Figure 1

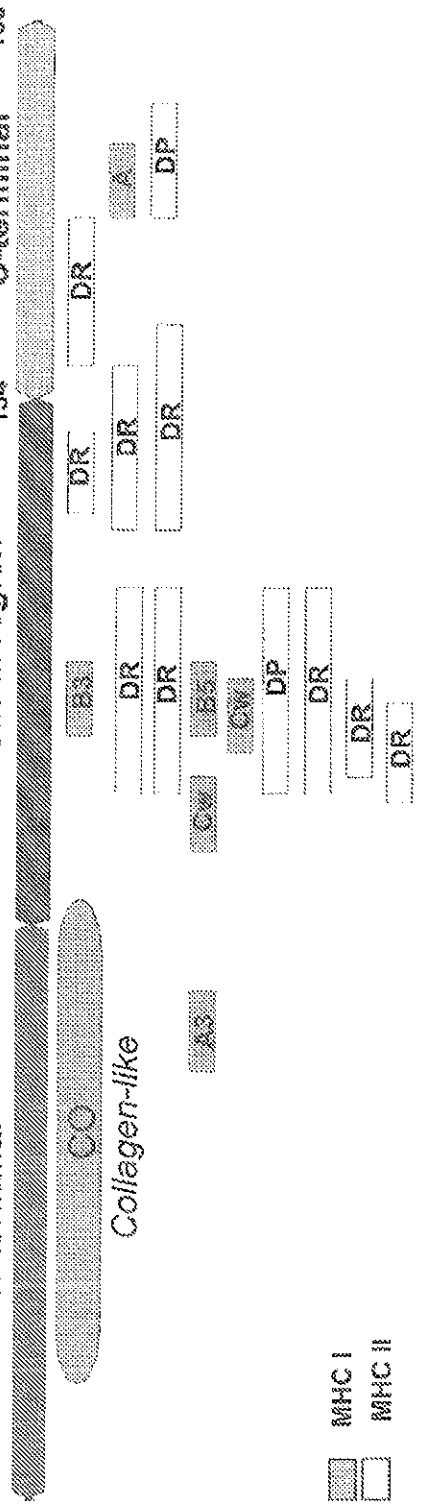

N-terminal
70
Central region
134
C-terminal
180
CO
Collagen-like
A3
C2
E5
C1
E3
DR
DR
DR
DR
DR
DR
DP
DP
DR
DR
A
MHC I
MHC II

Figure 2

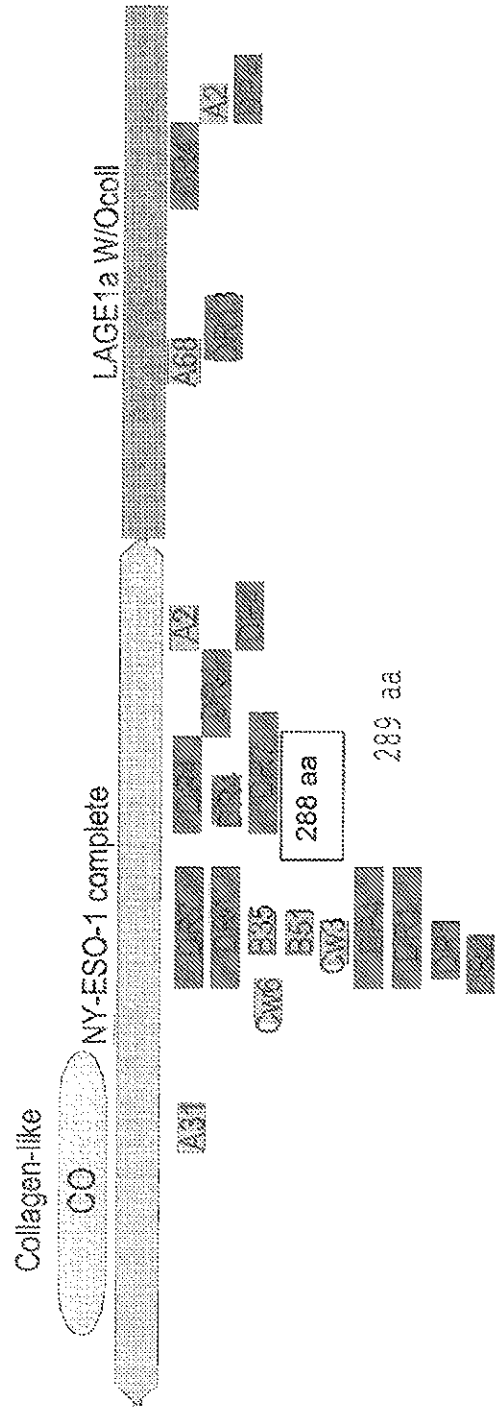

Collagen-like
GD
NY-ESO-1 complete
LAGE1a W/Ocoil
A2
A3
A2
288 aa
289 aa

Figure 3

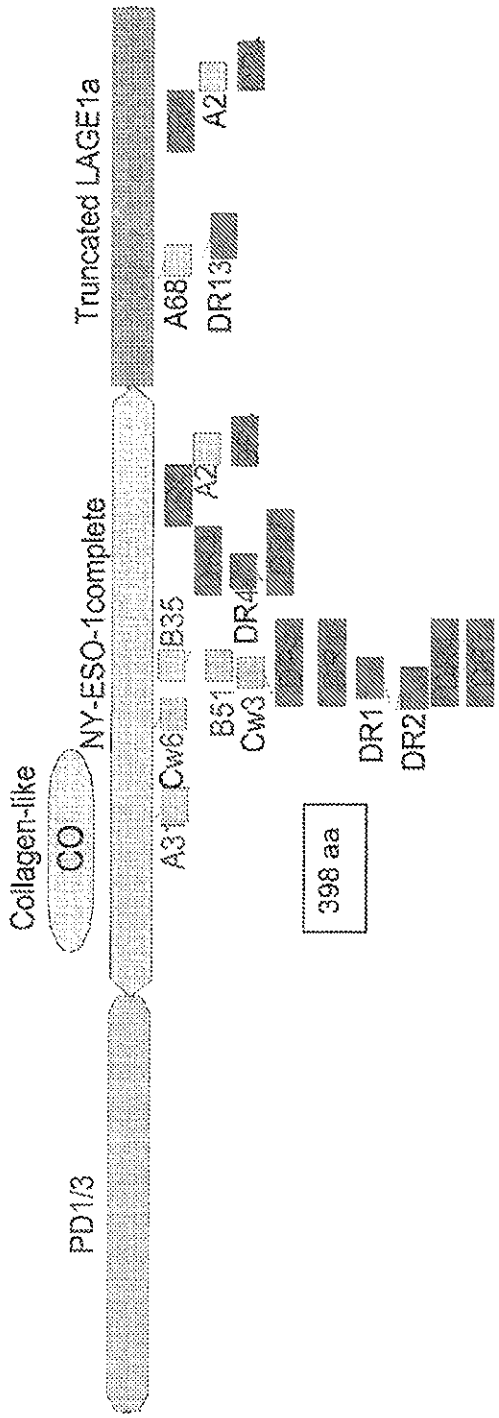

Collagen-like
CO
NY-ESO-1complete
Truncated LAGE1a
PD1/3
A31
Cw6
B35
A2
B51
Cw3
DR4
DR1
DR2
A68
DR13
A2
398 aa

