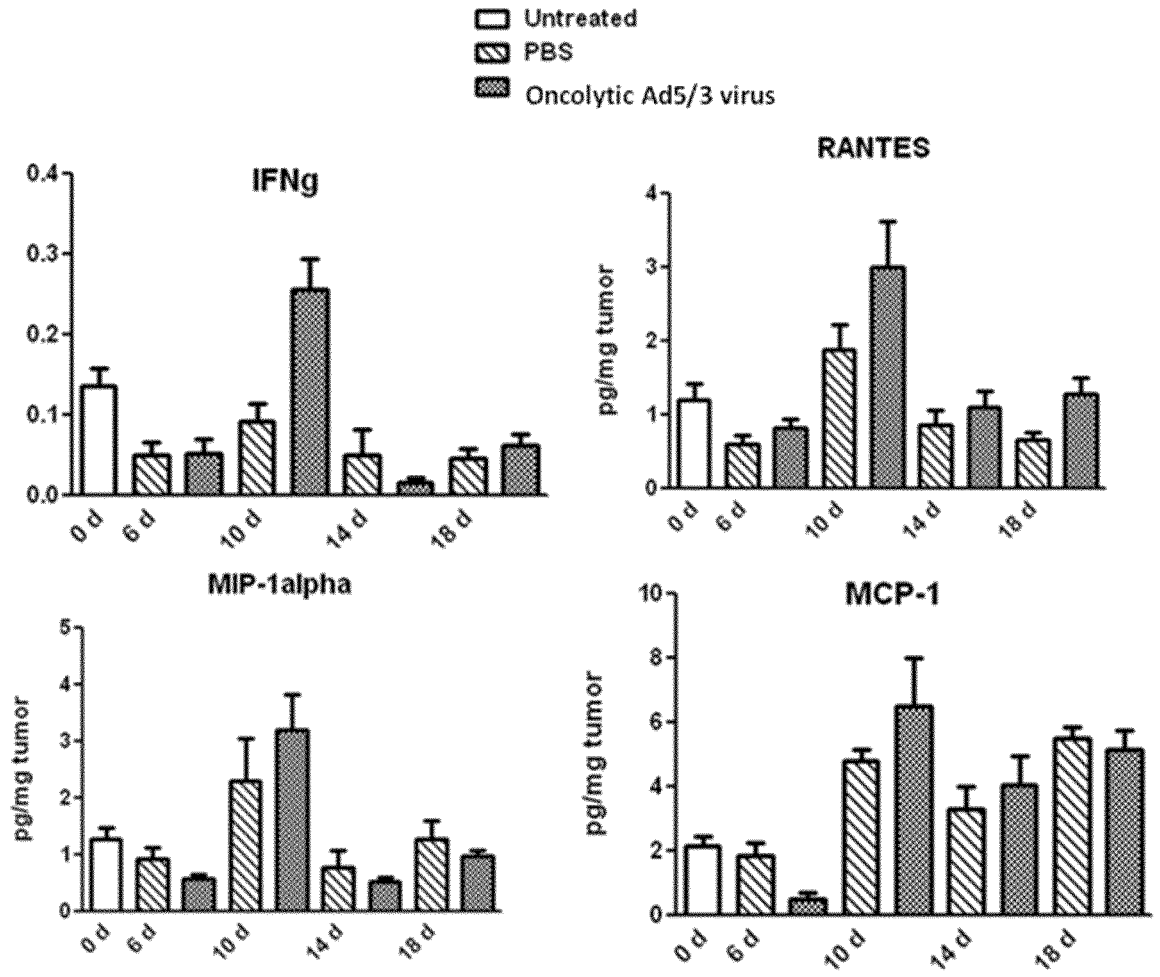

Untreated
PBS
Oncolytic Ad5/3 virus
IFNg
RANTES
MIP-1alpha
MCP-1
pg/mg tumor
0 d
6 d
10 d
14 d
18 d

Figure 1.

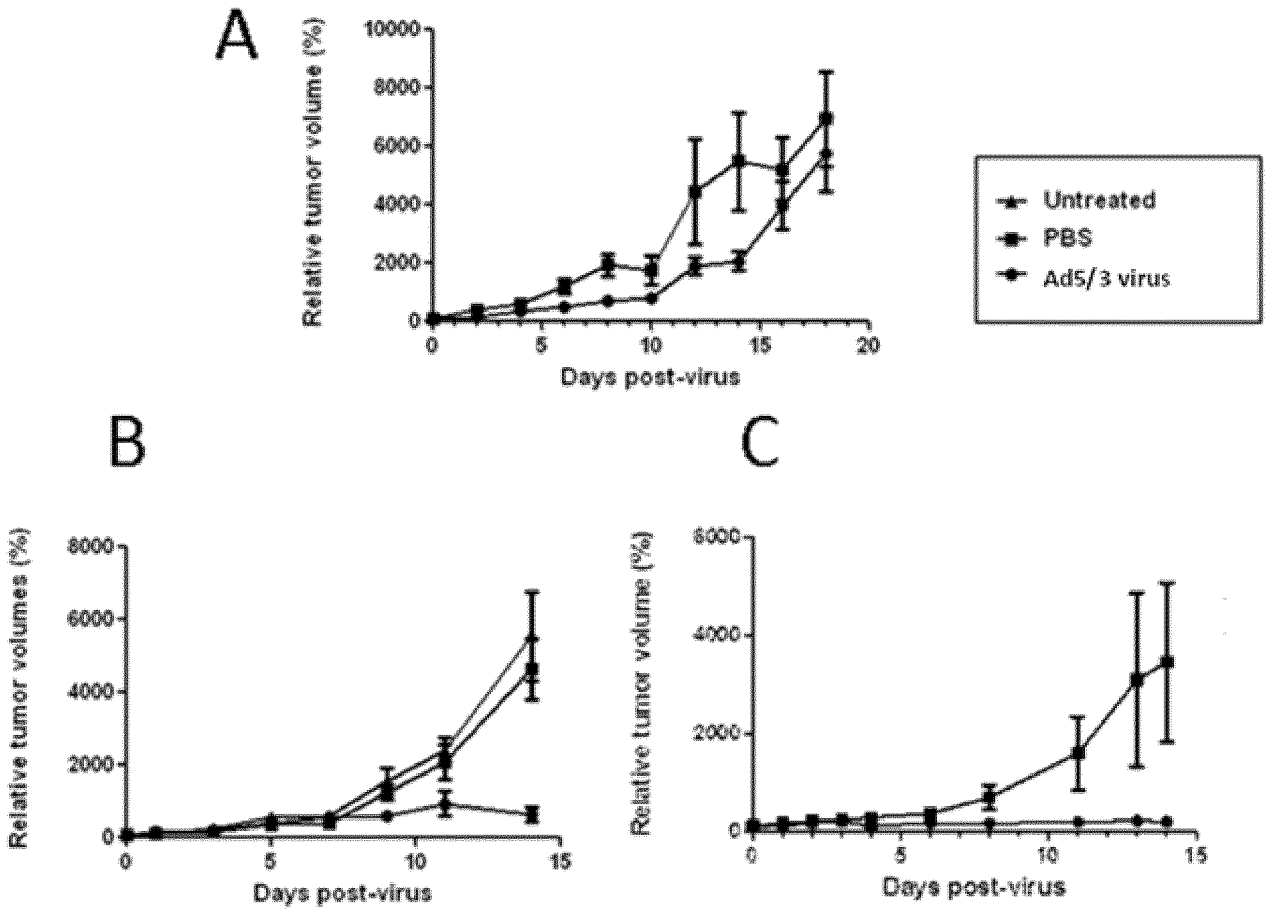

A
Relative tumor volume (%)
10000
8000
6000
4000
2000
0
0
5
10
15
20
Days post-virus
Untreated
PBS
Ad5/3 virus
B
Relative tumor volumes (%)
8000
6000
4000
2000
0
0
5
10
15
Days post-virus
C
Relative tumor volume (%)
6000
4000
2000
0
0
5
10
15
Days post-virus

Figure 2.

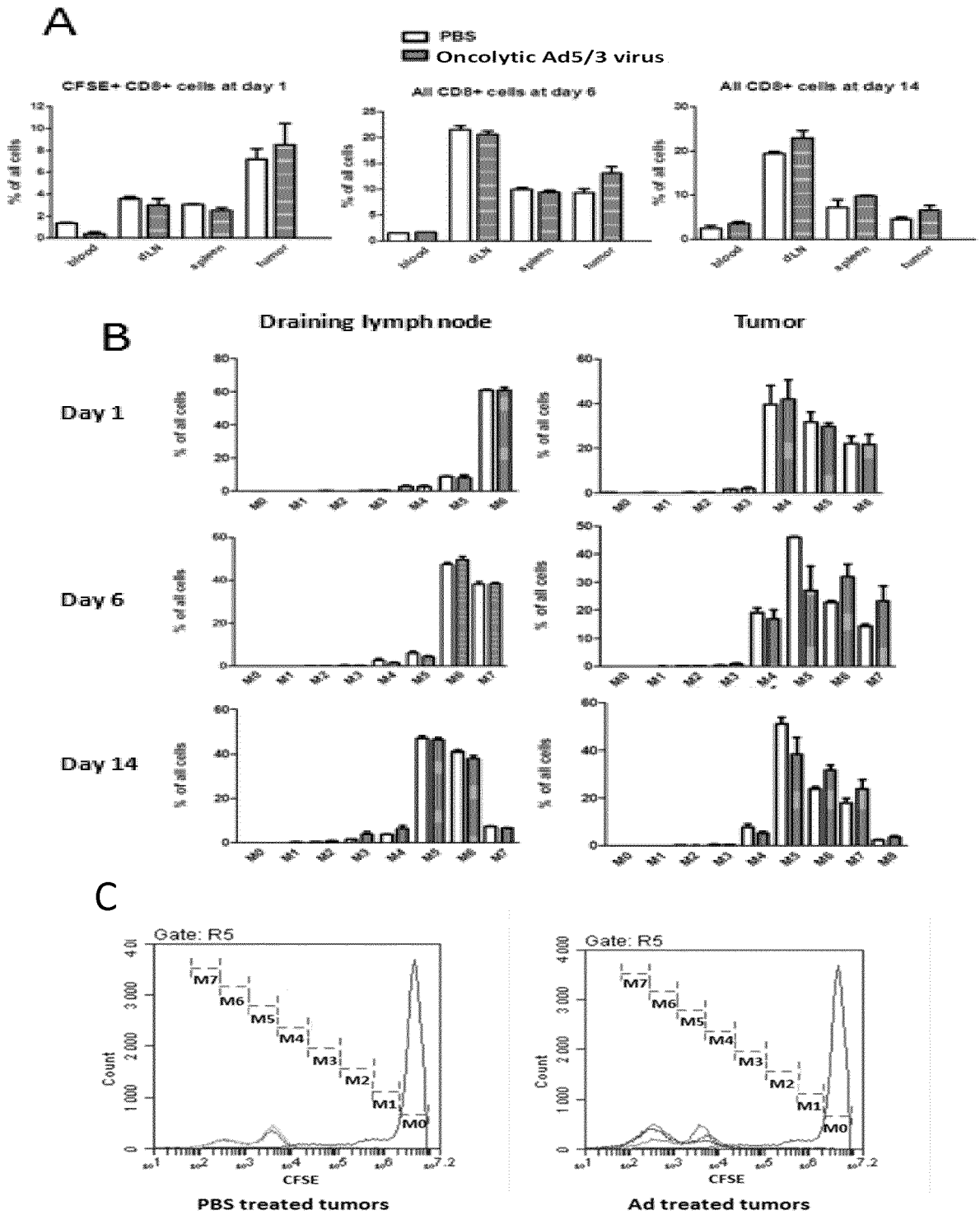

A
PBS
Oncolytic Ad5/3 virus
CFSE+ CD8+ cells at day 1
All CD8+ cells at day 6
All CD8+ cells at day 14
% of all cells
blood
dLN
spleen
tumor
B
Draining lymph node
Tumor
Day 1
Day 6
Day 14
% of all cells
C
Gate: R5
Count
CFSE
M0
M1
M2
M3
M4
M5
M6
M7
PBS treated tumors
Ad treated tumors

Figure 3.

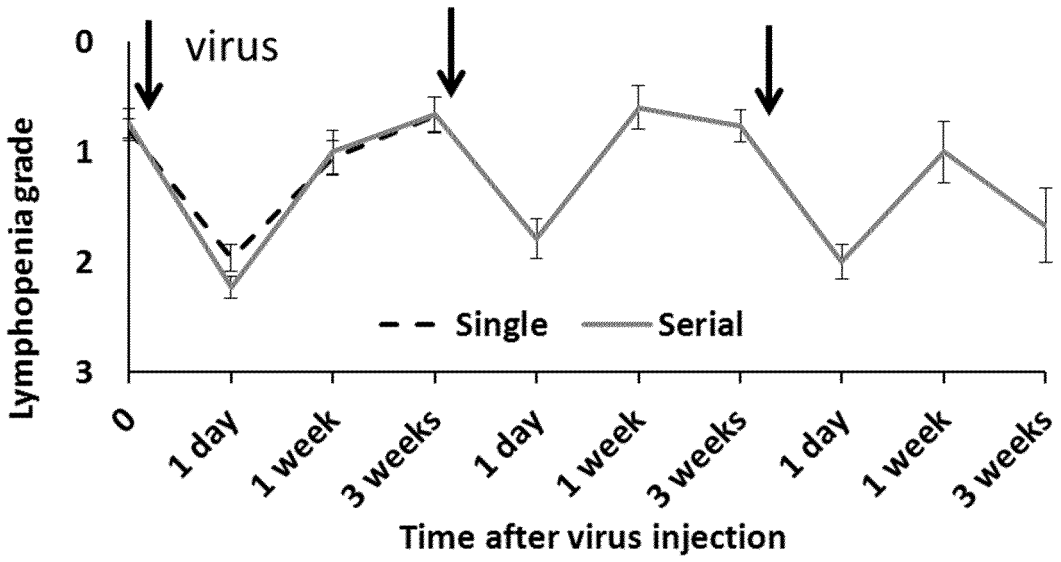



Figure 4.

