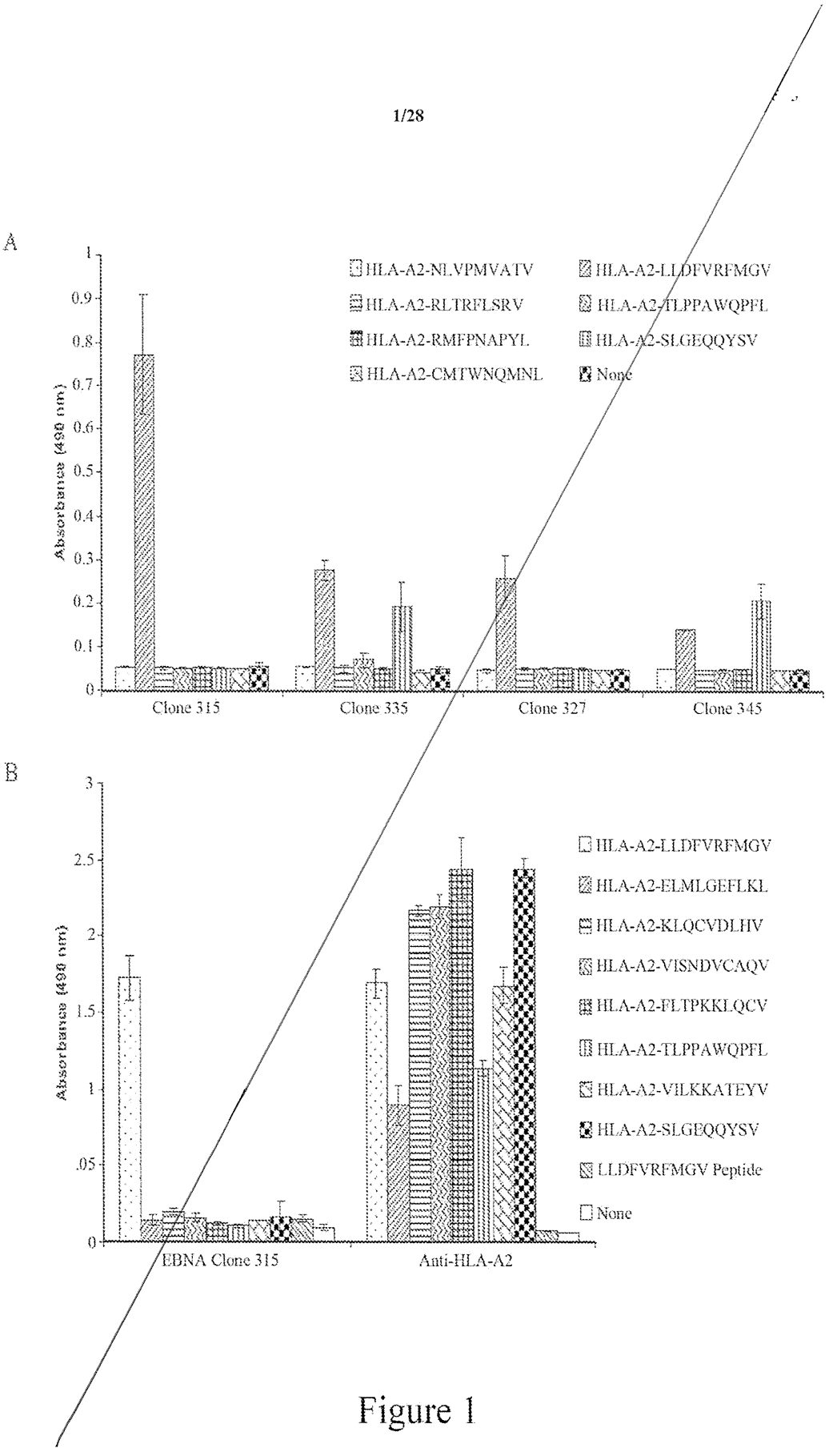

A
Absorbance (490 nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
HLA-A2-NLVPMVATV
HLA-A2-LLDFVRFMGV
HLA-A2-RLTRFLSRV
HLA-A2-TLPPAWQPFL
HLA-A2-RMFPNAPYL
HLA-A2-SLGEQQYSV
HLA-A2-CMTWNQMNL
None
Clone 315
Clone 335
Clone 327
Clone 345
B
Absorbance (490 nm)
3
2.5
2
1.5
1
.05
0
HLA-A2-LLDFVRFMGV
HLA-A2-ELMLGEFLKL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

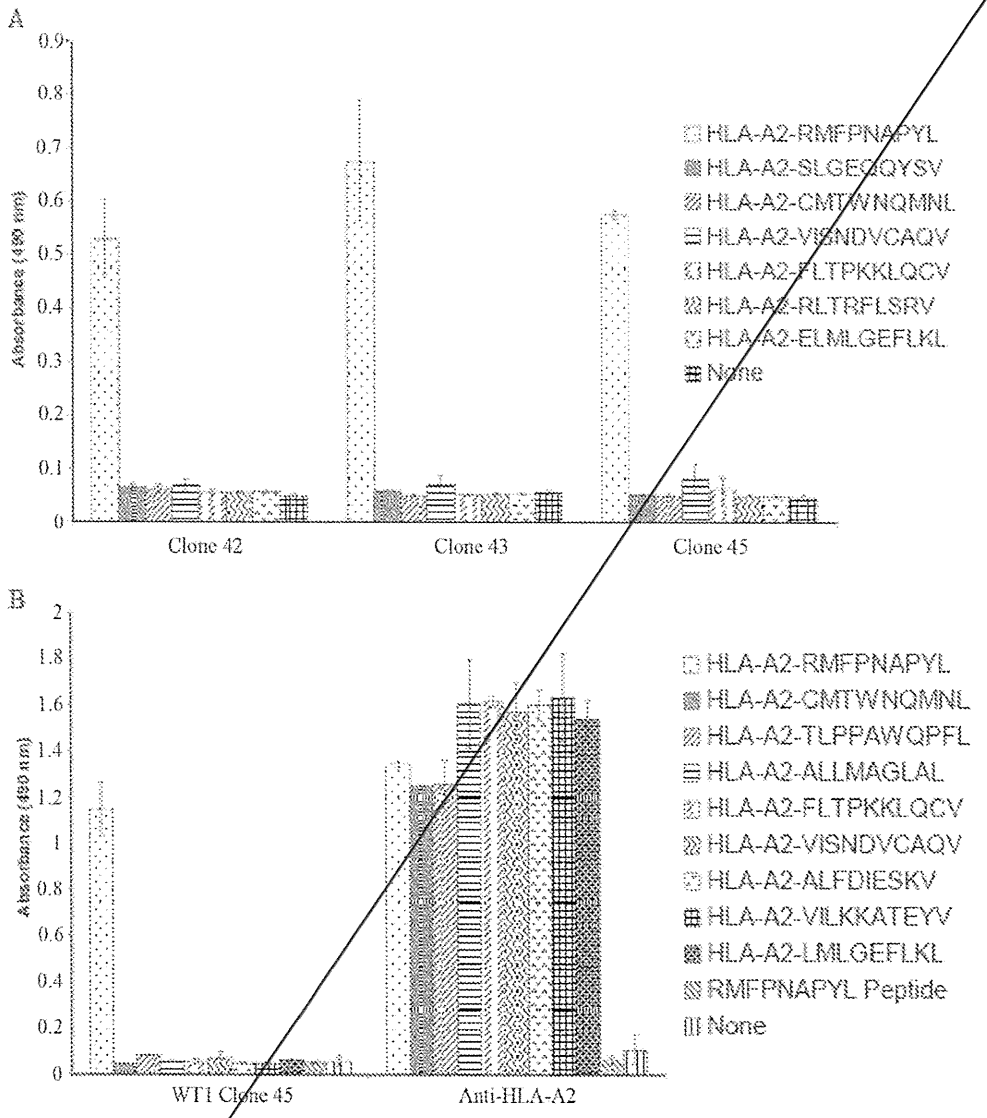

A
Absorbance (490 nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
HLA-A2-RMFPNAPYL
HLA-A2-SLGEQQYSV
HLA-A2-CMTWNQMNL
HLA-A2-VISNDVCAQV
HLA-A2-FLTPKKLQCV
HLA-A2-RLTRFLSRV
HLA-A2-ELMLGEFLKL
None
Clone 42
Clone 43
Clone 45
B
Absorbance (490 nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HLA-A2-RMFPNAPYL
HLA-A2-CMTWNQMNL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

Figure 2

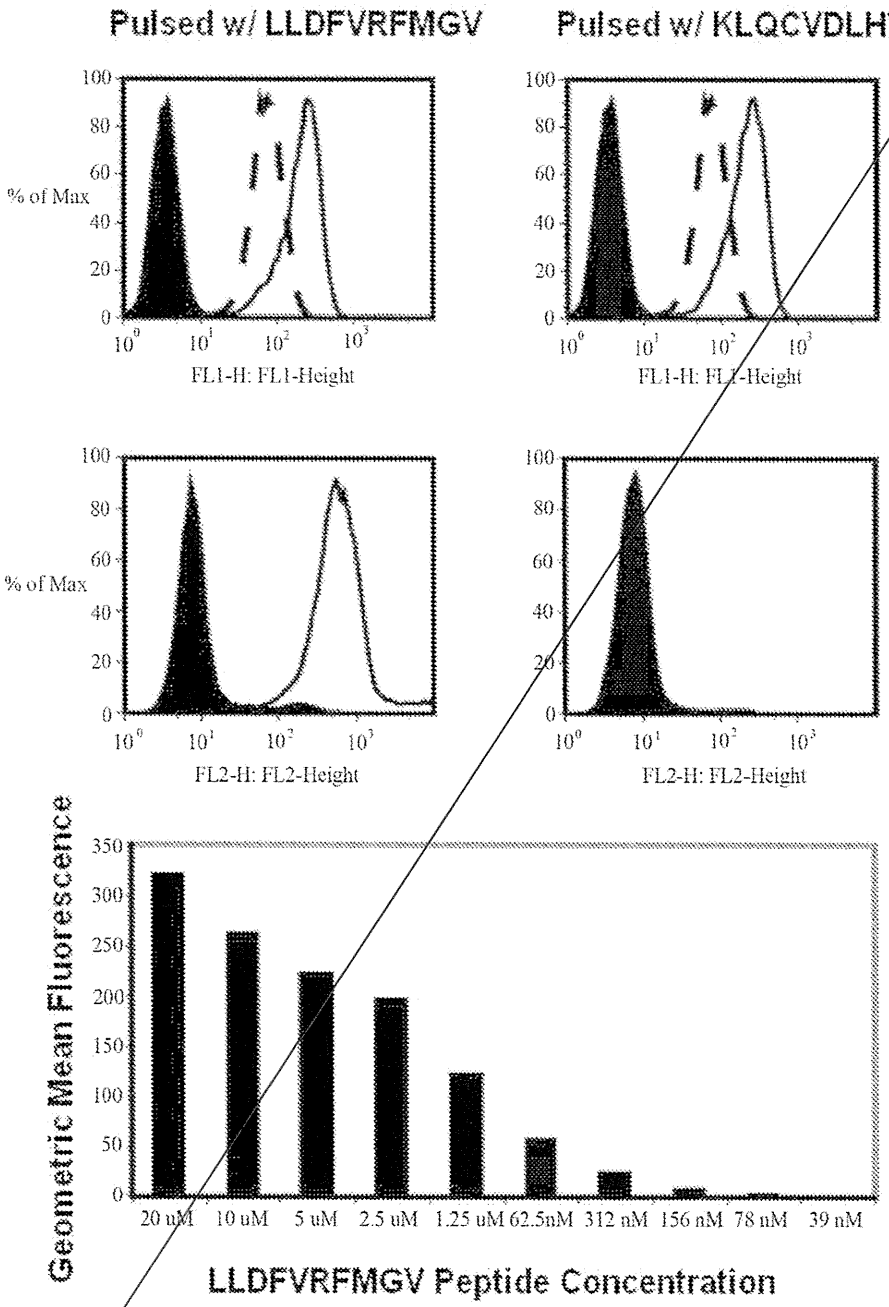

Pulsed w/ LLDFVRFMGV
Pulsed w/ KLQCVDLHV
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
Geometric Mean Fluorescence
20 uM
10 uM
5 uM
2.5 uM
1.25 uM
62.5nM
312 nM
156 nM
78 nM
39 nM
LLDFVRFMGV Peptide Concentration

Figure 3

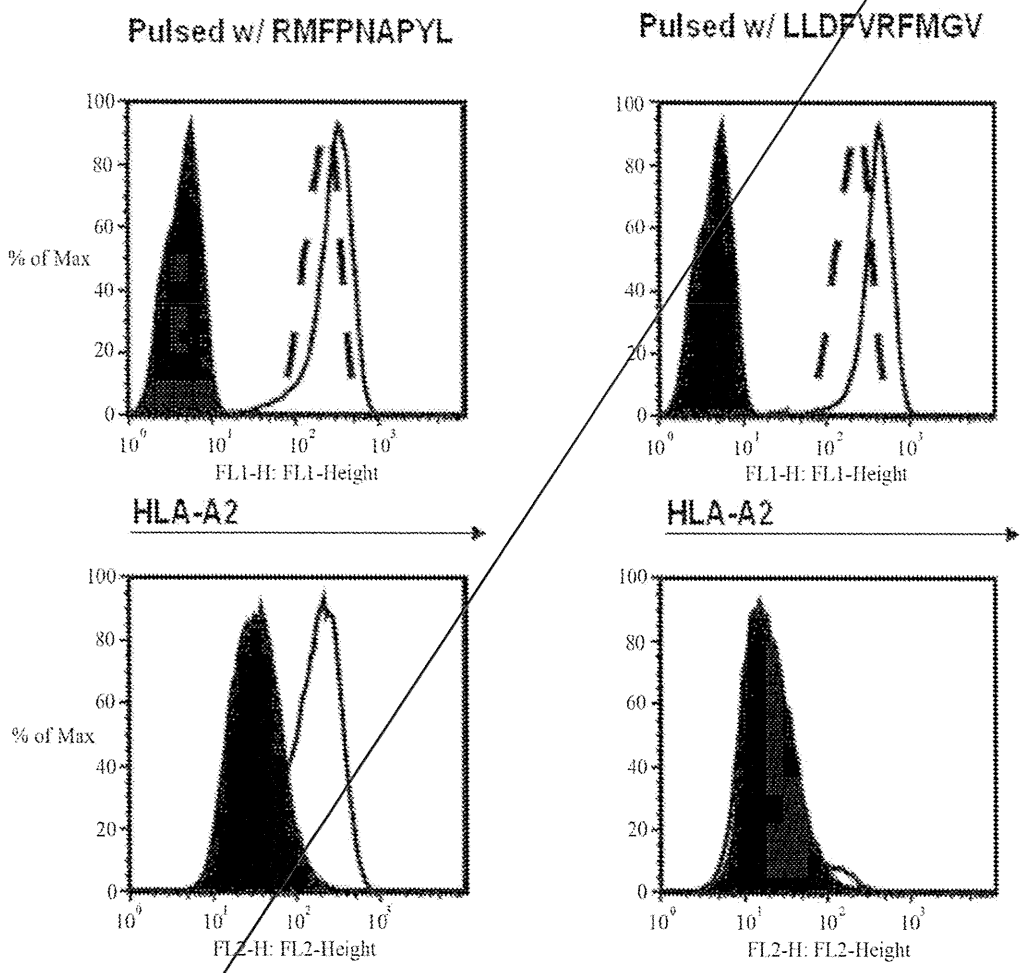

Pulsed w/ RMFPNAPYL
Pulsed w/ LLDFVRFMGV
% of Max
FL1-H: FL1-Height
HLA-A2
HLA-A2
% of Max
FL2-H: FL2-Height
FL2-H: FL2-Height

Figure 4

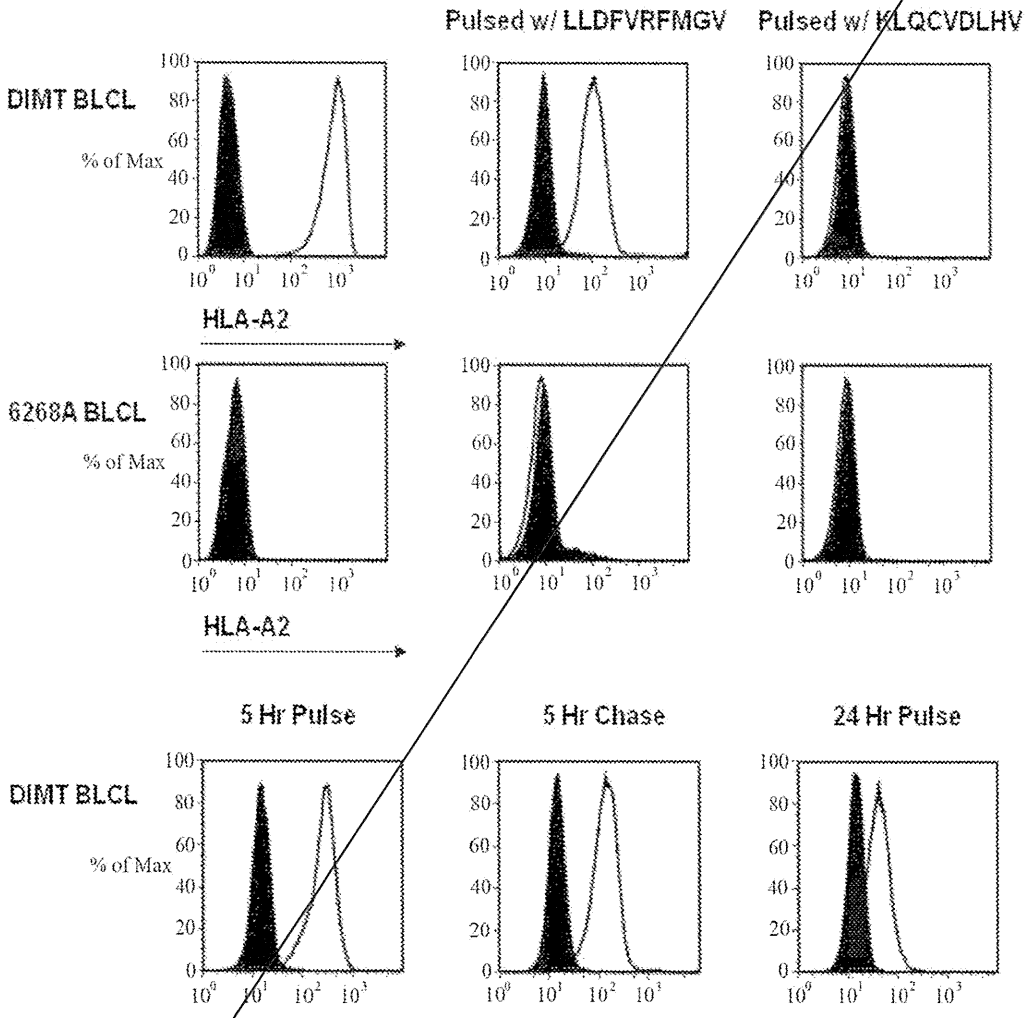

Pulsed w/ LLDFVRFMGV
Pulsed w/ KLQCVDLHV
DIMT BLCL
% of Max
HLA-A2
6268A BLCL
% of Max
HLA-A2
5 Hr Pulse
5 Hr Chase
24 Hr Pulse
DIMT BLCL
% of Max

Figure 5

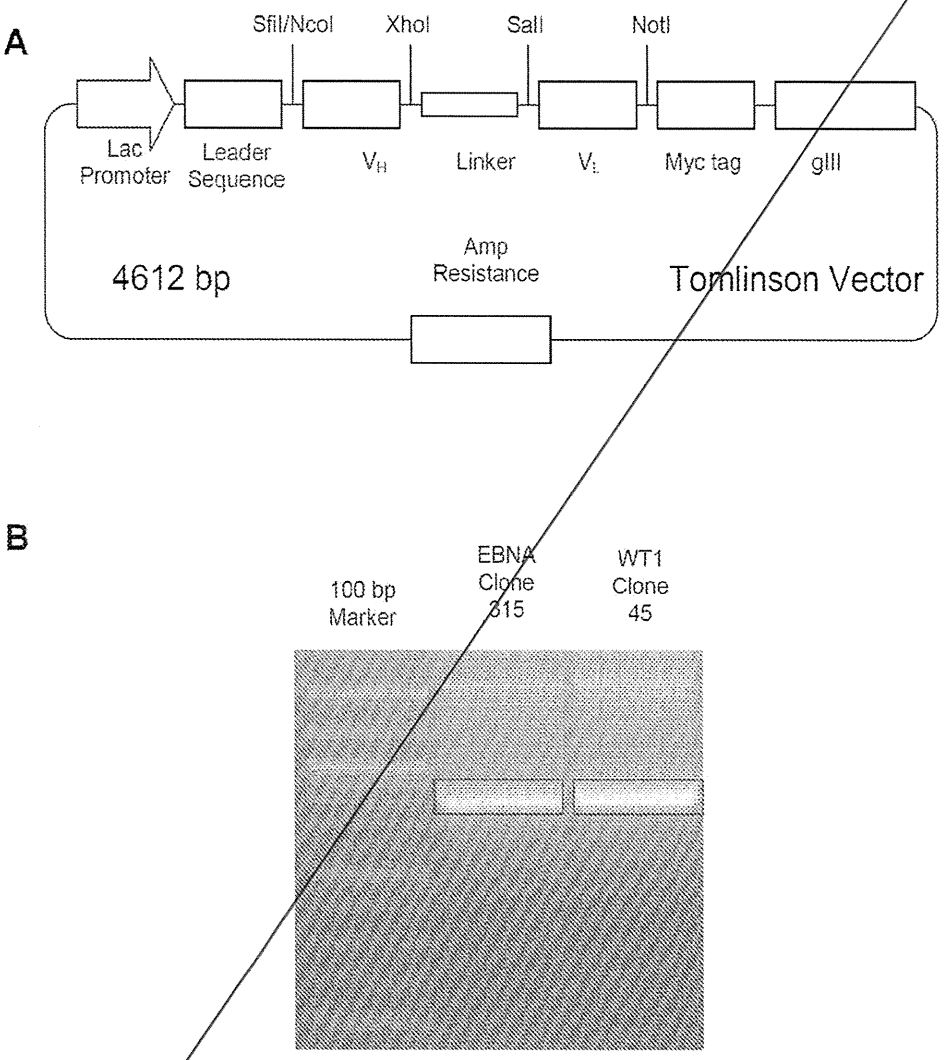

A
SfiI/NcoI
XhoI
SalI
NotI
Lac Promoter
Leader Sequence
V_H
Linker
V_L
Myc tag
gIII
4612 bp
Amp Resistance
Tomlinson Vector
B
100 bp Marker
EBNA Clone 315
WT1 Clone 45