Figure 4

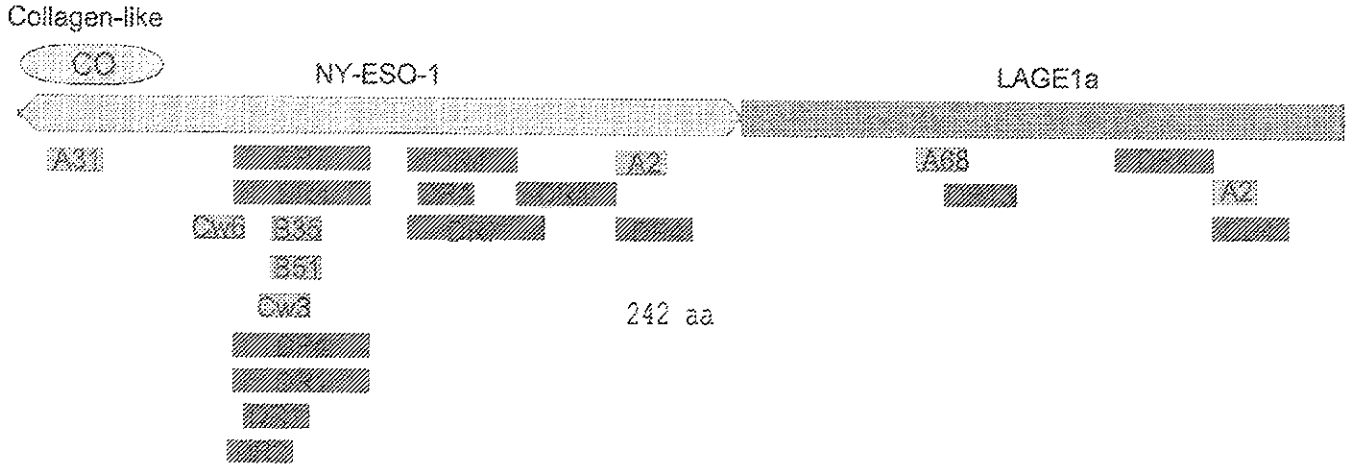

Collagen-like
CO
NY-ESO-1
LAGE1a
A31
A2
A68
A2
Cw6
B35
B51
Cw3
242 aa

Figure 5

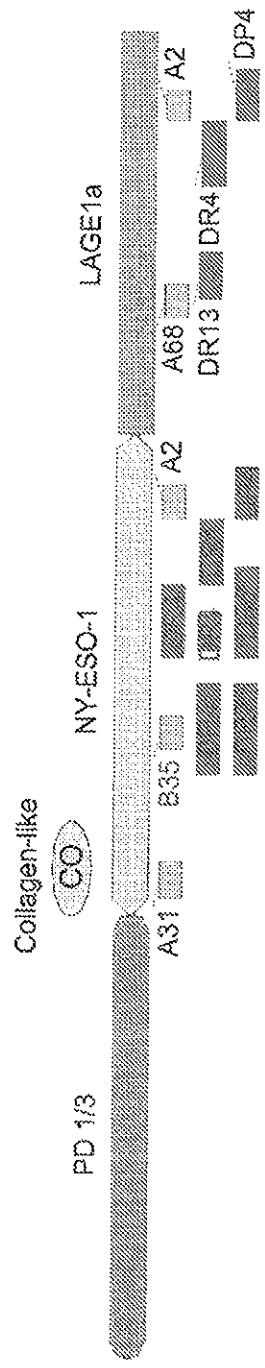

Collagen-like
CO
NY-ESO-1
LAGE1a
PD 1/3
A31
B35
A2
A68
A2
DR13
DR4
DP4
Cw6
B51
Cw3
352 aa
DR1
DR2

Figure 6

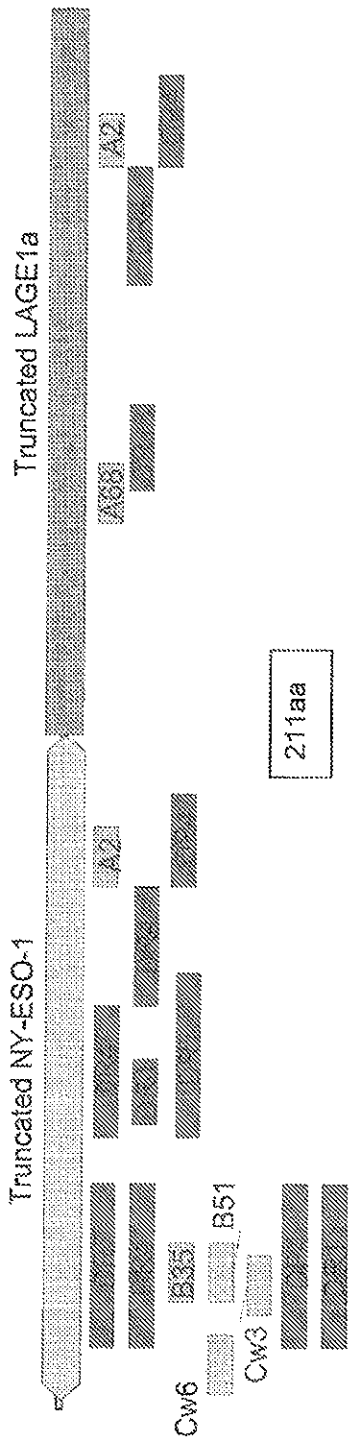

Truncated NY-ESO-1
Truncated LAGE1a
A2
A2
A*03
211aa
B35
B51
Cw6
Cw3

Figure 7

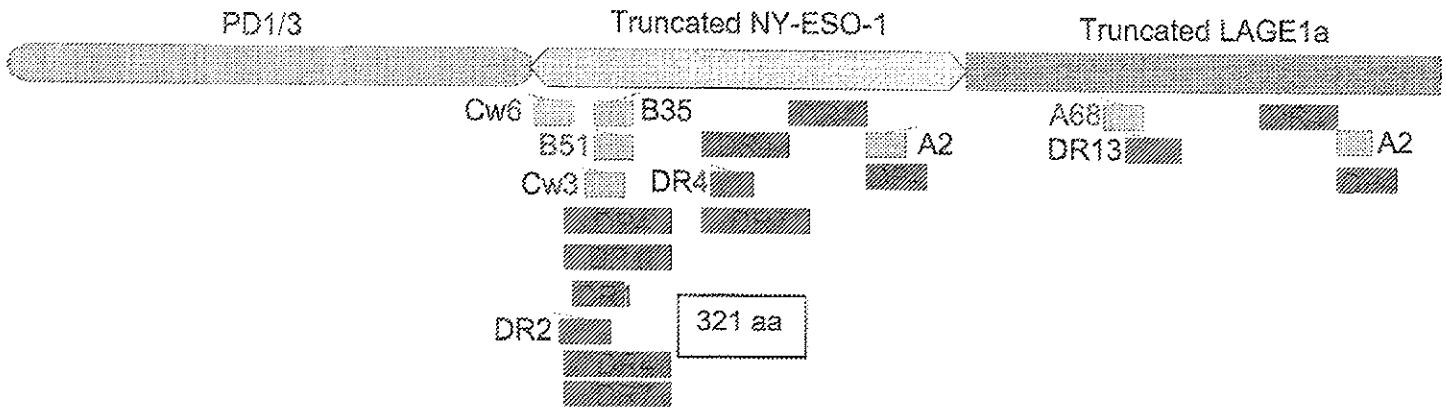

PD1/3
Truncated NY-ESO-1
Truncated LAGE1a
Cw6
B35
B51
A2
Cw3
DR4
DR2
321 aa
A68
DR13
A2