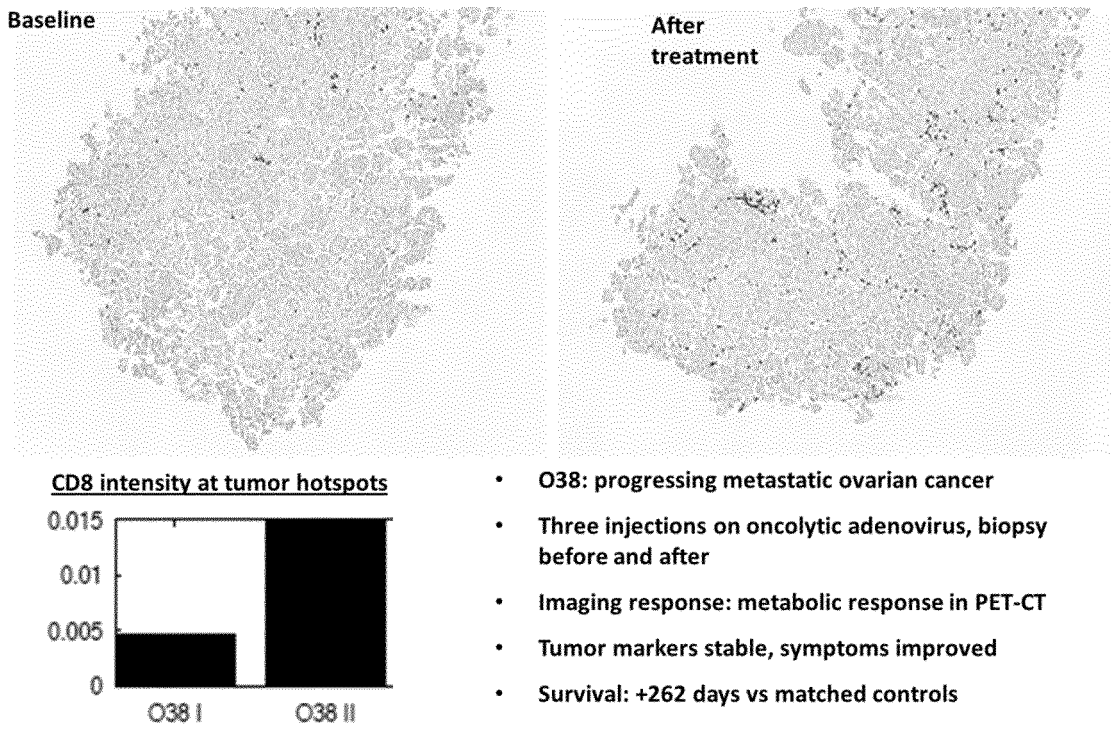
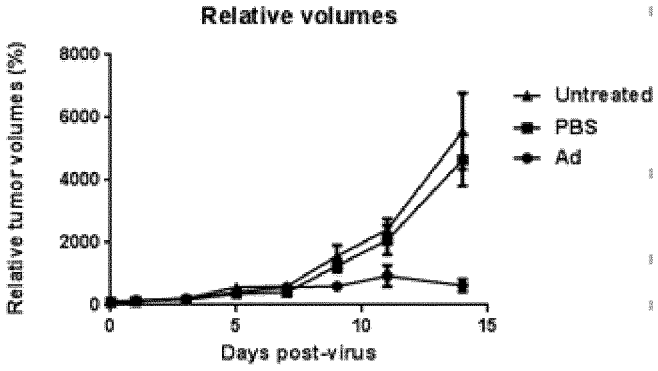
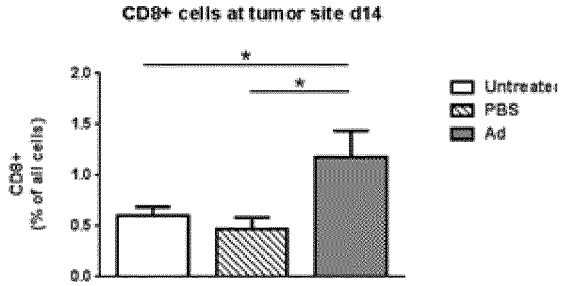



Figure 5.

a)


Relative volumes
Relative tumor volumes (%)
8000
6000
4000
2000
0
0
5
10
15
Days post-virus
Untreated
PBS
Ad

b)


CD8+ cells at tumor site d14
CD8+
(% of all cells)
2.0
1.5
1.0
0.5
0.0
*
*
Untreated
PBS
Ad

c)

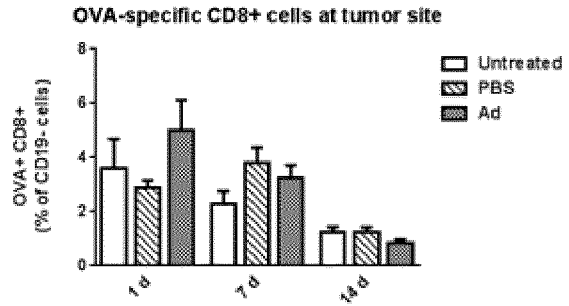

OVA-specific CD8+ cells at tumor site
OVA+ CD8+
(% of CD19- cells)
8
6
4
2
0
1 d
7 d
14 d
Untreated
PBS
Ad

Figure 6.

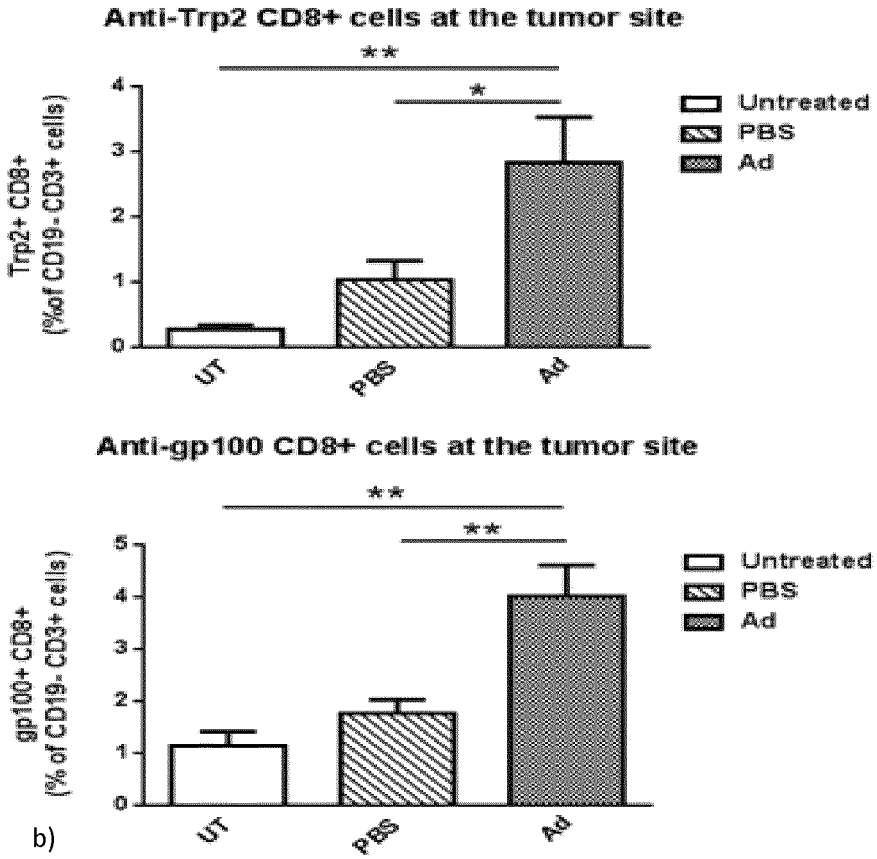



Figure 7.

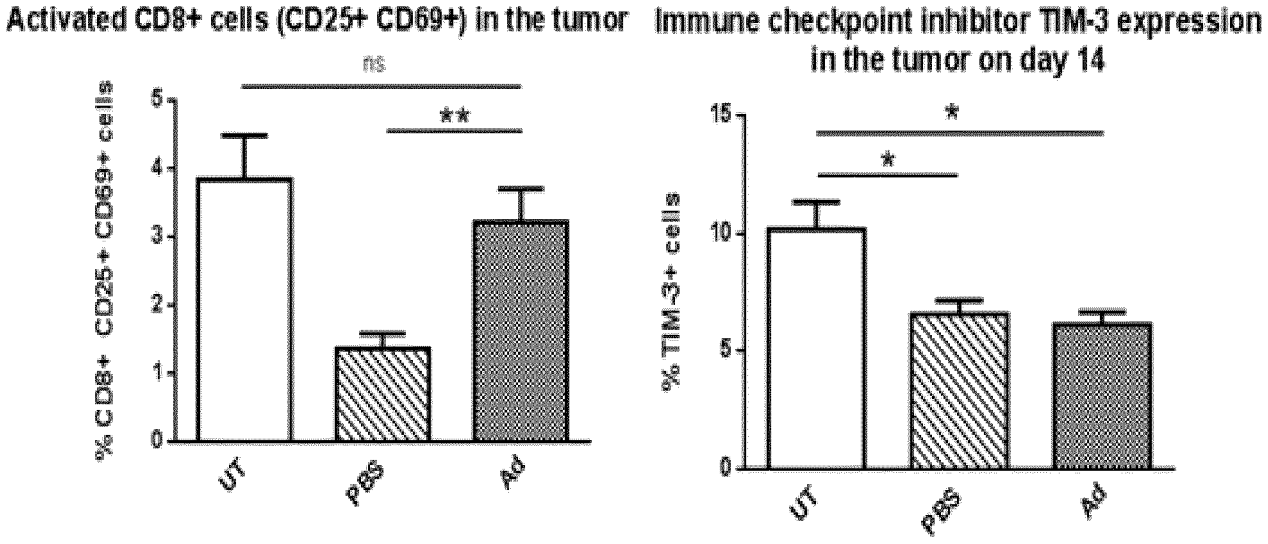
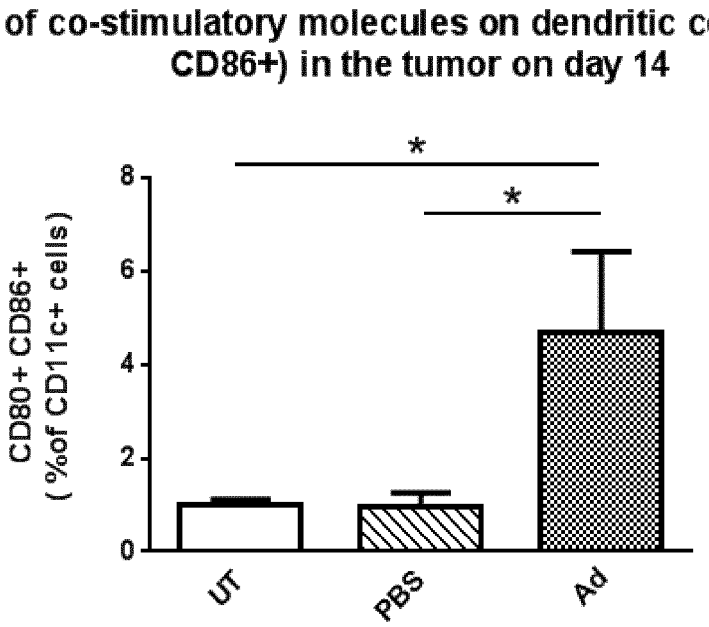



Figure 8.

a)


Expression of co-stimulatory molecules on dendritic cells (CD11c+ CD80+ CD86+) in the tumor on day 14
CD80+ CD86+ ( %of CD11c+ cells)
0
2
4
6
8
*
*
UT
PBS
Ad

b)

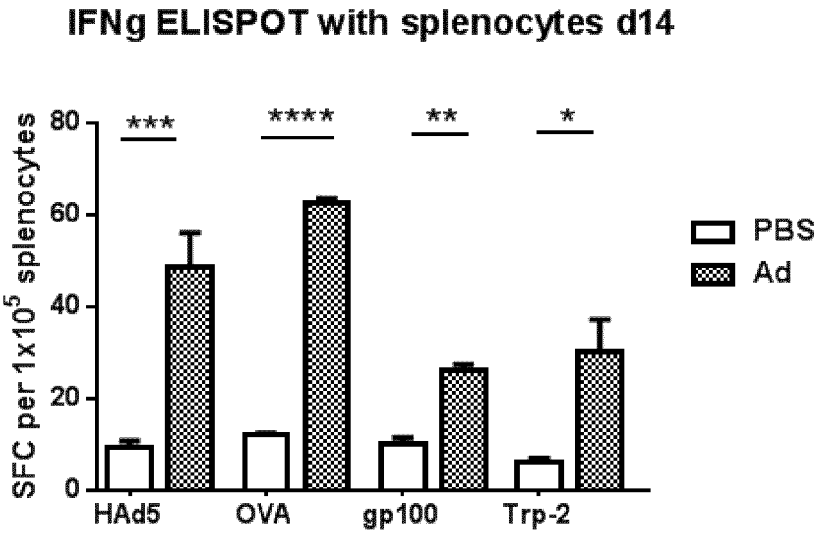
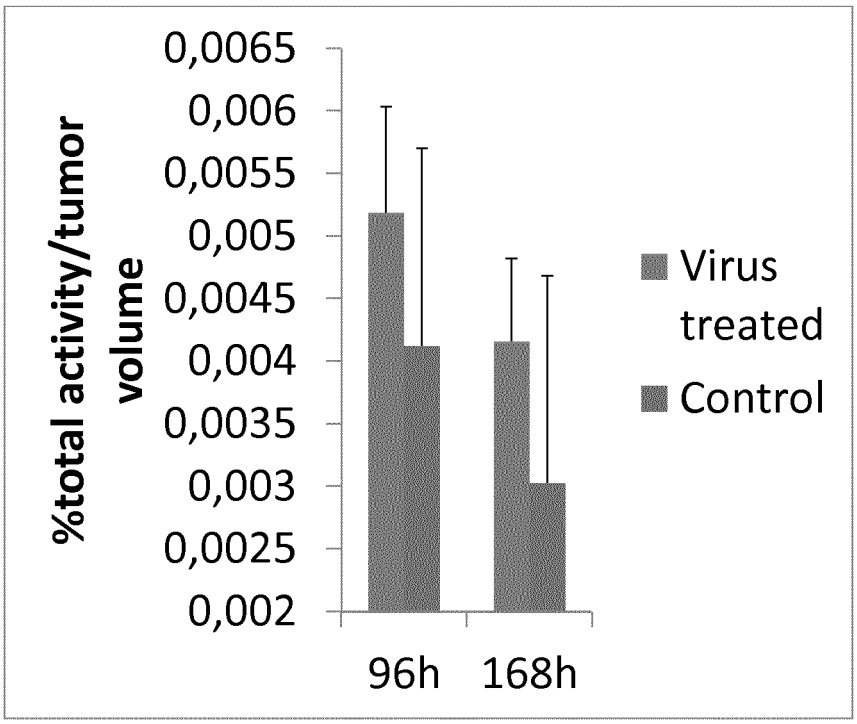

IFNg ELISPOT with splenocytes d14
SFC per 1x10^5 splenocytes
0
20
40
60
80
***
****
**
*
HAd5
OVA
gp100
Trp-2
PBS
Ad

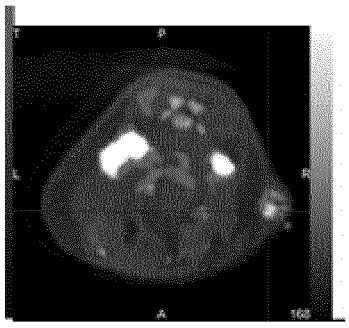
Figure 9.

a)






Uptake in normal lymphatic organs



Minor uptake in tumor

b) c)

Figures 10a-c.

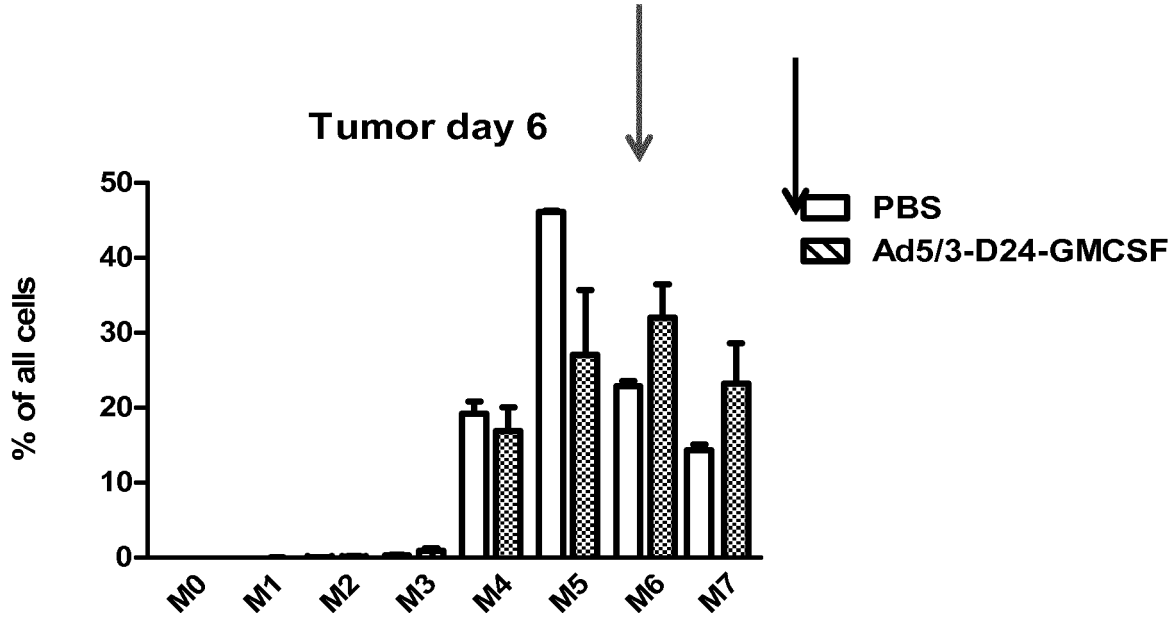



Figure 11.