Figure 6

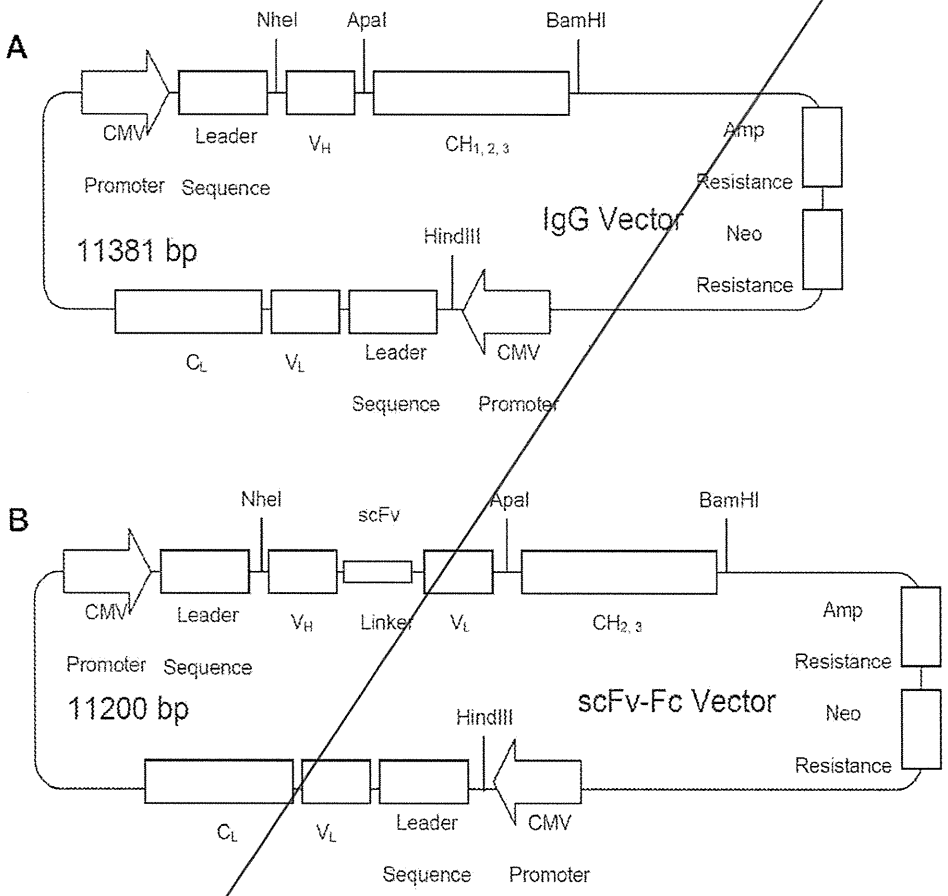

A
NheI
ApaI
BamHI
CMV
Promoter
Leader
Sequence
V_H
$CH_{1,2,3}$
Amp
Resistance
IgG Vector
Neo
Resistance
11381 bp
HindIII
C_L
V_L
Leader
Sequence
CMV
Promoter
B
NheI
scFv
ApaI
BamHI
CMV
Promoter
Leader
Sequence
V_H
Linker
V_L
$CH_{2,3}$
Amp
Resistance
11200 bp
scFv-Fc Vector
Neo
Resistance
HindIII
C_L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

Figure 7

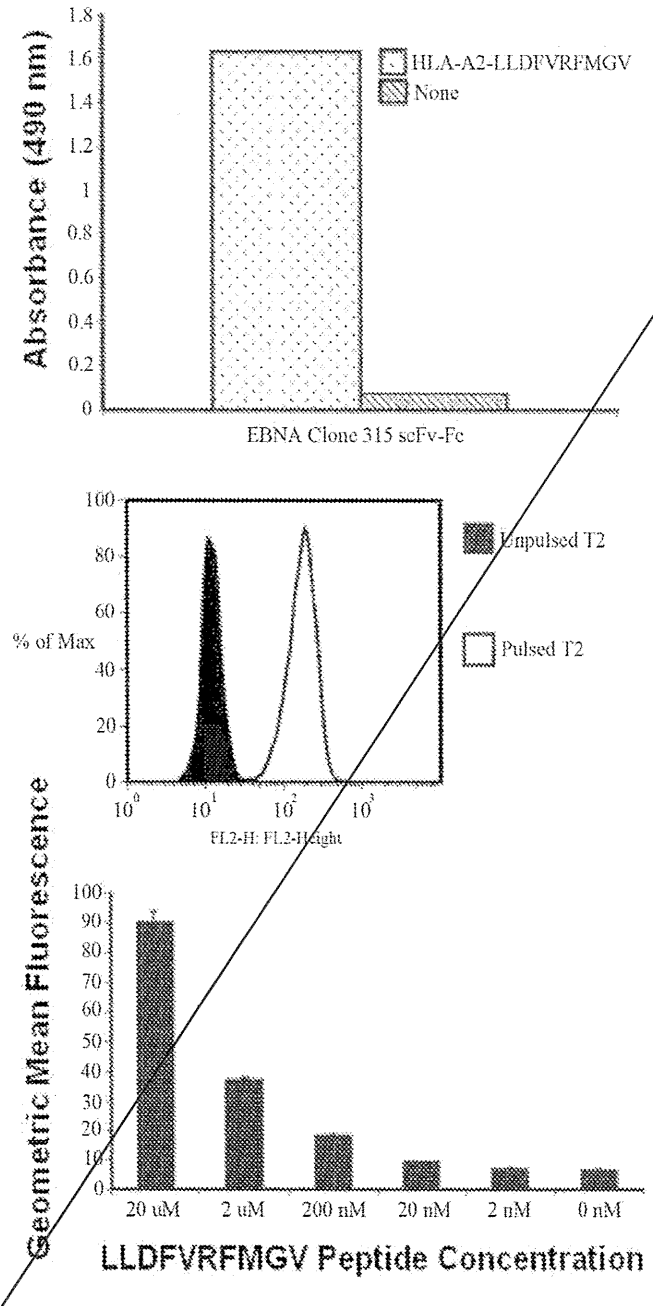
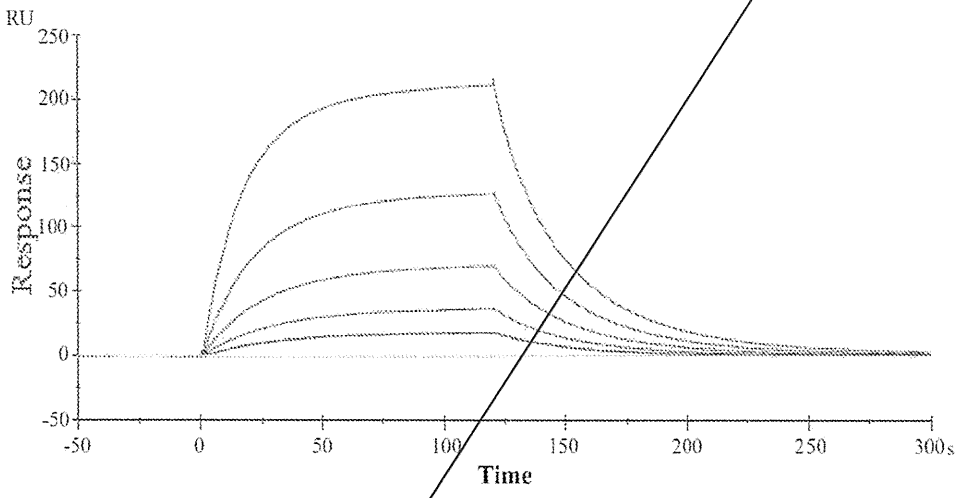
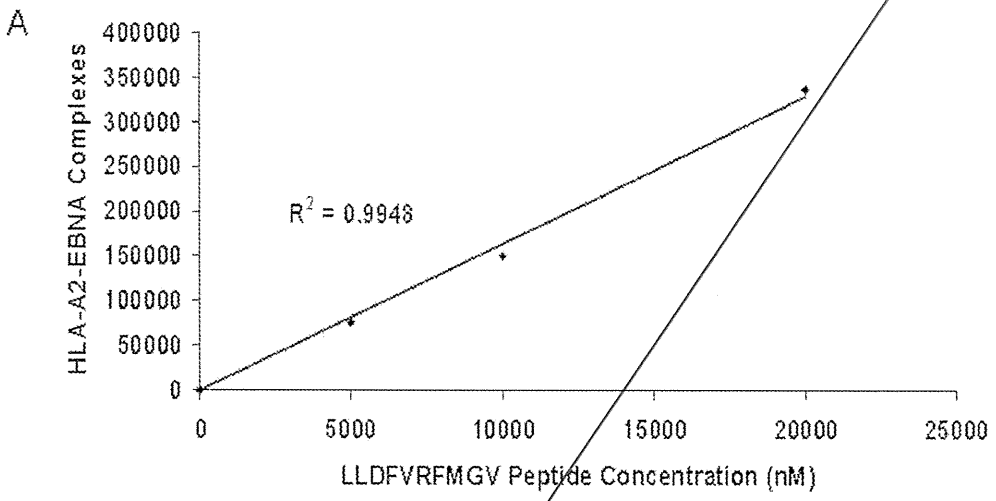

Absorbance (490 nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HLA-A2-LLDFVRFMGV
None
EBNA Clone 315 scFv-Fc
100
80
60
40
20
0
% of Max
Unpulsed T2
Pulsed T2
10^0
10^1
10^2
10^3
FL2-H: FL2-Height
Geometric Mean Fluorescence
100
90
80
70
60
50
40
30
20
10
0
20 uM
2 uM
200 nM
20 nM
2 nM
0 nM
LLDFVRFMGV Peptide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

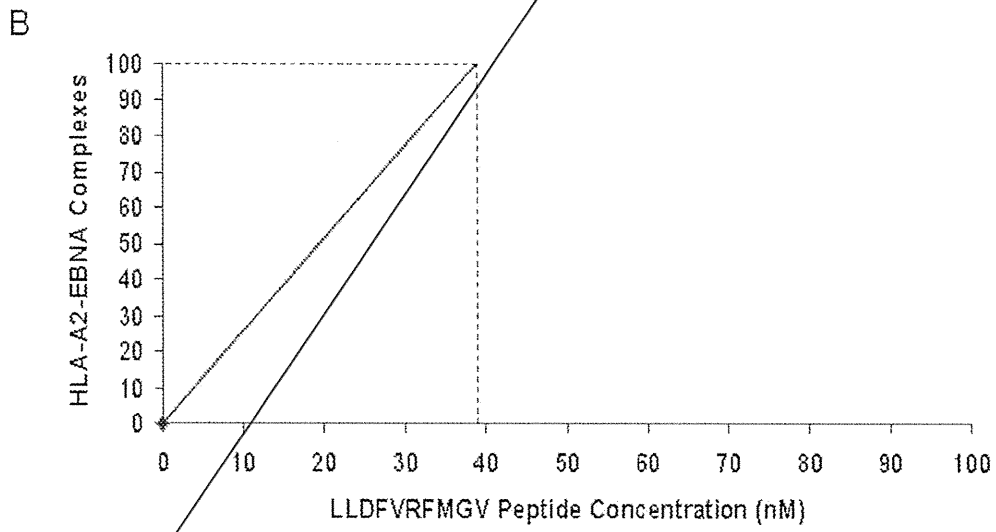

B
HLA-A2-EBNA Complexes
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
LLDFVRFMGV Peptide Concentration (nM)

Figure 10

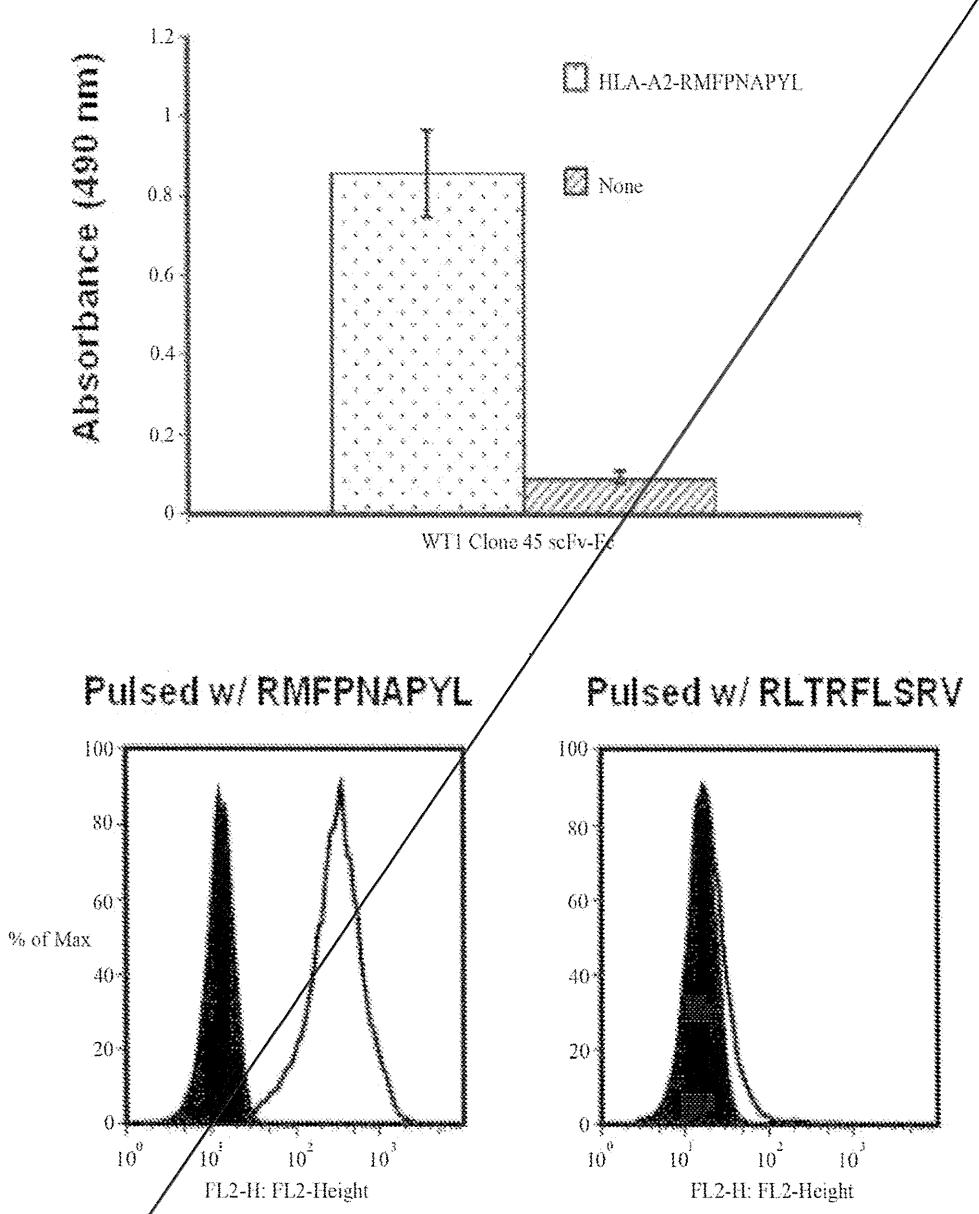

Absorbance (490 nm)
1.2
1
0.8
0.6
0.4
0.2
0
HLA-A2-RMFPNAPYL
None
WT1 Clone 45 scFv-Fc
Pulsed w/ RMFPNAPYL
Pulsed w/ RLTRFLSRV
% of Max
100
80
60
40
20
0
FL2-H: FL2-Height
FL2-H: FL2-Height

Figure 11

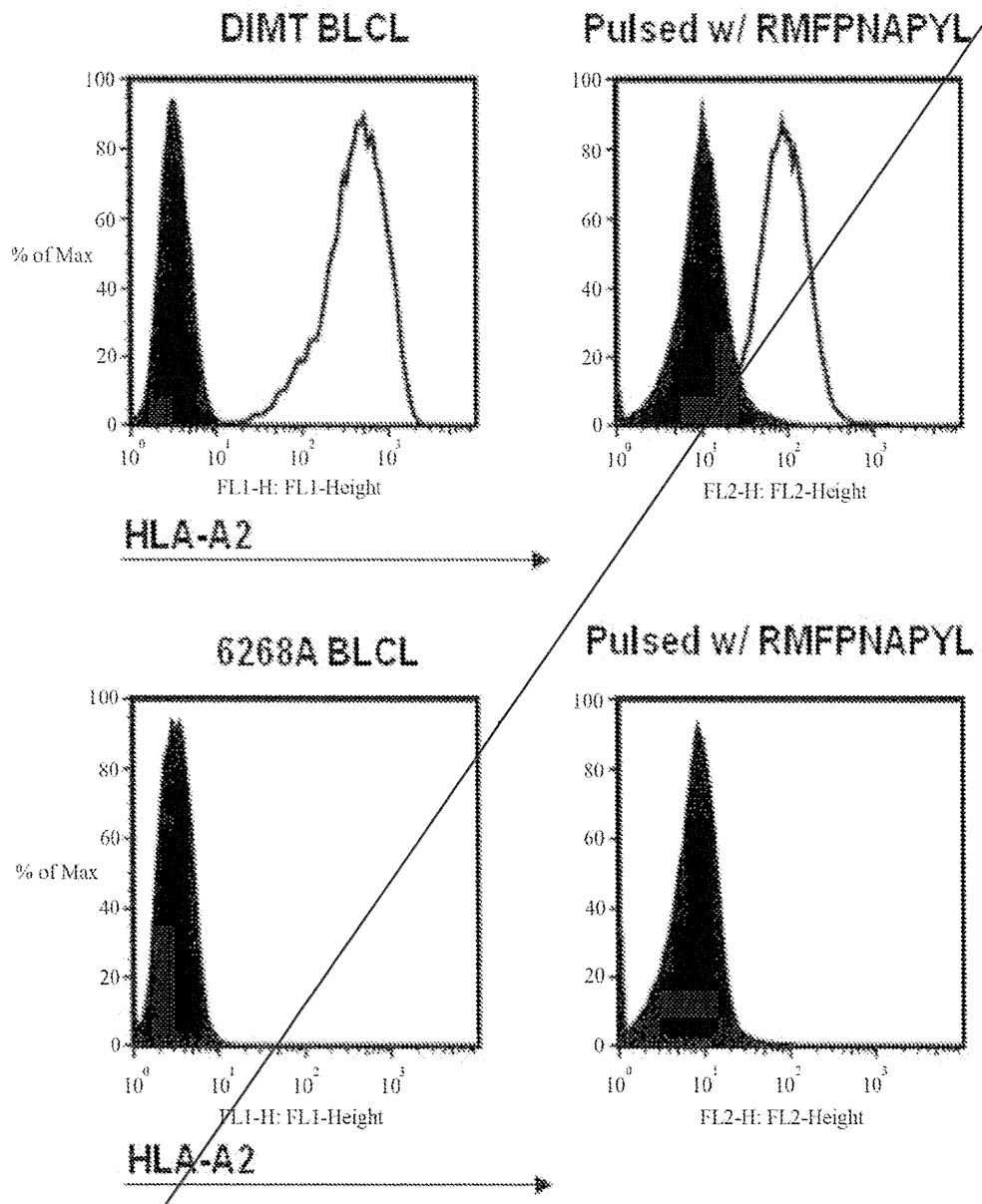
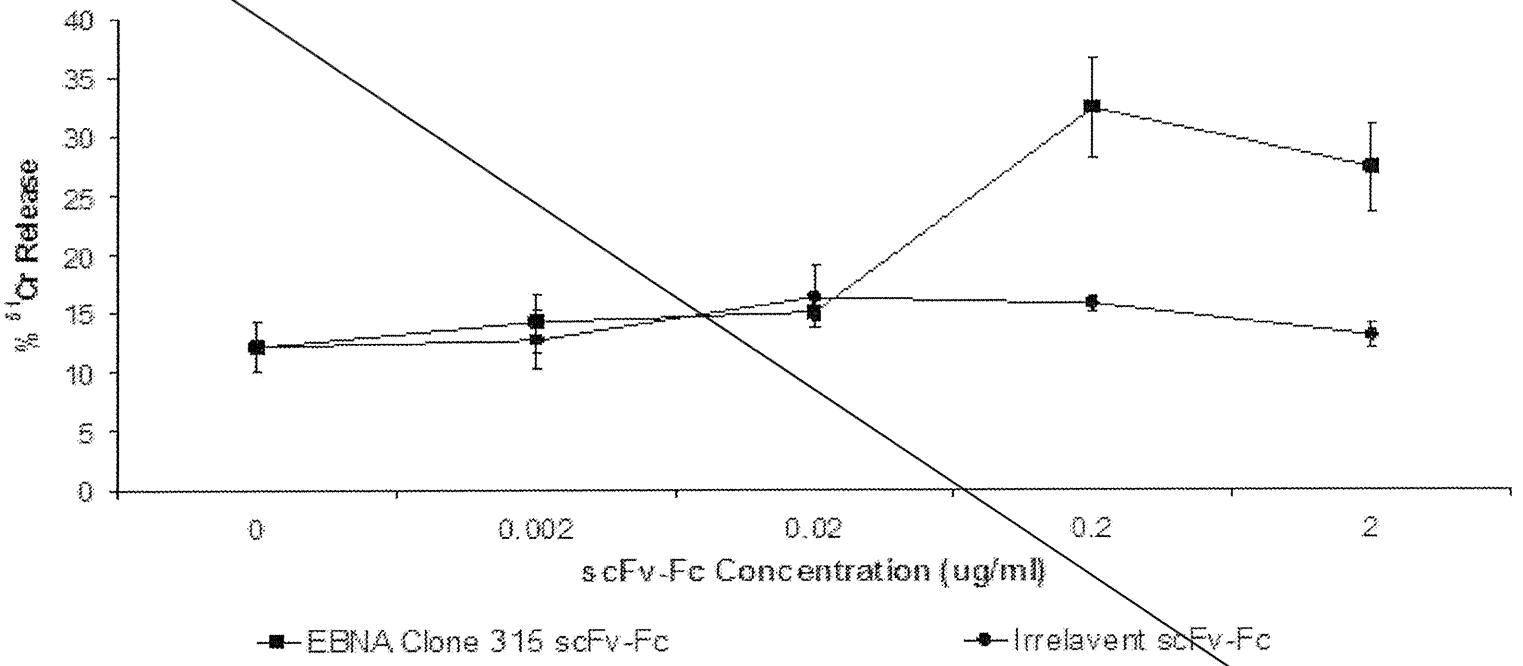

