US 20070243170A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0243170 A1**

Roizman et al. (43) **Pub. Date: Oct. 18, 2007**

(54) **TARGETING OF HERPES SIMPLEX VIRUS TO SPECIFIC RECEPTORS**

(75) Inventors: **Bernard Roizman**, Chicago, IL (US); **Guoying Zhou**, Chicago, IL (US)

Correspondence Address:
MARSHALL, GERSTEIN & BORUN LLP
233 S. WACKER DRIVE, SUITE 6300
SEARS TOWER
CHICAGO, IL 60606 (US)

(73) Assignee: **THE UNIVERSITY OF CHICAGO**, Chicago, IL (US)

(21) Appl. No.: **11/677,026**

(22) Filed: **Feb. 20, 2007**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US06/32291, filed on Aug. 18, 2006.
Continuation-in-part of application No. 11/215,636, filed on Aug. 30, 2005, which is a continuation-in-part of application No. 10/530,774, filed on Nov. 17, 2005, filed as 371 of international application No. PCT/US03/31598, filed on Oct. 6, 2003.

(60) Provisional application No. 60/709,597, filed on Aug. 19, 2005. Provisional application No. 60/416,716, filed on Oct. 7, 2002.

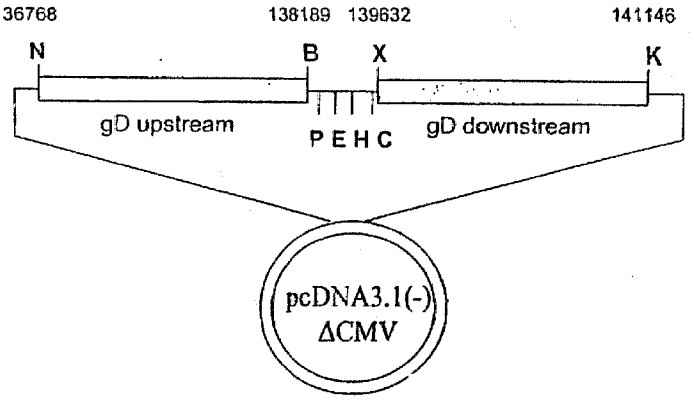
Publication Classification

(51) **Int. Cl.**
A61K 35/76 (2006.01)
C12N 7/00 (2006.01)

(52) **U.S. Cl.** **424/93.6**; 435/235.1

(57) **ABSTRACT**

The invention relates to engineered herpes simplex virus (HSV) particles that are targeted to one or more specific binding pair members, such as receptors. Also, recombinant vectors for producing such HSV particles are provided. By reducing the affinity of HSV for its natural receptor(s) and increasing the affinity for a selected receptor, the HSV particles of the invention are useful for targeting cells that express the selected receptor, which itself may be a product of genetic engineering. The ability to selectively target cells renders the HSV particles particularly useful in selectively diagnosing, treating, and imaging cells bearing the selected binding pair member, such as a receptor. The invention also provides for polynucleotide-based therapy to cells bearing the selected binding pair member such as a receptor.



pgD- in pcDNA3.1(-) with CMV promoter collapsed by NruI/NheI digestion. The fragment containing gD upstream at NotI/BamHI, and gD downstream at XhoI/KpnI in pBluescript II SK was excised by NotI/KpnIand ligated into pcDNA3.1(-) in which the CMV promoter had been collapsed. N, NotI, B, BamHI, P, PstI, E, EcoRI, H, HindIII, C, ClaI, X, XhoI, and K, KpnI.

Figure 1

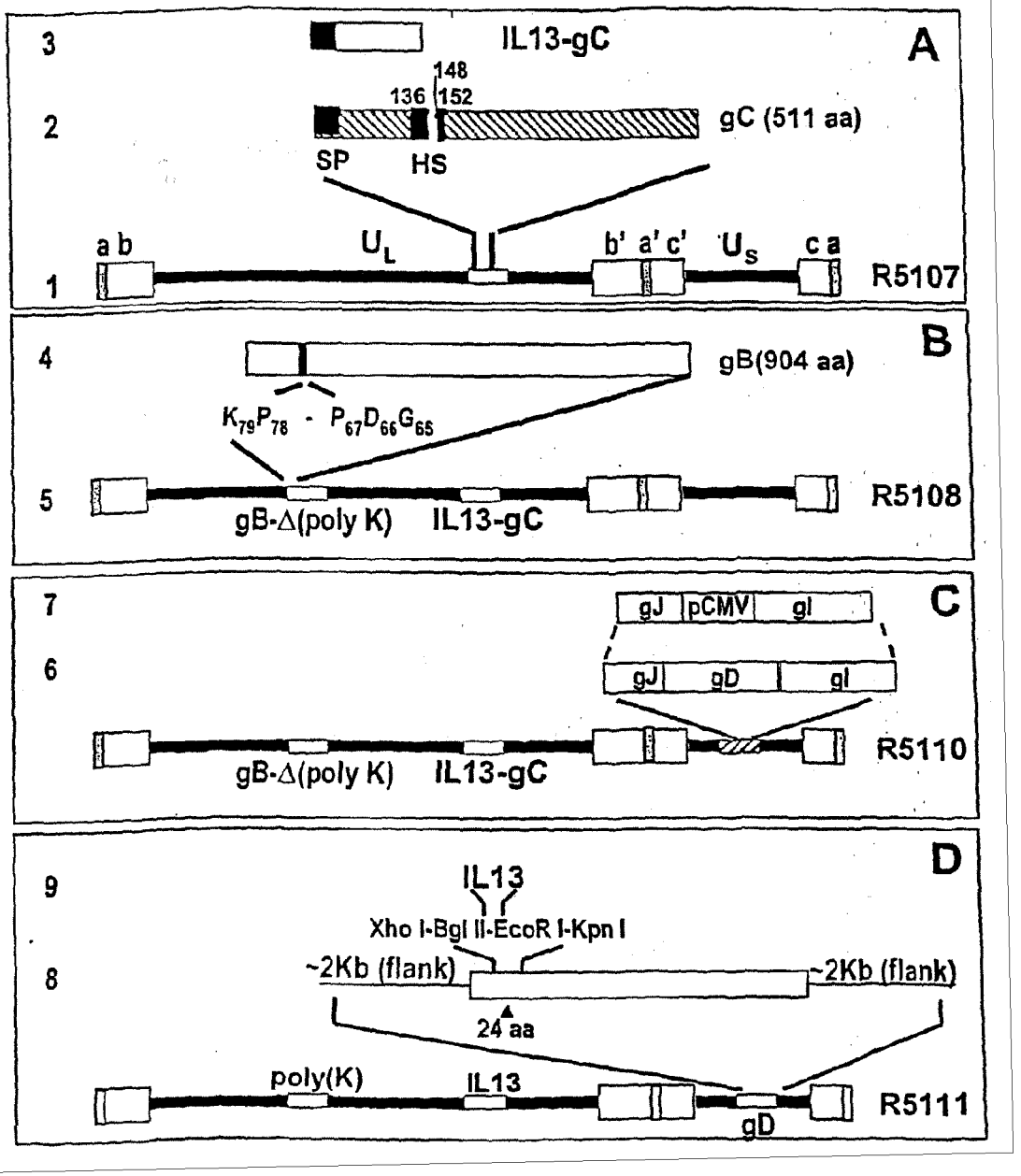

3
IL13-gC
A
148
136
152
2
gC (511 aa)
SP
HS
a b
U_L
b' a' c'
U_S
c a
1
R5107
4
gB(904 aa)
B
$K_{79}P_{78}$ - $P_{67}D_{66}G_{65}$
5
R5108
gB-Δ(poly K)
IL13-gC
7
gJ
pCMV
gI
C
6
gJ
gD
gI
R5110
gB-Δ(poly K)
IL13-gC
9
IL13
D
Xho I-Bgl II-EcoR I-Kpn I
8
~2Kb (flank)
~2Kb (flank)
24 aa
poly(K)
IL13
R5111
gD