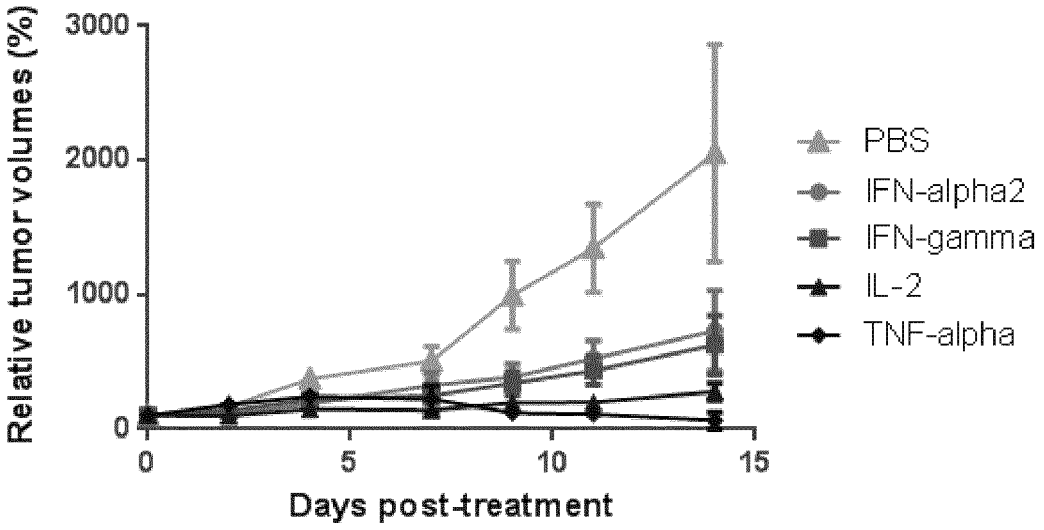
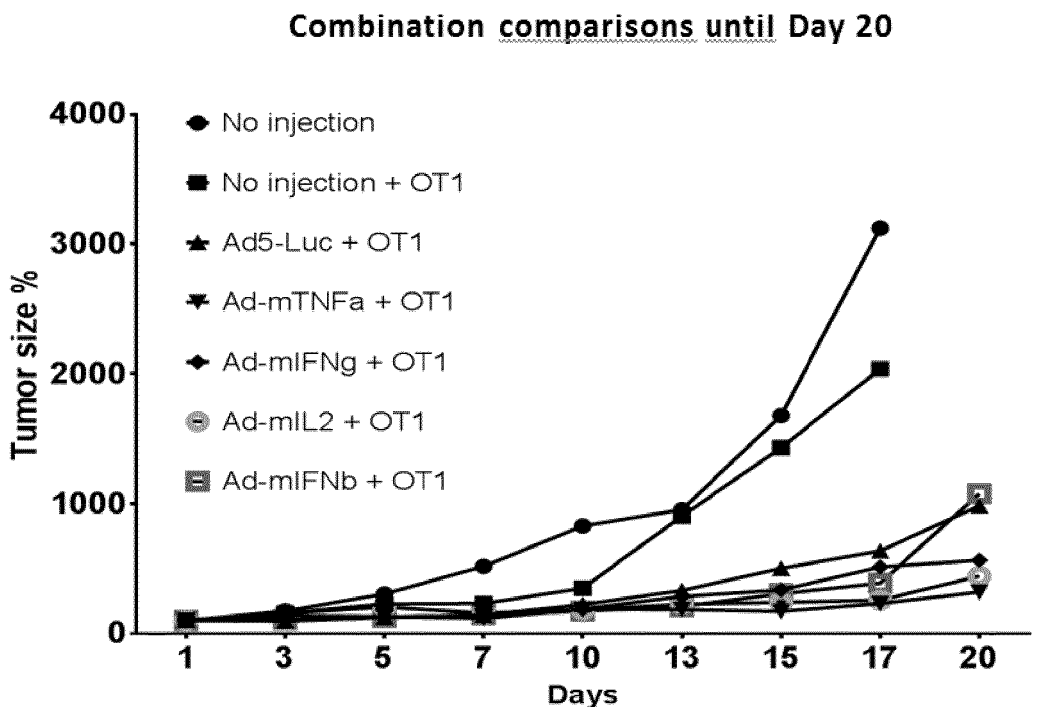
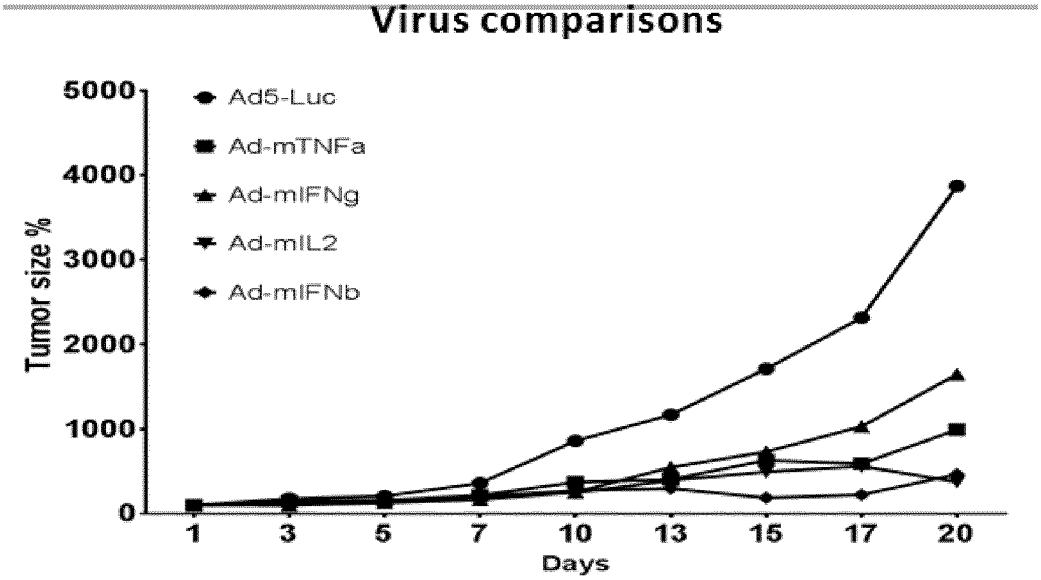



Figure 12.




| | Number at risk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 5 | Day 7 | Day 10 | Day 13 | Day 15 | Day 17 | Day 20 |
| No injection | 6 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 2 |
| No injection + OT1 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 5 | 3 |
| Ad5-Luc + OT1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 |
| Ad-mTNFa + OT1 | 6 | 6 | 6 | 6 | 5 | 5 | 4 | 4 | 4 |
| Ad-mIFNg + OT1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 |
| Ad-mIL2 + OT1 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 |
| Ad-mIFNb + OT1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

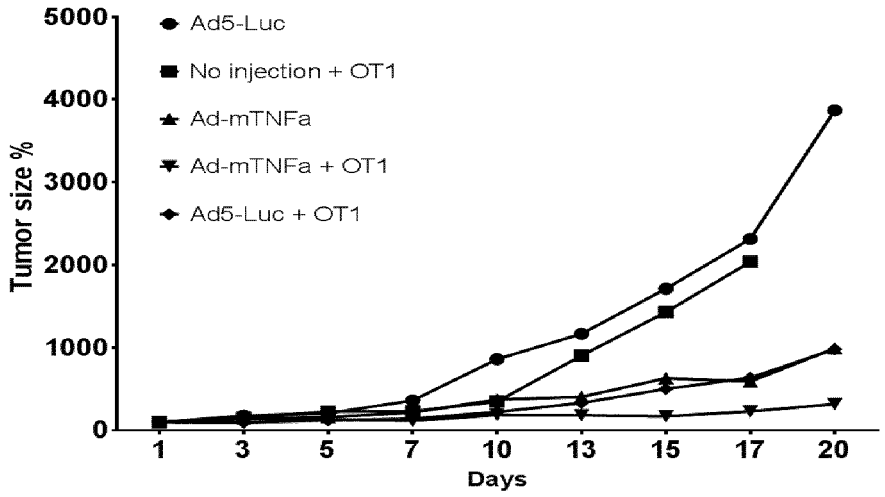
Figure 13.




| | Number at risk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 5 | Day 7 | Day 10 | Day 13 | Day 15 | Day 17 | Day 20 |
| Ad5-Luc | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 5 | 4 |
| Ad-mTNFa | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 3 | 3 |
| Ad-mIFNg | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Ad-mIL2 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 |
| Ad-mIFNb | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 4 |

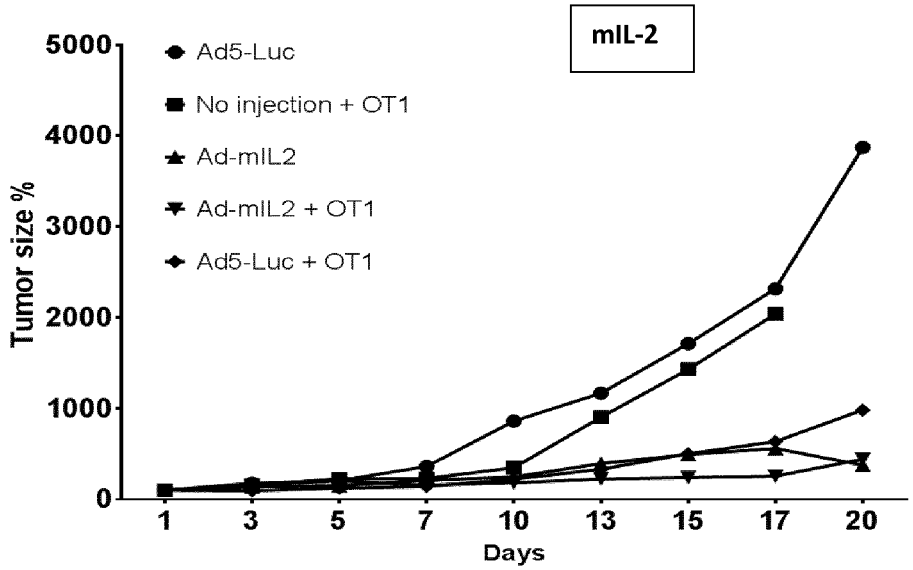
Figure 14.

**mTNFa**




| | Number at risk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 5 | Day 7 | Day 10 | Day 13 | Day 15 | Day 17 | Day 20 |
| Ad5-Luc | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 5 | 4 |
| No injection + OT1 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 5 | 3 |
| Ad-mTNFa | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 3 | 3 |
| Ad-mTNFa + OT1 | 6 | 6 | 6 | 6 | 5 | 5 | 4 | 4 | 4 |
| Ad5-Luc + OT1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 |

Figure 15.




| | Number at risk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 5 | Day 7 | Day 10 | Day 13 | Day 15 | Day 17 | Day 20 |
| Ad5-Luc | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 5 | 4 |
| No injection + OT1 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 5 | 3 |
| Ad-mIL2 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 |
| Ad-mIL2 + OT1 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 |
| Ad5-Luc + OT1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 |

Figure 16.

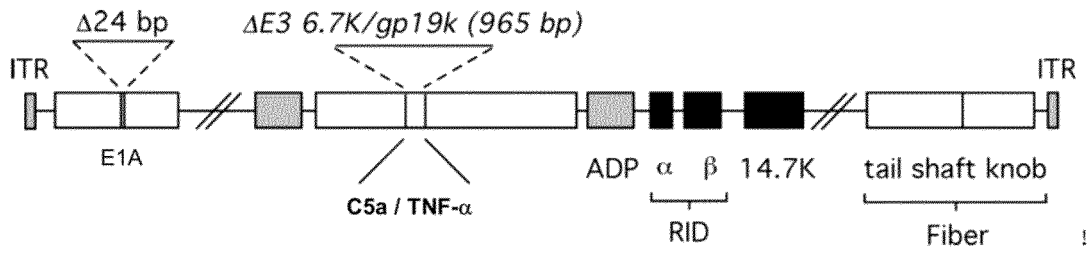



Figure 17.

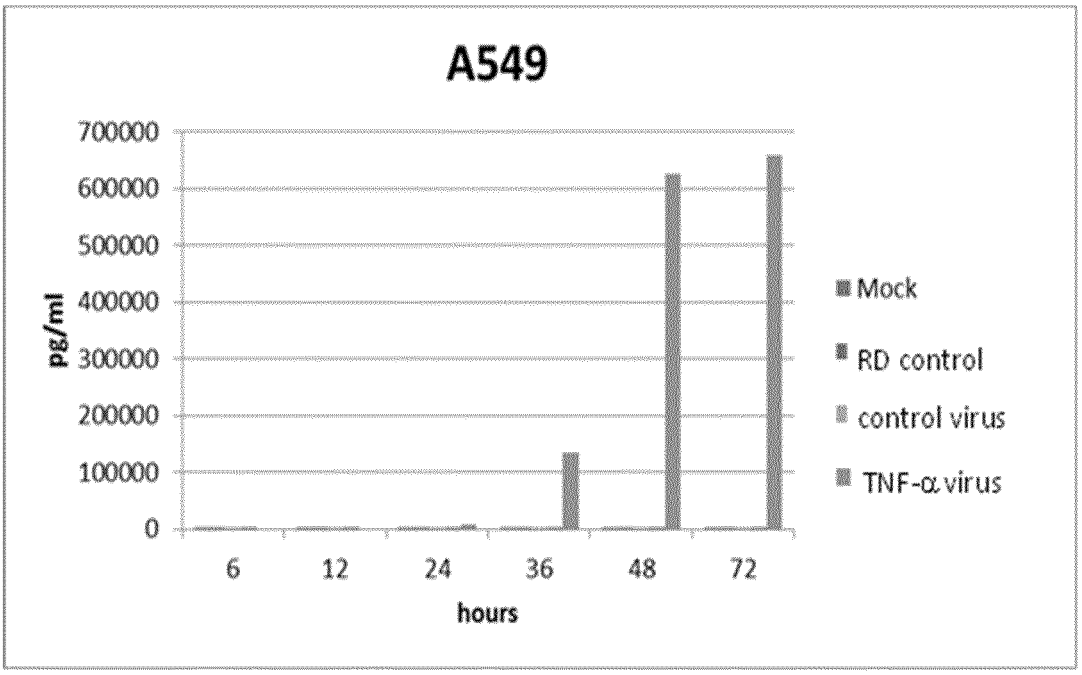



Figure 18.

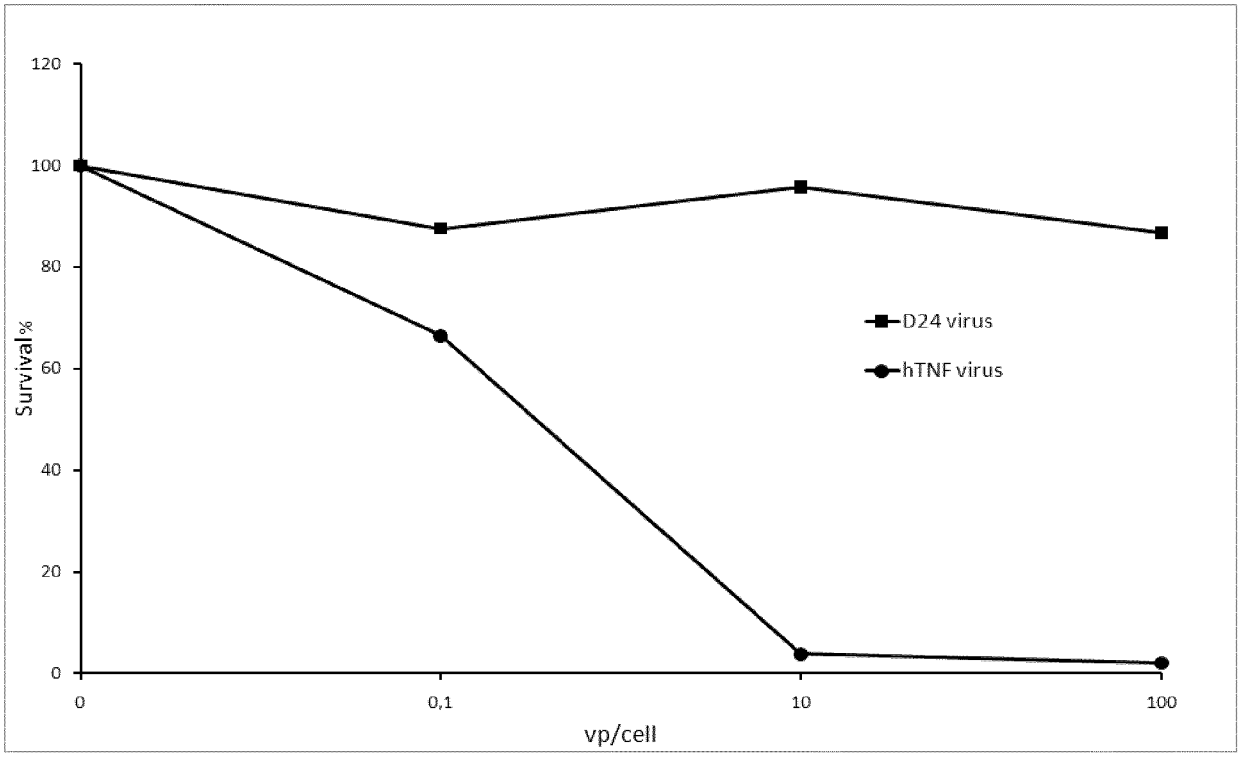



Figure 19.