DIMT BLCL
Pulsed w/ RMFPNAPYL
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
HLA-A2
6268A BLCL
Pulsed w/ RMFPNAPYL
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
HLA-A2

Figure 12



13/28

Figure 13

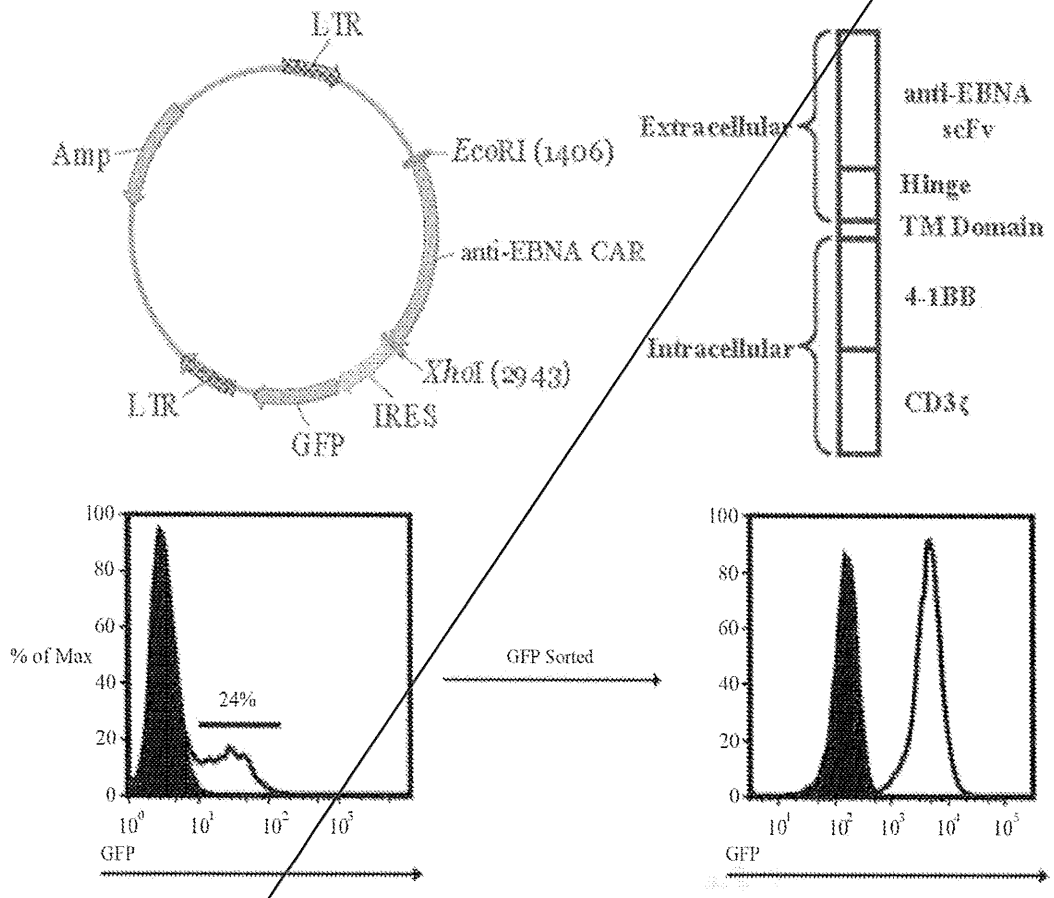

LTR
Amp
EcoRI (1406)
anti-EBNA CAR
XhoI (2943)
LTR
IRES
GFP
Extracellular
anti-EBNA scFv
Hinge
TM Domain
Intracellular
4-1BB
CD3ζ
100
80
60
40
20
0
% of Max
24%
10^0 10^1 10^2 10^3
GFP
GFP Sorted
100
80
60
40
20
0
10^1 10^2 10^3 10^4 10^5
GFP

Figure 14

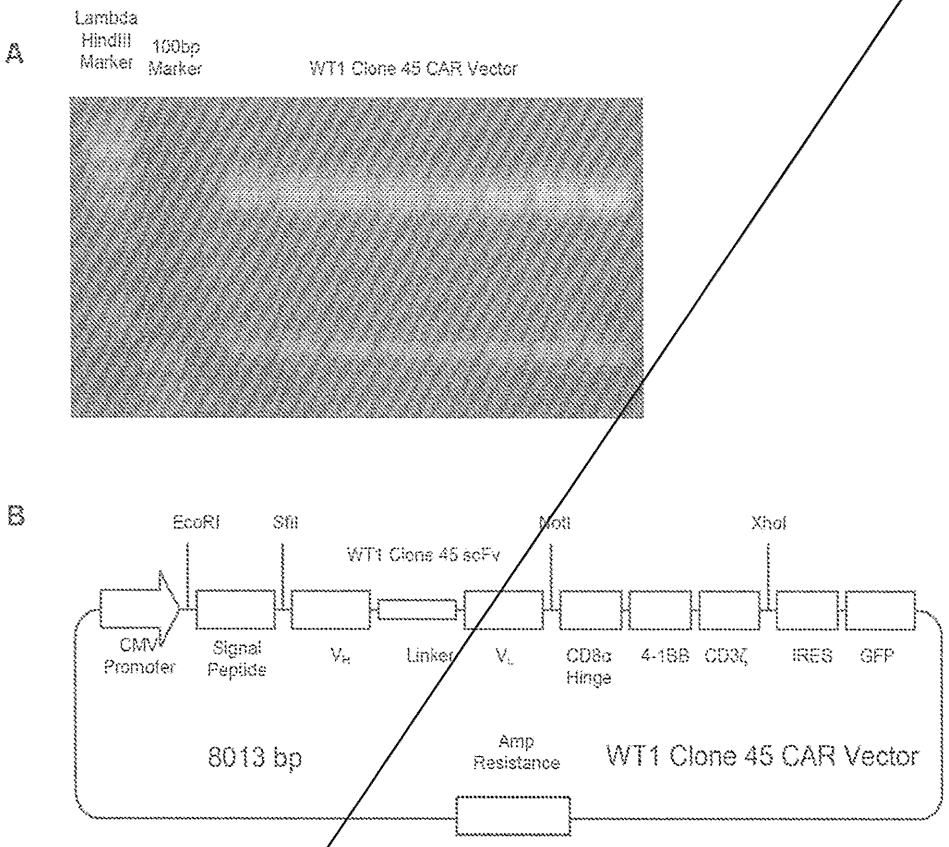

A
Lambda HindIII Marker
100bp Marker
WT1 Clone 45 CAR Vector
B
EcoRI
SfiI
WT1 Clone 45 scFv
NotI
XhoI
CMV Promoter
Signal Peptide
V_H
Linker
V_L
CD8α Hinge
4-1BB
CD3ζ
IRES
GFP
8013 bp
Amp Resistance
WT1 Clone 45 CAR Vector

Figure 15

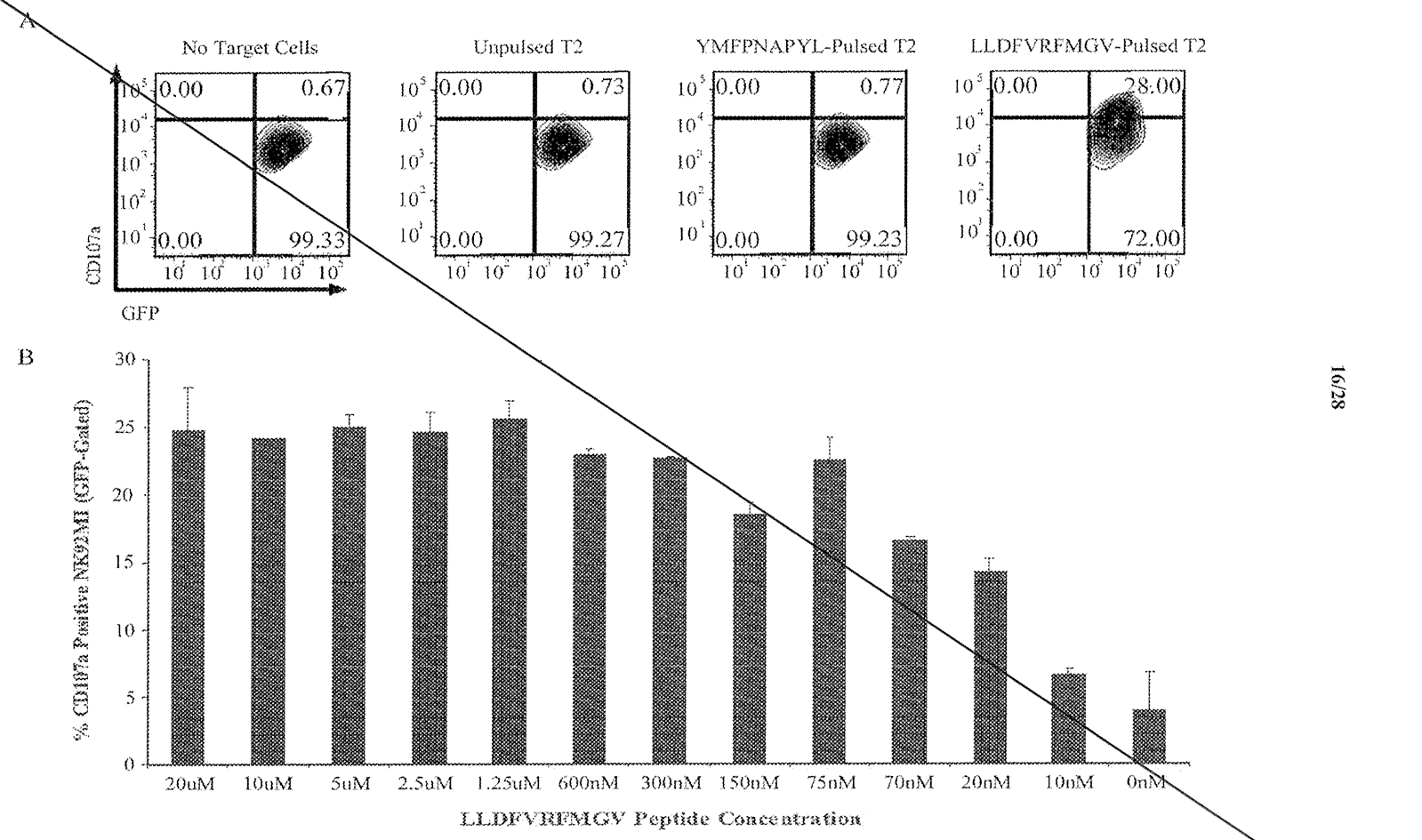

A
No Target Cells
Unpulsed T2
YMFPNAPYL-Pulsed T2
LLDFVRFMGV-Pulsed T2
CD107a
GFP
0.00
0.67
99.33
0.00
0.73
99.27
0.00
0.77
99.23
0.00
28.00
72.00
B
% CD107a Positive NK92MI (GFP-Gated)
0
5
10
15
20
25
30
20uM
10uM
5uM
2.5uM
1.25uM
600nM
300nM
150nM
75nM
70nM
20nM
10nM
0nM
LLDFVRFMGV Peptide Concentration

Figure 16

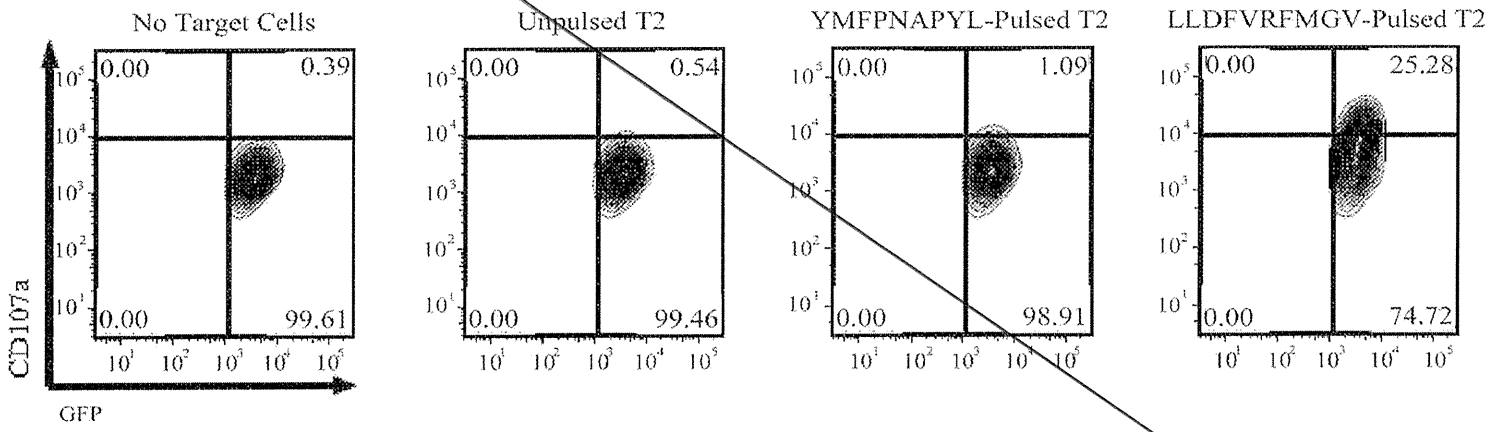

No Target Cells
Unpulsed T2
YMFPNAPYL-Pulsed T2
LLDFVRFMGV-Pulsed T2
CD107a
GFP
0.00
0.39
99.61
0.54
99.46
1.09
98.91
25.28
74.72

Figure 17

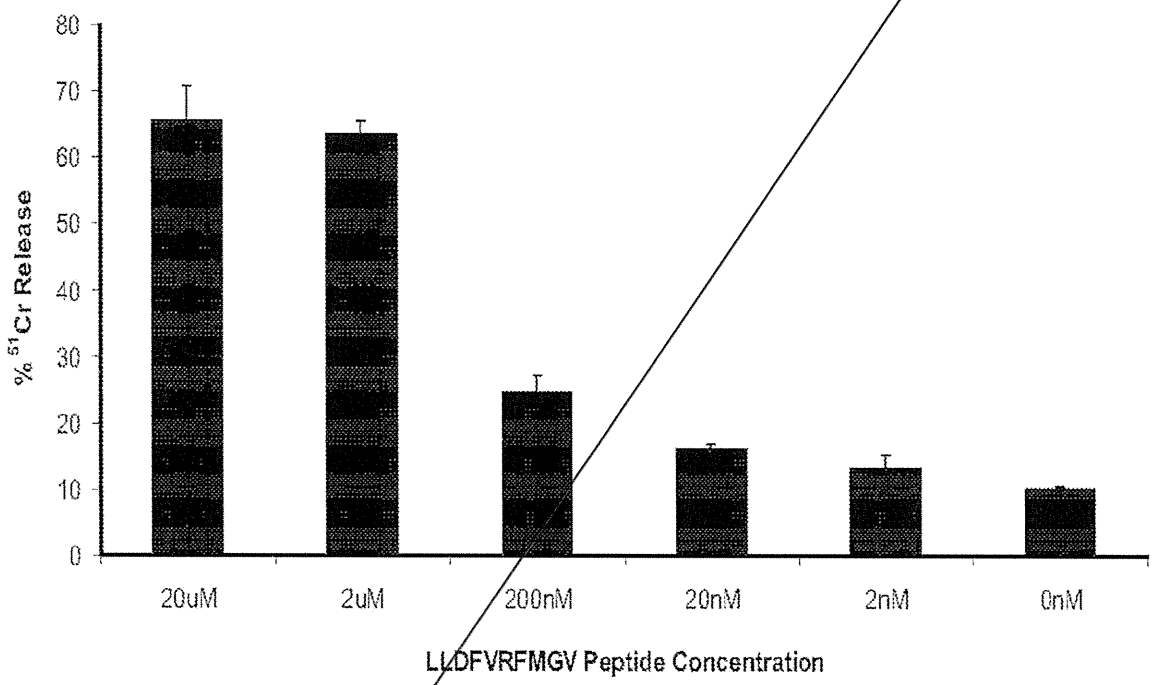

80
70
60
50
40
30
20
10
0
% 51Cr Release
20uM
2uM
200nM
20nM
2nM
0nM
LLDFVRFMGV Peptide Concentration

Figure 18

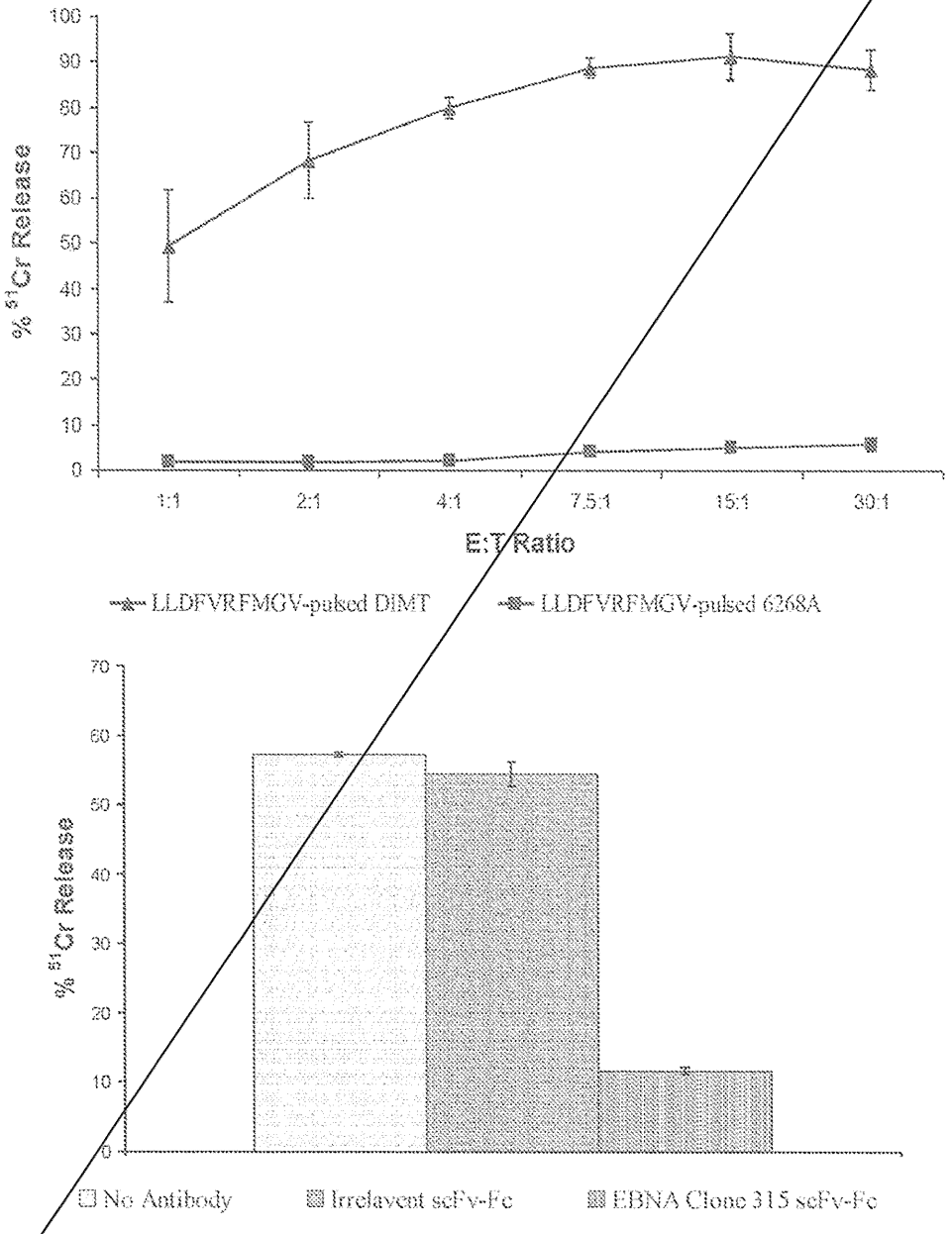
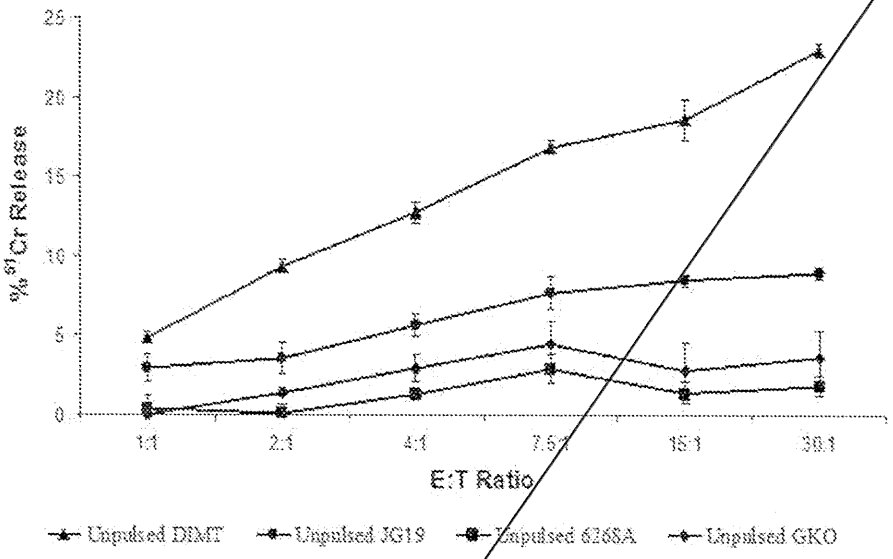