Figure 2

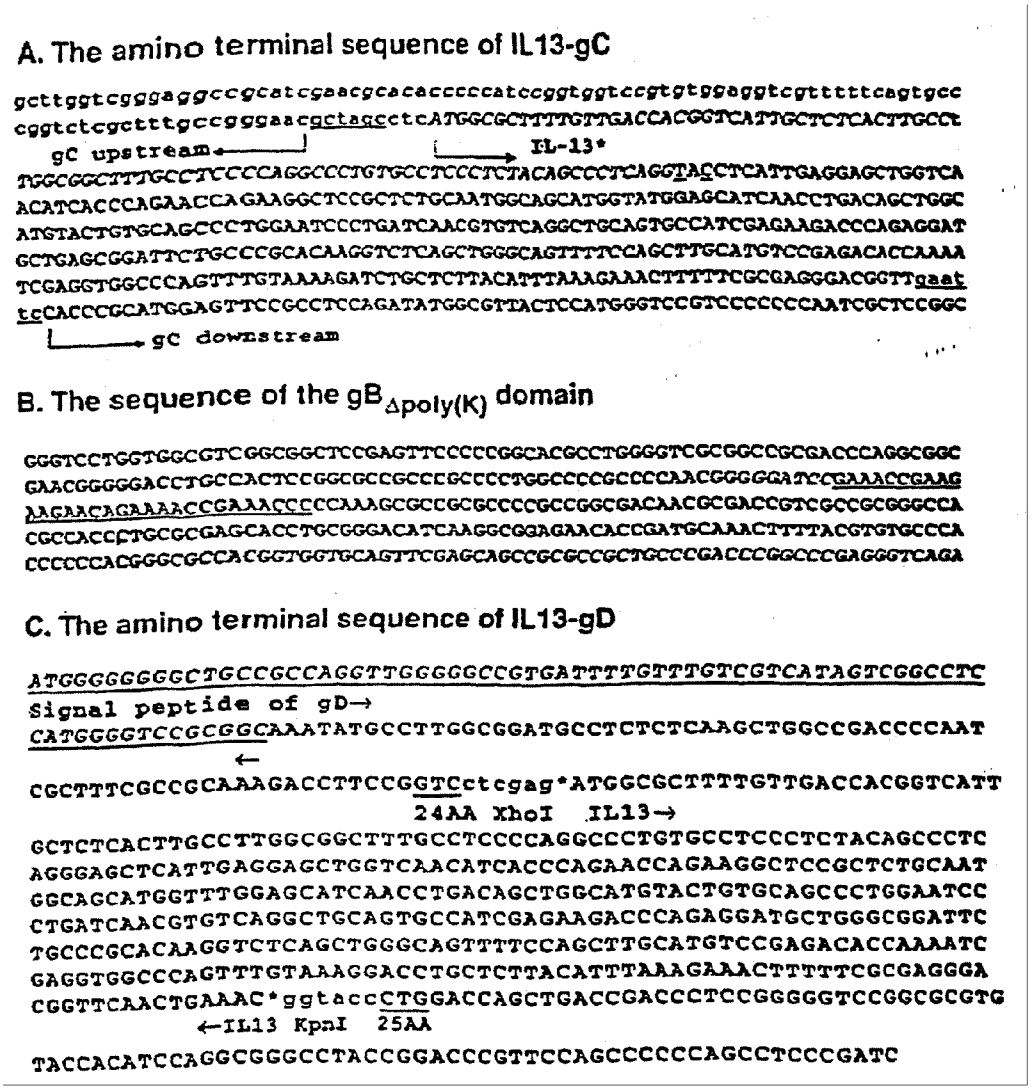

A. The amino terminal sequence of IL13-gC
gc upstream
IL-13*
gc downstream
B. The sequence of the gB Δpoly(K) domain
C. The amino terminal sequence of IL13-gD
Signal peptide of gD→
←
24AA XhoI IL13→
←IL13 KpnI 25AA

Figure 3

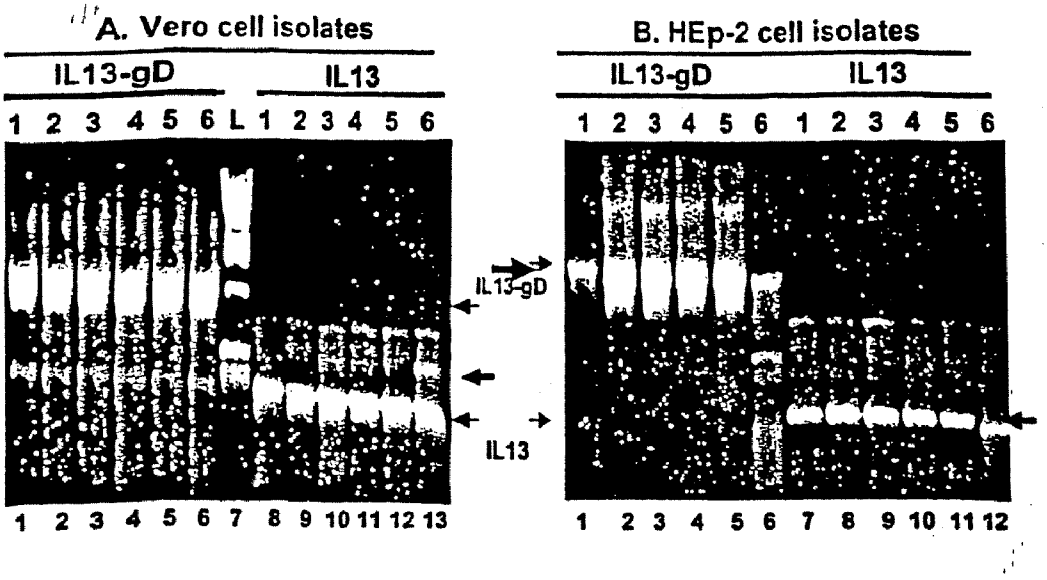

A. Vero cell isolates
IL13-gD
IL13
1 2 3 4 5 6 L 1 2 3 4 5 6
1 2 3 4 5 6 7 8 9 10 11 12 13
B. HEp-2 cell isolates
IL13-gD
IL13
1 2 3 4 5 6 1 2 3 4 5 6
1 2 3 4 5 6 7 8 9 10 11 12
IL13-gD
IL13