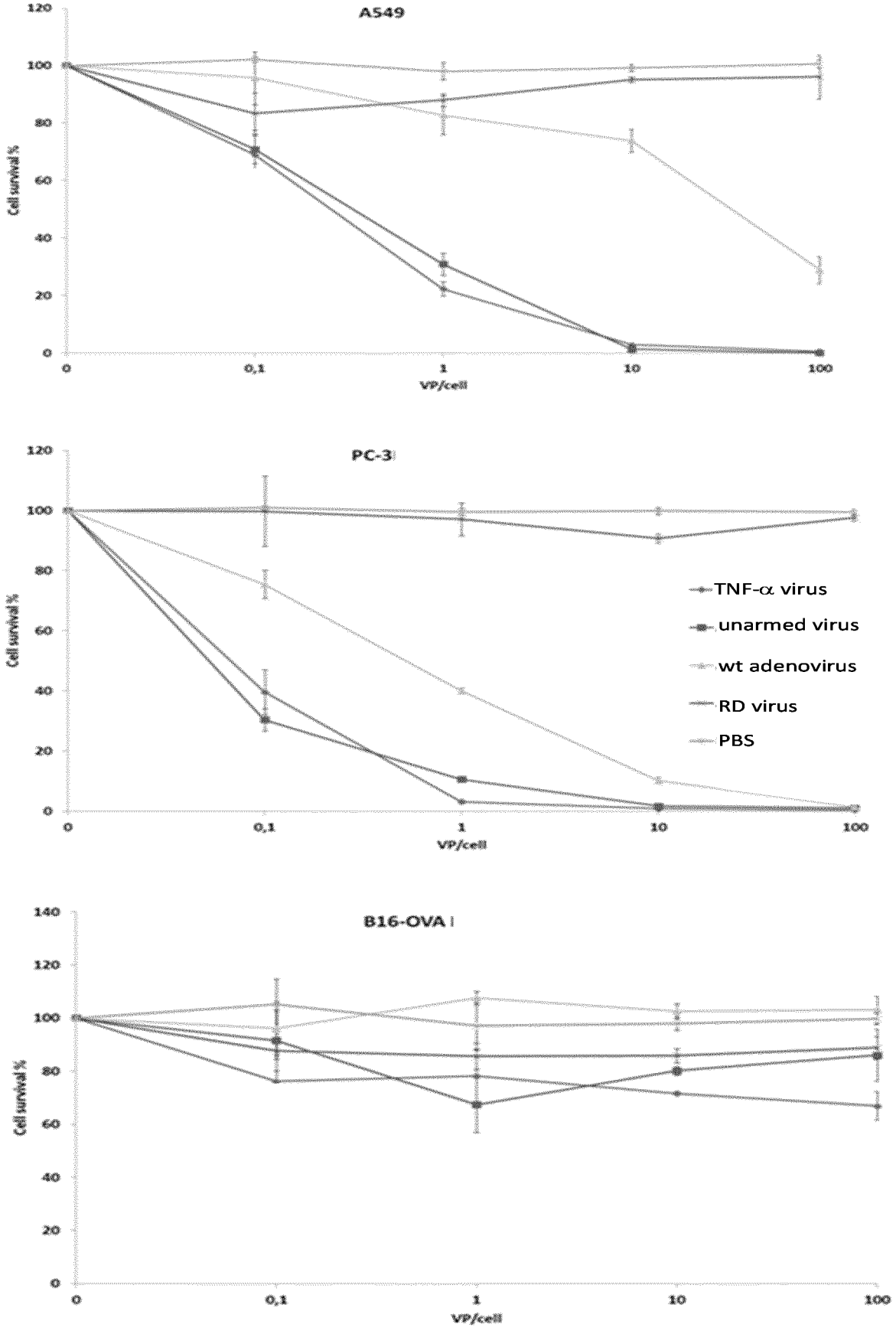

A549
Cell survival %
VP/cell
PC-3
TNF-α virus
unarmed virus
wt adenovirus
RD virus
PBS
B16-OVA
0
0,1
1
10
100

Figure 20.

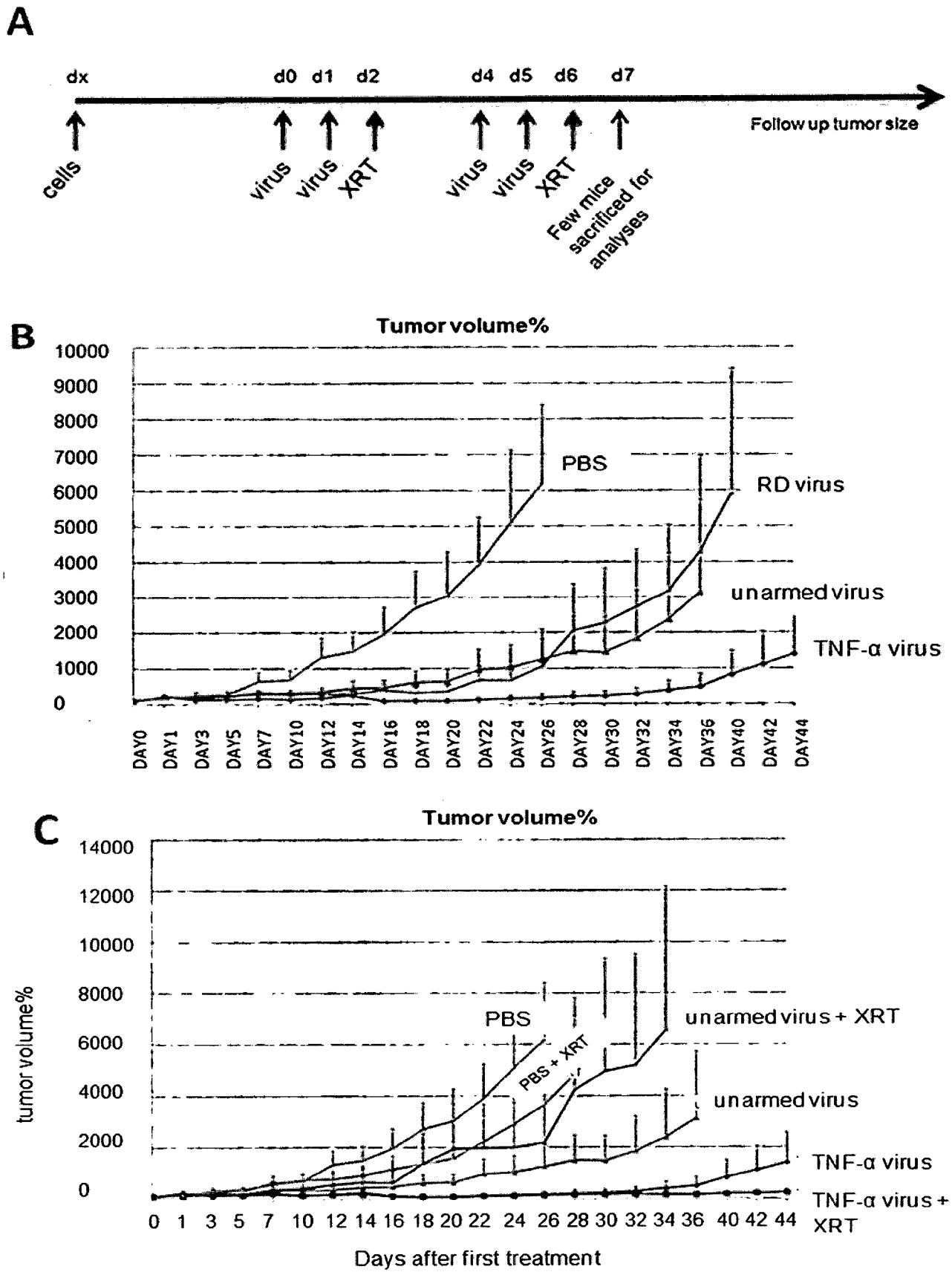

A
dx
d0 d1 d2
d4 d5 d6 d7
Follow up tumor size
cells
virus
virus
XRT
virus
virus
XRT
Few mice sacrificed for analyses
B
Tumor volume%
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
PBS
RD virus
unarmed virus
TNF-α virus
DAY0 DAY1 DAY3 DAY5 DAY7 DAY10 DAY12 DAY14 DAY16 DAY18 DAY20 DAY22 DAY24 DAY26 DAY28 DAY30 DAY32 DAY34 DAY36 DAY40 DAY42 DAY44
C
Tumor volume%
14000
12000
10000
8000
6000
4000
2000
0
tumor volume%
PBS
PBS + XRT
unarmed virus + XRT
unarmed virus
TNF-α virus
TNF-α virus + XRT
0 1 3 5 7 10 12 14 16 18 20 22 24 26 28 30 32 34 36 40 42 44
Days after first treatment

Figure 21.

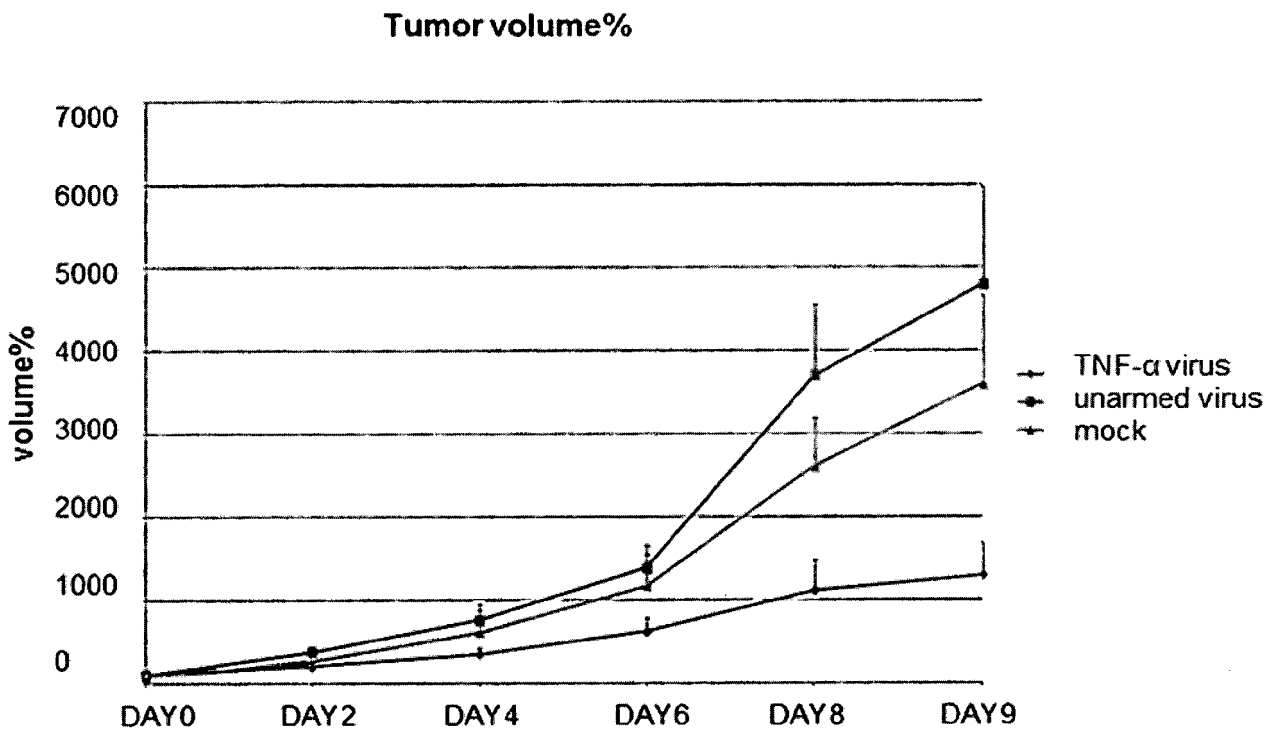



Figure 22.

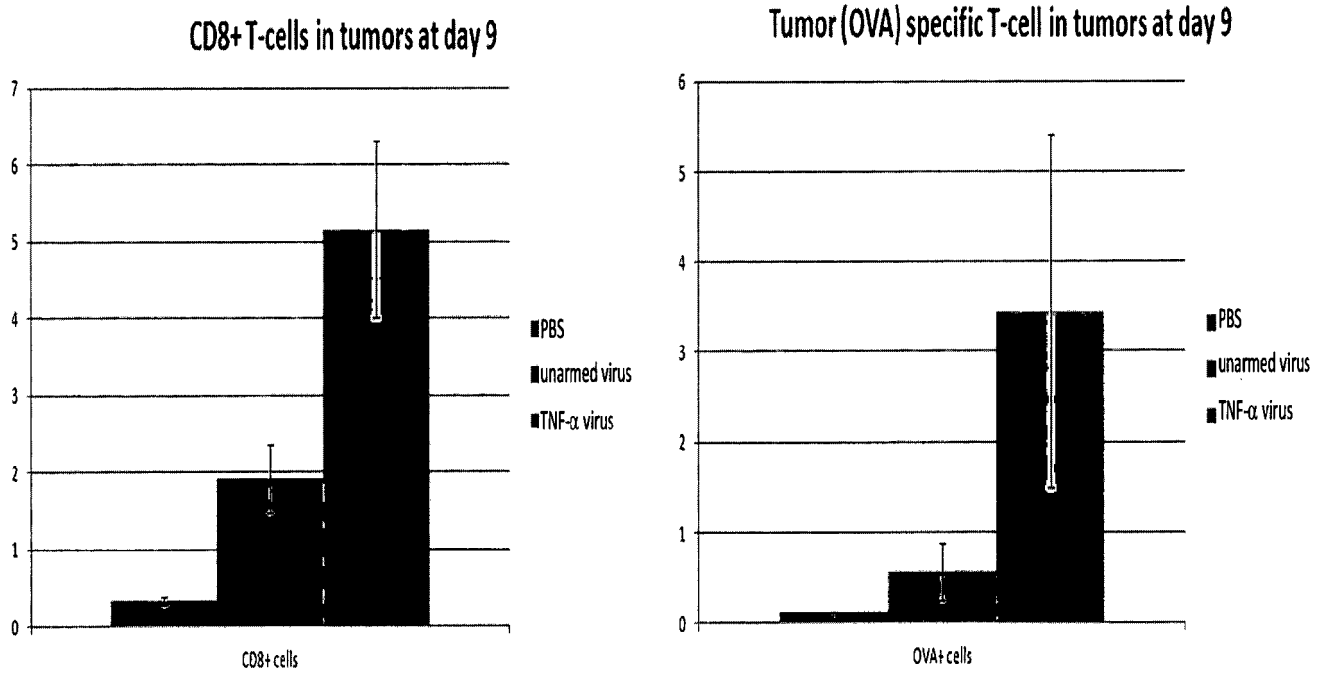



Figure 23.