100
90
80
70
60
50
40
30
20
10
0
% 51Cr Release
1:1
2:1
4:1
7.5:1
15:1
30:1
E:T Ratio
LLDFVRFMGV-pulsed DIMT
LLDFVRFMGV-pulsed 6268A
70
60
50
40
30
20
10
0
% 51Cr Release
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 19


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

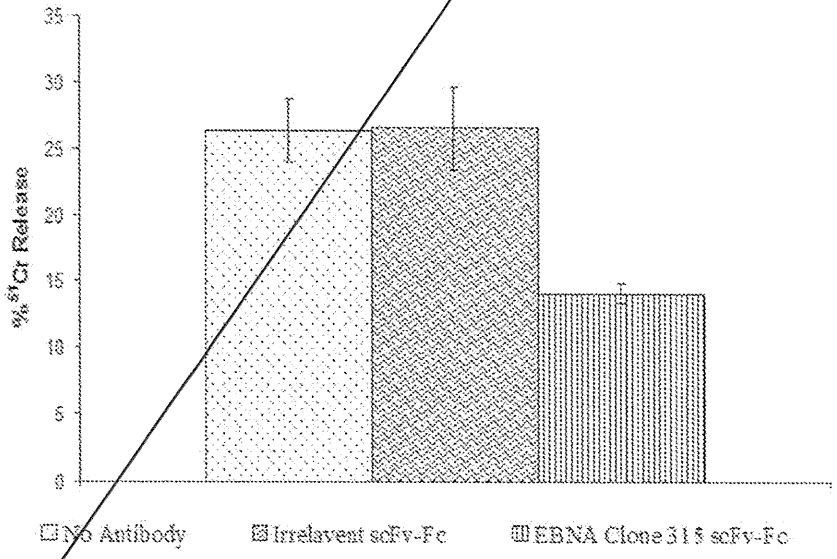

% 51Cr Release
35
30
25
20
15
10
5
0
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 20

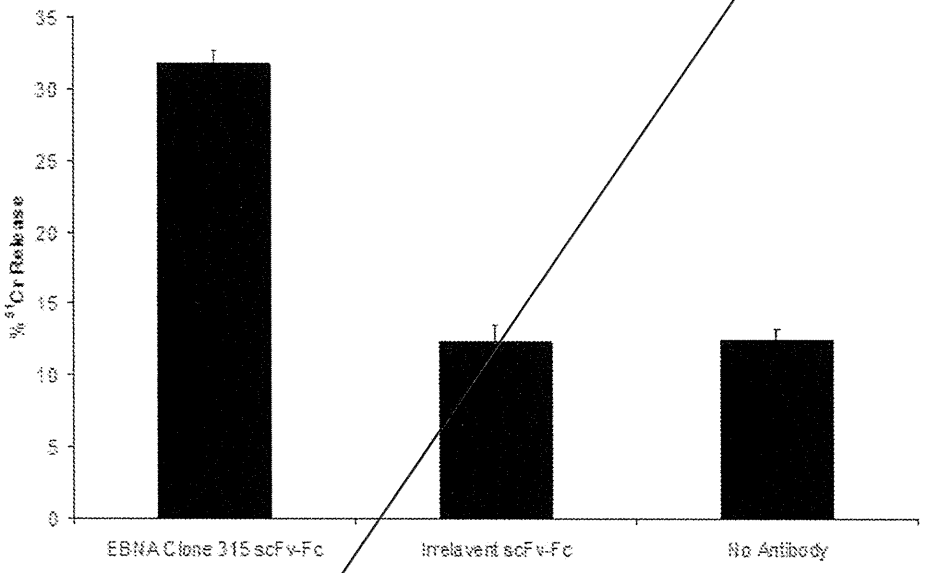

35
30
25
20
15
10
5
0
% ^{51}Cr Release
EBNA Clone 315 scFv-Fc
Irrelavent scFv-Fc
No Antibody

Figure 21

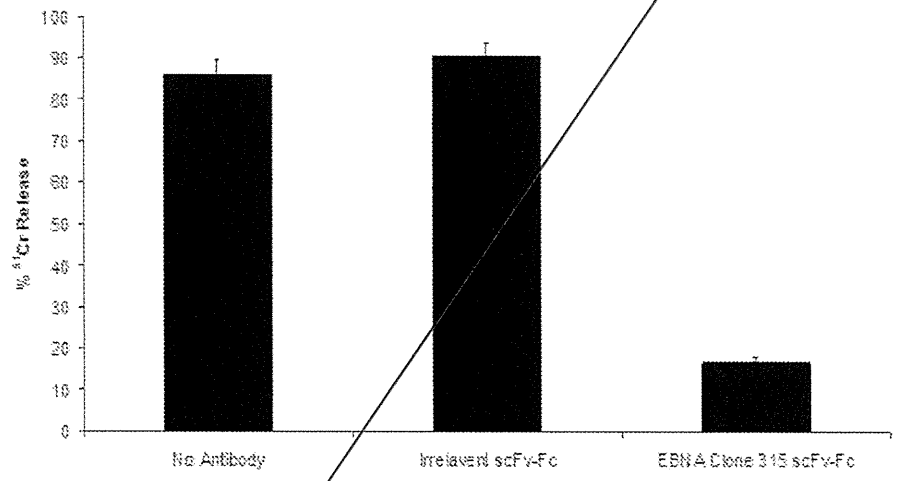

100
90
80
70
60
50
40
30
20
10
0
% ^{51}Cr Release
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 22

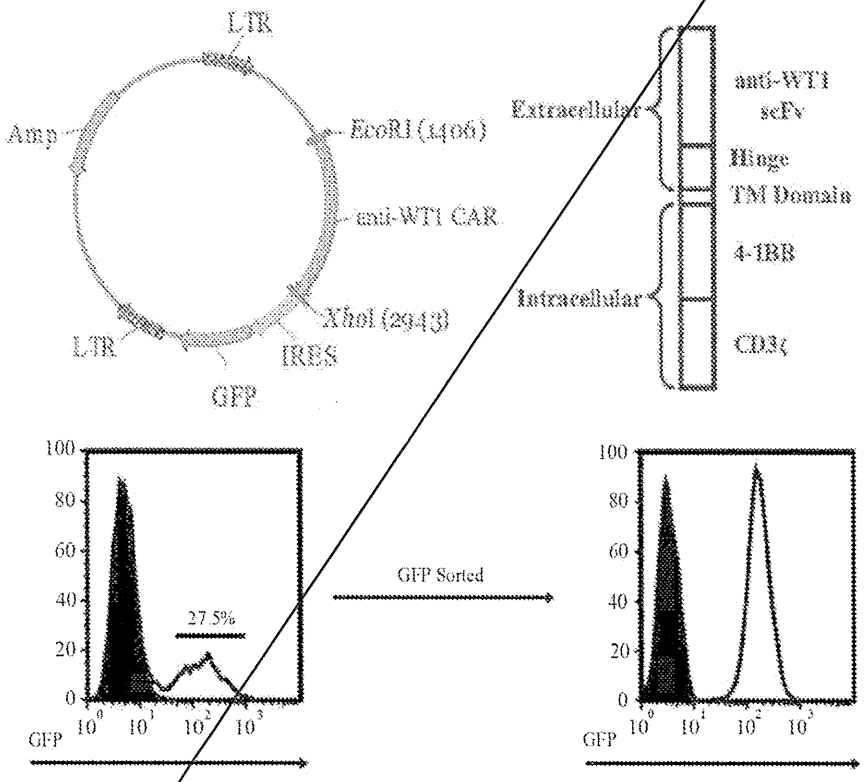

LTR
Amp
EcoRI (4406)
anti-WT1 CAR
XhoI (2943)
LTR
IRES
GFP
Extracellular
anti-WT1 scFv
Hinge
TM Domain
Intracellular
4-1BB
CD3ζ
100
80
60
40
20
0
27.5%
GFP
GFP Sorted

Figure 23

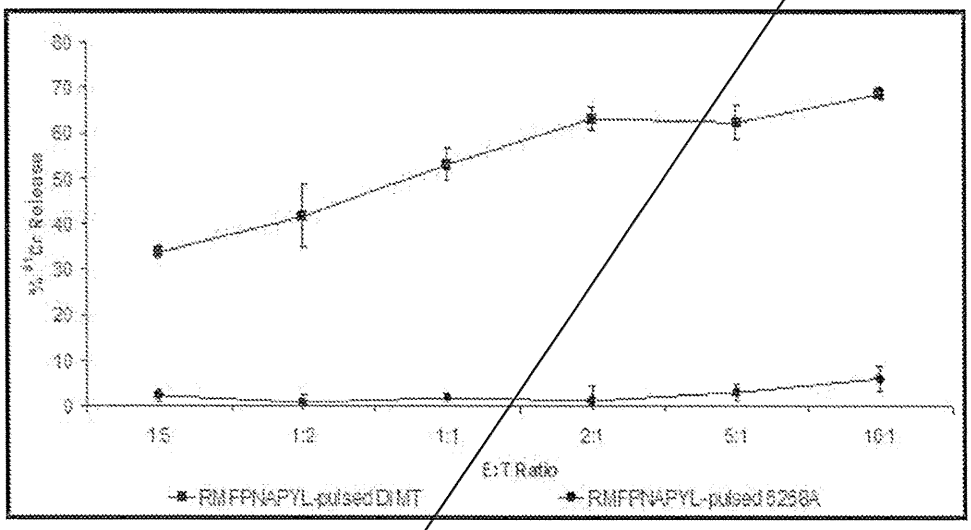

80
70
60
50
40
30
20
10
0
% ^{51}Cr Release
1:5
1:2
1:1
2:1
5:1
10:1
E:T Ratio
RMFPNAPYL-pulsed DMT
RMFPNAPYL-pulsed 6268A

Figure 24

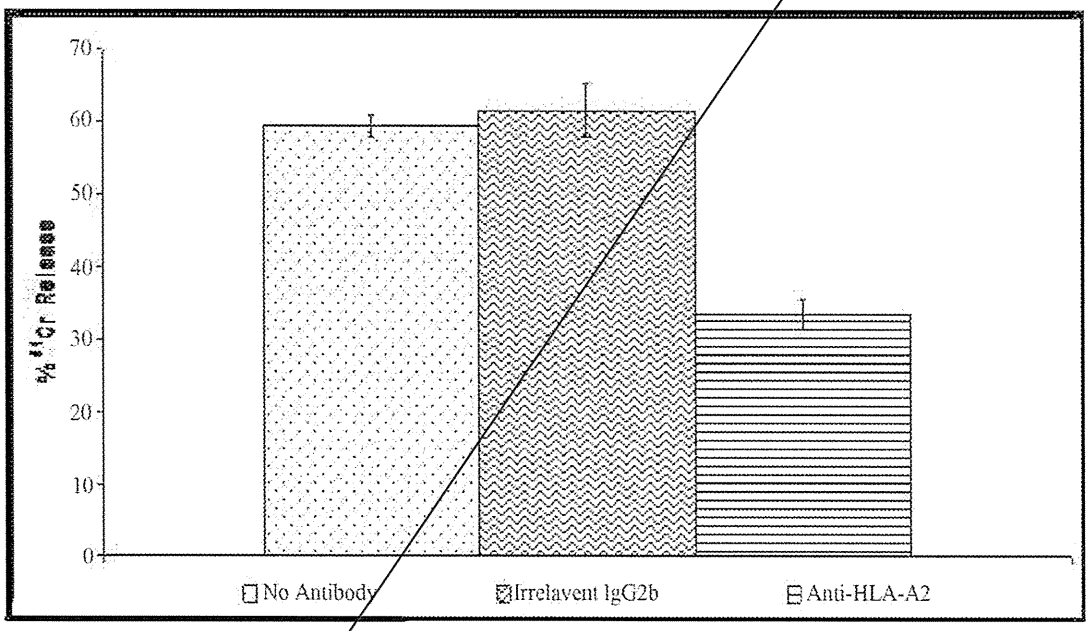

70
60
50
40
30
20
10
0
% 51Cr Release
No Antibody
Irrelavent IgG2b
Anti-HLA-A2

Figure 25

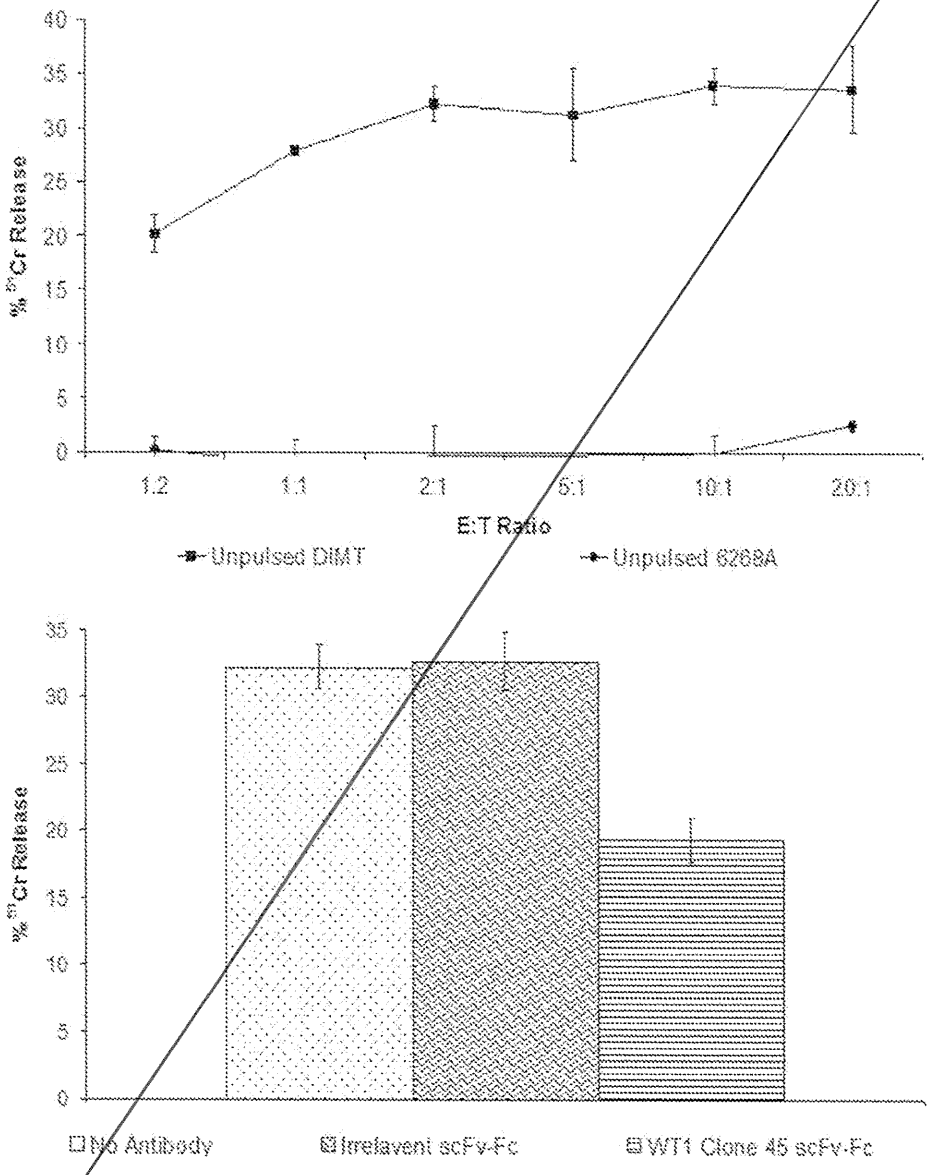

40
35
30
25
20
15
10
5
0
% 51Cr Release
1:2
1:1
2:1
5:1
10:1
20:1
E:T Ratio
Unpulsed DIMT
Unpulsed 6268A
35
30
25
20
15
10
5
0
% 51Cr Release
No Antibody
Irrelaveni scFv-Fc
WT1 Clone 45 scFv-Fc

Figure 26

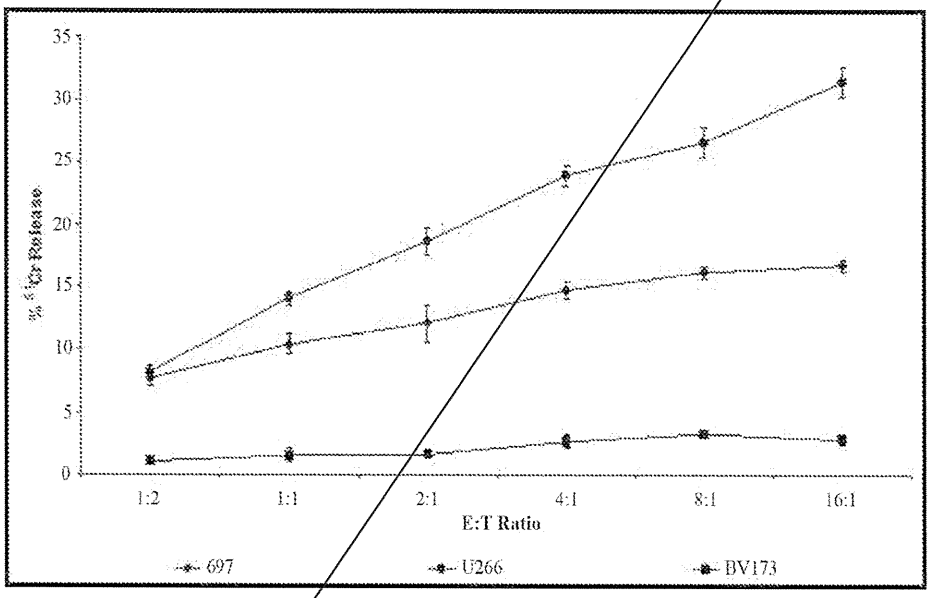

35
30
25
20
15
10
5
0
% ^{51}Cr Release
1:2
1:1
2:1
4:1
8:1
16:1
E:T Ratio
697
U266
BV173

Figure 27

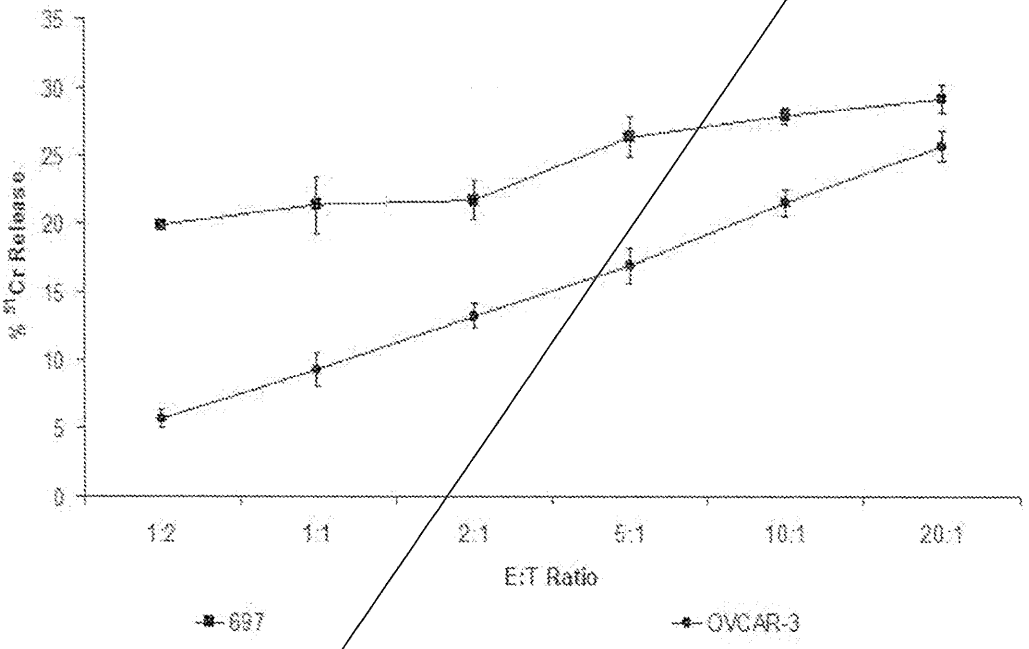

35
30
25
20
15
10
5
0
% ^{51}Cr Release
1:2
1:1
2:1
5:1
10:1
20:1
E:T Ratio
697
OVCAR-3