Figure 4

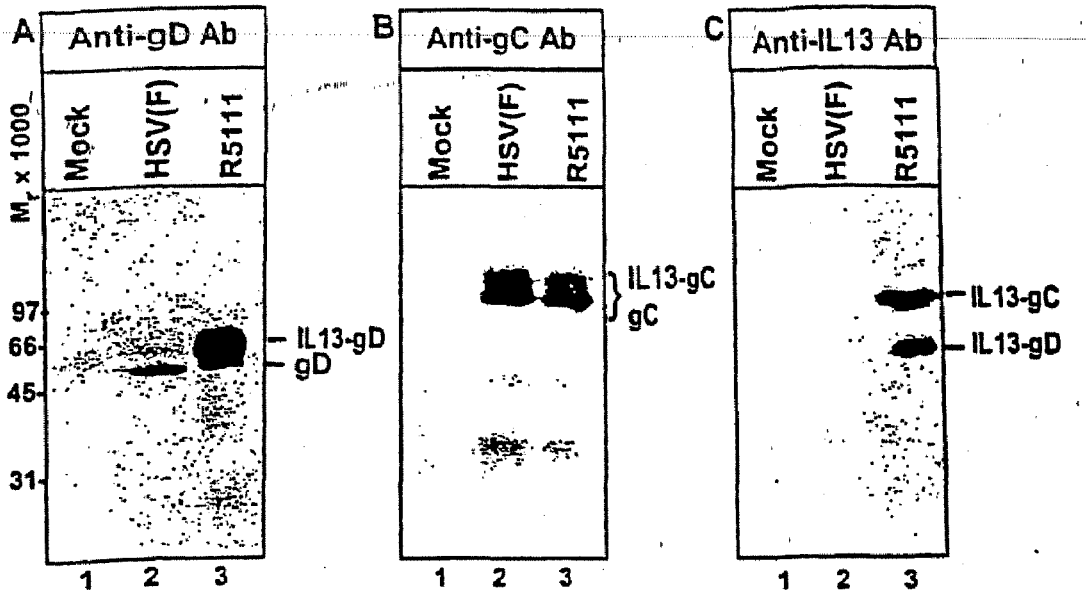

A
Anti-gD Ab
Mock
HSV(F)
R5111
M_r x 1000
97
66
45
31
IL13-gD
gD
1 2 3
B
Anti-gC Ab
Mock
HSV(F)
R5111
IL13-gC
gC
1 2 3
C
Anti-IL13 Ab
Mock
HSV(F)
R5111
IL13-gC
IL13-gD
1 2 3

Figure 5

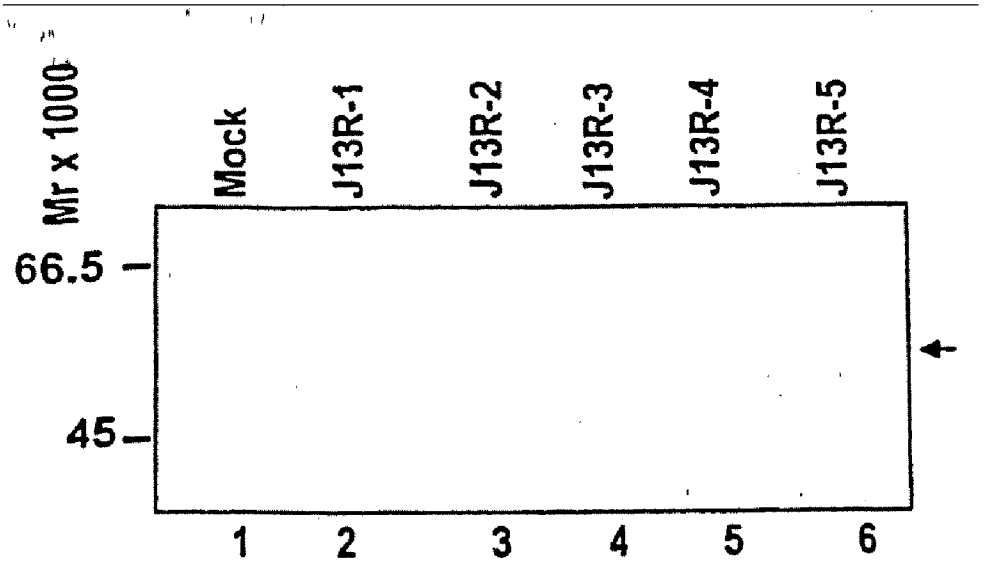
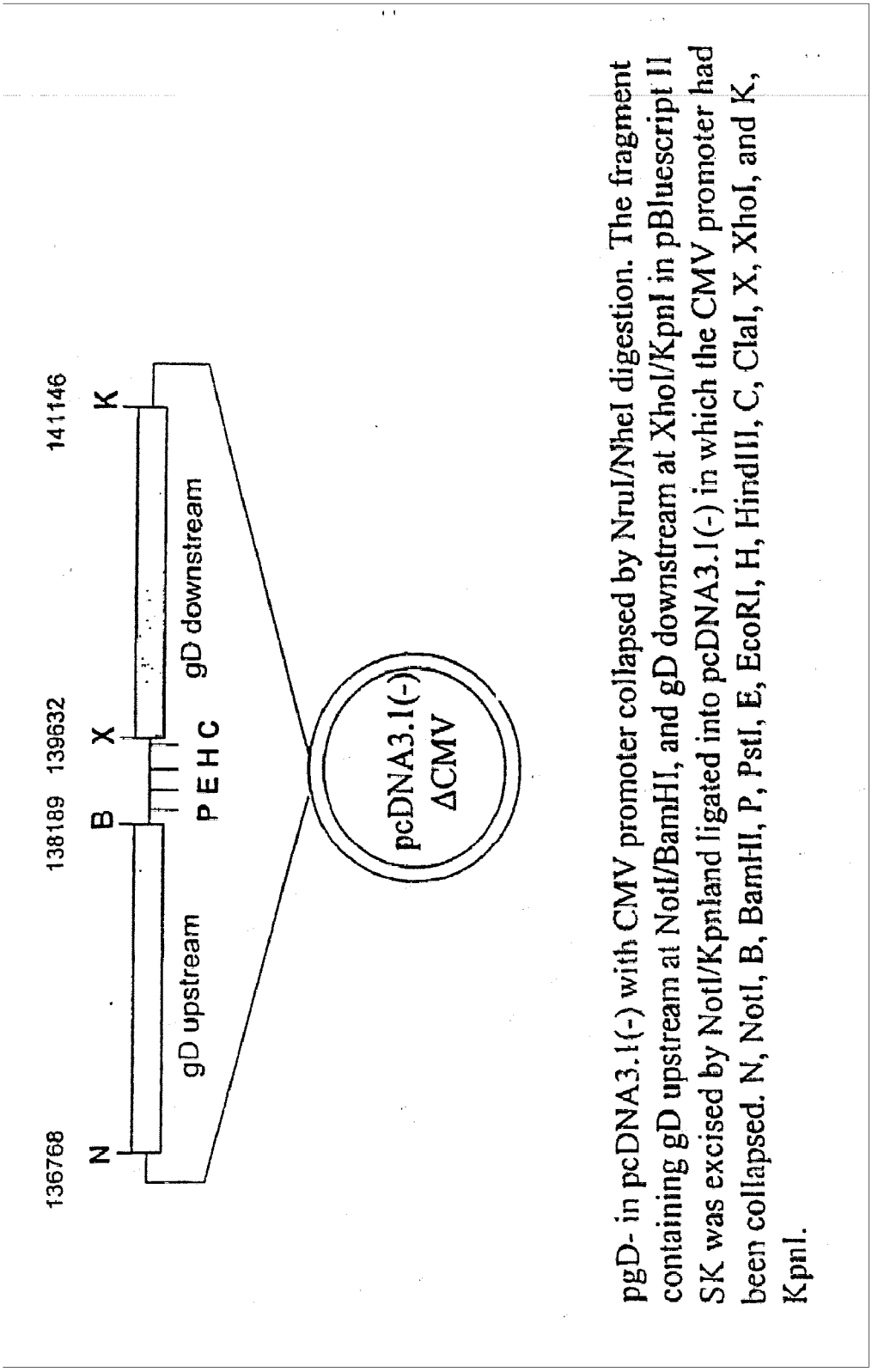


Figure 6



pgD- in pcDNA3.1(-) with CMV promoter collapsed by NruI/NheI digestion. The fragment containing gD upstream at NotI/BamHI, and gD downstream at XhoI/KpnI in pBluescript II SK was excised by NotI/KpnIand ligated into pcDNA3.1(-) in which the CMV promoter had been collapsed. N, NotI, B, BamHI, P, PstI, E, EcoRI, H, HindIII, C, ClaI, X, XhoI, and K, KpnI.