Figure 8

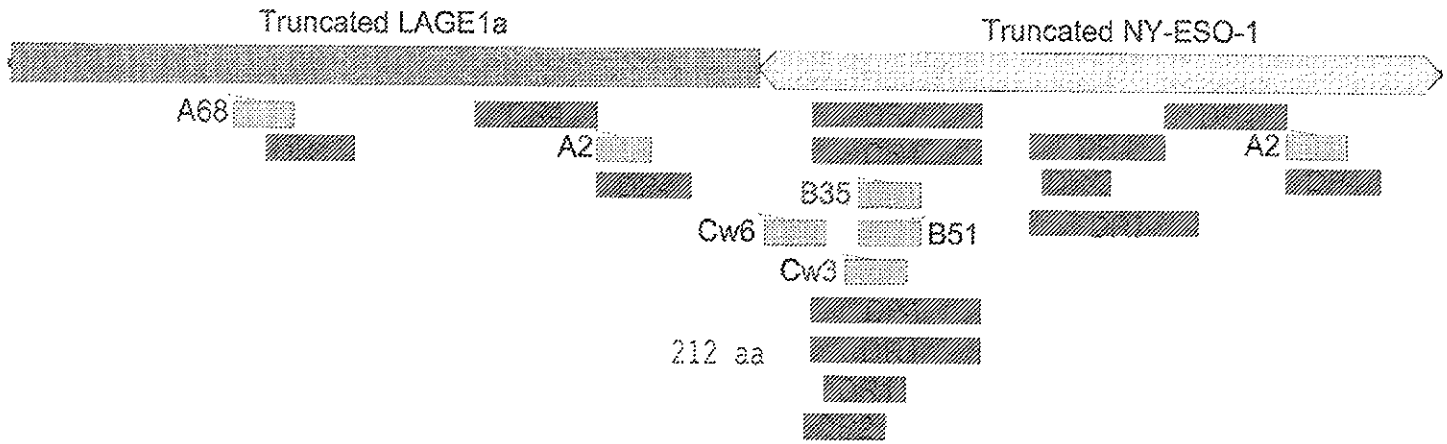

Truncated LAGE1a
Truncated NY-ESO-1
A68
A2
A2
B35
Cw6
B51
Cw3
212 aa

Figure 9

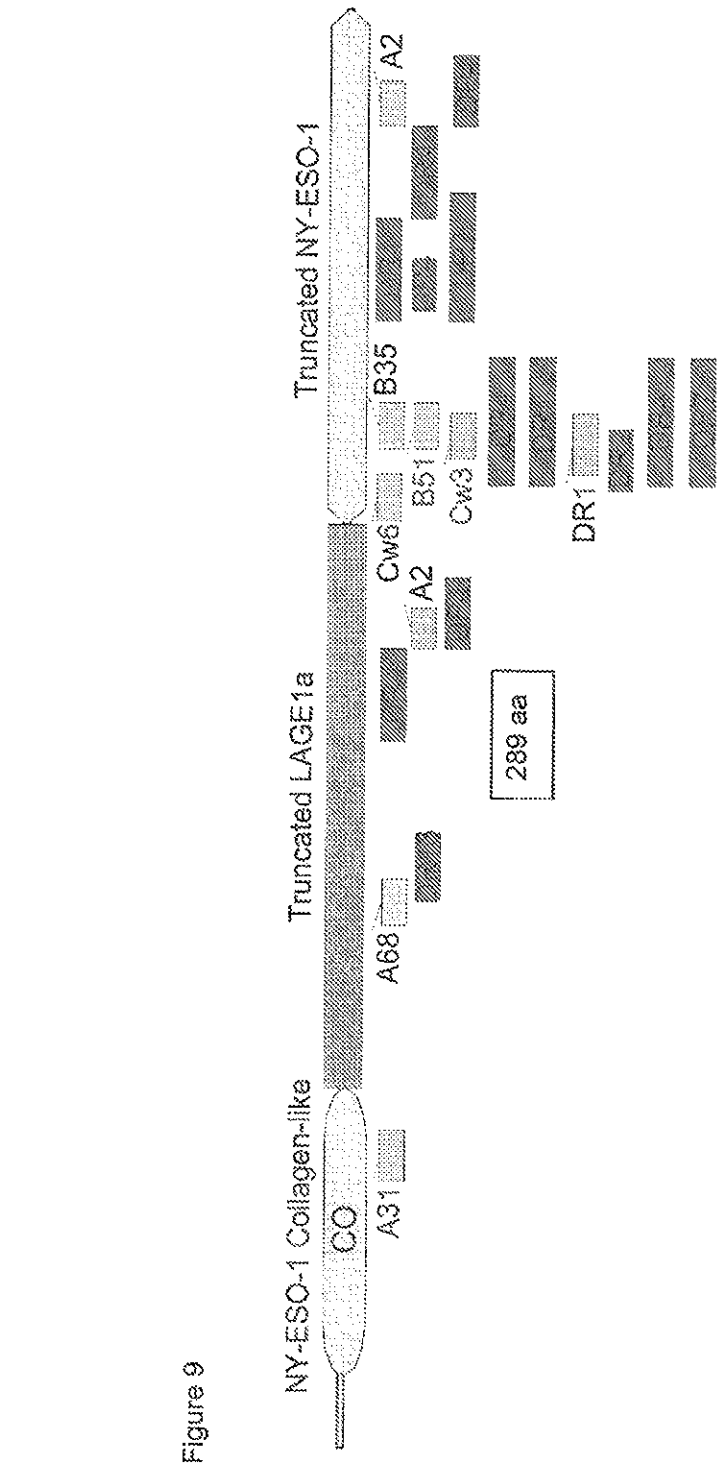

NY-ESO-1 Collagen-like
CO
A31
Truncated LAGE1a
A68
A2
Cw6
B51
Cw3
B35
289 aa
Truncated NY-ESO-1
A2
DR1