Figure 28

FIG 1A

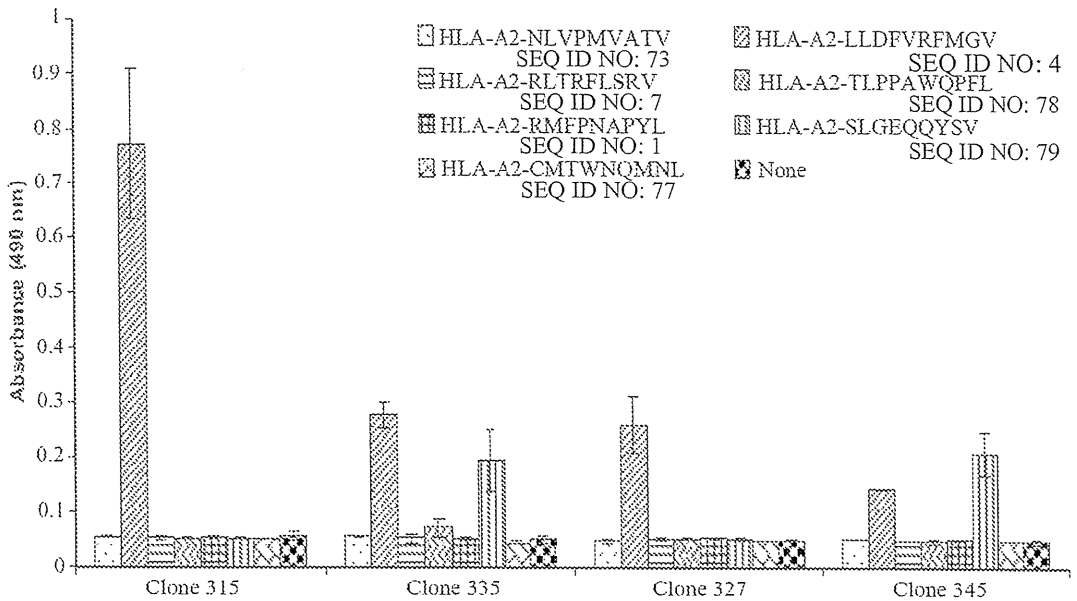


FIG 1B

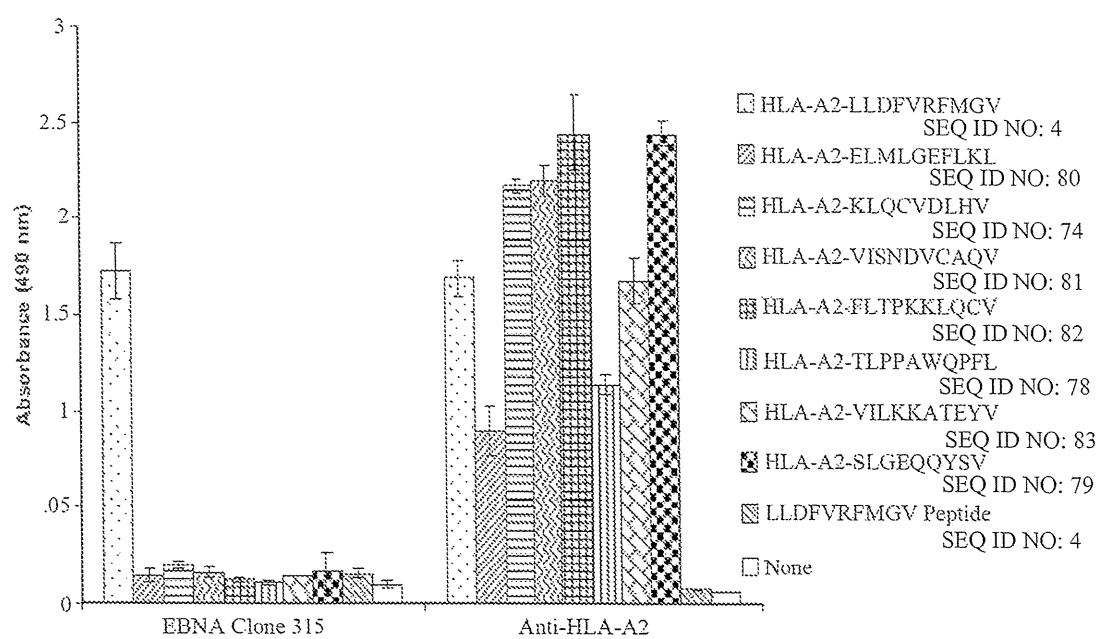


Figure 1

FIG 2A

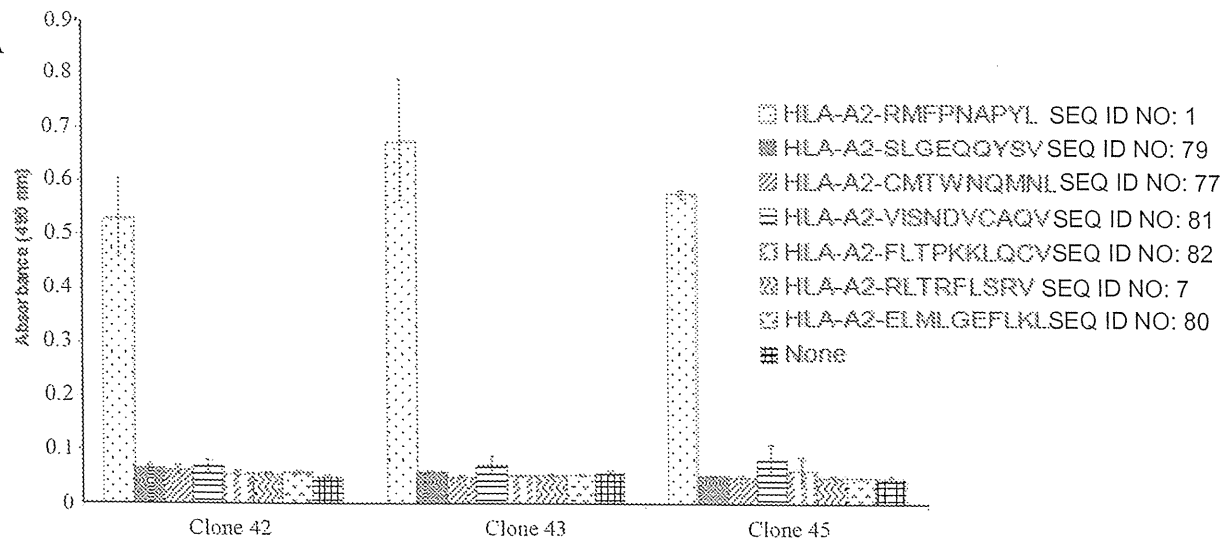


FIG 2B

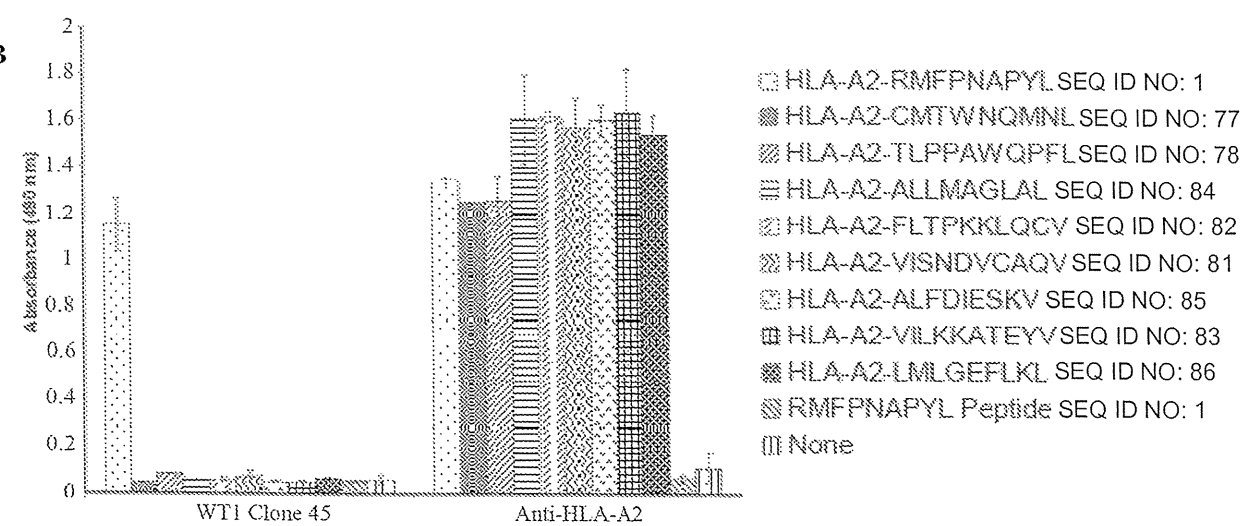


Figure 2

FIG 3A

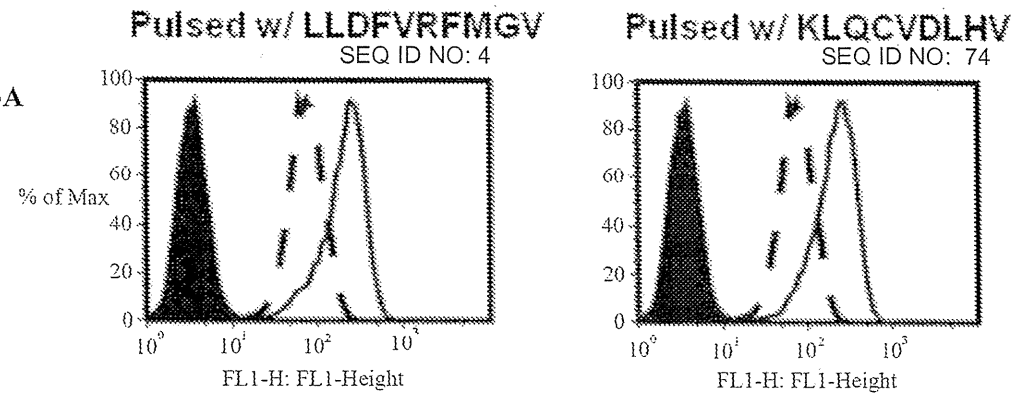


FIG 3B

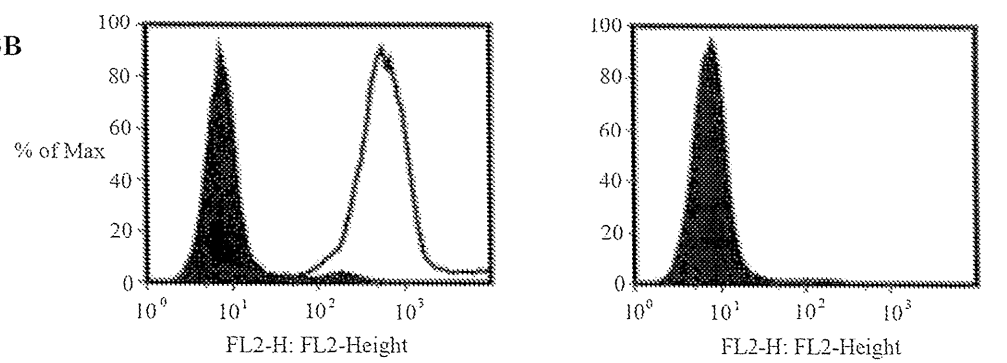


FIG 3C

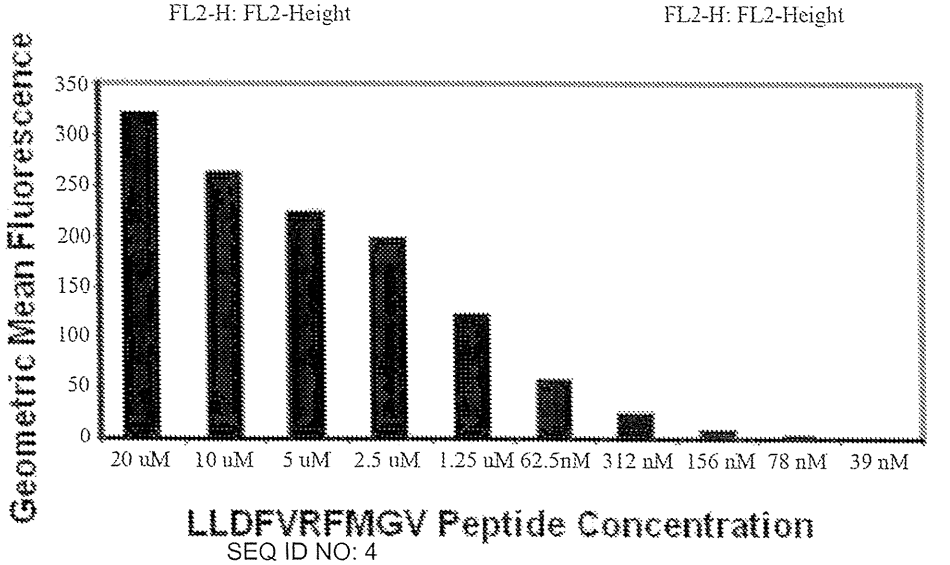
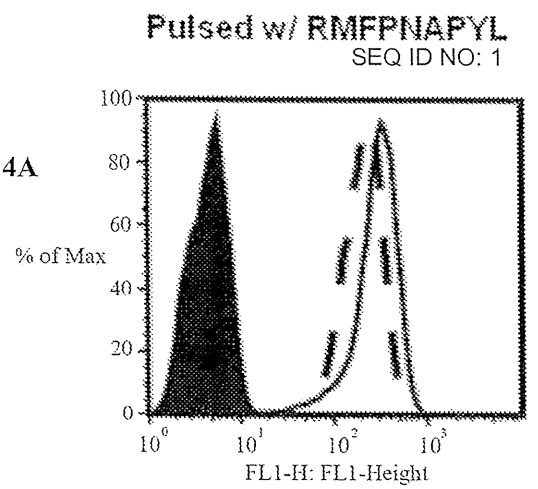


Figure 3

FIG 4A



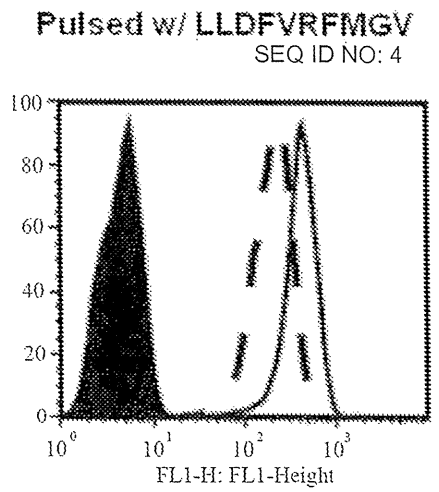
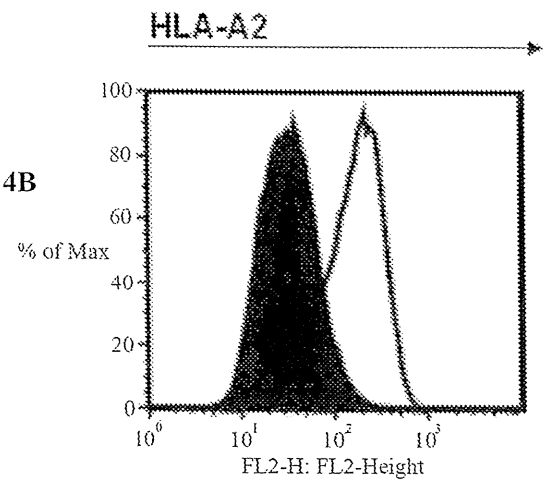