Figure 7

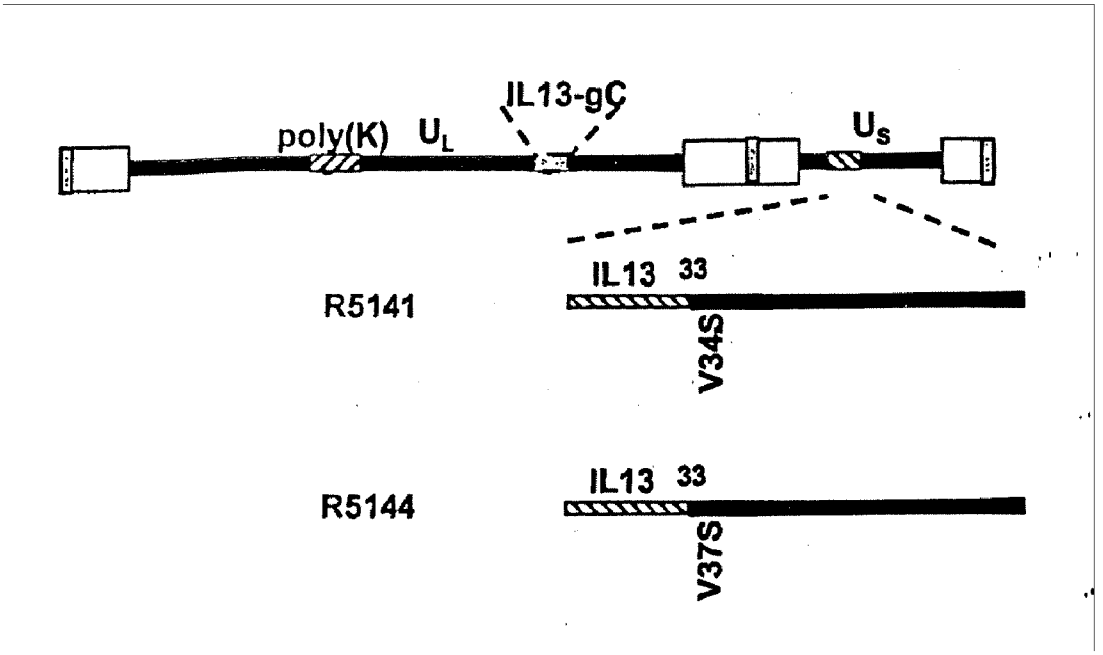


Figure 8

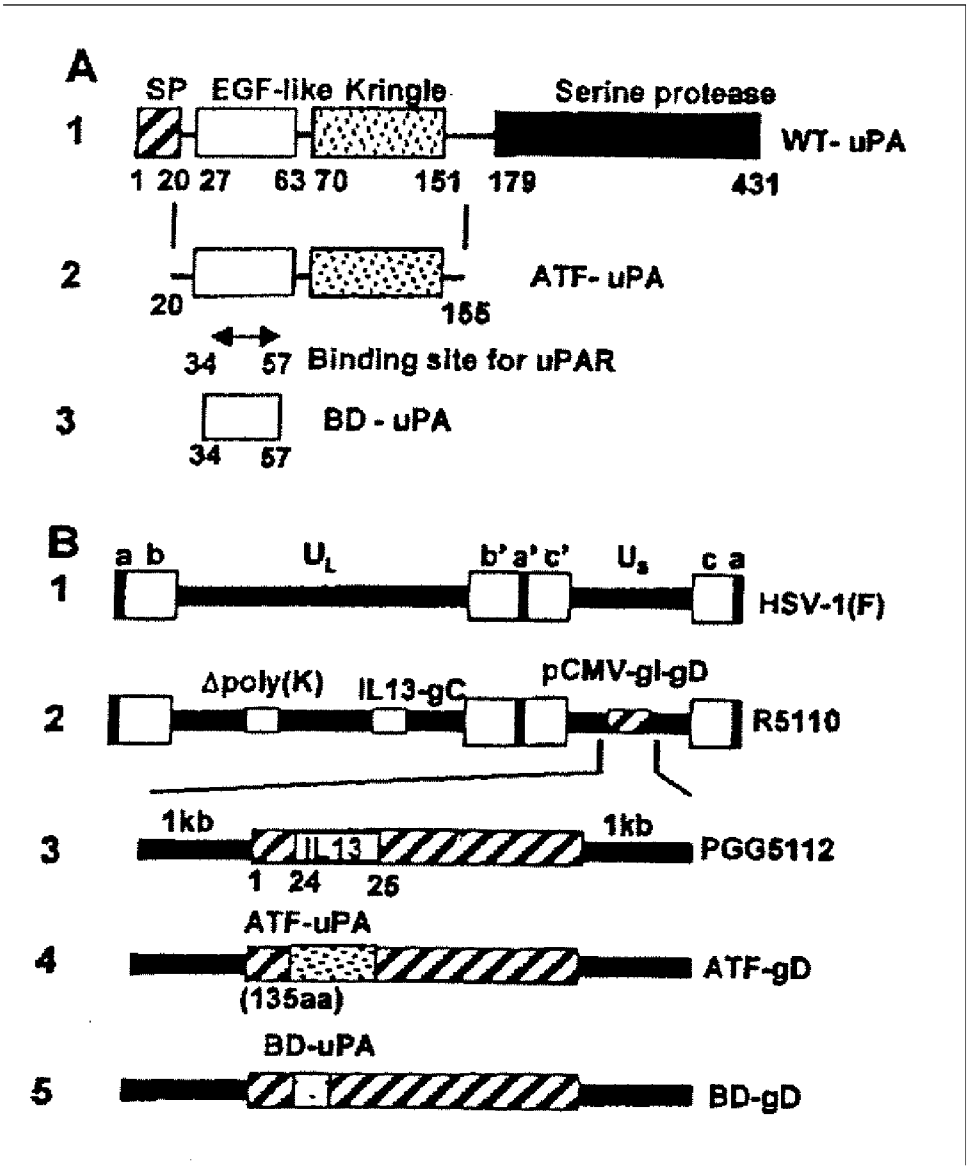
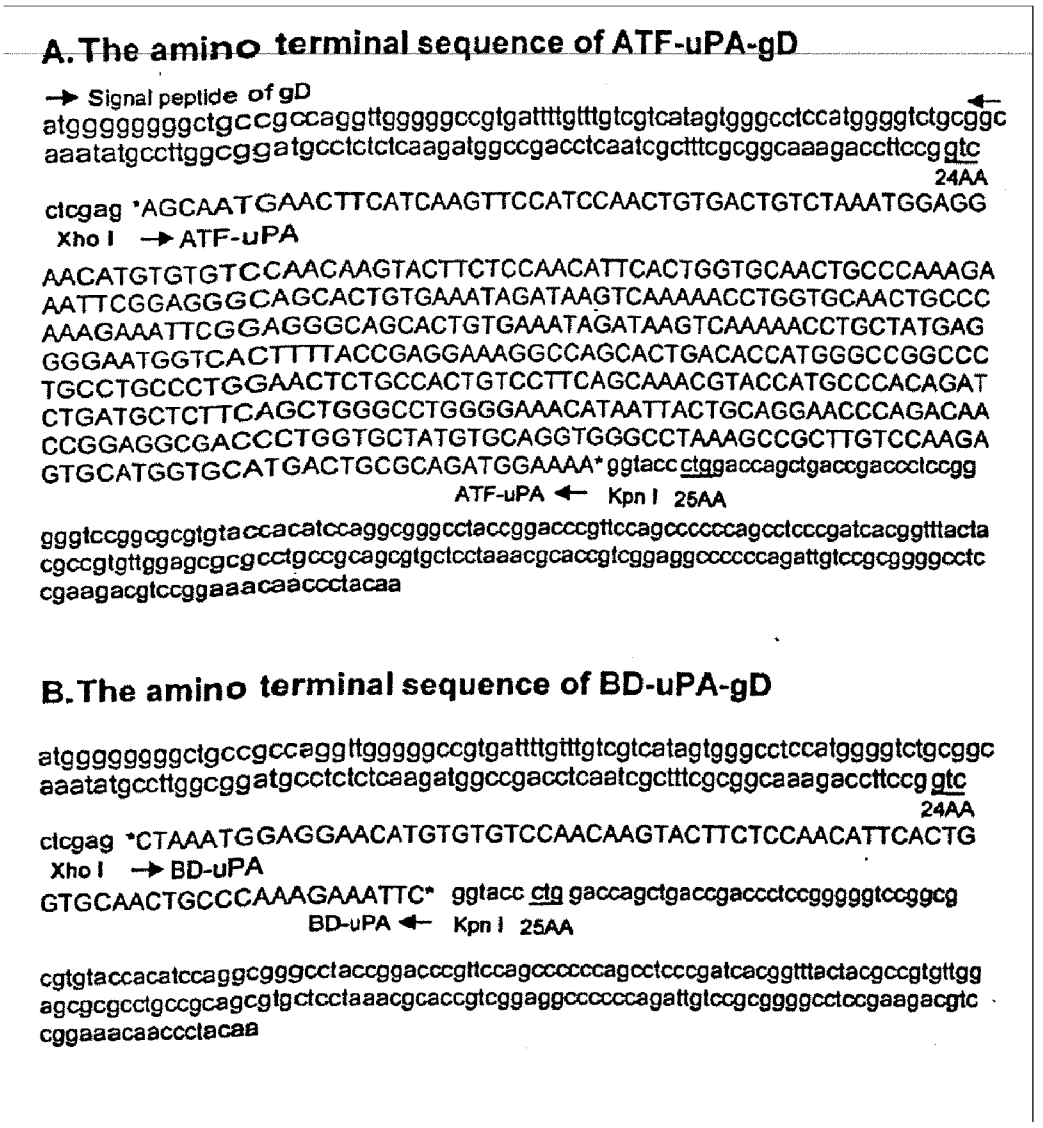