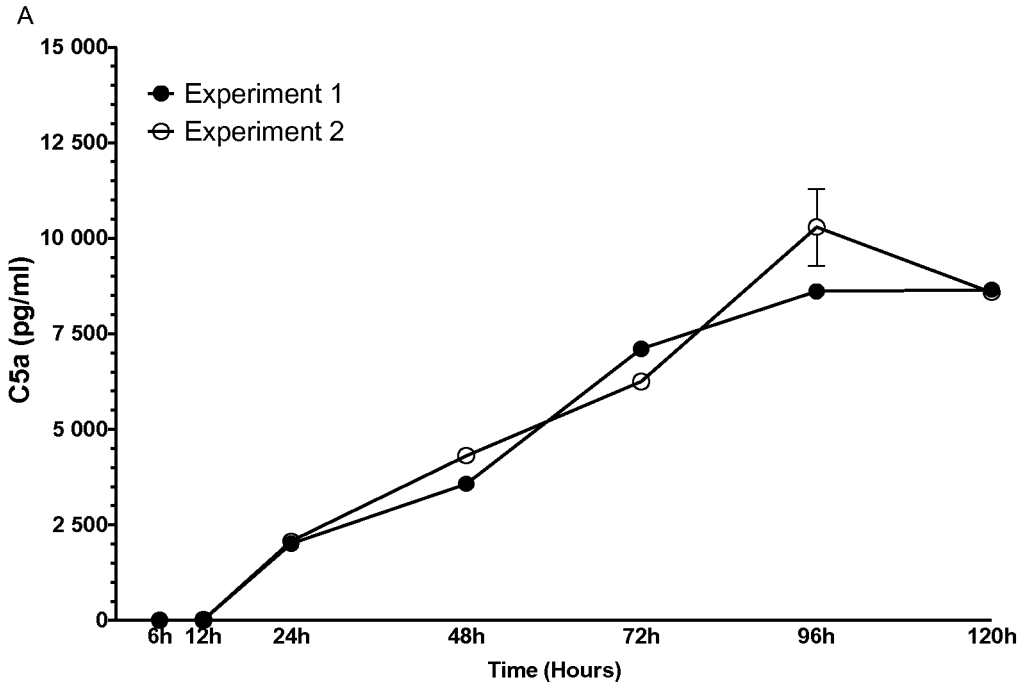



Figure 24.

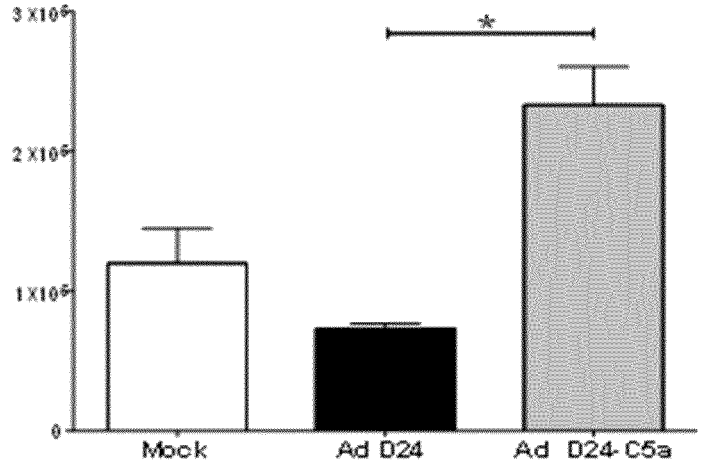



Figure 25.

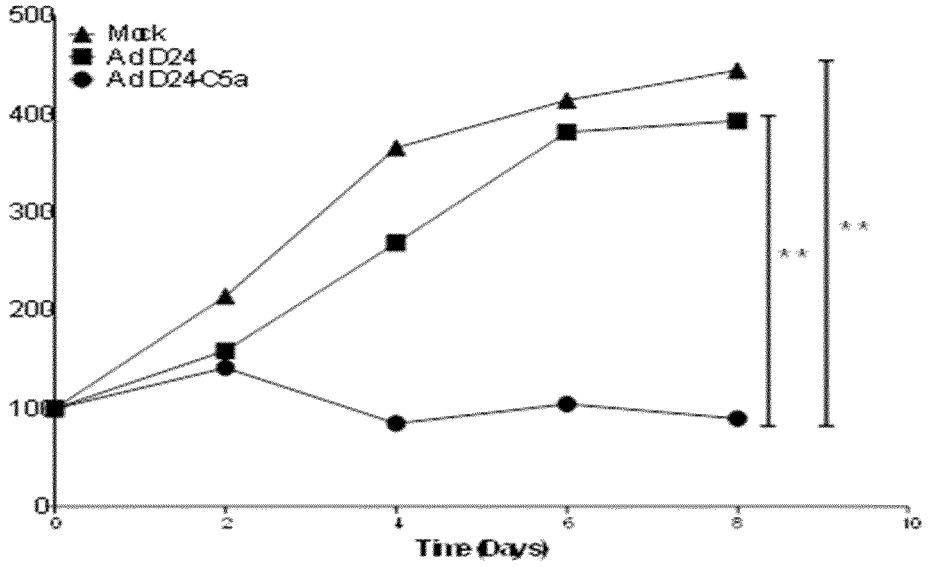



Figure 26.

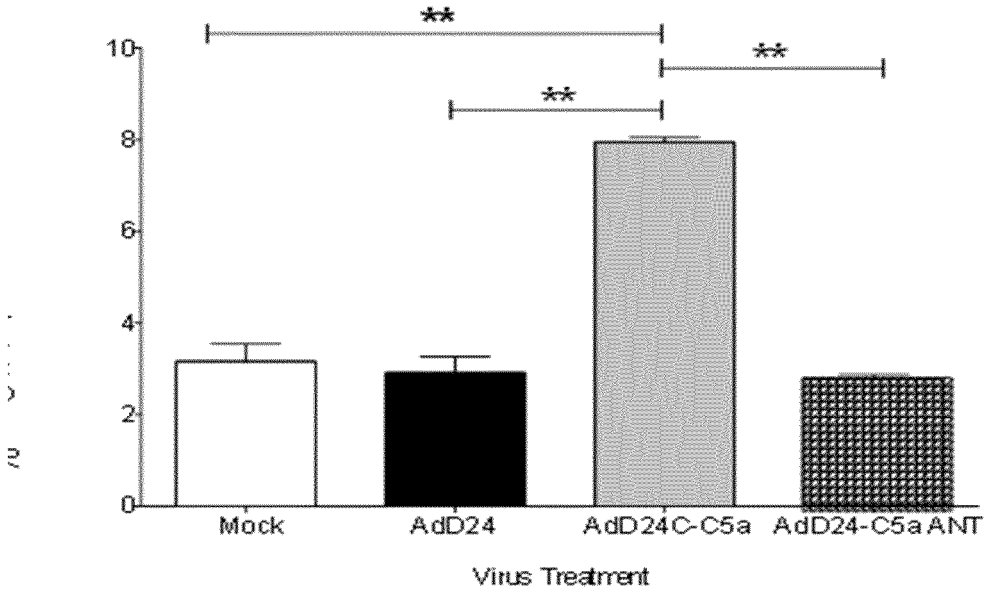



Figure 27.

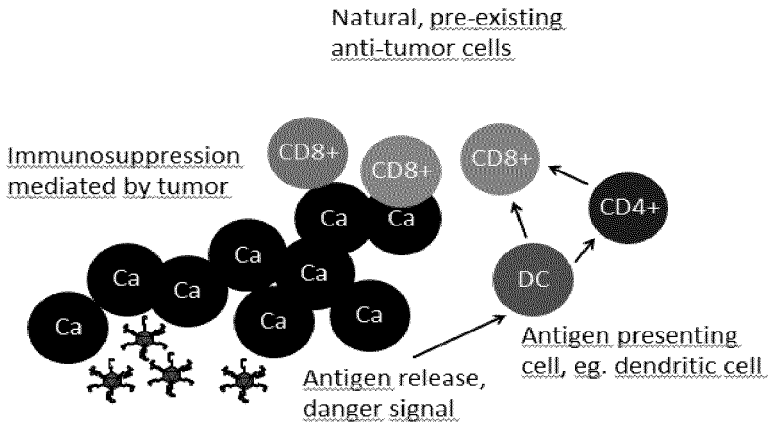



Figure 28.

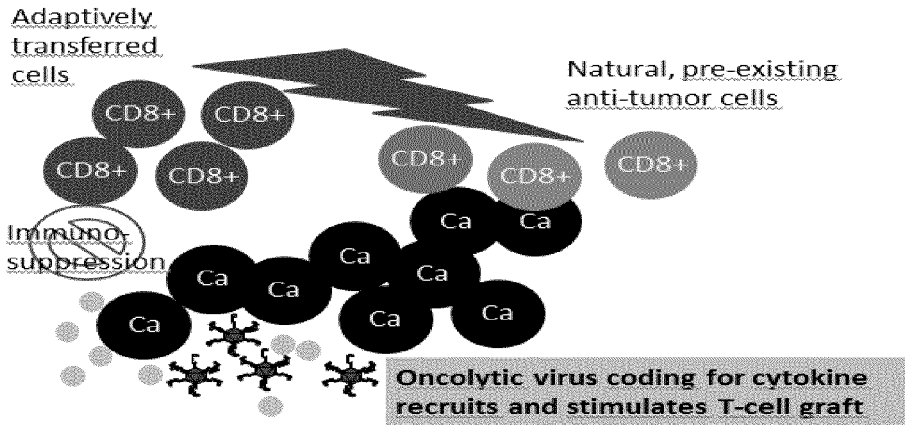



Figure 29.

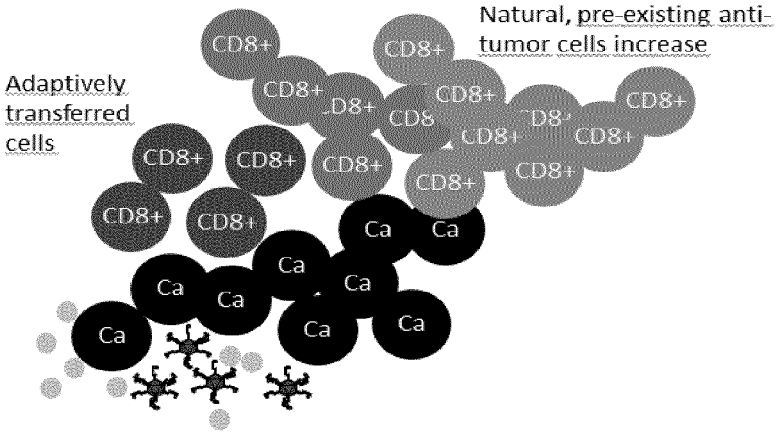



Figure 30.

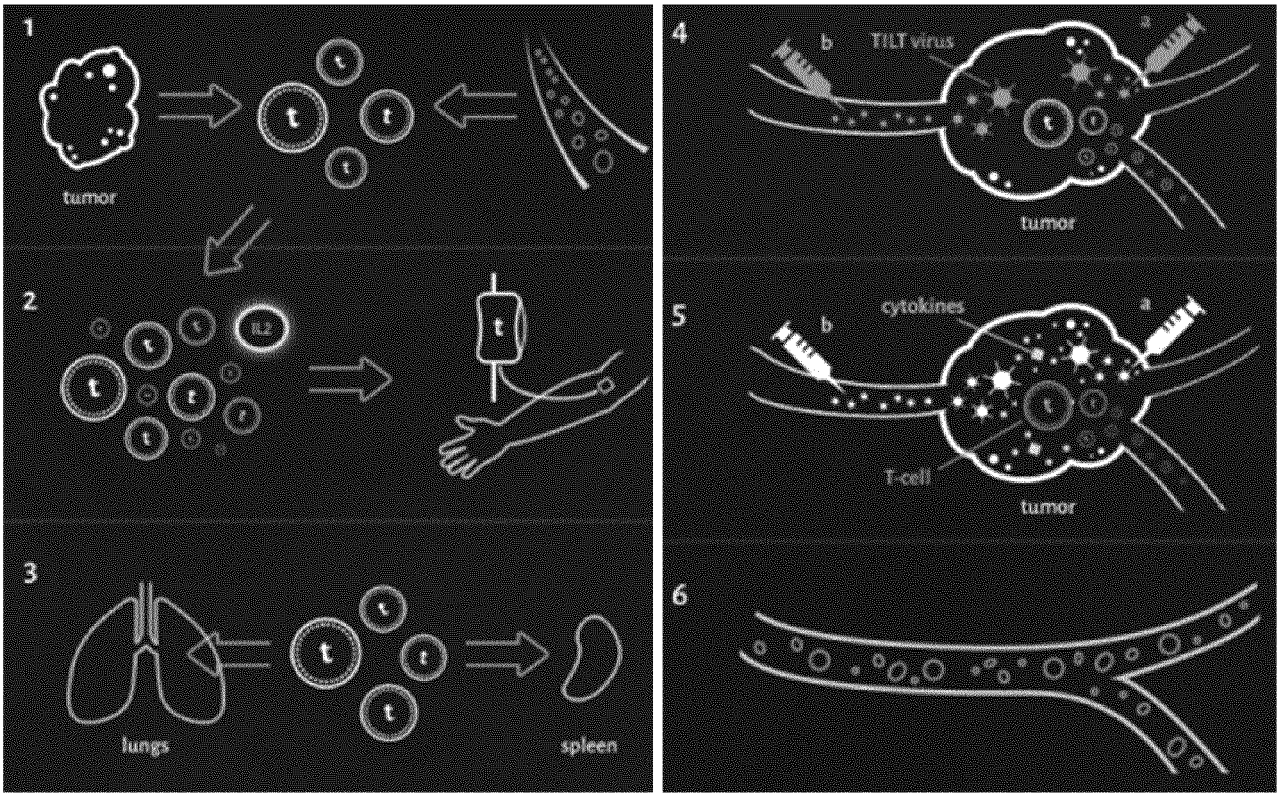



Figure 31.

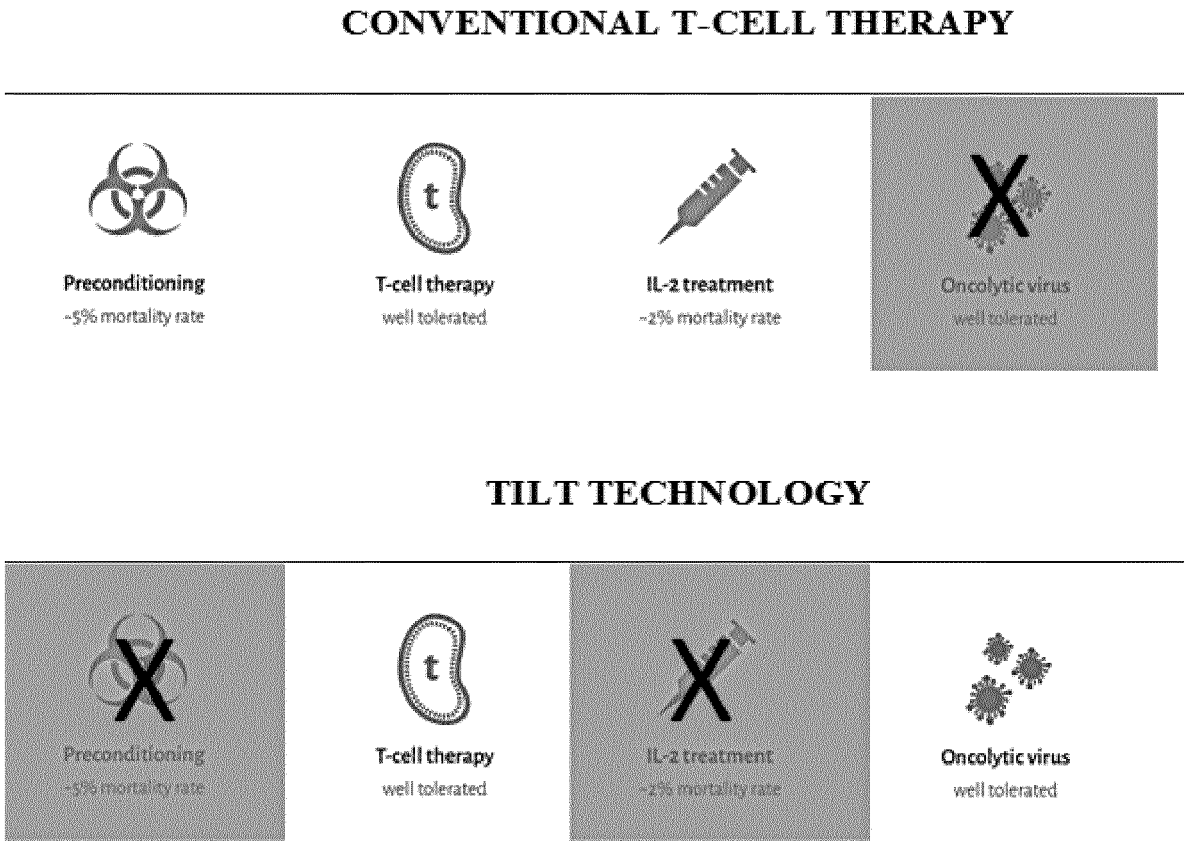



Figure 32.