Figure 10

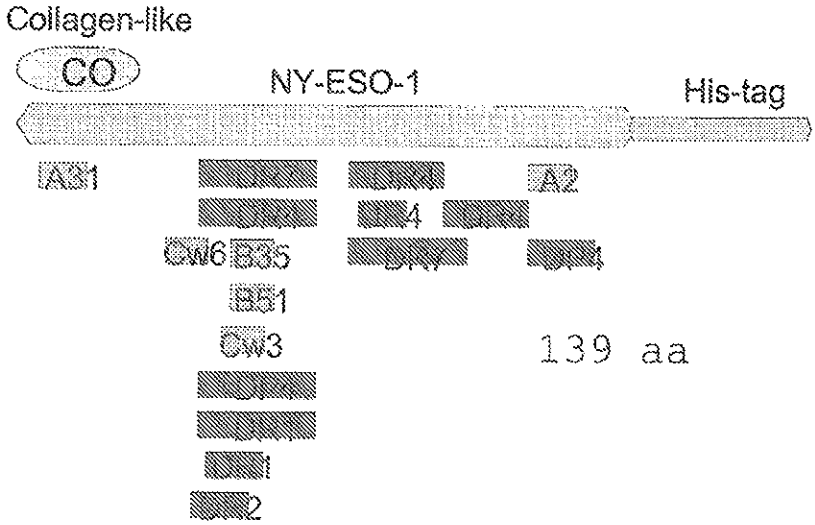


Figure 11

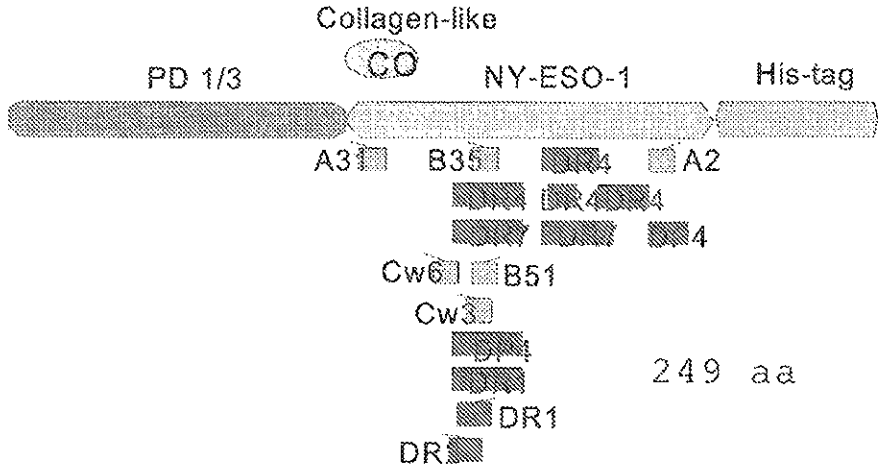

Collagen-like
CO
PD 1/3
NY-ESO-1
His-tag
A3
B35
A2
Cw6
B51
Cw3
DR1
DR
249 aa

Figure 12

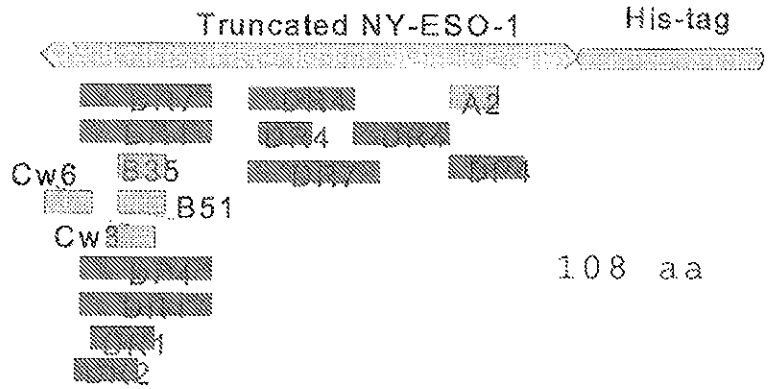


Figure 13

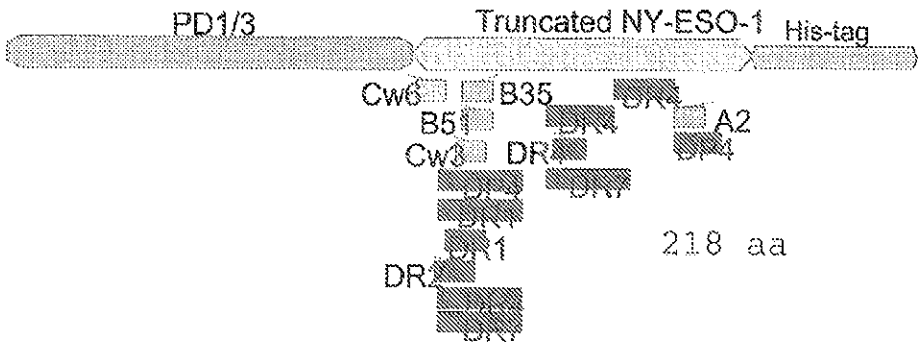

PD1/3
Truncated NY-ESO-1
His-tag
Cw6
B35
B51
A2
Cw3
DR
218 aa
DR1
DR2

Figure 14

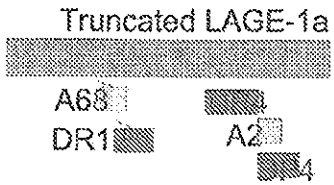


Figure 15

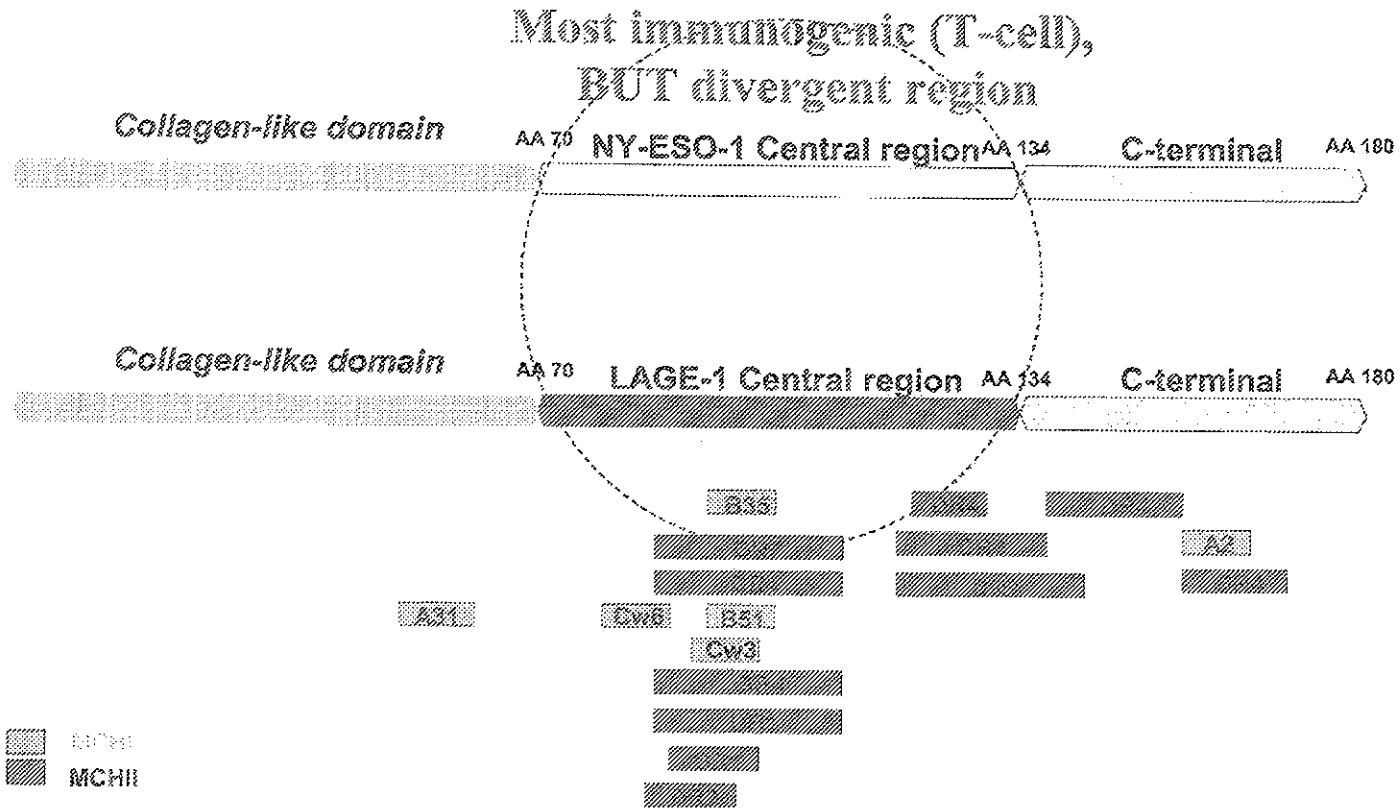
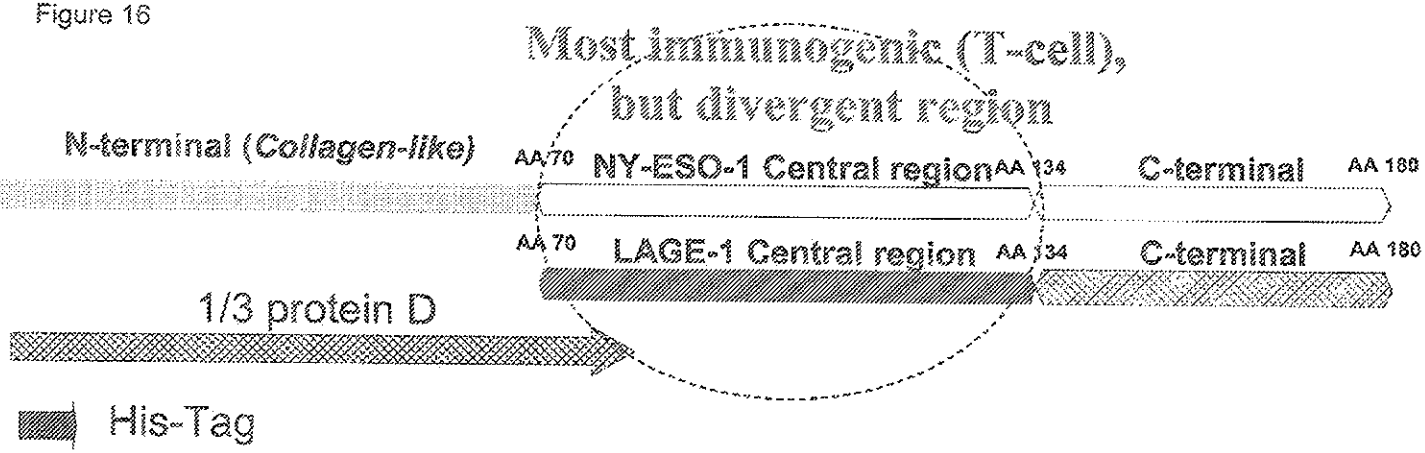

Most immunogenic (T-cell),
BUT divergent region
Collagen-like domain
AA 70
NY-ESO-1 Central region
AA 134
C-terminal
AA 180
Collagen-like domain
AA 70
LAGE-1 Central region
AA 134
C-terminal
AA 180
B35
A2
A31
Cw6
B51
Cw3
MCHII

Figure 16


Most immunogenic (T-cell), but divergent region
N-terminal (Collagen-like)
AA 70
NY-ESO-1 Central region
AA 134
C-terminal
AA 180
AA 70
LAGE-1 Central region
AA 134
C-terminal
AA 180
1/3 protein D
His-Tag