A
SP
EGF-like
Kringle
Serine protease
1
WT- uPA
1 20 27 63 70 151 179 431
2
ATF- uPA
20
155
34 57 Binding site for uPAR
3
BD - uPA
34 57
B
a b
U_L
b' a' c'
U_s
c a
1
HSV-1(F)
Δpoly(K)
IL13-gC
pCMV-gI-gD
2
R5110
1kb
1kb
3
IL13
PGG5112
1 24 26
ATF-uPA
4
ATF-gD
(135aa)
BD-uPA
5
BD-gD

Figure 9


A. The amino terminal sequence of ATF-uPA-gD
→ Signal peptide of gD
atgggggggctgccgccaggttgggggccgtgattttgtttgtcgtcatagtgggcctccatggggtctgcggc
aaatatgccttggcggatgcctctctcaagatggccgacctcaatcgctttcgcggcaaagaccttccg gtc
24AA
ctcgag *AGCAATGAACTTCATCAAGTTCCATCCAACTGTGACTGTCTAAATGGAGG
Xho I → ATF-uPA
AACATGTGTGTCCAACAAGTACTTCTCCAACATTCACTGGTGCAACTGCCCAAAGA
AATTCGGAGGGCAGCACTGTGAAATAGATAAGTCAAAAACCTGGTGCAACTGCCC
AAAGAAATTCGGAGGGCAGCACTGTGAAATAGATAAGTCAAAAACCTGCTATGAG
GGGAATGGTCACTTTTACCGAGGAAAGGCCAGCACTGACACCATGGGCCGGCCC
TGCCTGCCCTGGAACTCTGCCACTGTCCTTCAGCAAACGTACCATGCCCACAGAT
CTGATGCTCTTCAGCTGGGCCTGGGGAAACATAATTACTGCAGGAACCCAGACAA
CCGGAGGCGACCCTGGTGCTATGTGCAGGTGGGCCTAAAGCCGCTTGTCCAAGA
GTGCATGGTGCATGACTGCGCAGATGGAAAA* ggtacc ctggaccagctgaccgaccctccgg
ATF-uPA ← Kpn I 25AA
gggtccggcgcgtgtaccacatccaggcgggcctaccggacccgttccagcccccagcctcccgatcacggtttacta
cgccgtgttggagcgcgcctgccgcagcgtgctcctaaacgcaccgtcggaggcccccagattgtccgcgggcctc
cgaagacgtccggaaacaaccctacaa

B. The amino terminal sequence of BD-uPA-gD

atgggggggctgccgccaggttgggggccgtgattttgtttgtcgtcatagtgggcctccatggggtctgcggc
aaatatgccttggcggatgcctctctcaagatggccgacctcaatcgctttcgcggcaaagaccttccg gtc
24AA
ctcgag *CTAAATGGAGGAACATGTGTGTCCAACAAGTACTTCTCCAACATTCACTG
Xho I → BD-uPA
GTGCAACTGCCCAAAGAAATTC* ggtacc ctg gaccagctgaccgaccctccgggggtccggcg
BD-uPA ← Kpn I 25AA
cgtgtaccacatccaggcgggcctaccggacccgttccagcccccagcctcccgatcacggtttactacgccgtgttgg
agcgcgcctgccgcagcgtgctcctaaacgcaccgtcggaggcccccagattgtccgcgggcctccgaagacgtc
cggaaacaaccctacaa