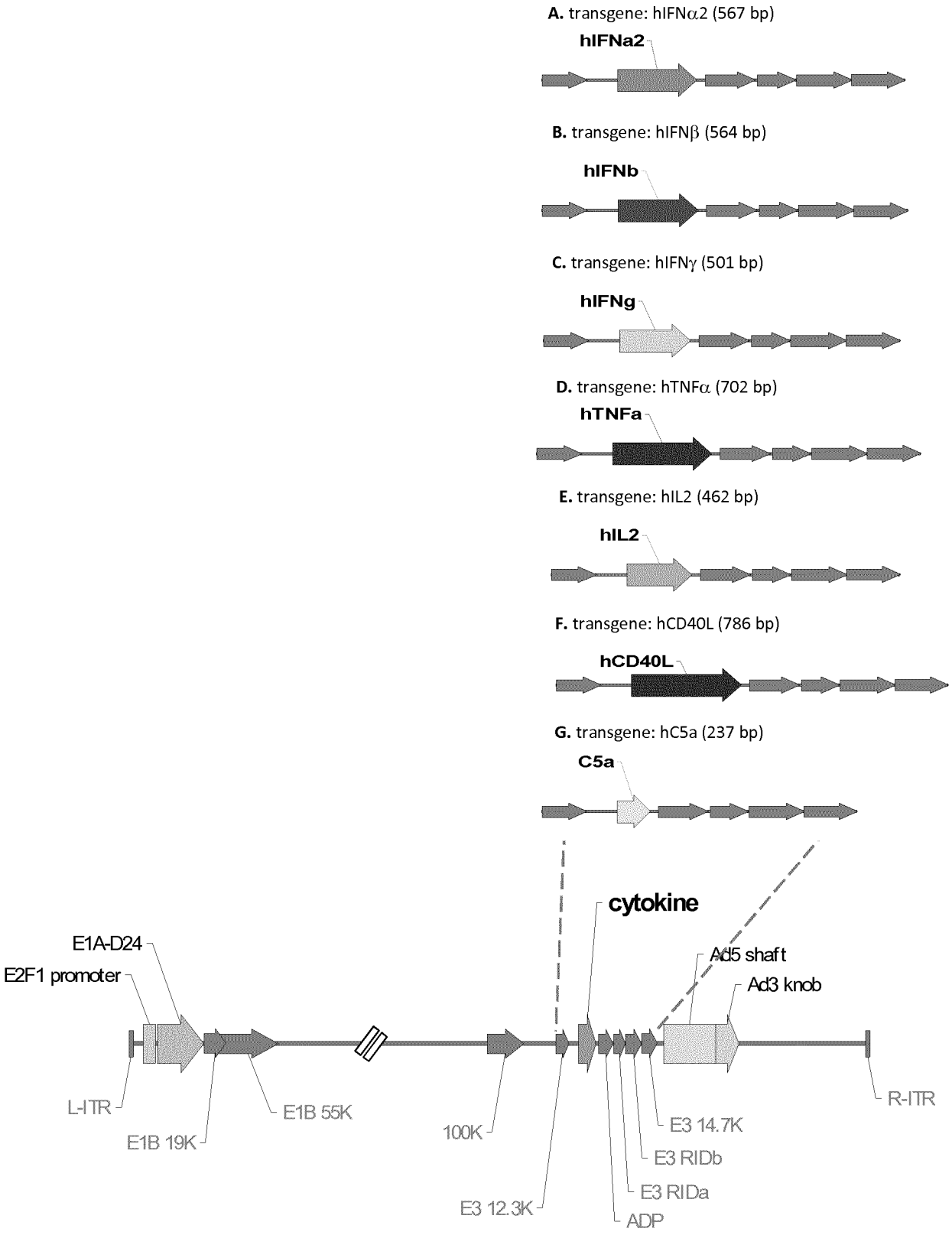

A. transgene: hIFNα2 (567 bp)
hIFNa2
B. transgene: hIFNβ (564 bp)
hIFNb
C. transgene: hIFNγ (501 bp)
hIFNg
D. transgene: hTNFα (702 bp)
hTNFa
E. transgene: hIL2 (462 bp)
hIL2
F. transgene: hCD40L (786 bp)
hCD40L
G. transgene: hC5a (237 bp)
C5a
cytokine
E1A-D24
E2F1 promoter
Ad5 shaft
Ad3 knob
L-ITR
E1B 19K
E1B 55K
100K
E3 12.3K
ADP
E3 RIDa
E3 RIDb
E3 14.7K
R-ITR

Figure 33.

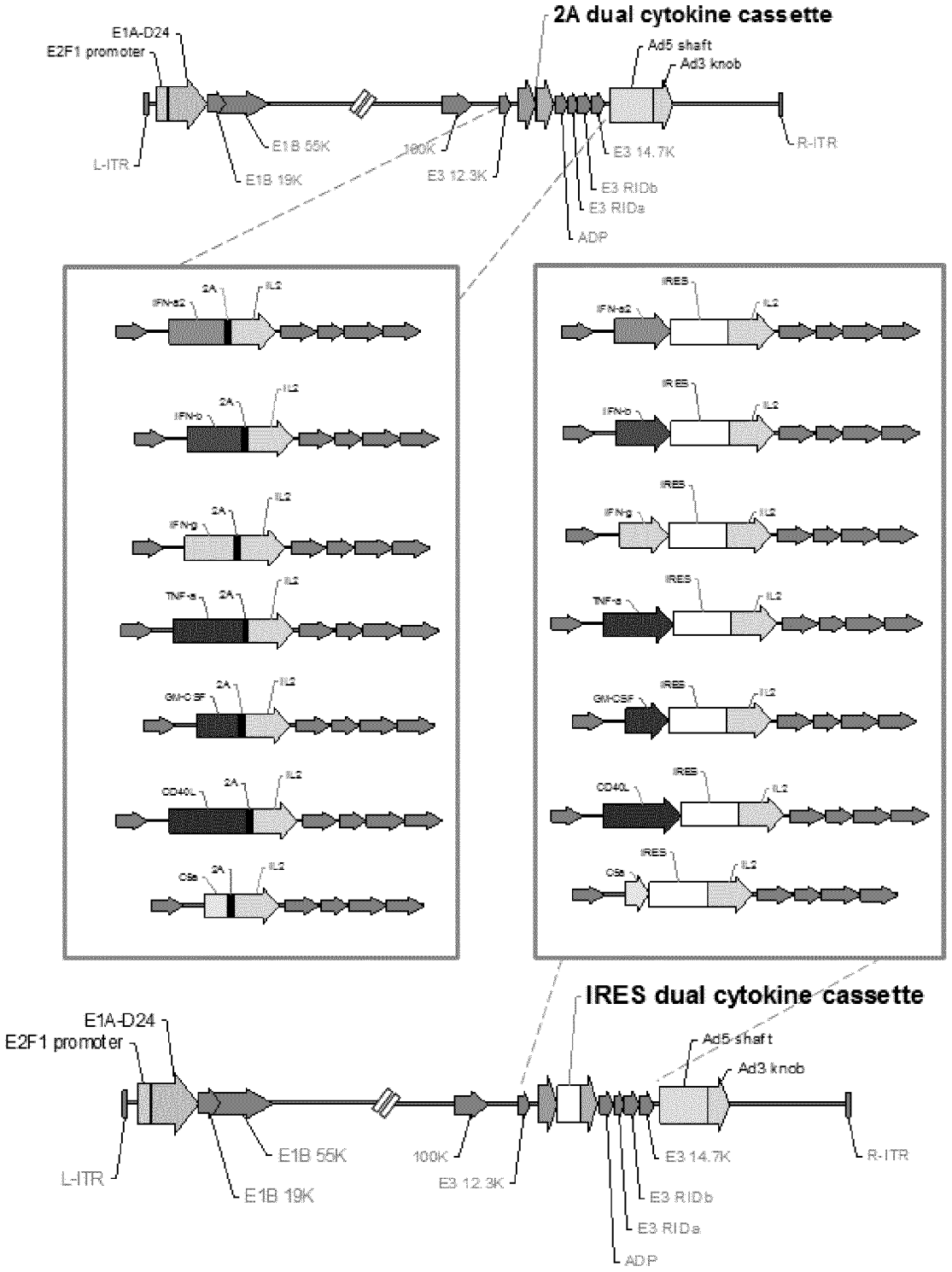

2A dual cytokine cassette
E1A-D24
E2F1 promoter
L-ITR
E1B 19K
E1B 55K
100K
E3 12.3K
E3 14.7K
E3 RIDb
E3 RIDa
ADP
Ad5 shaft
Ad3 knob
R-ITR
IFN-α2
IFN-b
IFN-g
TNF-a
GM-CSF
CD40L
OSt
2A
IL2
IRES
IRES dual cytokine cassette

Figure 34.

**'ribosome shunt site', 'ribosome skipping site', 'cis-acting hydrolase element' (CHYSEL), '2A-like':**

**Foot & mouth disease virus (FMDV) 2A peptide:**

nucleotide sequence: gtgaaaCAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCCGGGCCC

translation: VKQLLNFDLLKLAGDVESNPG P

**Porcine teshcovirus 1 (TV1) 2A peptide:**

nucleotide sequence: ggcagcggcGCCACGAACTTCTCTCTGTTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGGCCT

translation: GSGATNFSLLKQAGDVEENPG P (The GSG spacer improves cleavage; Holst et al., 2006, Nat Prot)

**Thosea asigna virus (TAV) 2A peptide:**

nucleotide sequence: GAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCT

EGRGSLLTCGDVEENPG P

Figure 35.

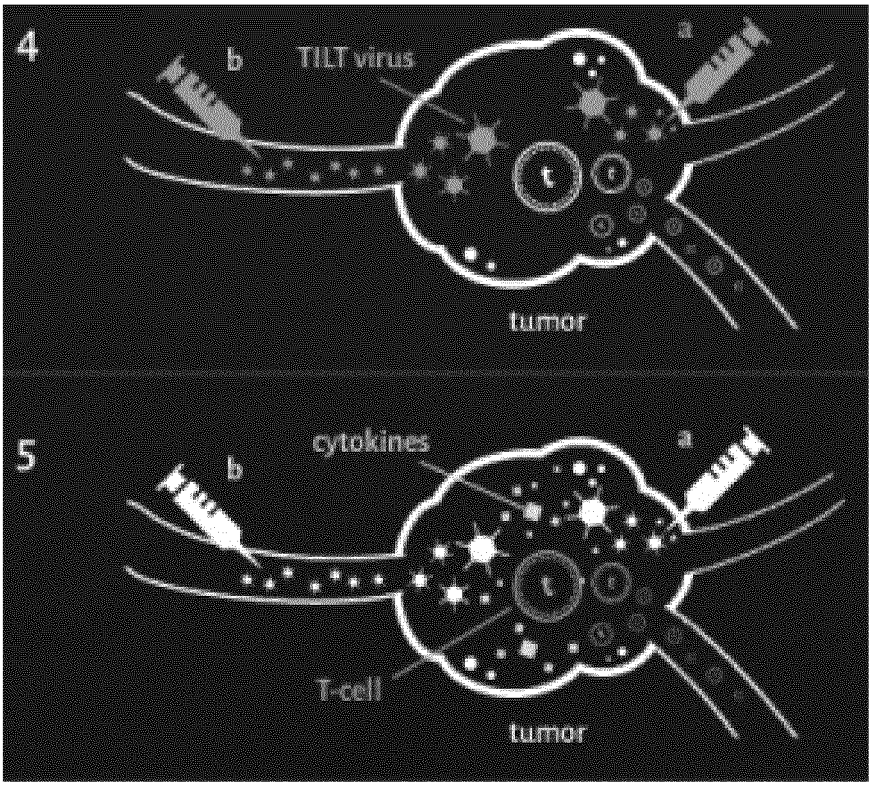
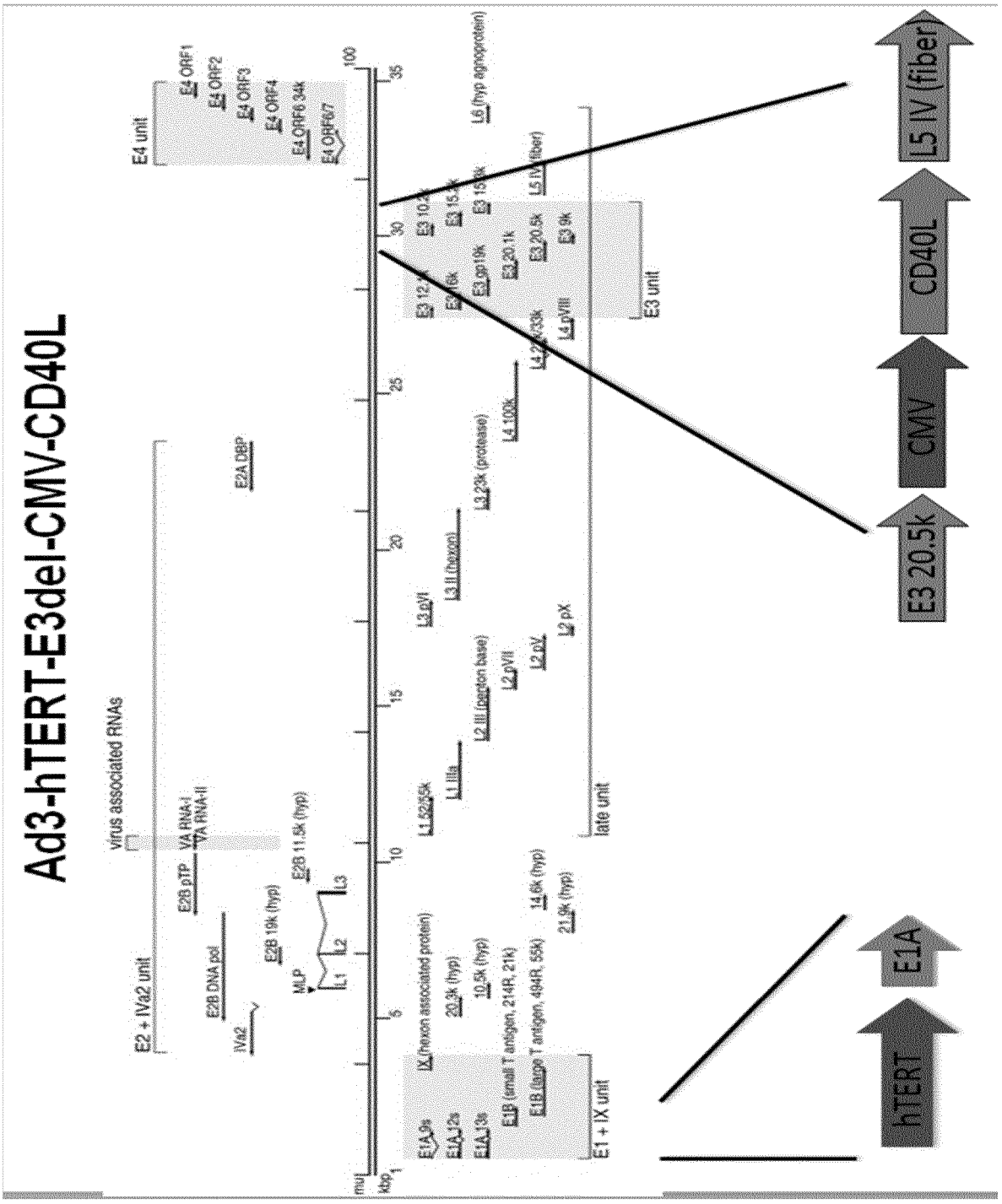