FIG 4B



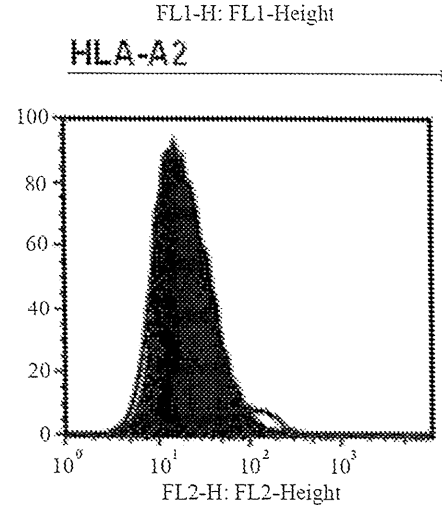


Figure 4

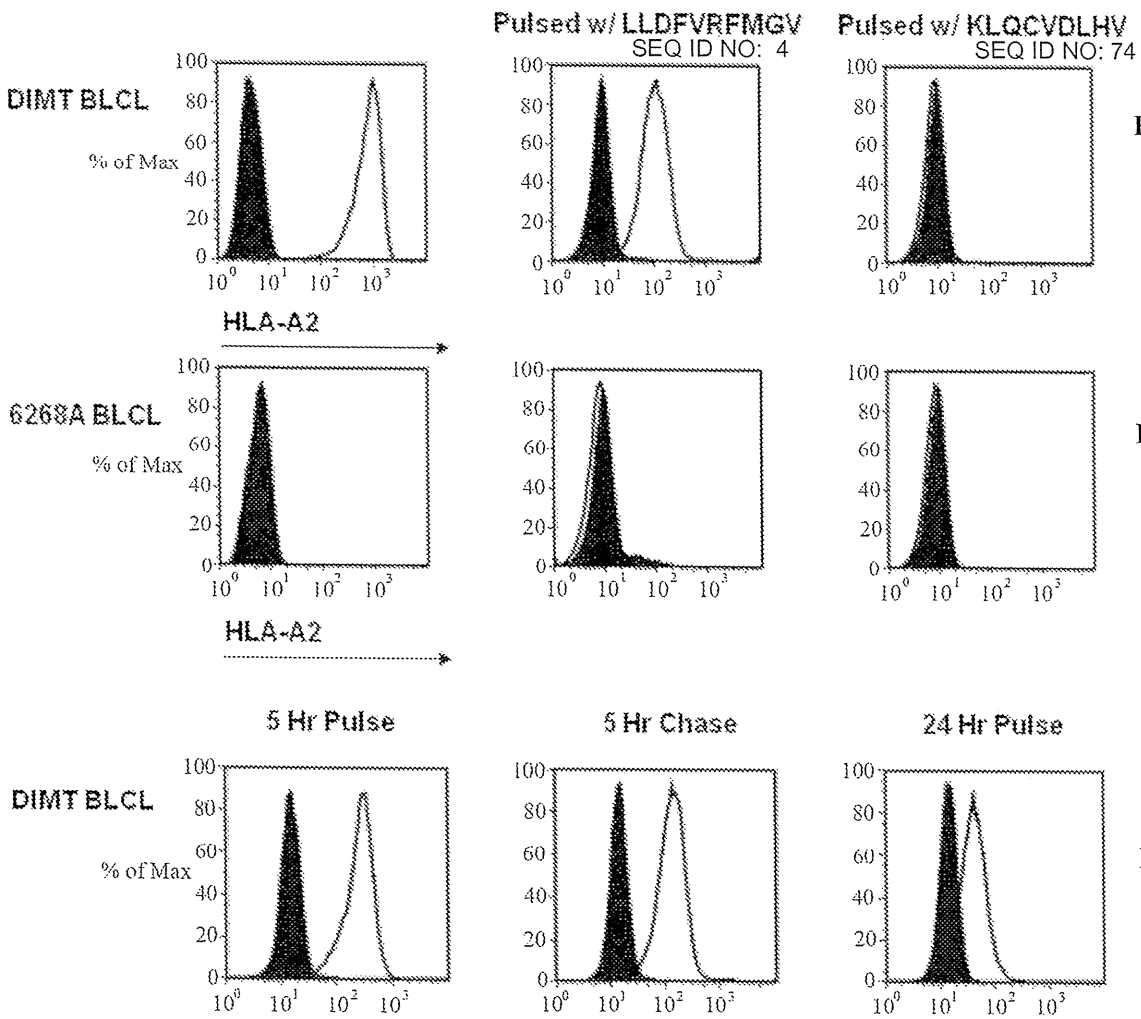


FIG 5A

FIG 5B

FIG 5C

Figure 5

FIG 6A

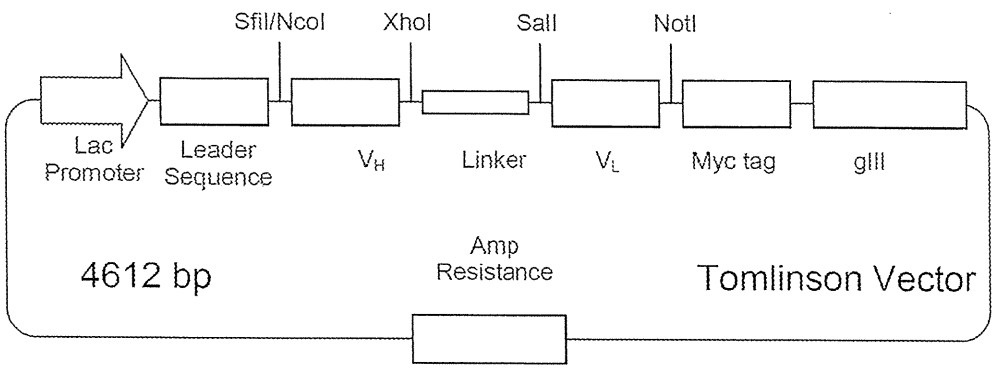


FIG 6B

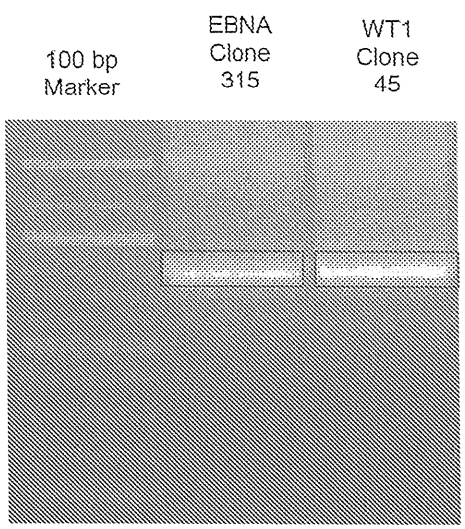


Figure 6

FIG 7A

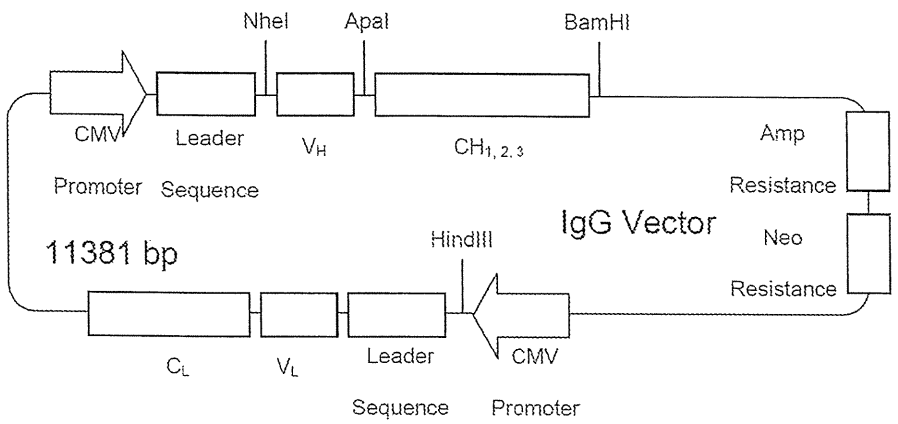


FIG 7B

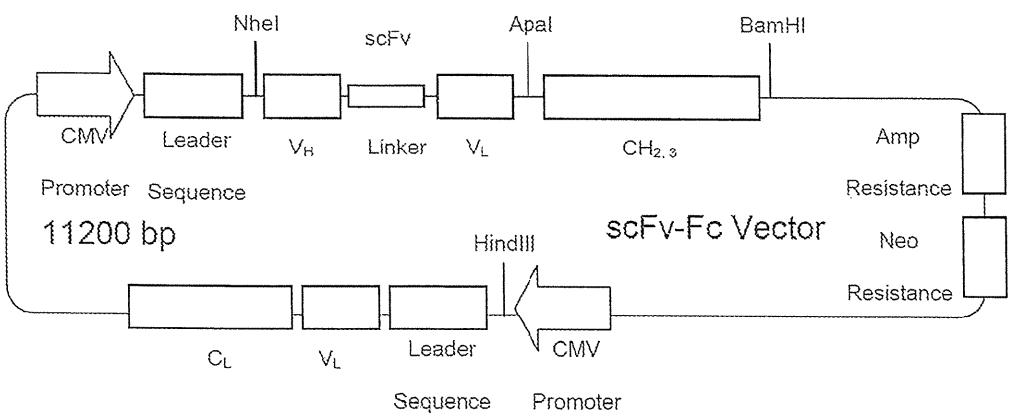


Figure 7

FIG 8A

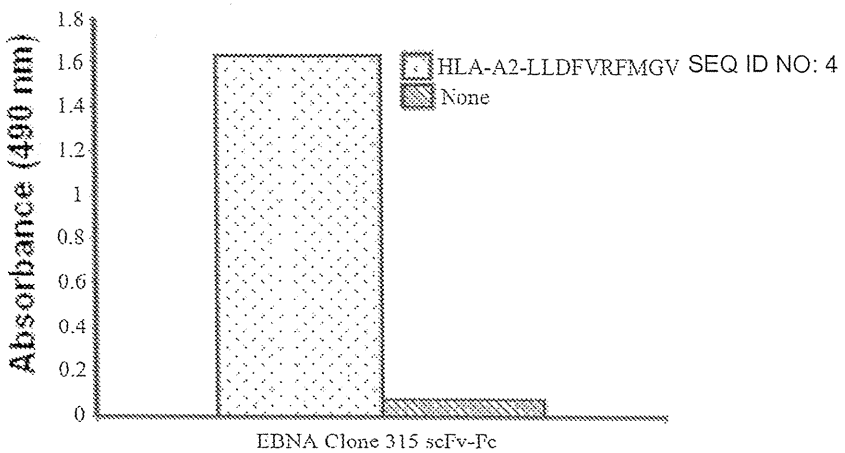


FIG 8B

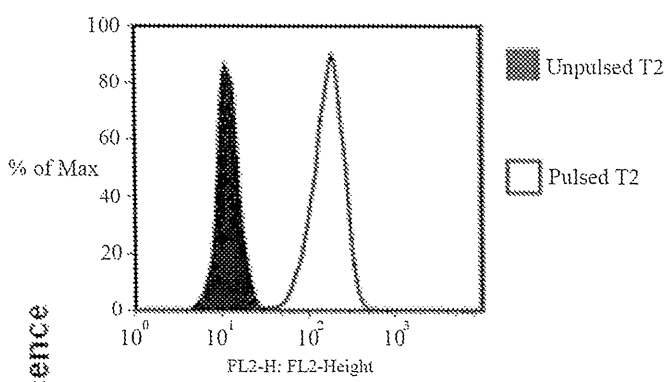


FIG 8C

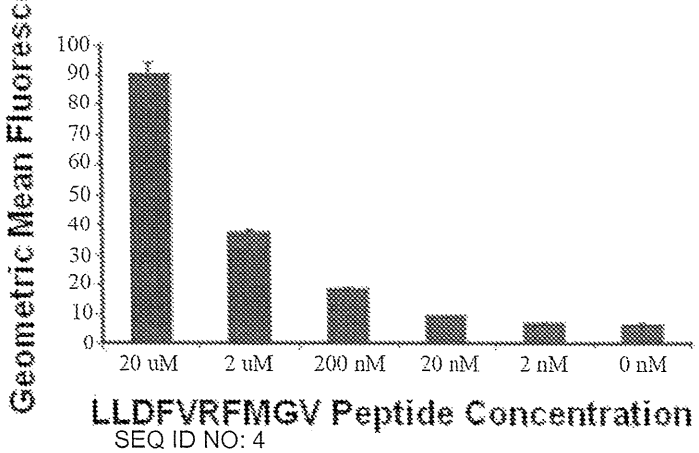
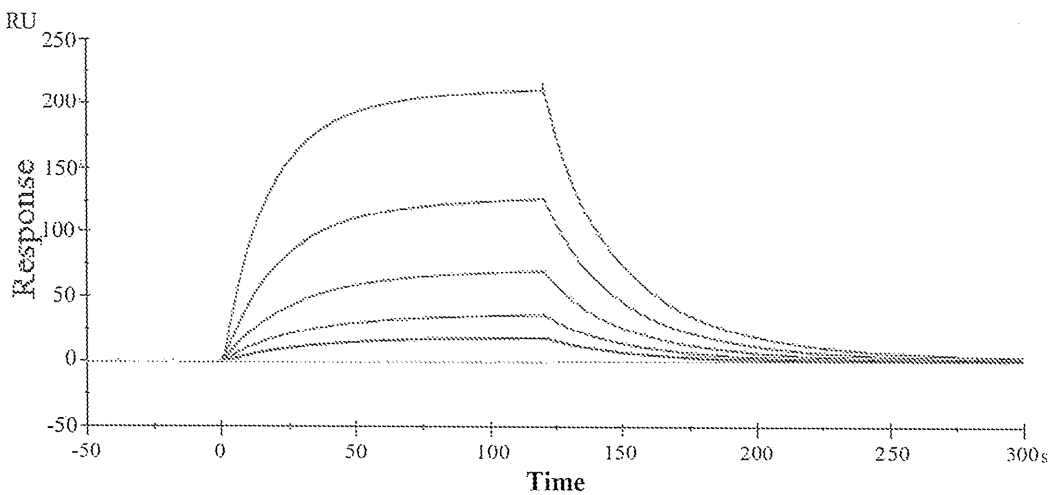


Figure 8



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

FIG 10A

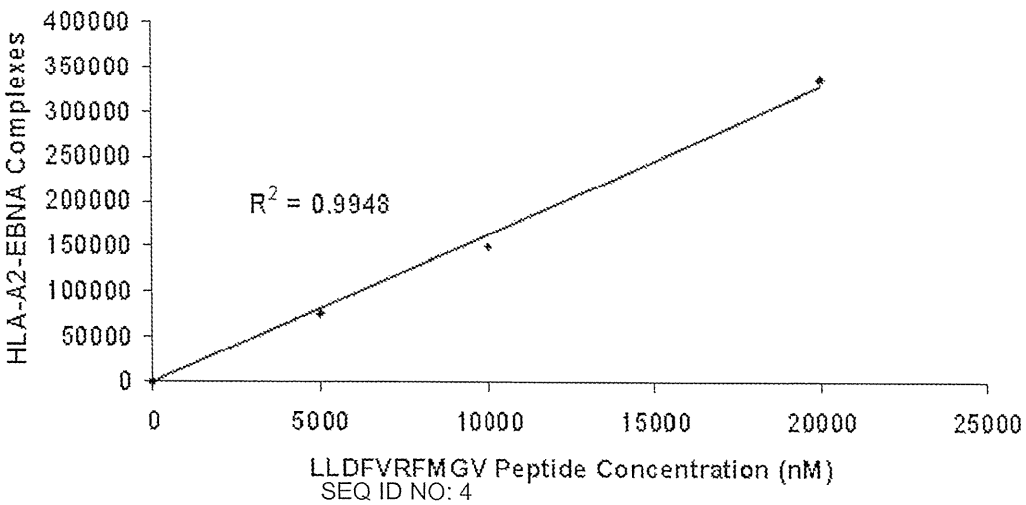


FIG 10B

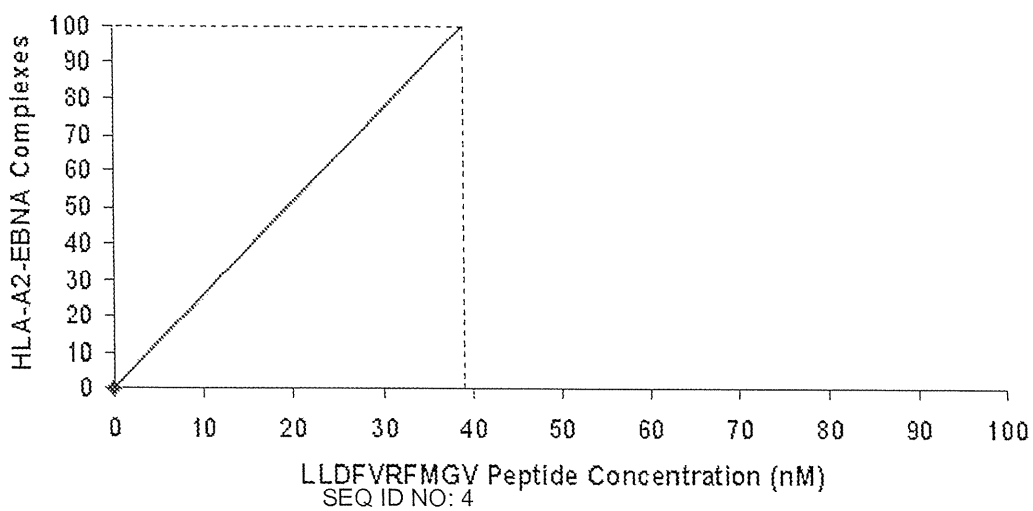


Figure 10

FIG 11A

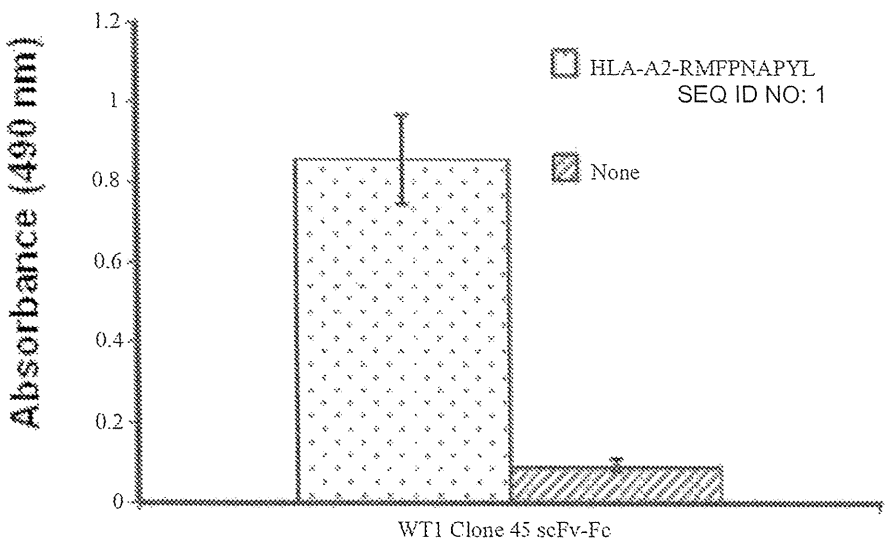


FIG 11B

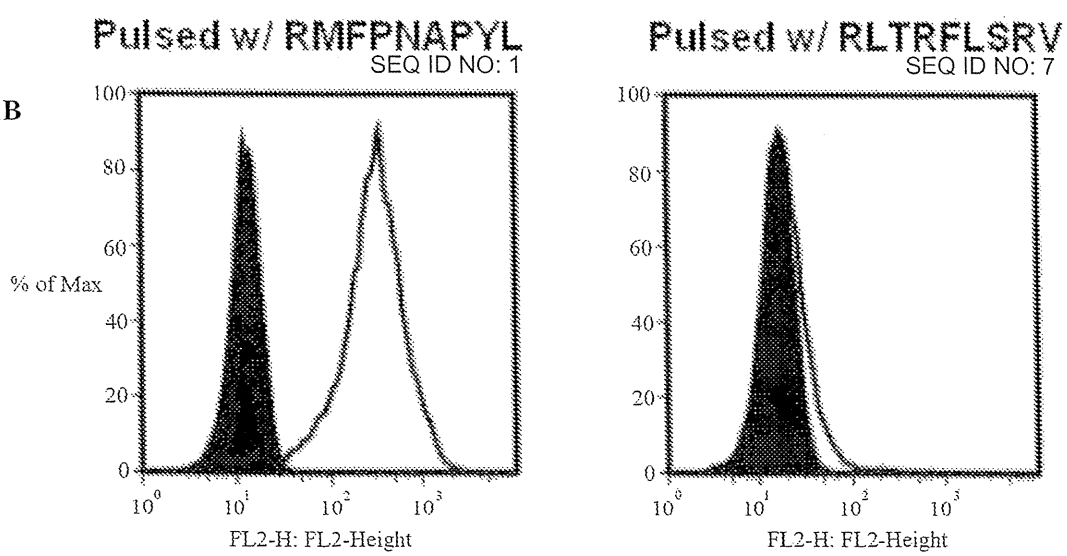


Figure 11

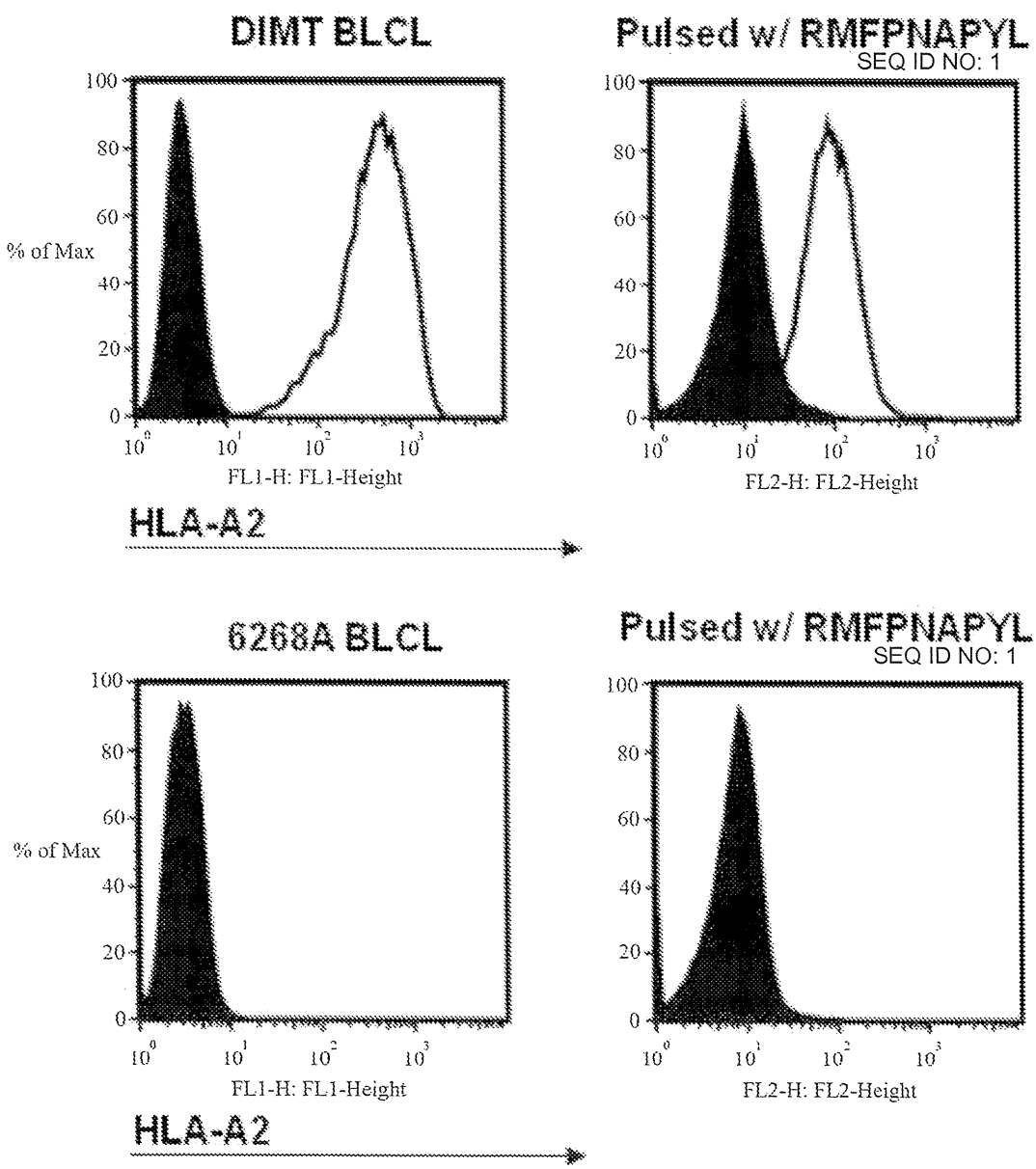

DIMT BLCL
Pulsed w/ RMFPNAPYL
SEQ ID NO: 1
% of Max
100
80
60
40
20
0
10^0
10^1
10^2
10^3
FL1-H: FL1-Height
FL2-H: FL2-Height
HLA-A2
6268A BLCL
Pulsed w/ RMFPNAPYL
SEQ ID NO: 1
% of Max
FL1-H: FL1-Height
FL2-H: FL2-Height
HLA-A2

Figure 12

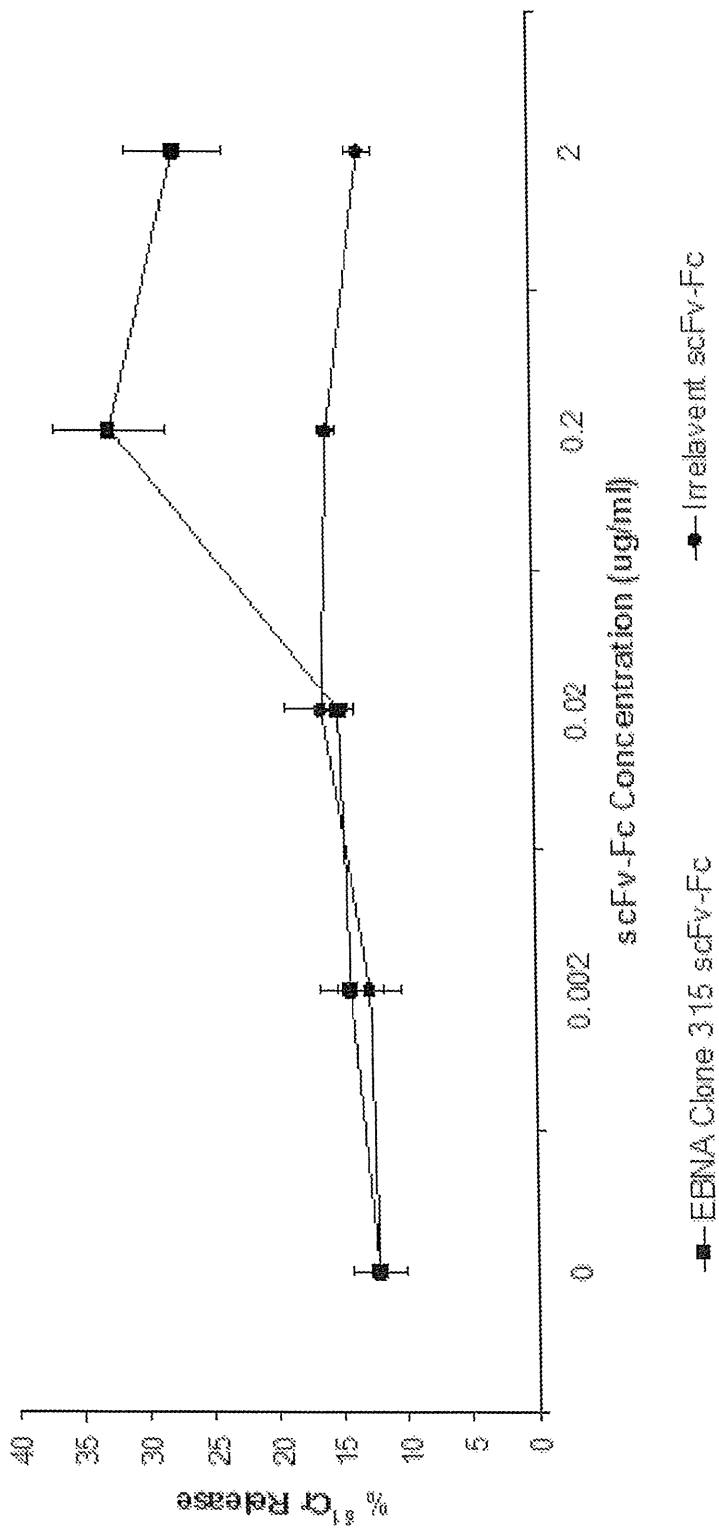

% ^{51}Cr Release
40
35
30
25
20
15
10
5
0
0
0.002
0.02
0.2
2
scFv-Fc Concentration (ug/ml)
EBNA Clone 315 scFv-Fc
Irrelavent scFv-Fc