Figure 10

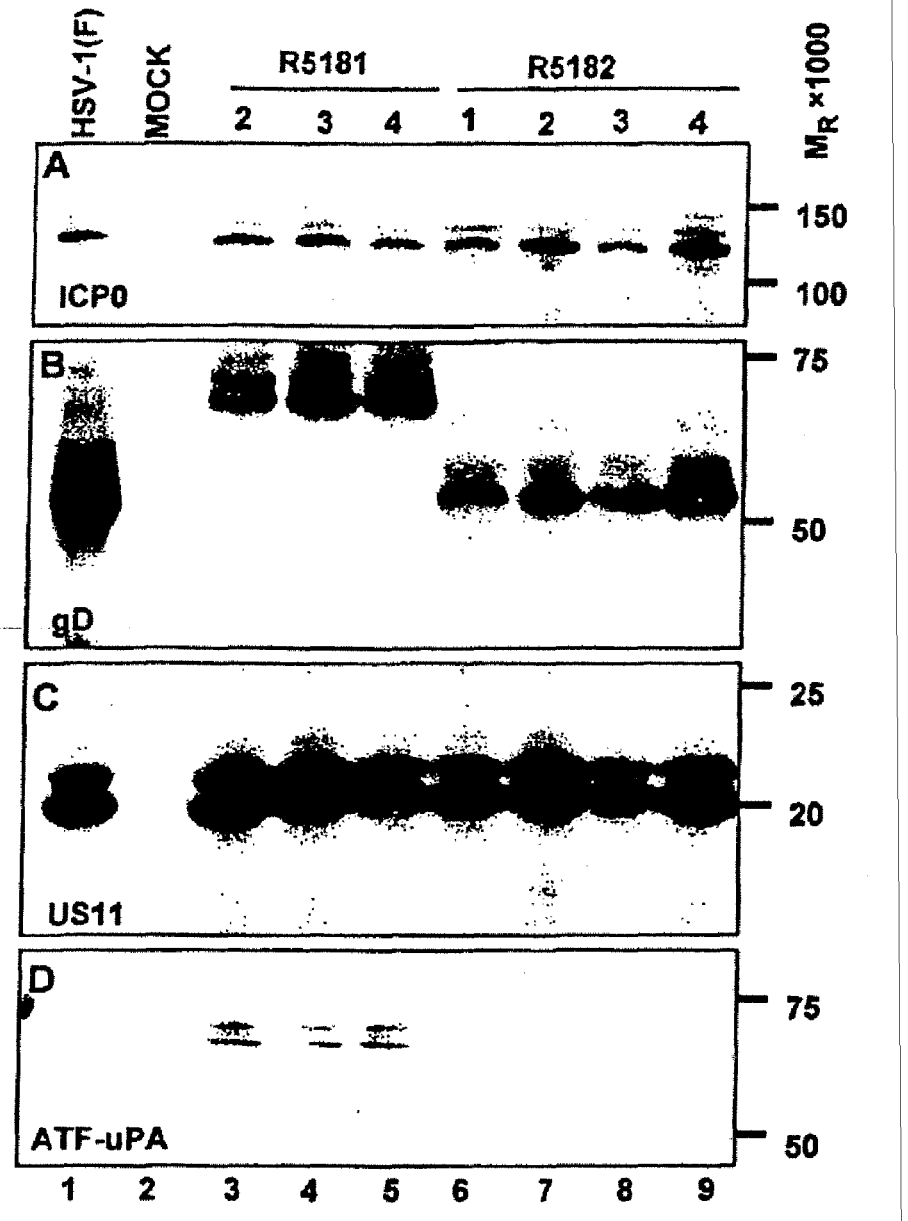

HSV-1(F)
MOCK
R5181
R5182
2 3 4 1 2 3 4
M_R ×1000
A
ICP0
150
100
B
gD
75
50
C
US11
25
20
D
ATF-uPA
75
50
1 2 3 4 5 6 7 8 9

Figure 11

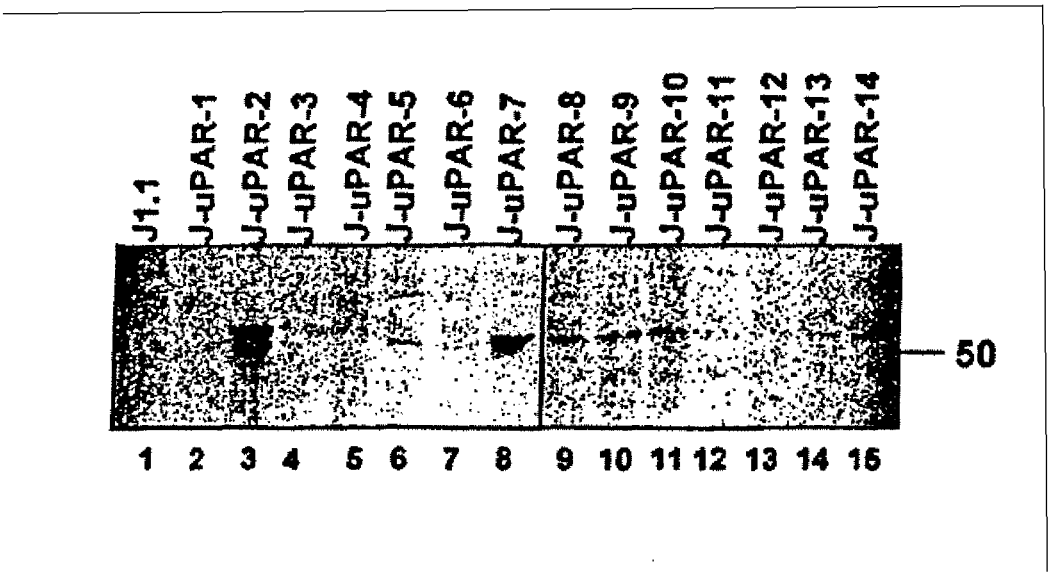

J1.1
J-uPAR-1
J-uPAR-2
J-uPAR-3
J-uPAR-4
J-uPAR-5
J-uPAR-6
J-uPAR-7
J-uPAR-8
J-uPAR-9
J-uPAR-10
J-uPAR-11
J-uPAR-12
J-uPAR-13
J-uPAR-14
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure 12

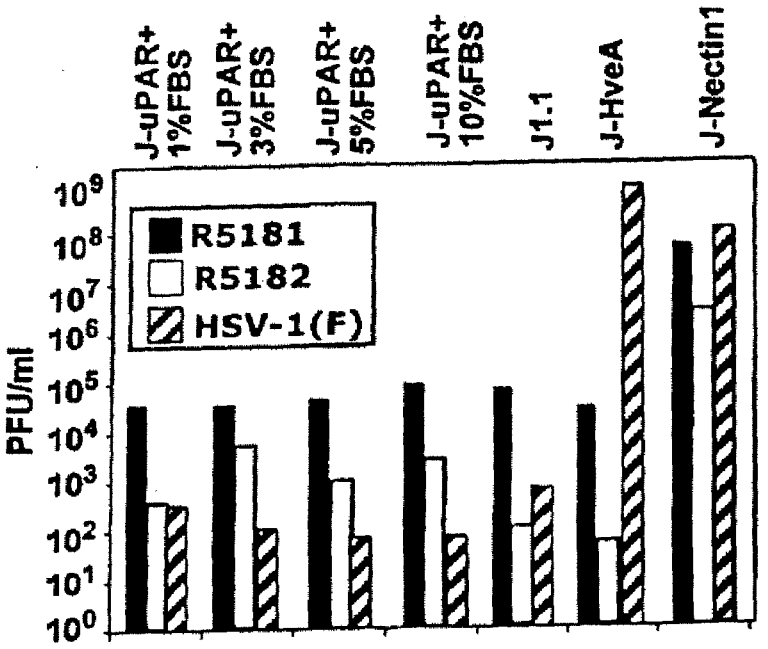

J-uPAR+ 1%FBS
J-uPAR+ 3%FBS
J-uPAR+ 5%FBS
J-uPAR+ 10%FBS
J1.1
J-HveA
J-Nectin1
PFU/ml
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
R5181
R5182
HSV-1(F)