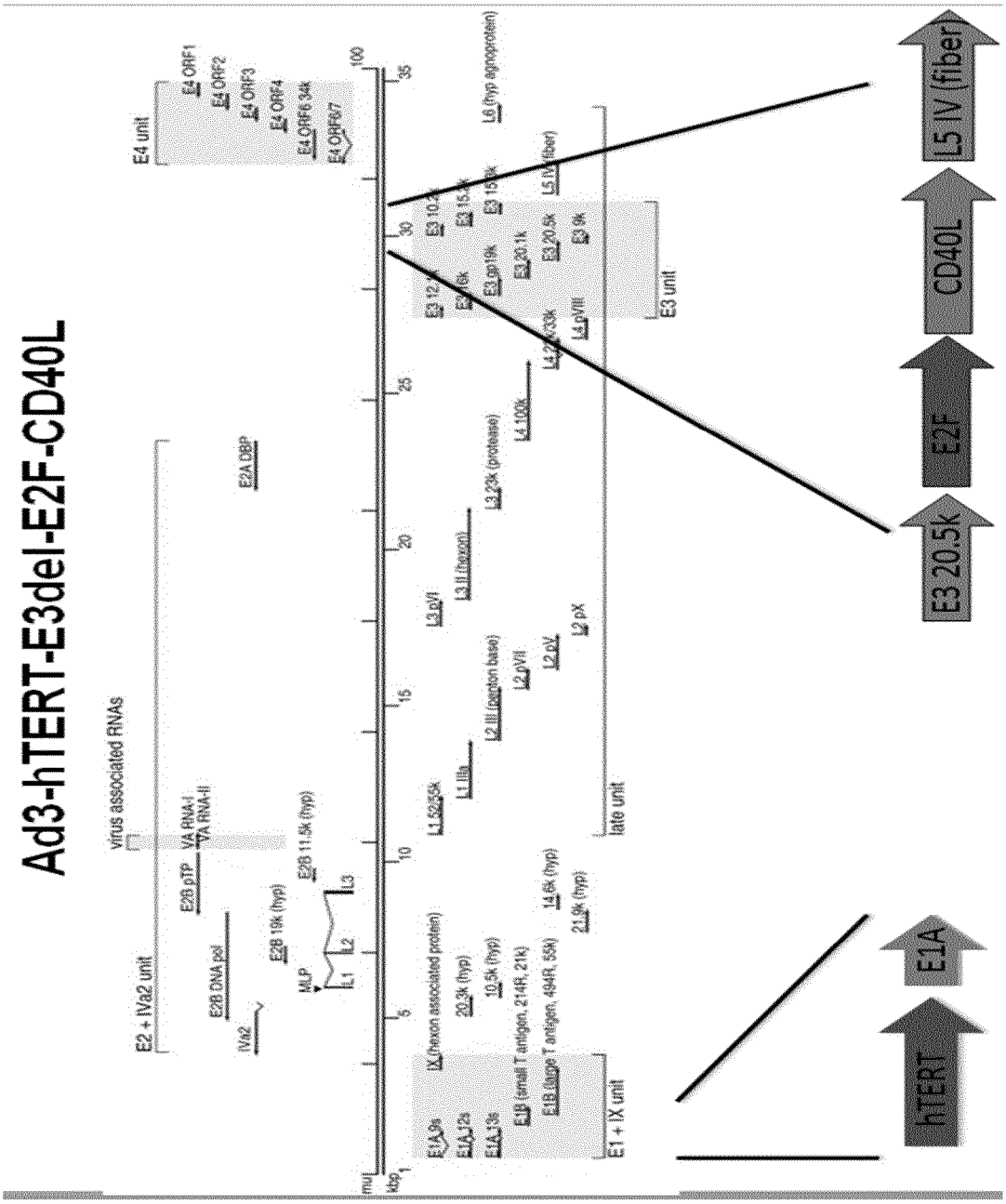

Figure 36.




hTERT promoter has replaced the native E1A promoter

Deletion of area 29892-30947 (Genbank accession no. DQ086466) affects the following features in the E3 unit: E3 9-kDa, E3 10.2-kDa, E3 15.2-kDa, E3 15.3-kDa

Figure 37.




hTERT promoter has replaced the native E1A promoter

Deletion of area 29892-30947 (Genbank accession no. DQ086466) affects the following features in the E3 unit: E3 9-kDa, E3 10.2-kDa, E3 15.2-kDa, E3 15.3-kDa

Figure 38.

pWEA-Ad3-hTERT-CMV-CD40L

1. BamHI
2. BglI
3. BglII
4. NcoI
5. SacI
6. HindIII
7. EcoRI
8. FseI
9. uncut

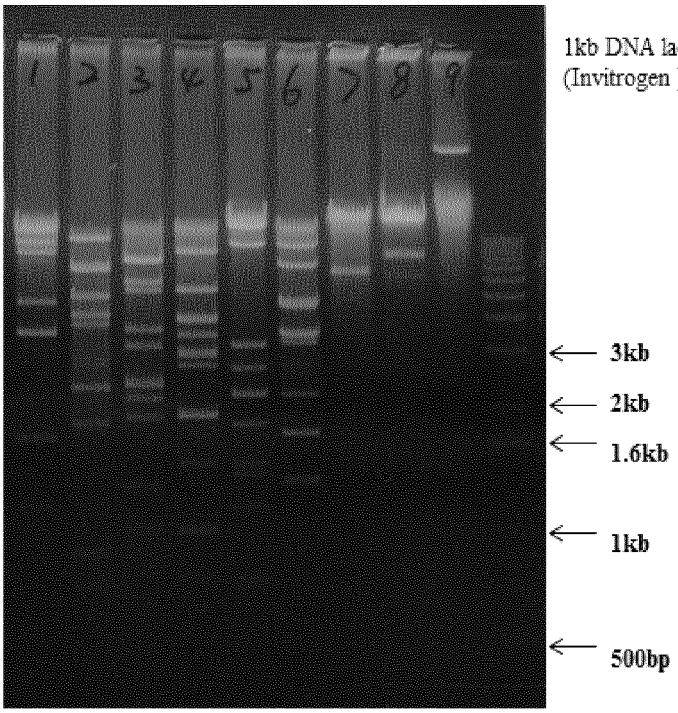



Figure 39.

pWEA-Ad3-hTERT-E2F-CD40L

1. BamHI
2. BglI
3. BglII
4. NcoI
5. SacI
6. HindIII
7. EcoRI
8. FseI
9. uncut

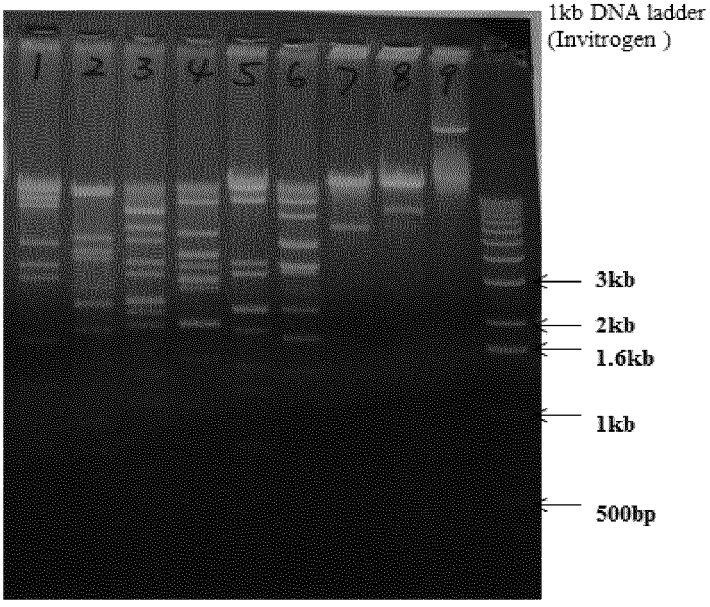



Figure 40.

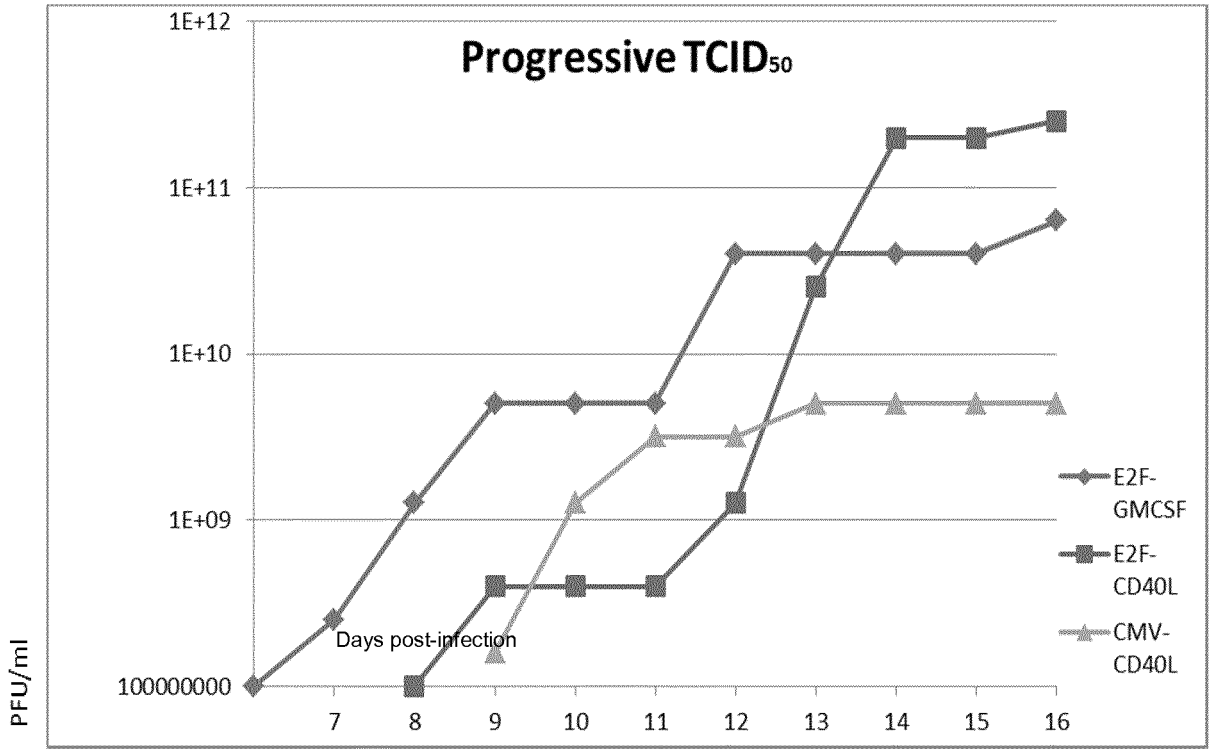



Figure 41.

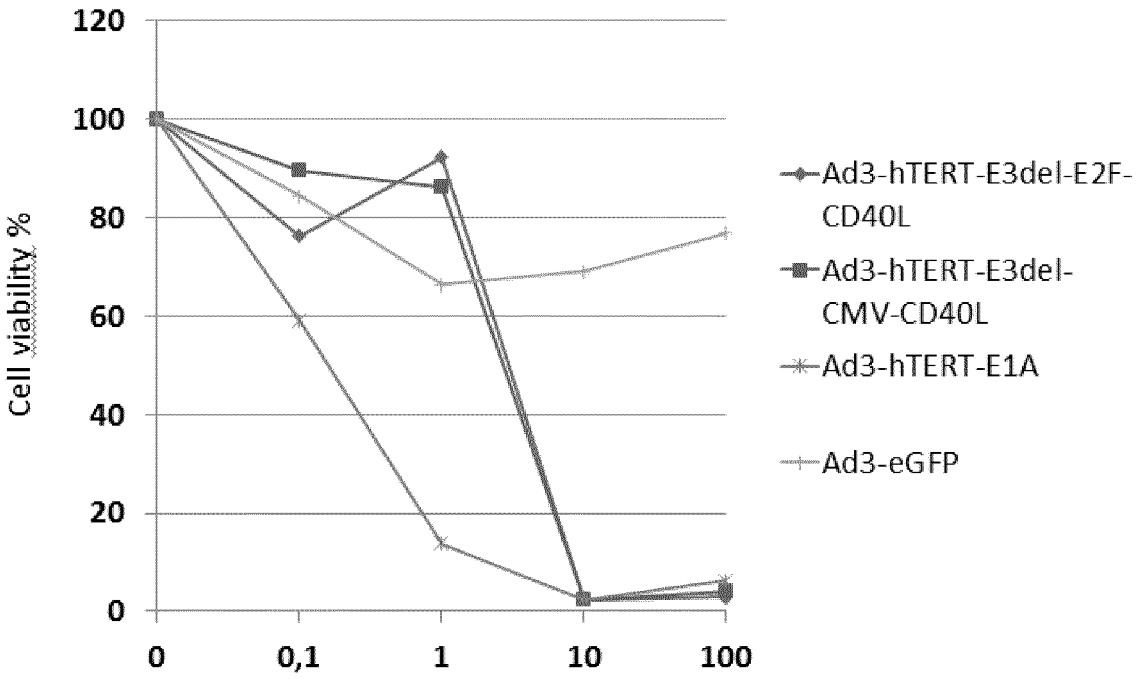



Figure 42.

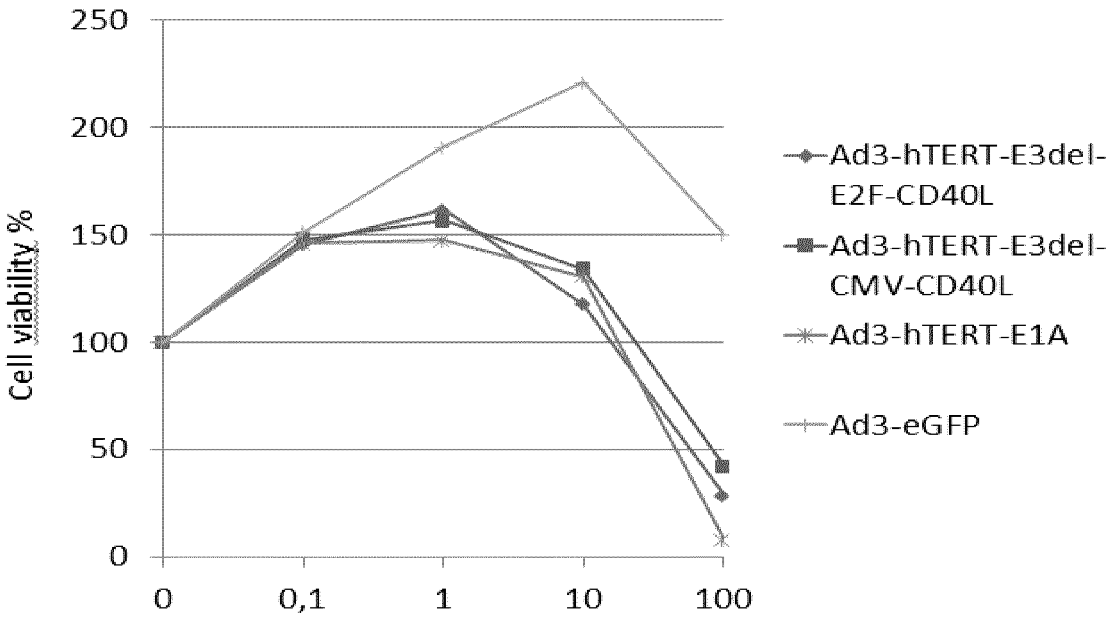



Figure 43.

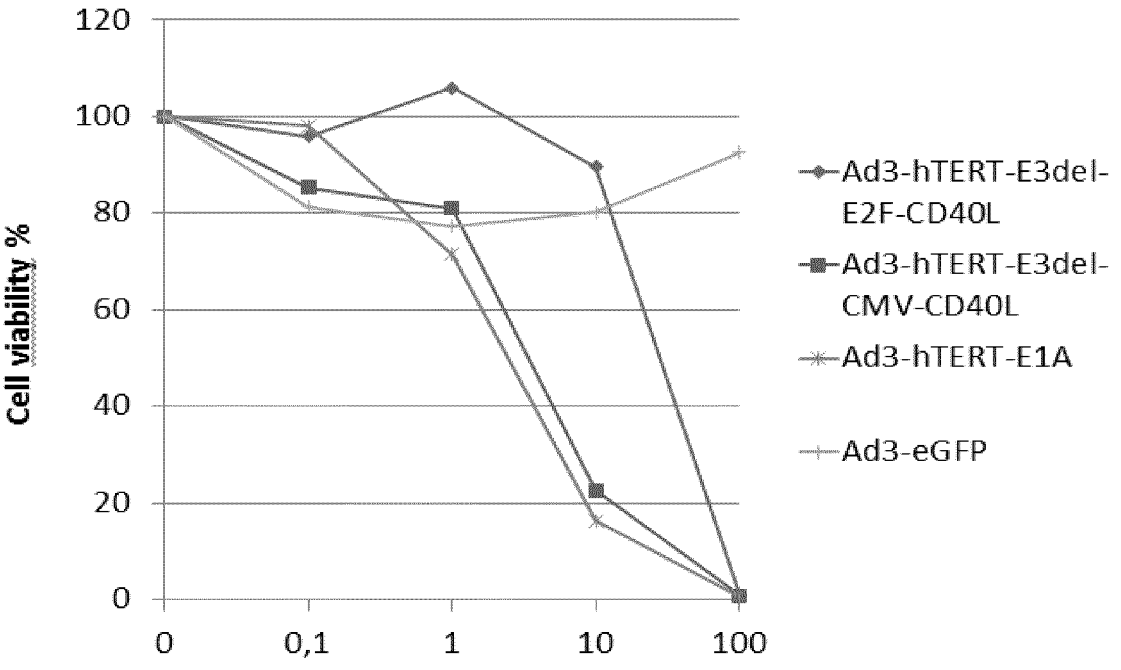



Figure 44.