Figure 13

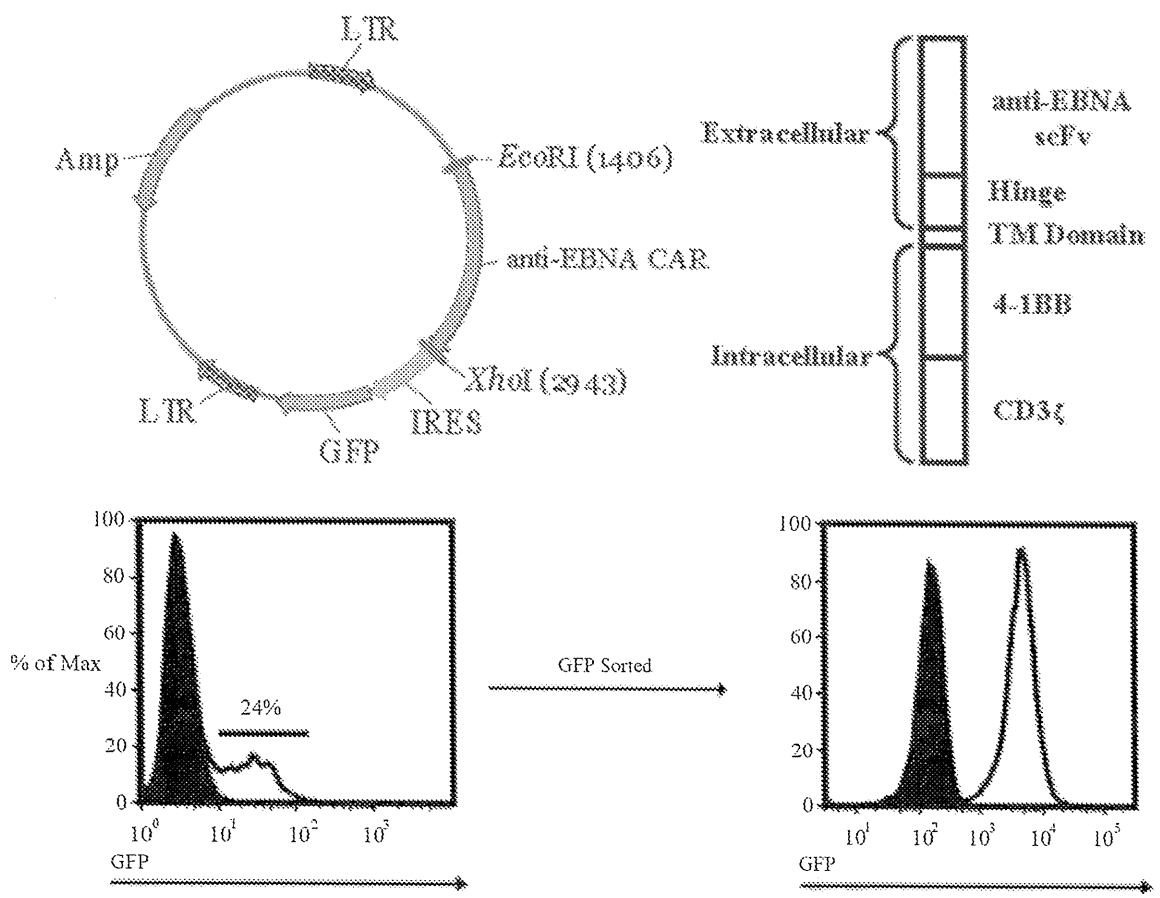
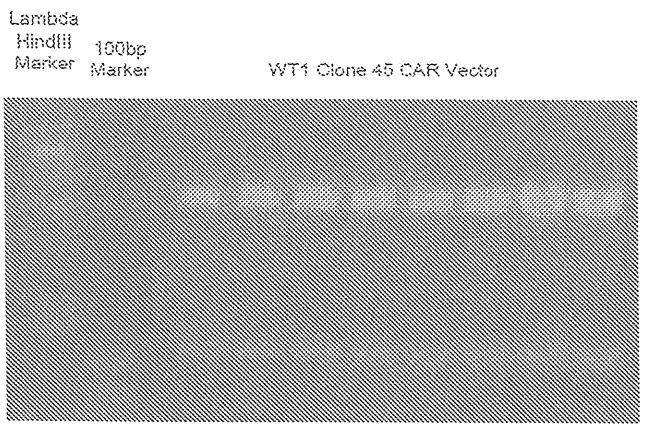

LTR
Amp
EcoRI (1406)
anti-EBNA CAR
XhoI (2943)
LTR
IRES
GFP
Extracellular
anti-EBNA scFv
Hinge
TM Domain
Intracellular
4-1BB
CD3ζ
100
80
60
% of Max
40
20
0
24%
10^0 10^1 10^2 10^3
GFP
GFP Sorted
100
80
60
40
20
0
10^1 10^2 10^3 10^4 10^5
GFP

Figure 14

FIG 15A



FUG 15B

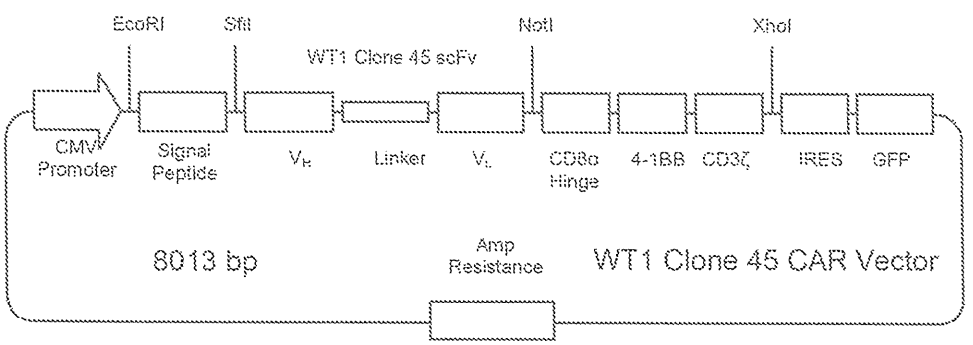


Figure 15

FIG 16A

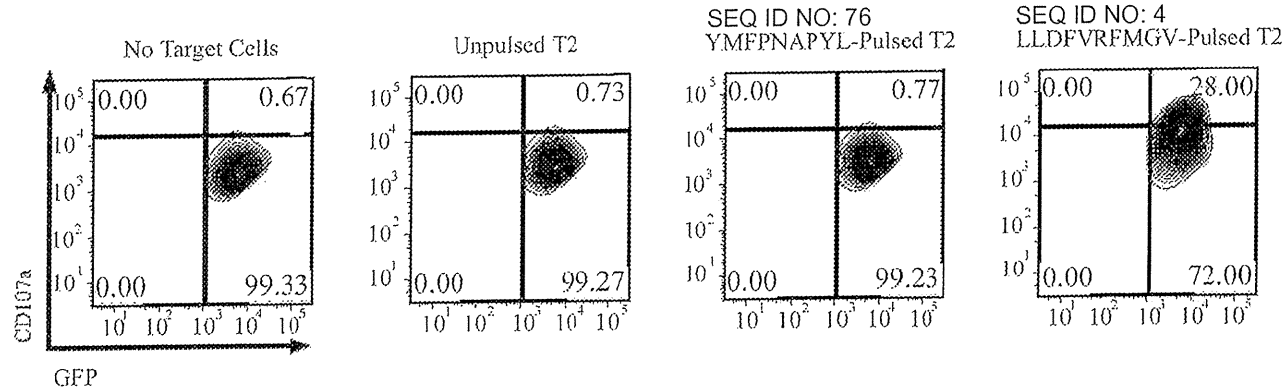


FIG 16B

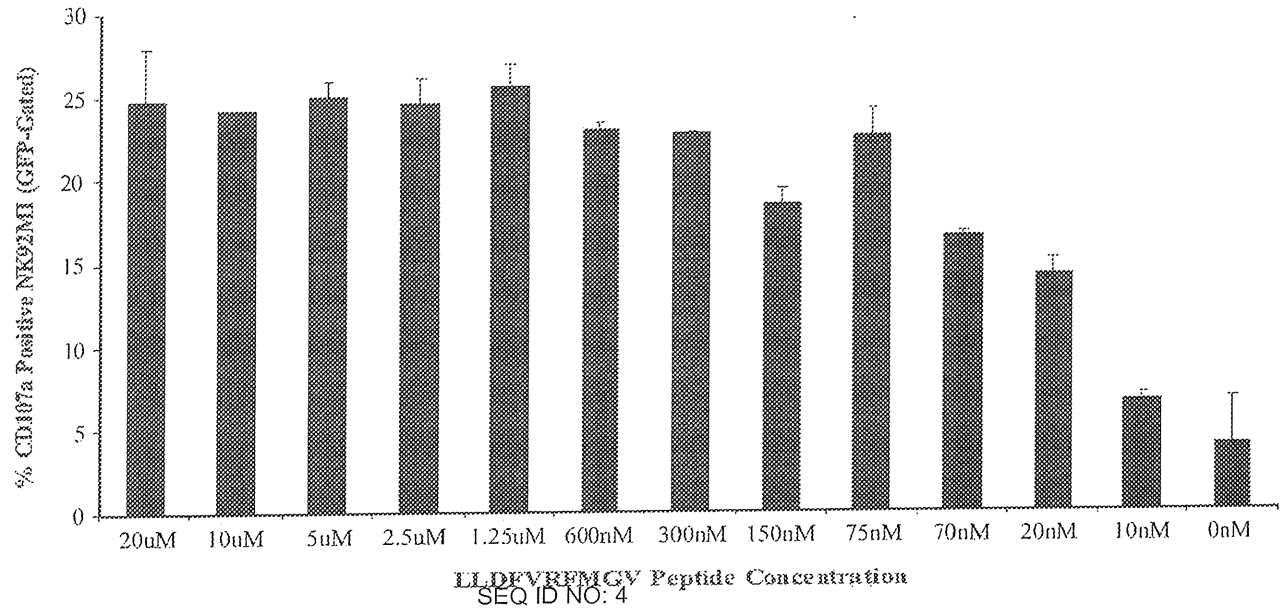


Figure 16

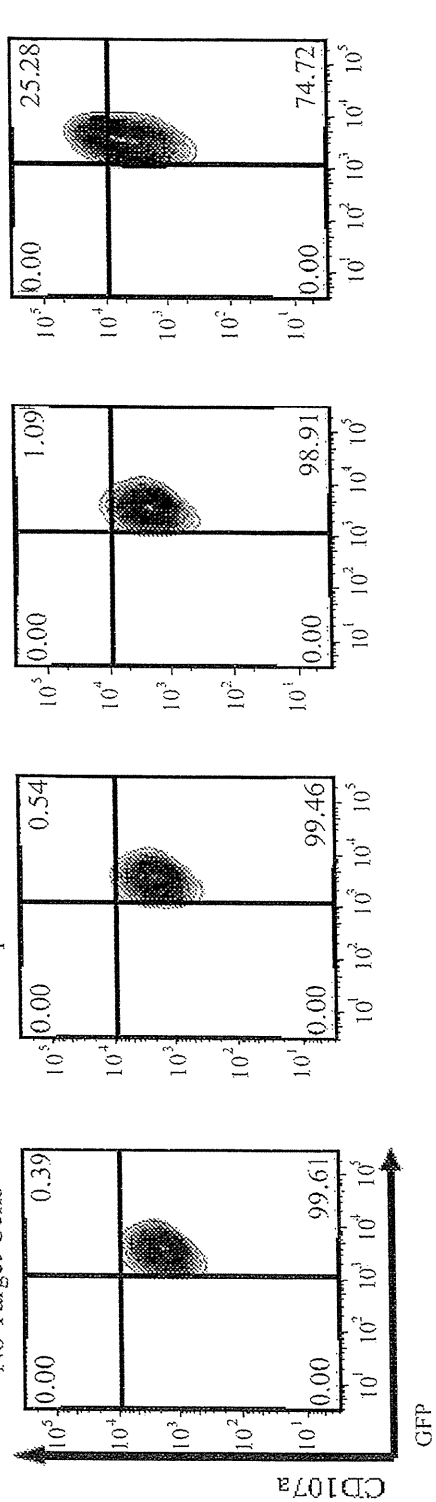

No Target Cells
Unpulsed T2
SEQ ID NO: 76
YMFPNAPYL-Pulsed T2
SEQ ID NO: 4
LLDFVRFMGV-Pulsed T2
0.00
0.39
99.61
0.00
0.54
99.46
0.00
1.09
98.91
0.00
25.28
74.72
CD107a
GFP

Figure 17

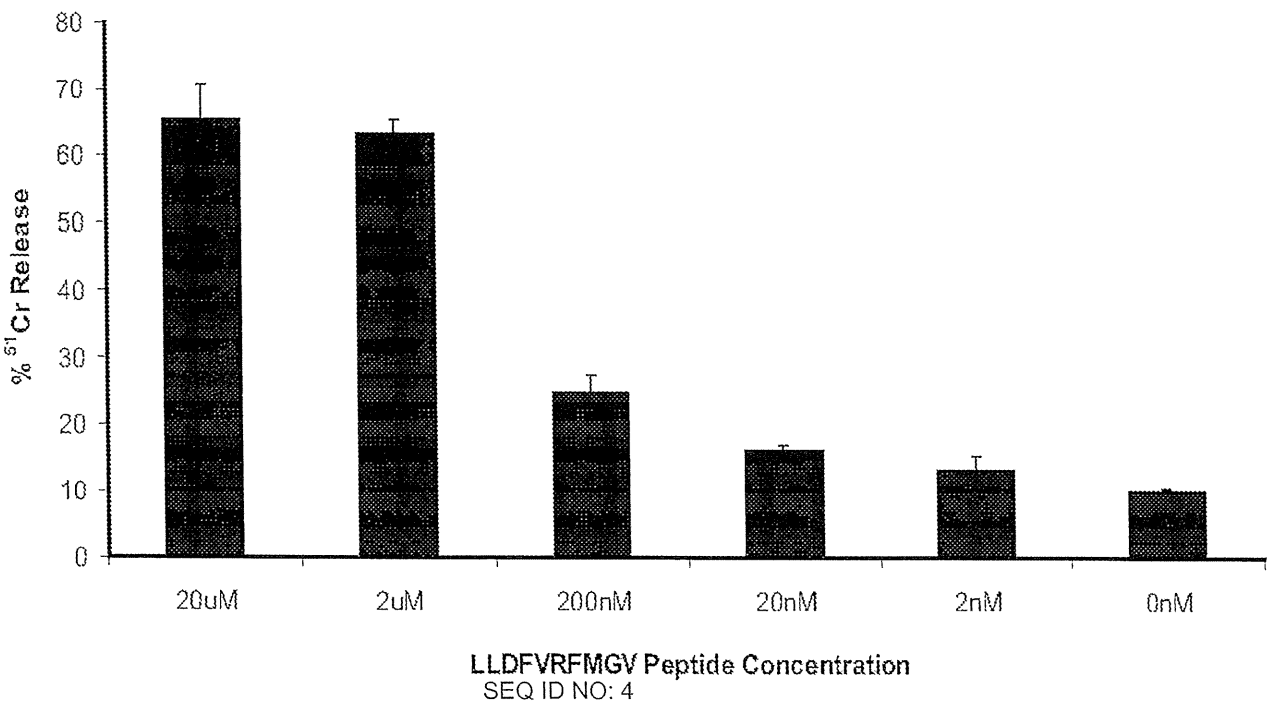

80
70
60
50
40
30
20
10
0
% 51Cr Release
20uM
2uM
200nM
20nM
2nM
0nM
LLDFVRFMGV Peptide Concentration
SEQ ID NO: 4

