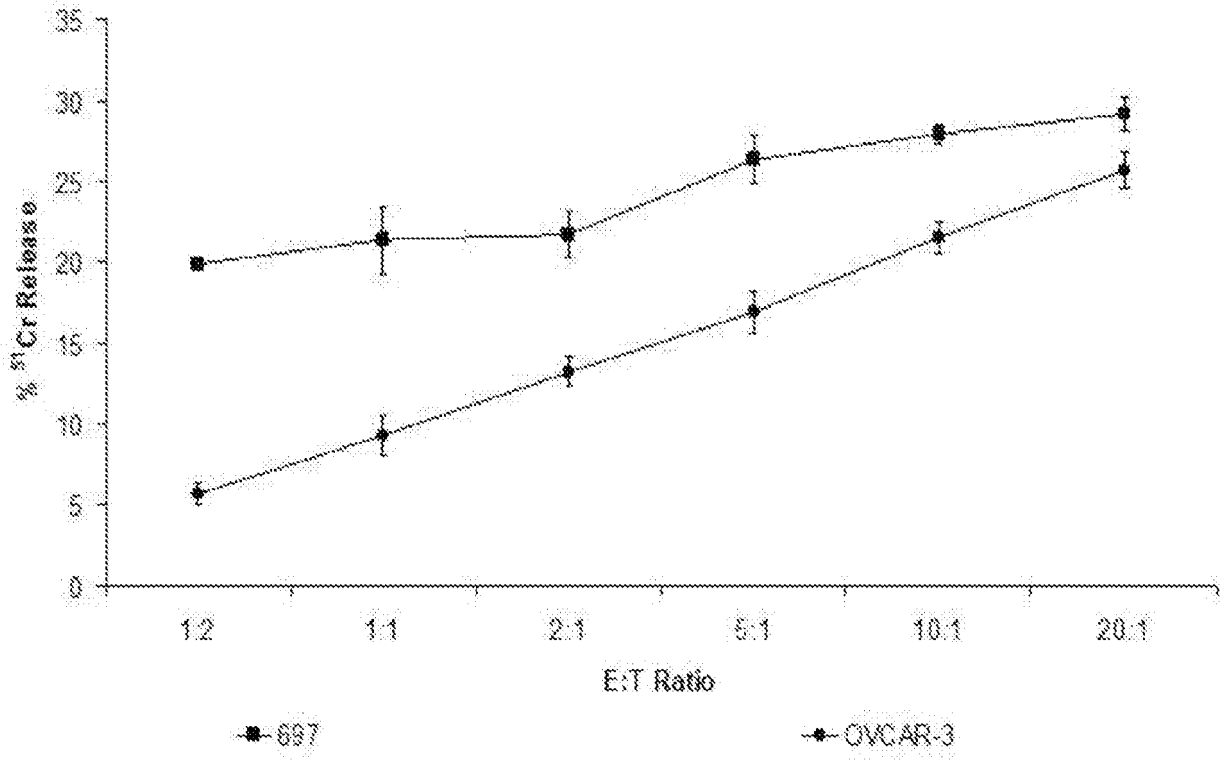

35
30
25
20
15
10
5
0
% 51Cr Release
1:2
1:1
2:1
5:1
10:1
20:1
E:T Ratio
697
OVCAR-3

Figure 28