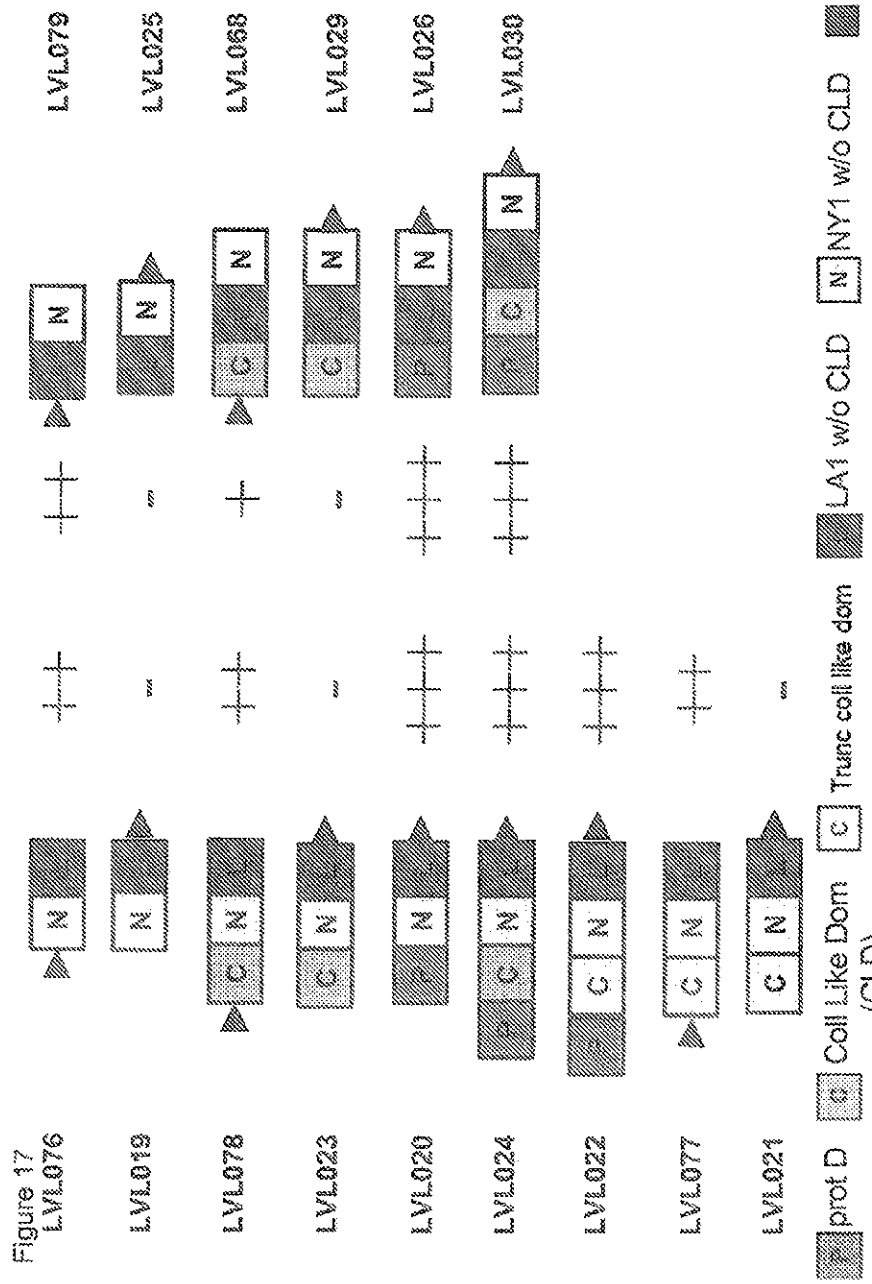

Figure 17
LVL076
LVL019
LVL078
LVL023
LVL020
LVL024
LVL022
LVL077
LVL021
LVL079
LVL025
LVL068
LVL029
LVL026
LVL030
N
C
prot D
Coil Like Dom (CLD)
Trunc coil like dom
LA1 w/o CLD
NY1 w/o CLD
His tag

Figure 18

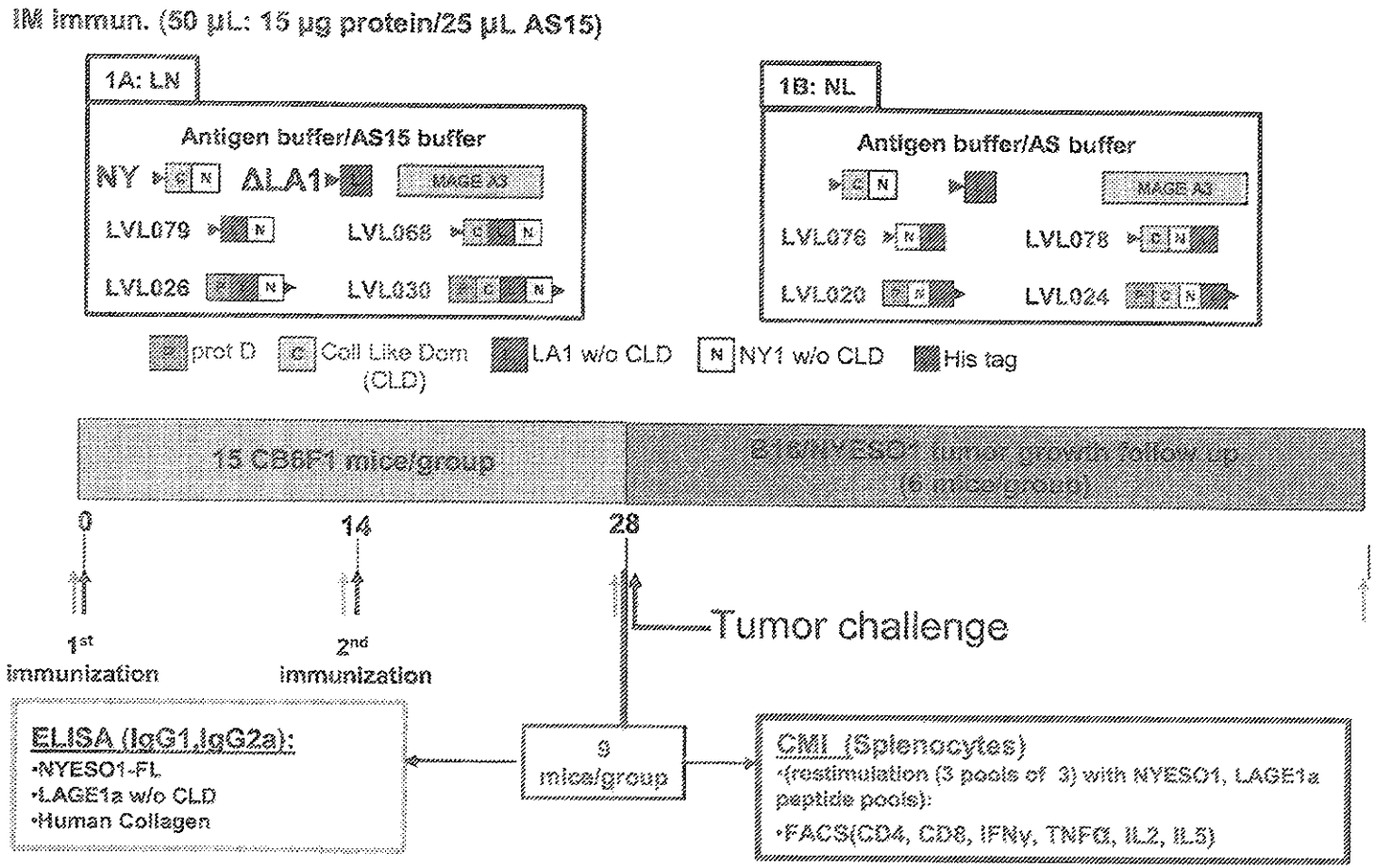

IM immun. (50 µL: 15 µg protein/25 µL AS15)
1A: LN
Antigen buffer/AS15 buffer
NY
ΔLA1
MAGE A3
LVL079
LVL068
LVL026
LVL030
1B: NL
Antigen buffer/AS buffer
MAGE A3
LVL076
LVL078
LVL020
LVL024
prot D
Coil Like Dom (CLD)
LA1 w/o CLD
NY1 w/o CLD
His tag
15 CB6F1 mice/group
0
14
28
Tumor challenge
1st immunization
2nd immunization
ELISA (IgG1,IgG2a):
•NYESO1-FL
•LAGE1a w/o CLD
•Human Collagen
9 mice/group
CMI (Splenocytes)
•(restimulation (3 pools of 3) with NYESO1, LAGE1a peptide pools):
•FACS(CD4, CD8, IFNγ, TNFα, IL2, IL5)