Figure 13

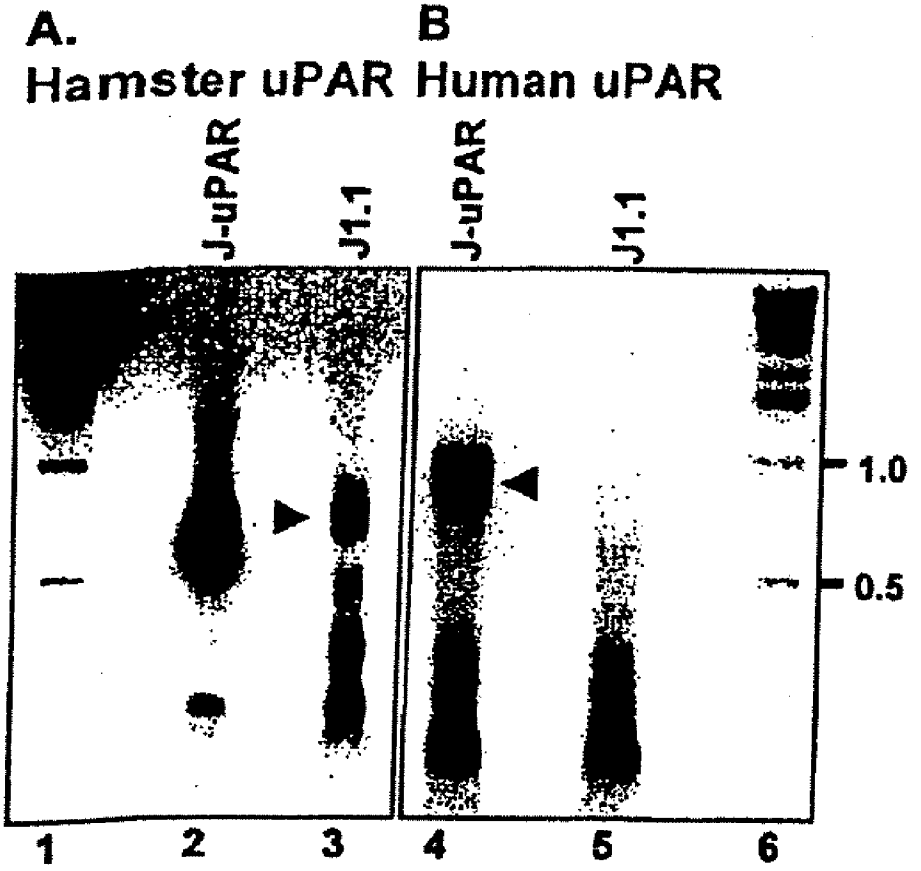


Figure 14

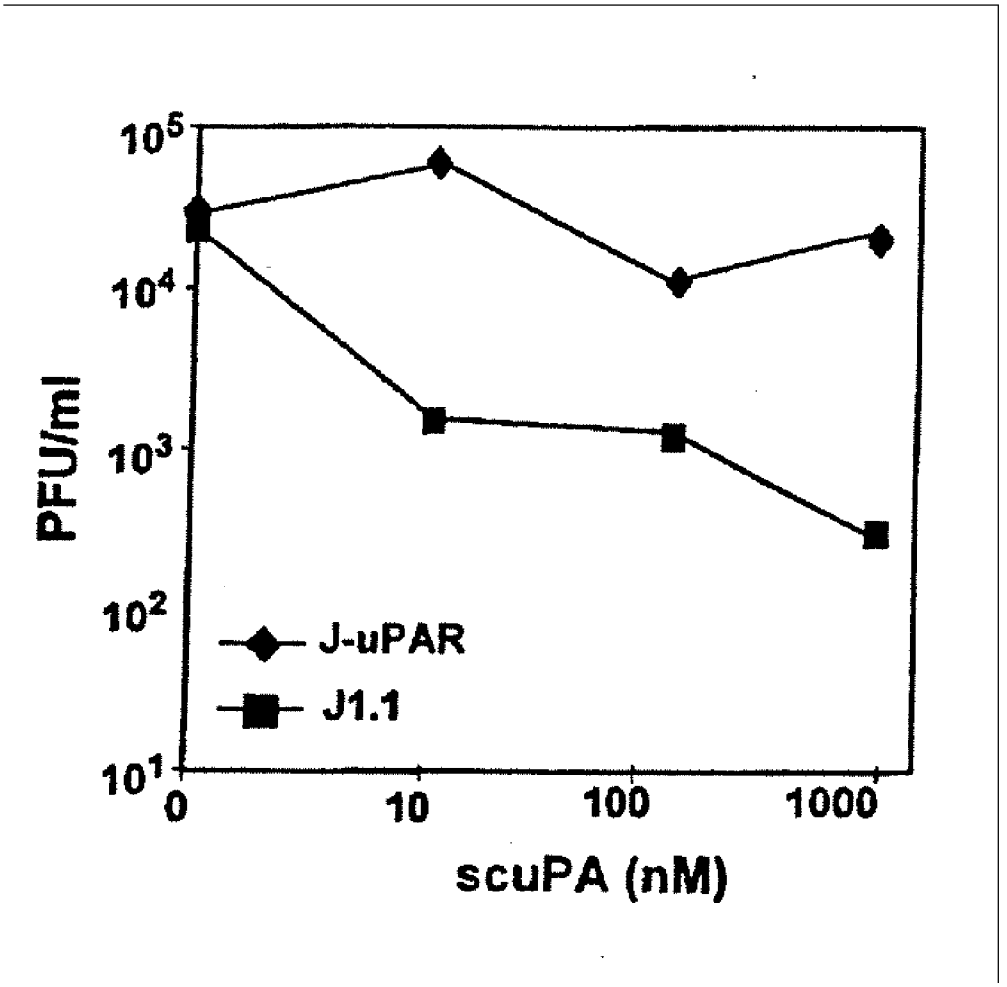


Figure 15

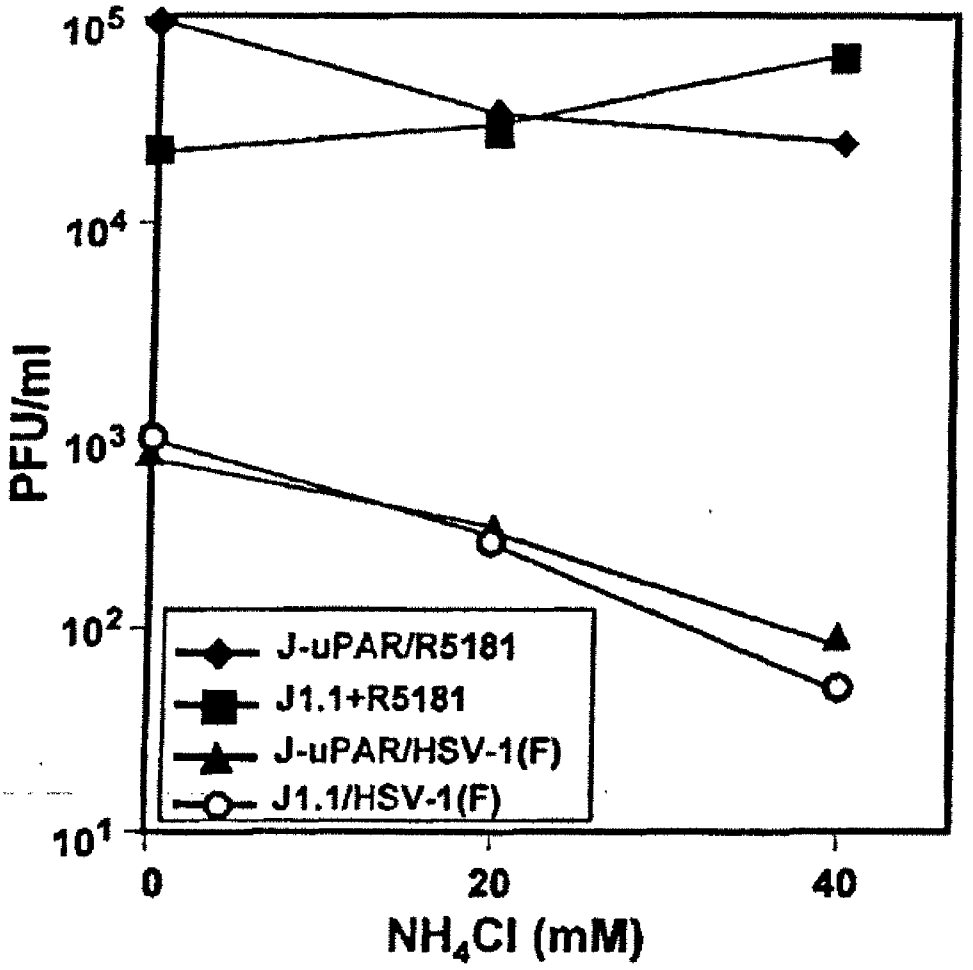

10^5
10^4
10^3
10^2
10^1
PFU/ml
J-uPAR/R5181
J1.1+R5181
J-uPAR/HSV-1(F)
J1.1/HSV-1(F)
0
20
40
NH_4Cl (mM)