Figure 18

FIG 19A

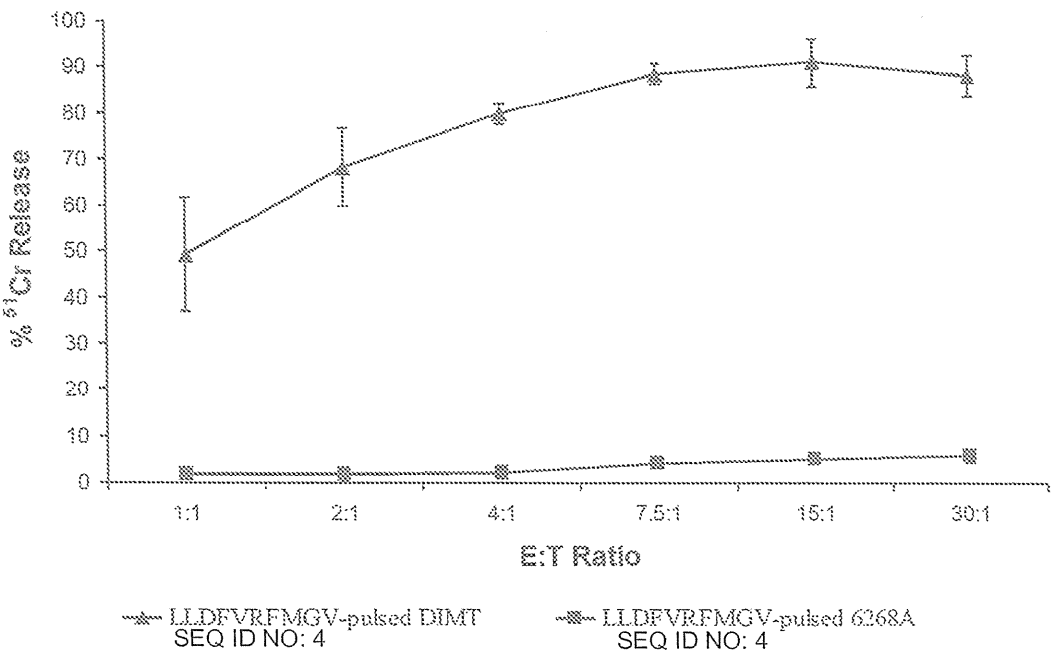


FIG 19B

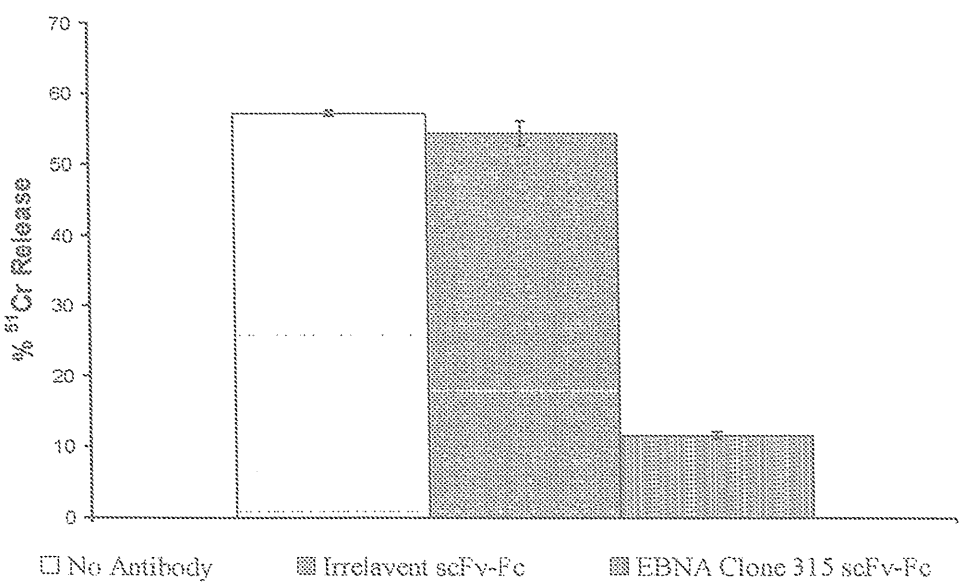


Figure 19

FIG 20A

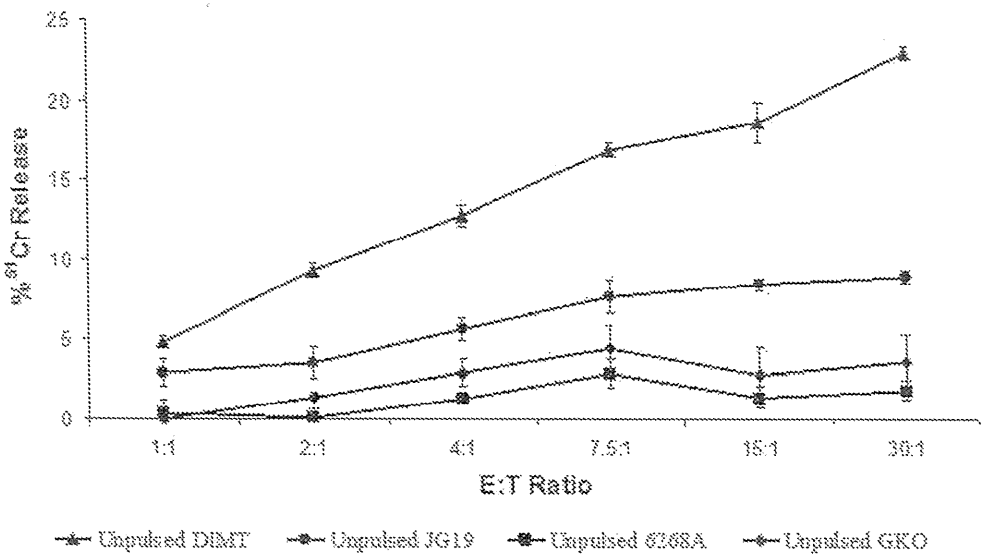


FIG 20B

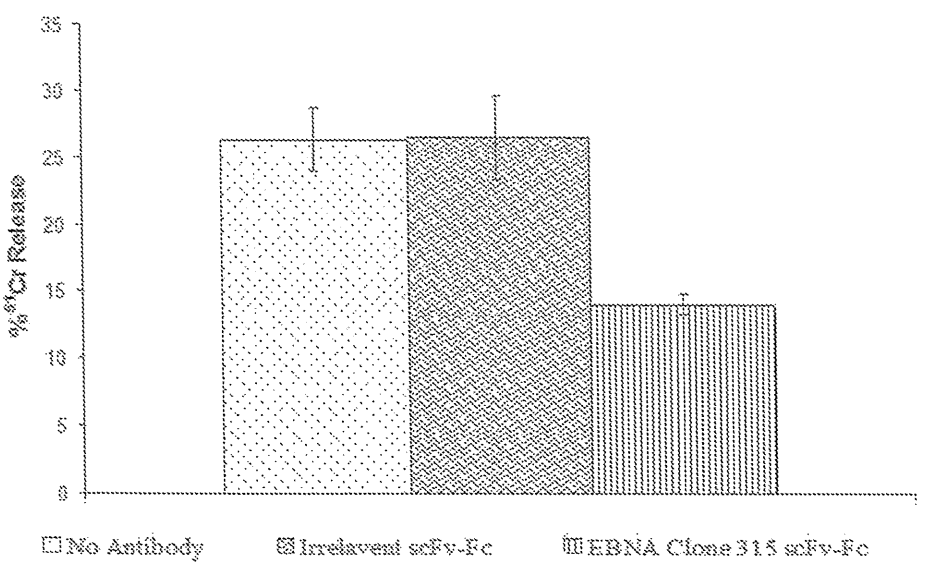


Figure 20

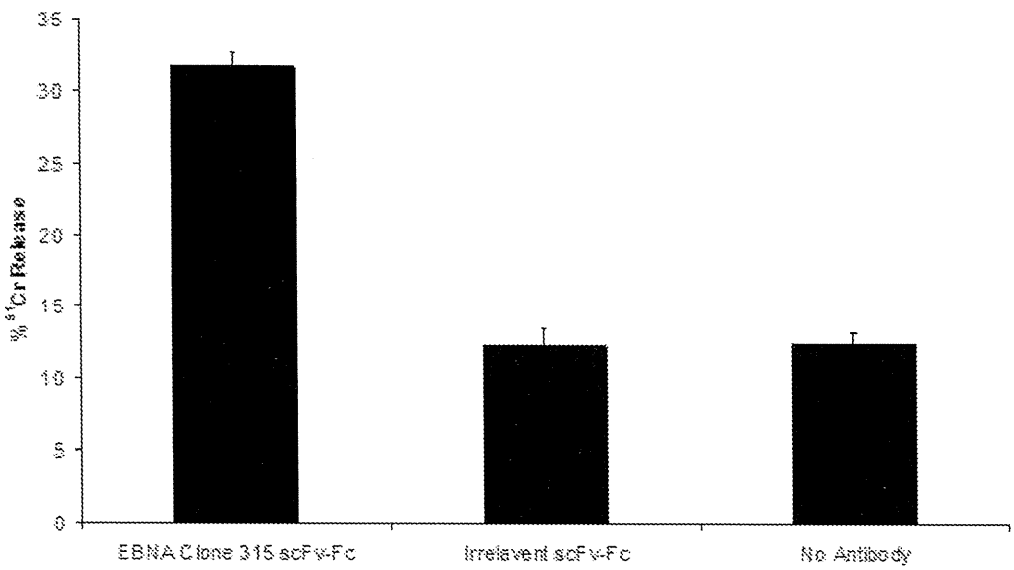

35
30
25
20
15
10
5
0
% 51Cr Release
EBNA Clone 315 scFv-Fc
Irrelavent scFv-Fc
No Antibody

Figure 21

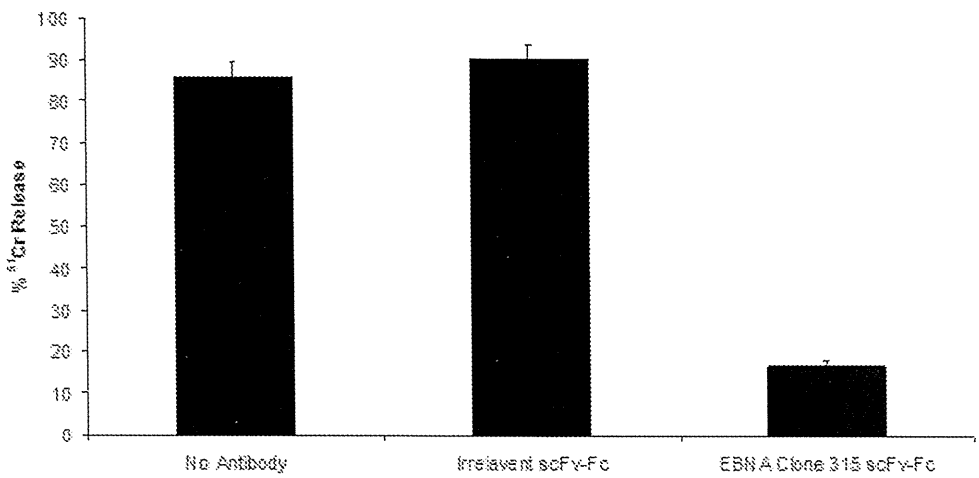

100
90
80
70
60
50
40
30
20
10
0
% 51Cr Release
No Antibody
Irrelavent scFv-Fc
EBNA Clone 315 scFv-Fc

Figure 22

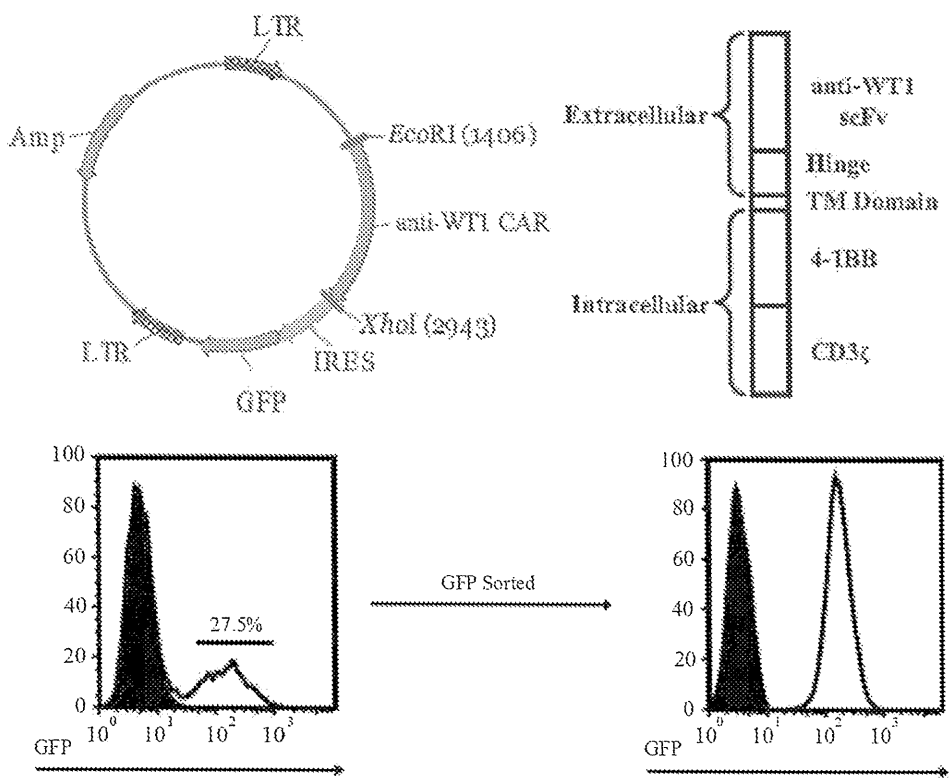

LTR
Amp
EcoRI (1406)
anti-WT1 CAR
XhoI (2943)
LTR
IRES
GFP
Extracellular
anti-WT1 scFv
Hinge
TM Domain
Intracellular
4-1BB
CD3ζ
100
80
60
40
20
0
27.5%
GFP
GFP Sorted

Figure 23

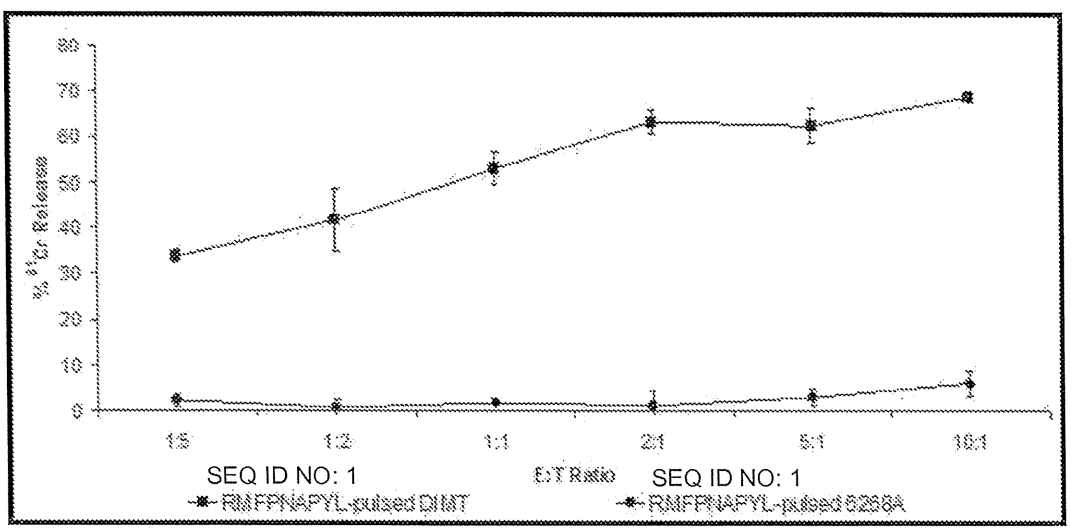

80
70
60
50
40
30
20
10
0
1:5
1:2
1:1
2:1
5:1
10:1
E:T Ratio
SEQ ID NO: 1
RMFPNAPYL-pulsed DMT
SEQ ID NO: 1
RMFPNAPYL-pulsed 8258A

Figure 24

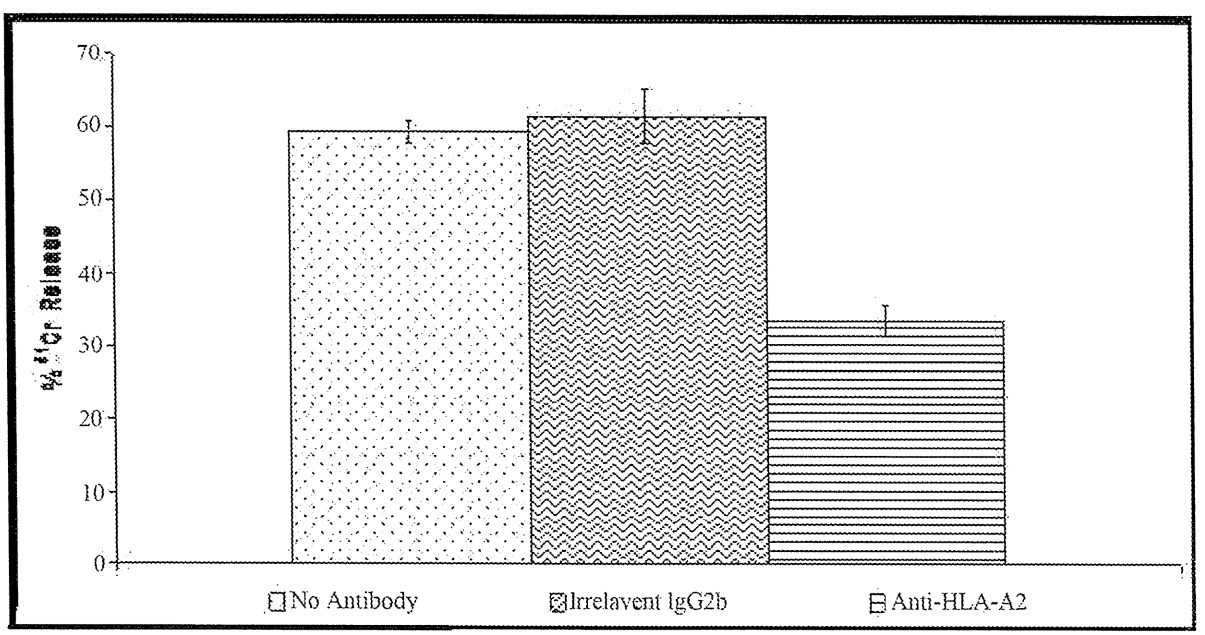


Figure 25

FIG 26A

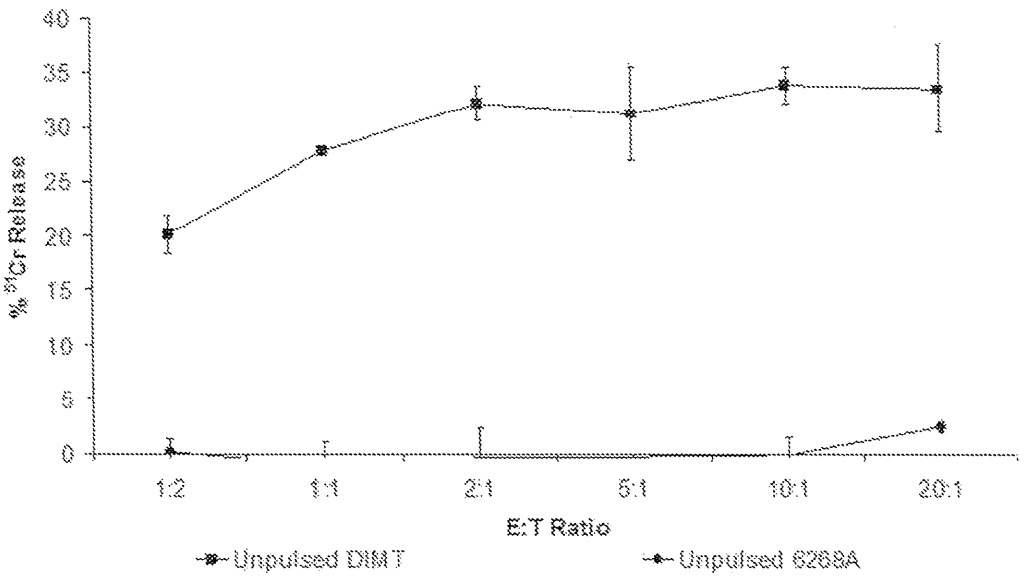


FIG 26B

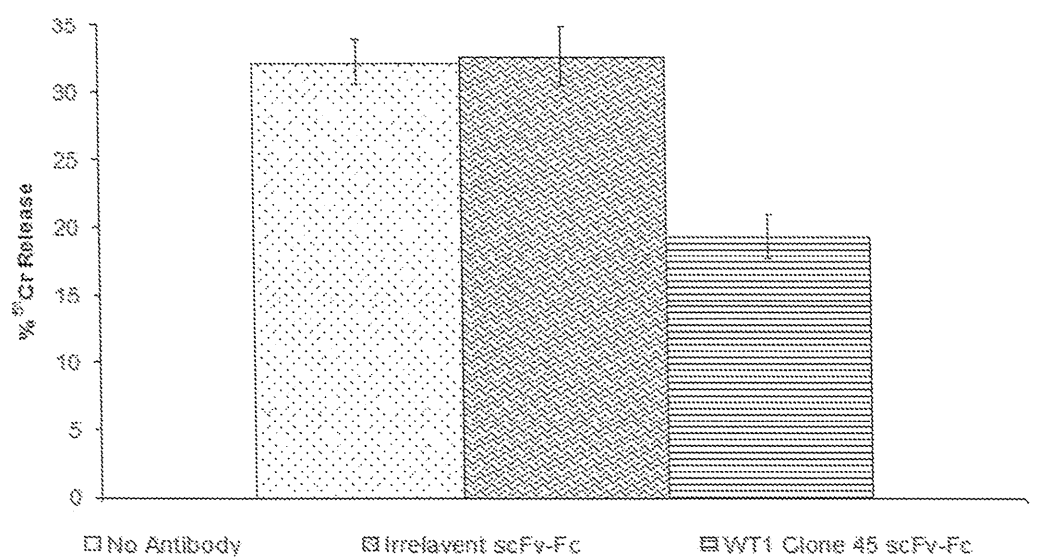


Figure 26

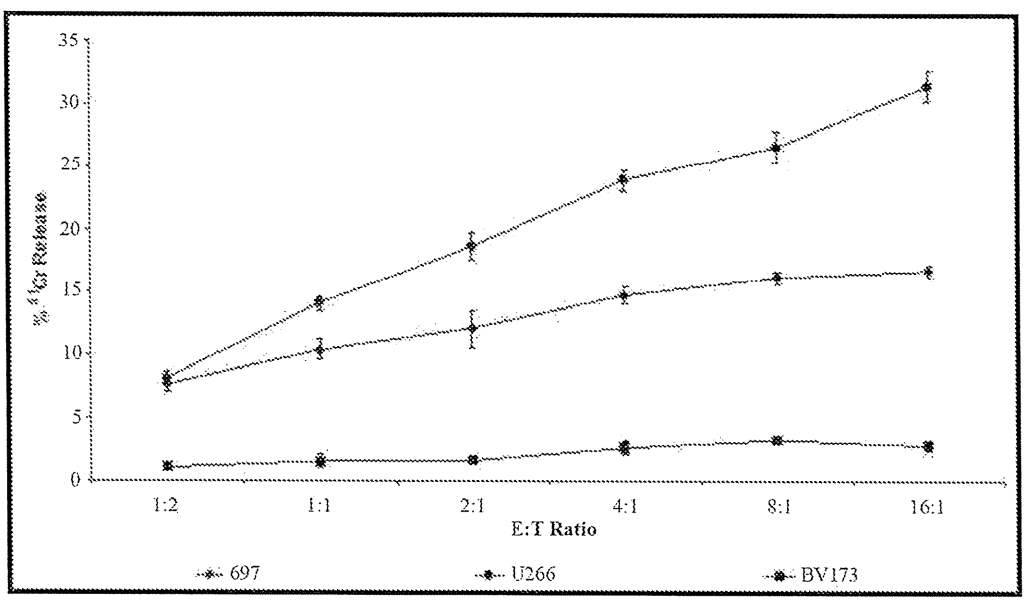

35
30
25
20
15
10
5
0
% 51Cr Release
1:2
1:1
2:1
4:1
8:1
16:1
E:T Ratio
697
U266
BV173

Figure 27

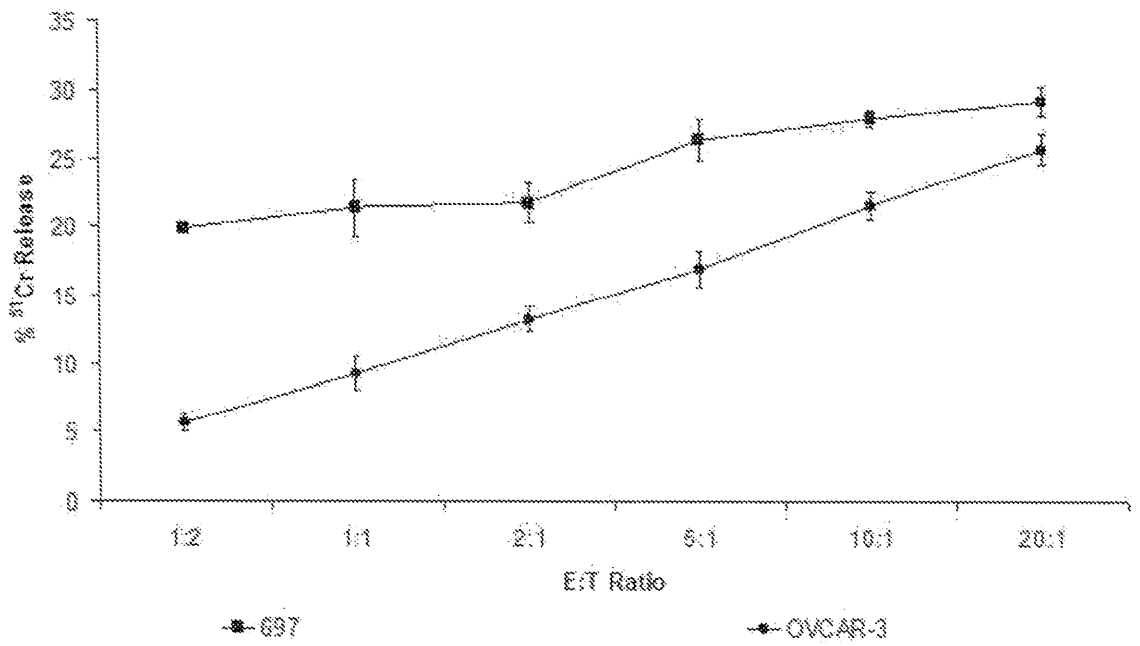

35
30
25
20
15
10
5
0
% ^{51}Cr Release
1:2
1:1
2:1
5:1
10:1
20:1
E:T Ratio
697
OVCAR-3

Figure 28