Figure 19

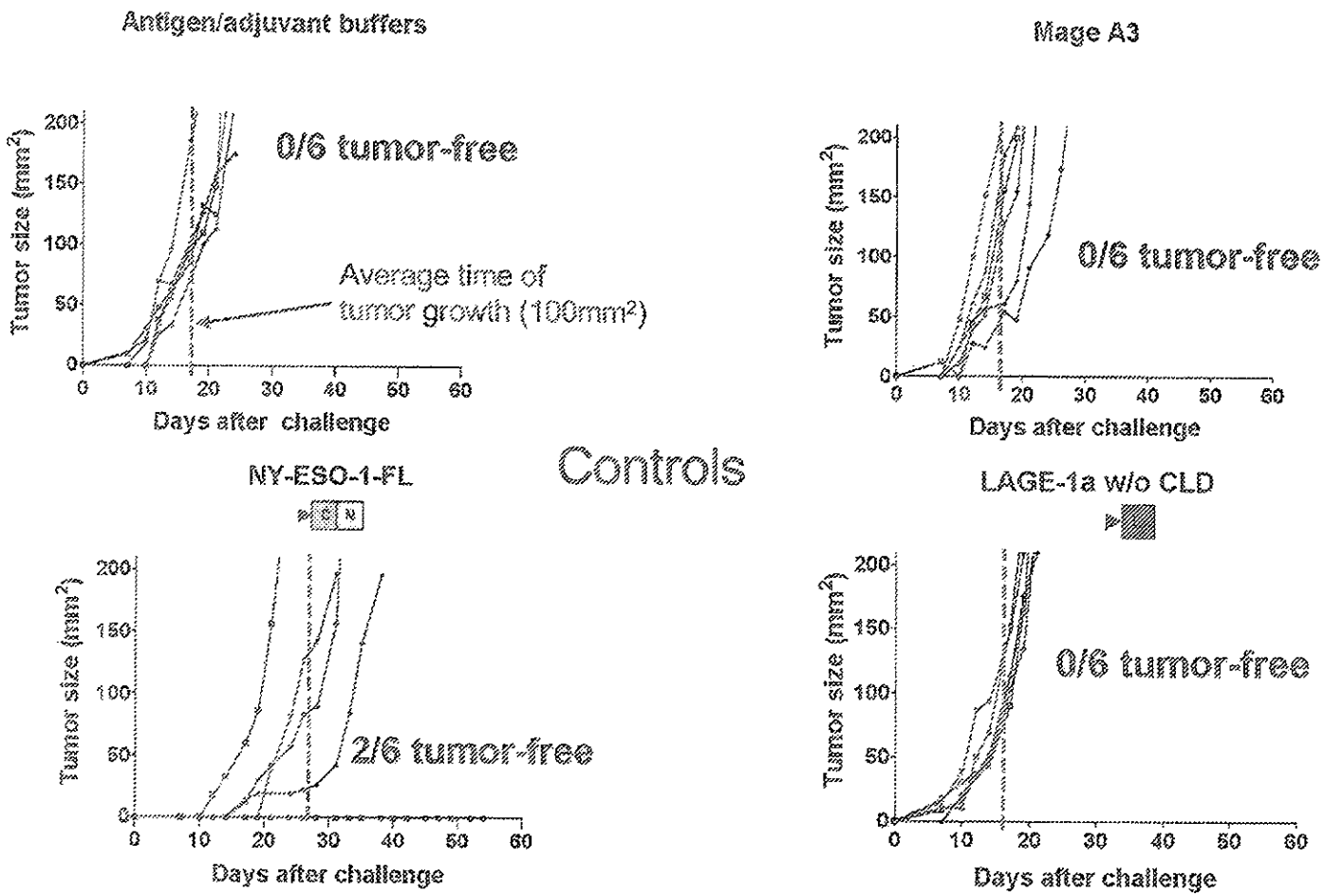

Antigen/adjuvant buffers
Mage A3
Tumor size (mm2)
200
150
100
50
0
0/6 tumor-free
Average time of
tumor growth (100mm2)
0 10 20 30 40 50 60
Days after challenge
Controls
NY-ESO-1-FL
C
N
2/6 tumor-free
LAGE-1a w/o CLD
0/6 tumor-free

Figure 20

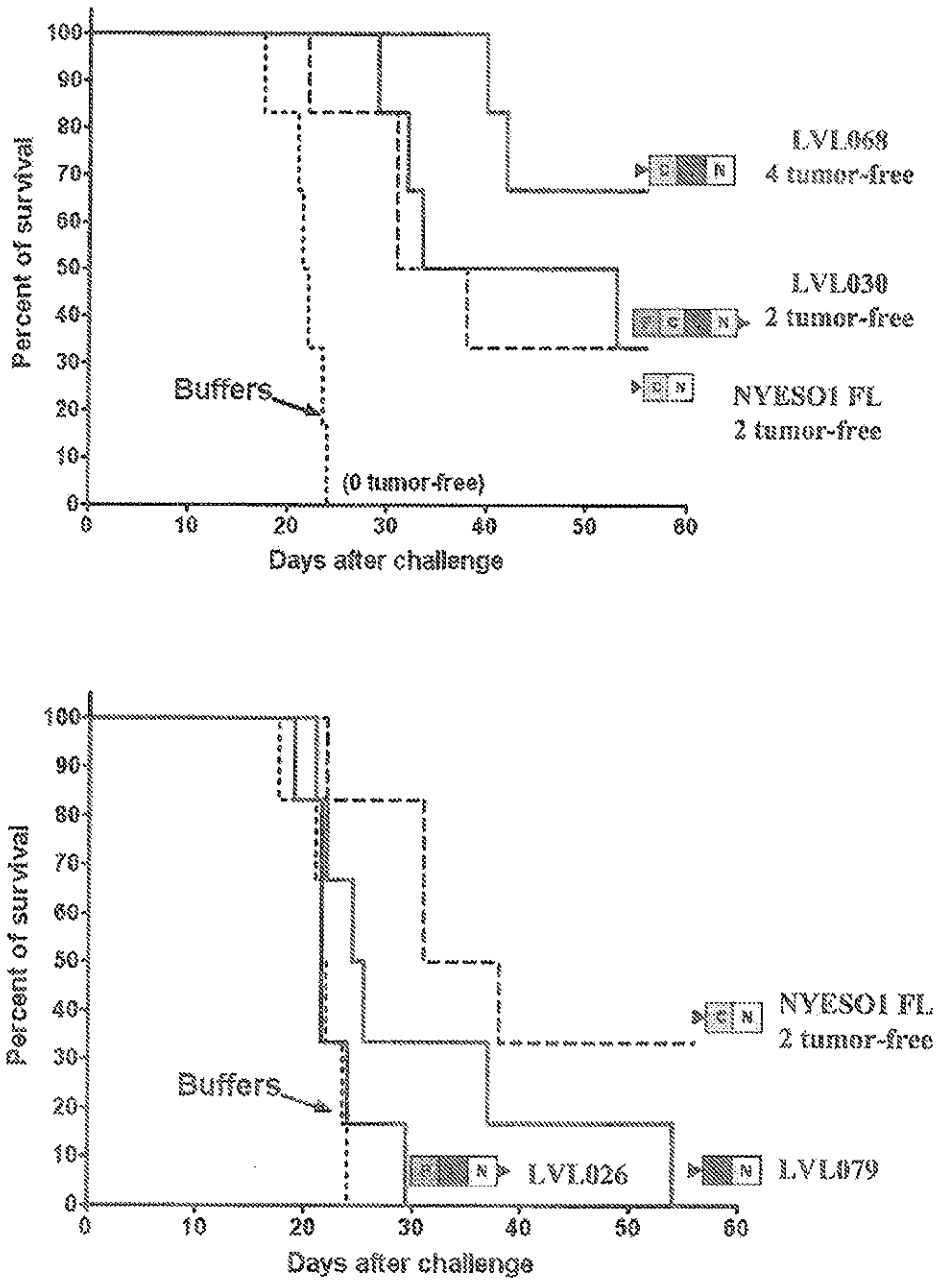

Percent of survival
Days after challenge
Buffers
(0 tumor-free)
LVL068
4 tumor-free
LVL030
2 tumor-free
NYESO1 FL
2 tumor-free
NYESO1 FL
2 tumor-free
LVL026
LVL079

Figure 21

Screening #1: NYESO1-specific Immune Responses

Immunogens	IgG2a (ng/mL)	% CD4 (INF-γ+/TNF-α+)	WB anti-NYESO1 w/o CLD
LVL030 	509,499	0.23	+
LVL 068 	231,255	0.27	+++
LVL078 	159,471	0.30	+++
LVL024 	155,384	0.23	+
LVL076 	109,041	0.30	+
LVL020 	18,410	0.13	+
LVL079	16,309	0.20	+
LVL026 	10,520	0.07	+
LVL075 	36	-	-
NYESOFL 	531,385	0.87	+++

Screening #1: LAGE 1a w/o CLD -specific immune responses

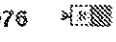
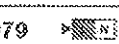
Immunogens	IgG2a (ng/mL)	% CD4 (INF-γ+/TNF-α+)
LVL 068 	315,092	0.20
LVL078 	206,300	0.13
NYESO FL 	64,959	0.37
LVL076 	28,803	0.03
LVL079	16,445	0.17
LVL024 	10,832	0.10
LVL030 	11,625	0.13
LVL026 	4,671	-
LVL020 	4,067	0.03
LVL075 	10,447	0.07