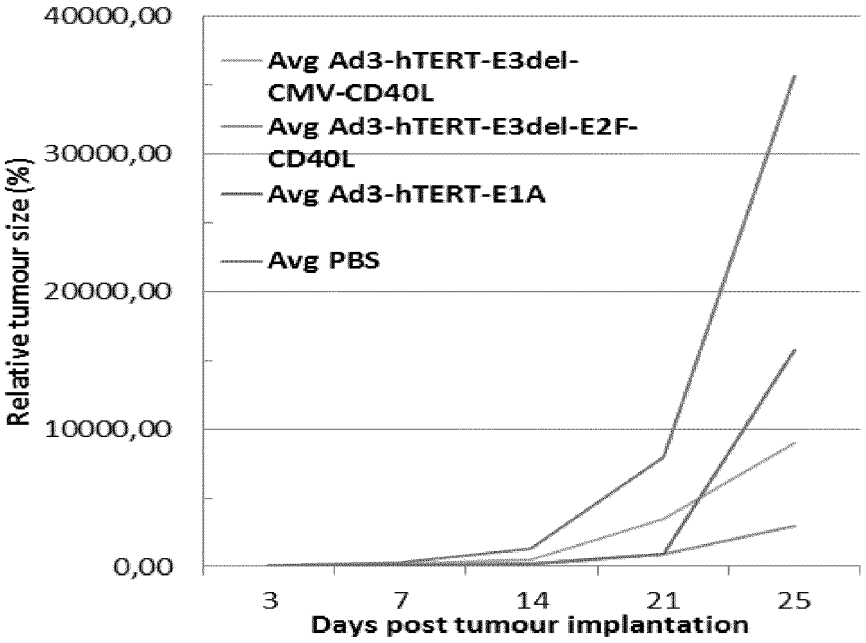



Figure 45.

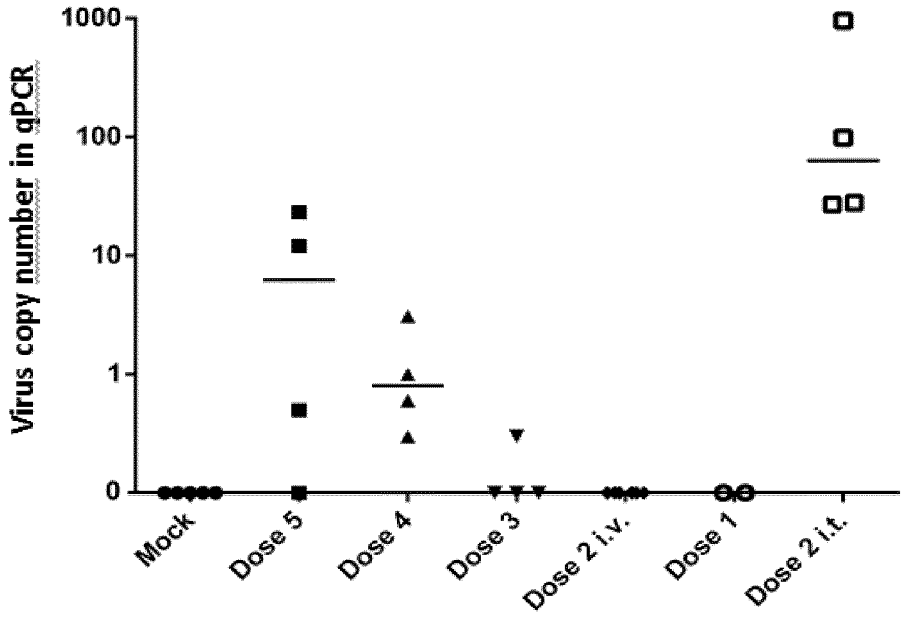



Figure 46.

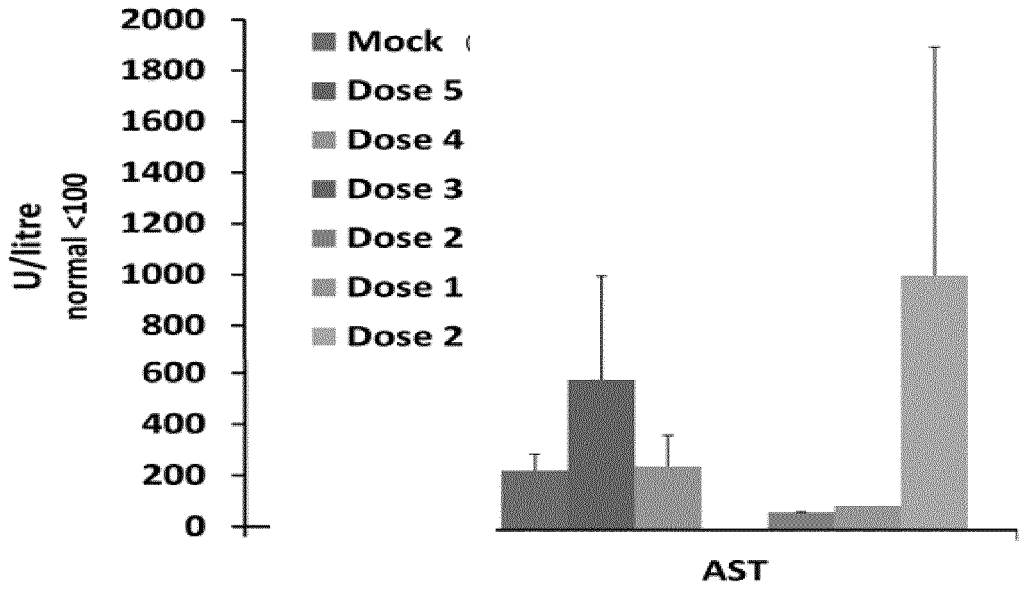



Figure 47.

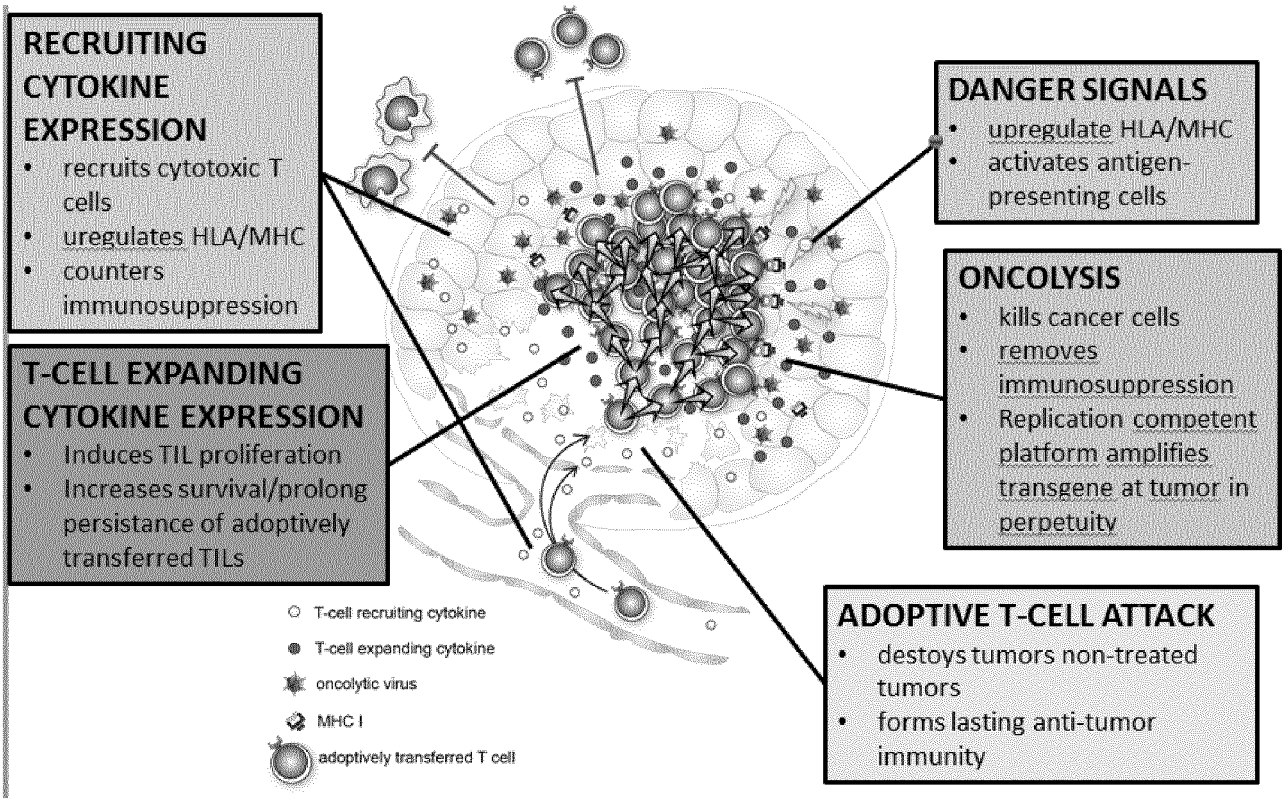



Figure 48.

| Groups | SAC 1 | SAC 2 |
|---|---|---|
| PBS | 5 | 5 |
| IFN-α2a | 5 | 5 |
| IFN-β | 5 | 5 |
| IFN-γ | 5 | 5 |
| IL-2 | 5 | 5 |
| GMCSF | 5 | 5 |
| CD40L | 5 | 5 |
| TNF-α | 5 | 5 |
| C5a | 5 | 5 |
| | 45 | 45 |

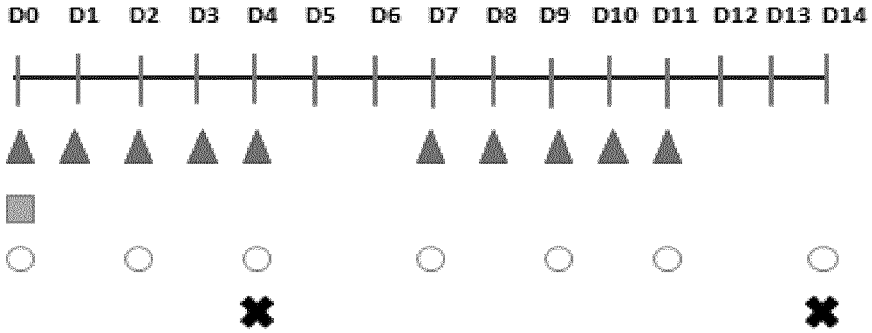



Figure 49.

| GROUP | SAC1 | SAC2 | SAC3 |
|---|---|---|---|
| PBS | 5 | 5 | 5 |
| unarmed RD control virus | 5 | 5 | 5 |
| unarmed oncolytic virus | 5 | 5 | 5 |
| C5a oncolytic virus | 5 | 5 | 5 |
| TNFa oncolytic virus | 5 | 5 | 5 |
| CD40L RD virus | 5 | 5 | 5 |
| IFNg RD virus | 5 | 5 | 5 |
| | **35** | **35** | **35** |

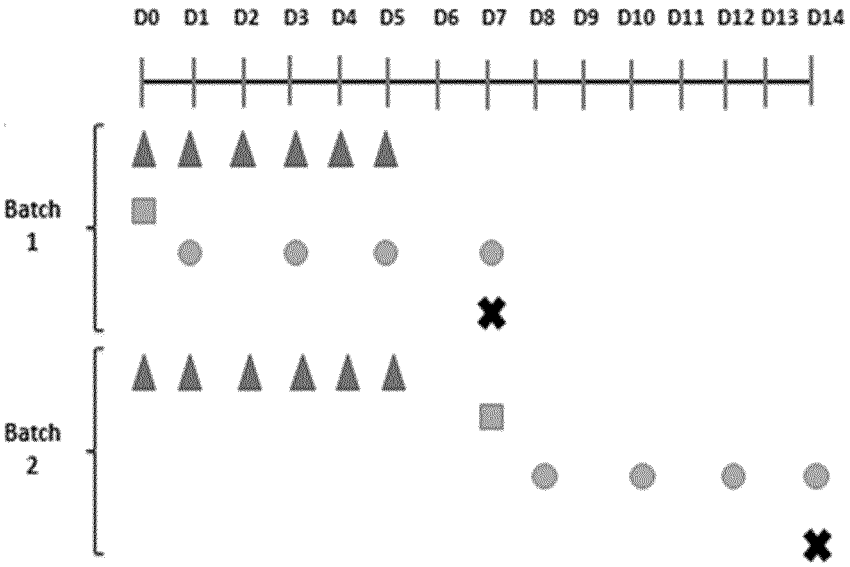



Figure 50.

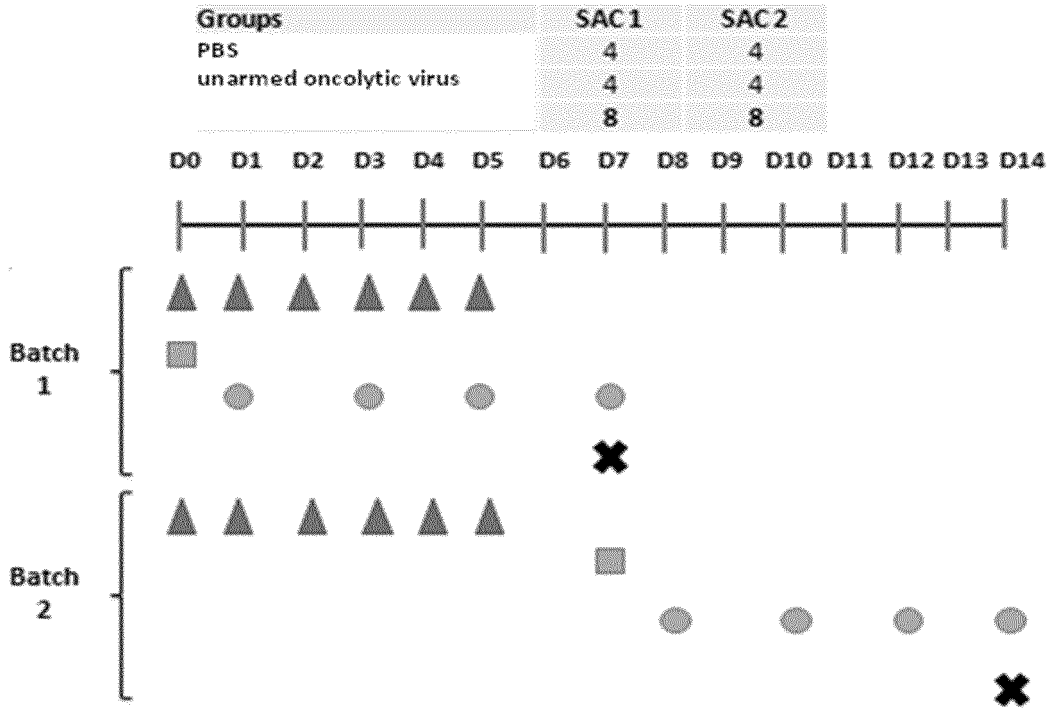

Groups
SAC 1
SAC 2
PBS
4
4
unarmed oncolytic virus
4
4
8
8
D0 D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
Batch 1
Batch 2

Figure 51.