Figure 22

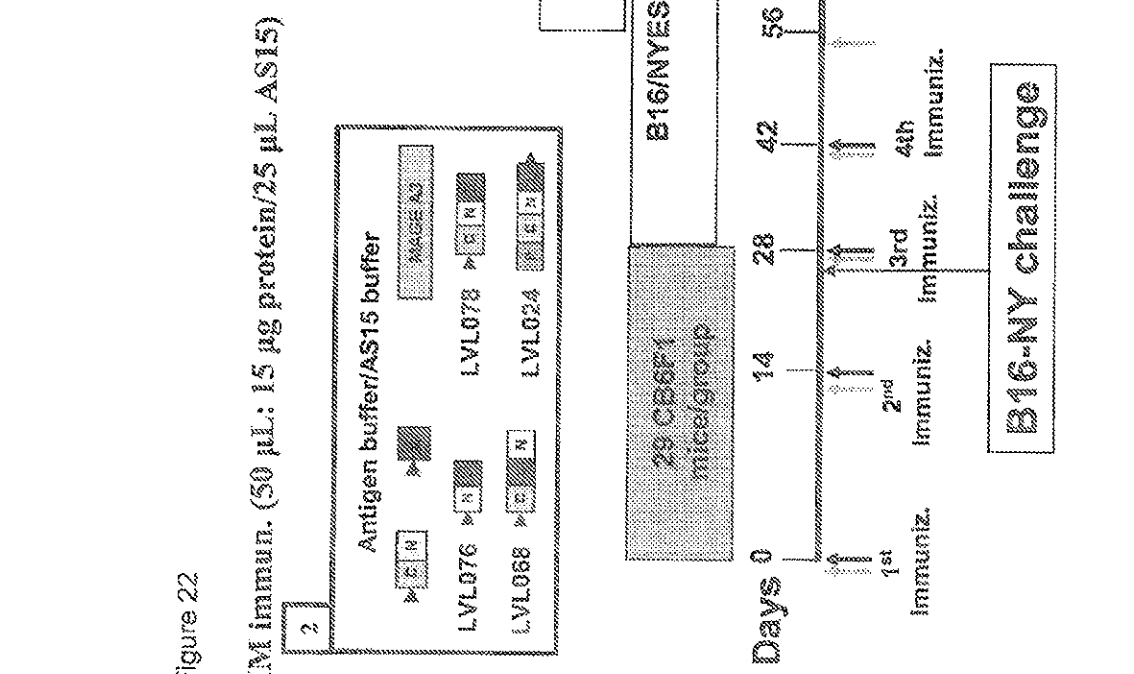

IM immun. (50 µL: 15 µg protein/25 µL AS15)
2
Antigen buffer/AS15 buffer
MAGE A3
LVL076
LVL068
LVL078
LVL024
29 CB6F1 mice/group
B16/NYESO1 tumor growth follow-up (10 mice/group)
B16/Lage 1a tumor growth follow-up (9 mice/group)
Days 0 14 28 42 56 84 105
1st Immuniz.
2nd Immuniz.
3rd Immuniz.
4th Immuniz.
B16-NY challenge
ELISA (sera): IgG1,IgG2a
•NYESO1-FL
•LAGE1a w/o CLD (LVL75)
•Human Collagen

Figure 23

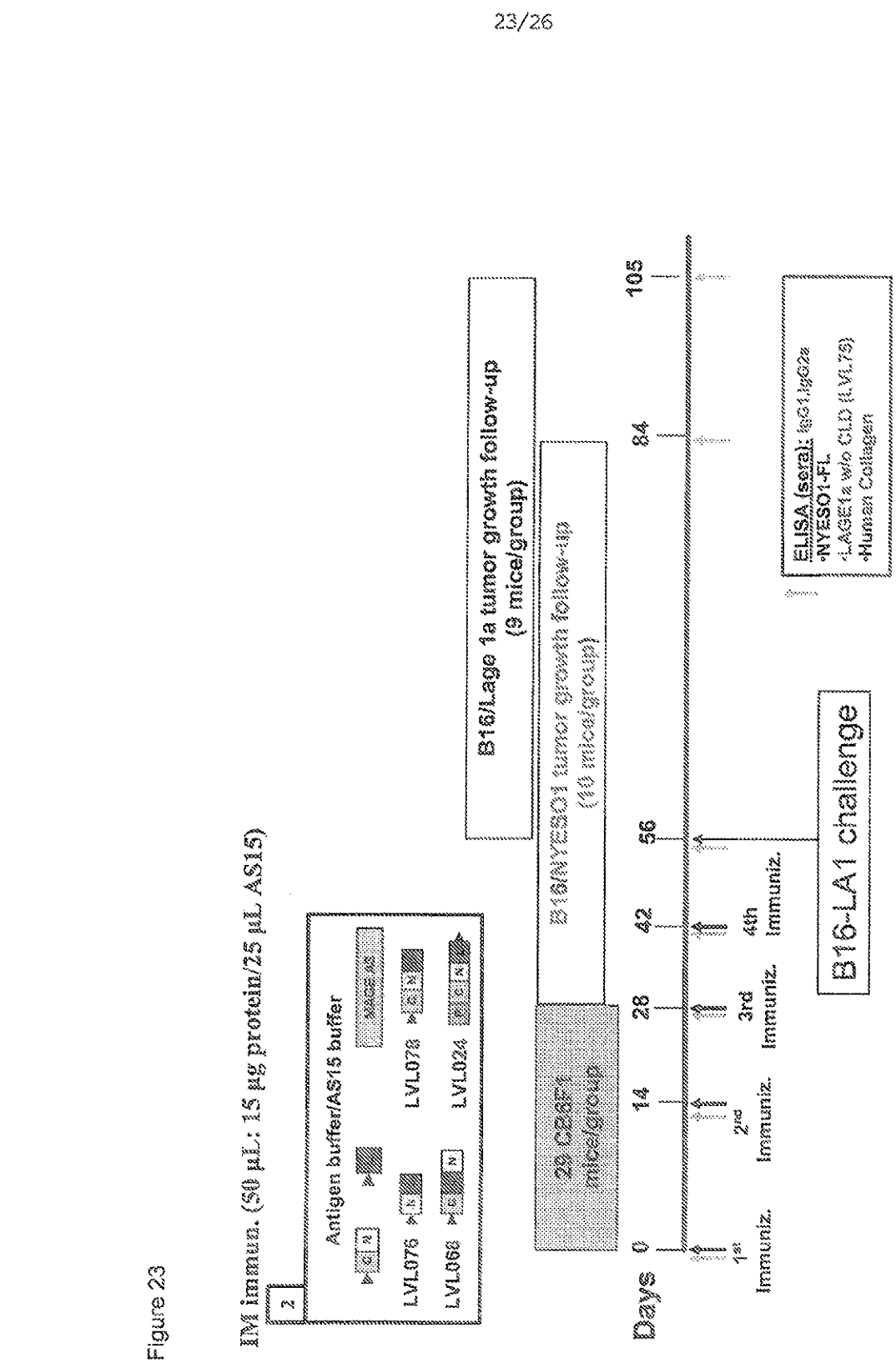

IM immun. (50 µL: 15 µg protein/25 µL AS15)
2
Antigen buffer/AS15 buffer
LVL076
LVL068
LVL078
LVL024
B16/Lage 1a tumor growth follow-up
(9 mice/group)
B16NYESO1 tumor growth follow-up
(10 mice/group)
20 C57
mice/group
Days
0
14
28
42
56
84
105
1st
Immuniz.
2nd
Immuniz.
3rd
Immuniz.
4th
Immuniz.
B16-LA1 challenge
ELISA (sera): IgG1/IgG2a
•NYESO1-FL
•LAGE1a w/o CLD (LVL76)
•Human Collagen

Figure 24

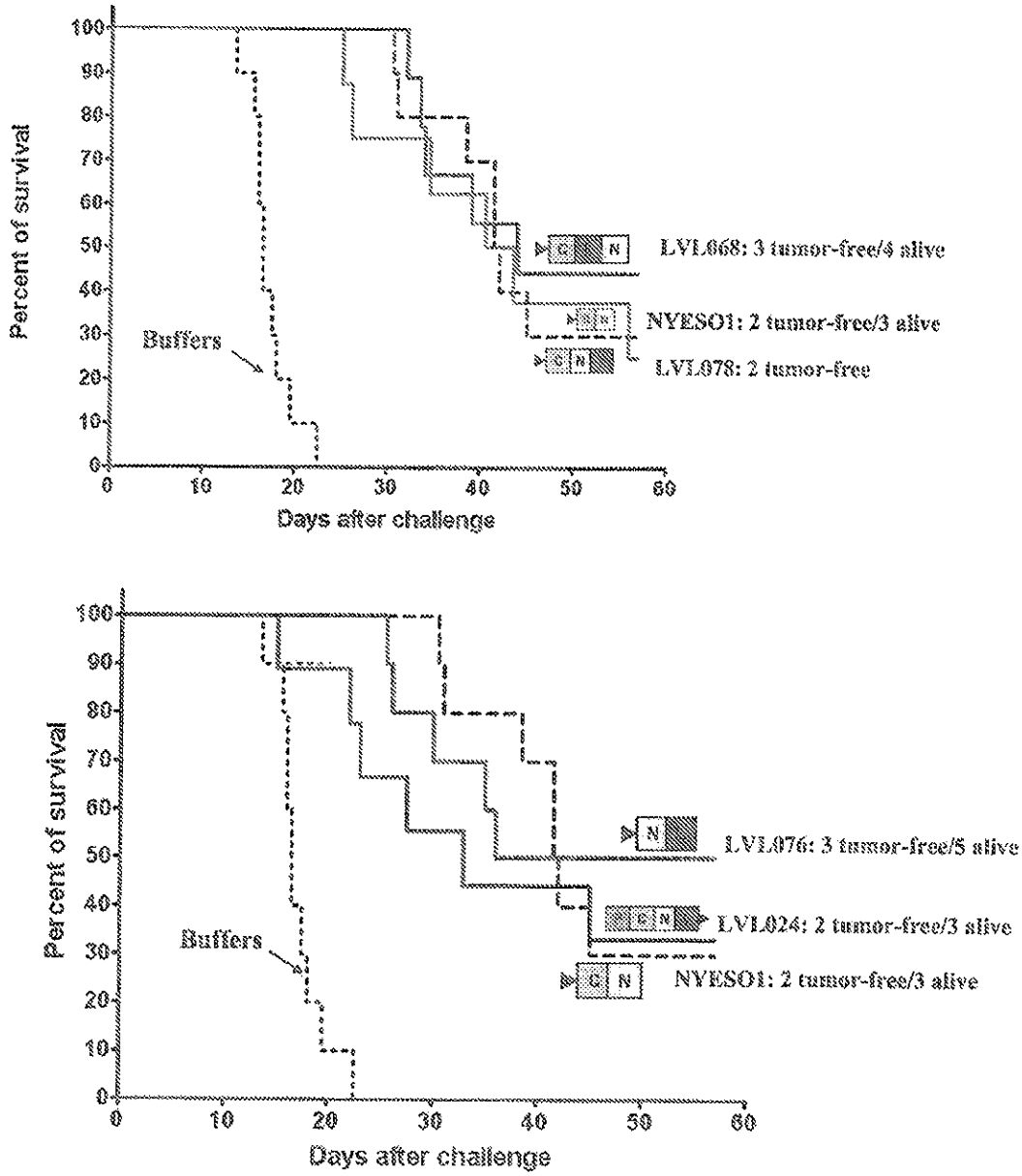
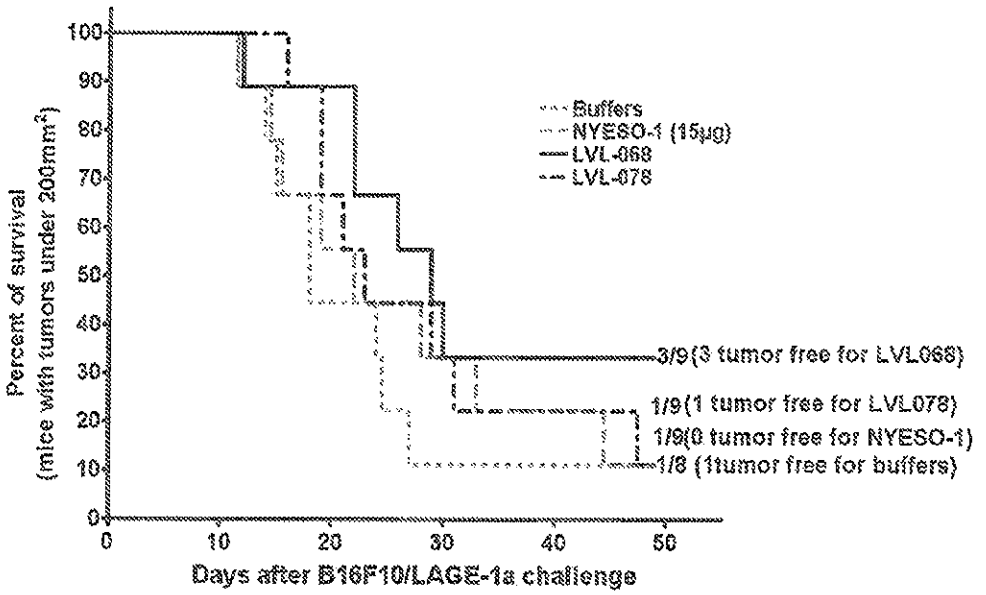

Percent of survival
Days after challenge
Buffers
LVL668: 3 tumor-free/4 alive
NYESO1: 2 tumor-free/3 alive
LVL078: 2 tumor-free
LVL076: 3 tumor-free/5 alive
LVL024: 2 tumor-free/3 alive
NYESO1: 2 tumor-free/3 alive

Figure 25


Percent of survival
(mice with tumors under 200mm²)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Days after B16F10/LAGE-1a challenge
Buffers
NYESO-1 (15µg)
LVL-068
LVL-078
3/9(3 tumor free for LVL068)
1/9(1 tumor free for LVL078)
1/9(0 tumor free for NYESO-1)
1/8 (1tumor free for buffers)

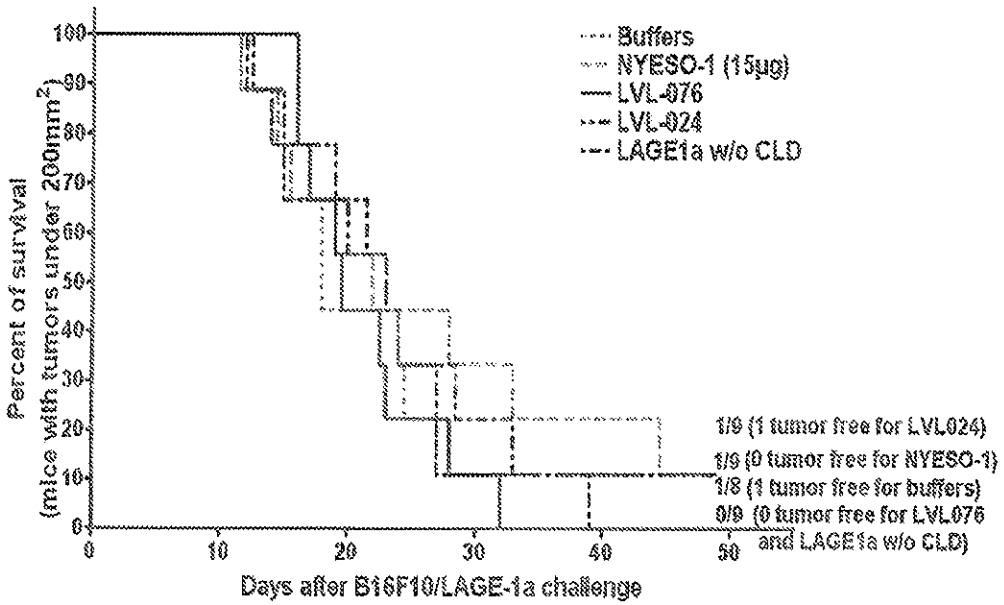
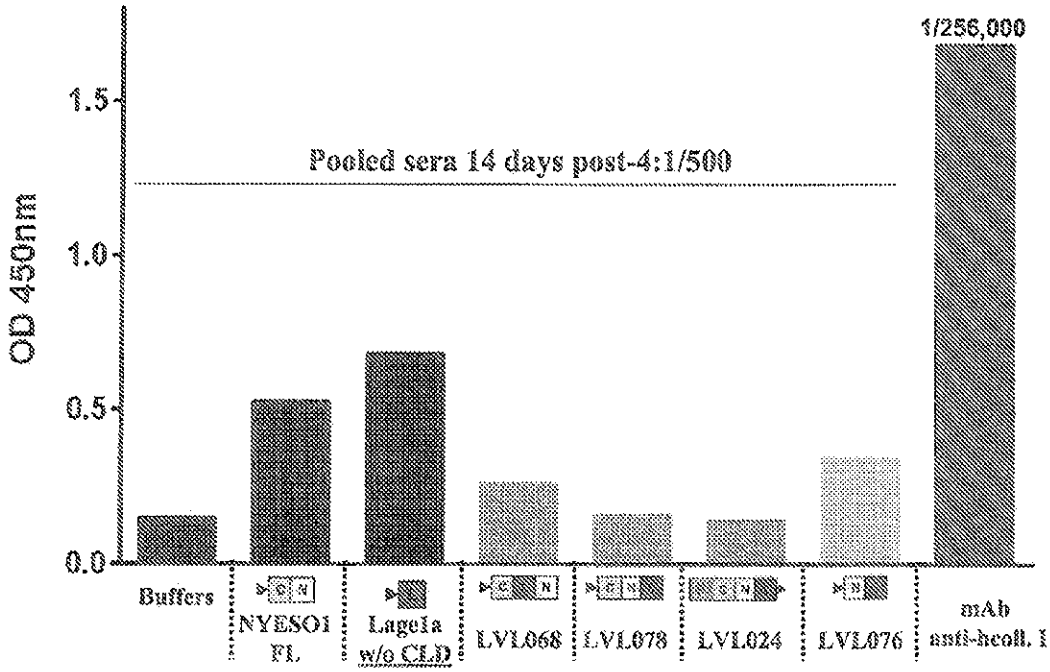

Percent of survival
(mice with tumors under 200mm²)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Days after B16F10/LAGE-1a challenge
Buffers
NYESO-1 (15µg)
LVL-076
LVL-024
LAGE1a w/o CLD
1/9 (1 tumor free for LVL024)
1/9 (0 tumor free for NYESO-1)
1/8 (1 tumor free for buffers)
0/9 (0 tumor free for LVL076
and LAGE1a w/o CLD)

Figure 26


1/256,000
1.5
Pooled sera 14 days post-4:1/500
OD 450nm
1.0
0.5
0.0
Buffers
NYESO1
FL
Lagela
w/o CLD
LVL068
LVL078
LVL024
LVL076
mAb
anti-hcoll. I