Figure 16

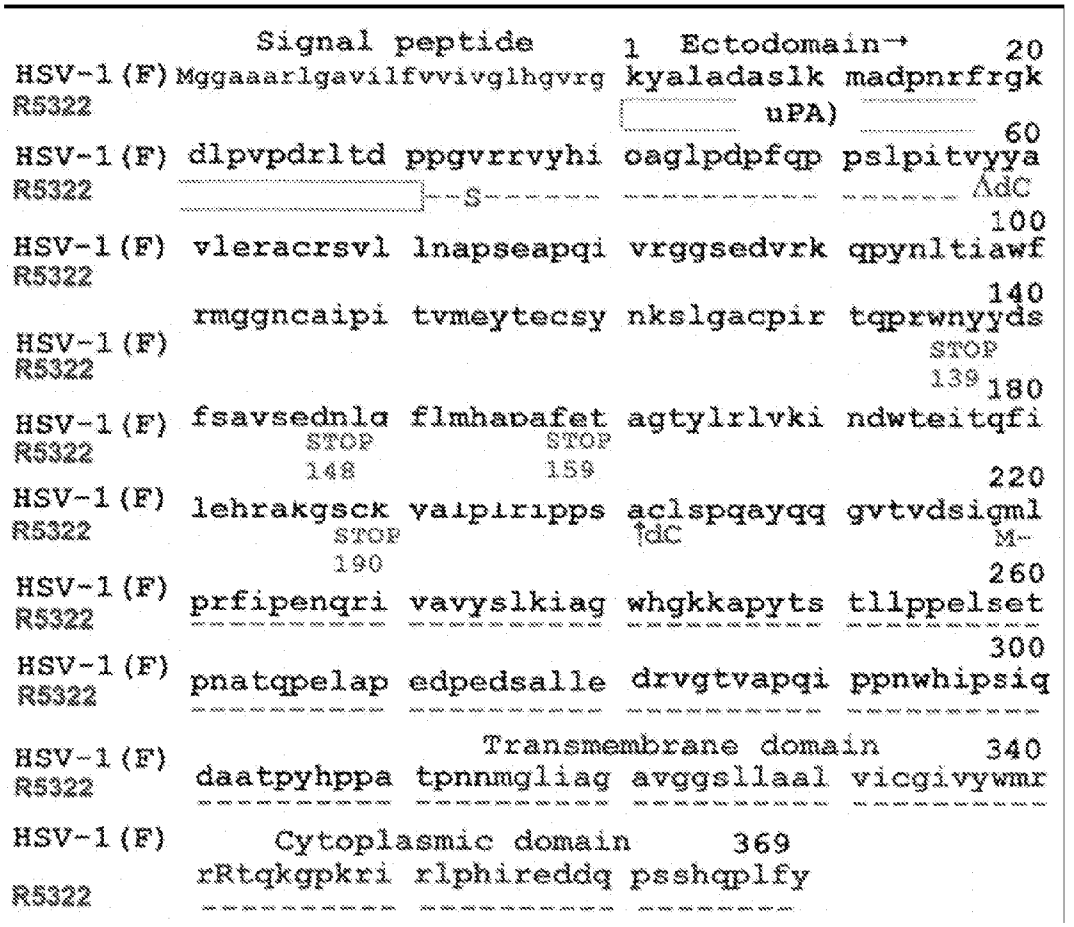

Signal peptide
1 Ectodomain→
20
HSV-1(F) Mggaaarlgavilfvvivglhgvrg kyaladaslk madpnrfrgk
R5322
uPA)
60
HSV-1(F) dlpvpdrltd ppgvrrvyhi oaglpdpfqp pslpitvyya
R5322 S ΔdC
100
HSV-1(F) vleracrsvl lnapseapqi vrggsedvrk qpynltiawf
R5322
140
HSV-1(F) rmggncaipi tvmeytecsy nkslgacpir tqprwnyyds
R5322 STOP 139
180
HSV-1(F) fsavsednlq flmhapafet agtylrlvki ndwteitqfi
R5322 STOP 148 STOP 159
220
HSV-1(F) lehrakgsck vaipiripps aclspqayqq gvtvdsigml
R5322 STOP 190 ↑dC M-
260
HSV-1(F) prfipenqri vavyslkiag whgkkapyts tllppelset
R5322
300
HSV-1(F) pnatqpelap edpedsalle drvgtvapqi ppnwhipsiq
R5322
Transmembrane domain
340
HSV-1(F) daatpyhppa tpnnmgliag avggsllaal vicgivywmr
R5322
HSV-1(F) Cytoplasmic domain 369
R5322 rRtqkgpkri rlphireddq psshqplfy

Figure 17

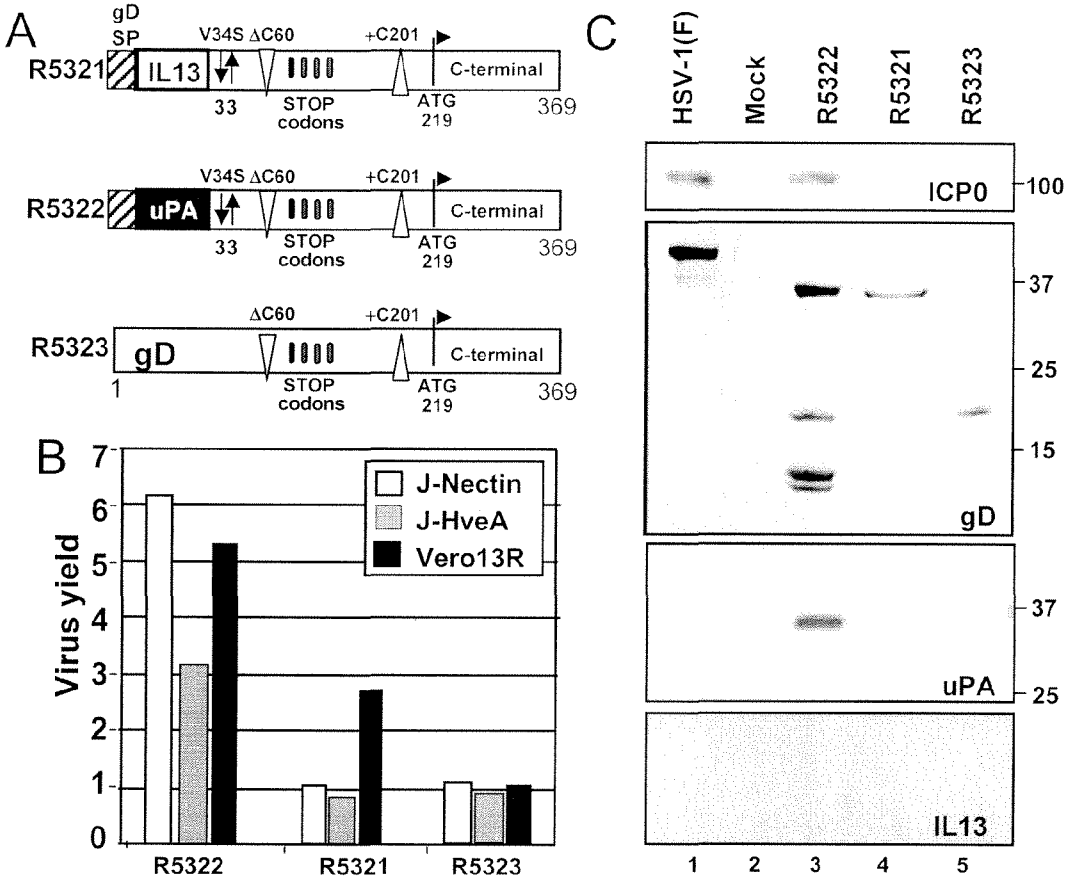

A
gD
SP
V34S ΔC60
+C201
R5321
IL13
C-terminal
33
STOP codons
ATG 219
369
R5322
uPA
ΔC60
R5323
gD
1
B
Virus yield
J-Nectin
J-HveA
Vero13R
R5322
R5321
R5323
C
HSV-1(F)
Mock
R5322
R5321
R5323
ICP0
gD
uPA
IL13
100
37
25
15
1
2
3
4
5

Figure 18

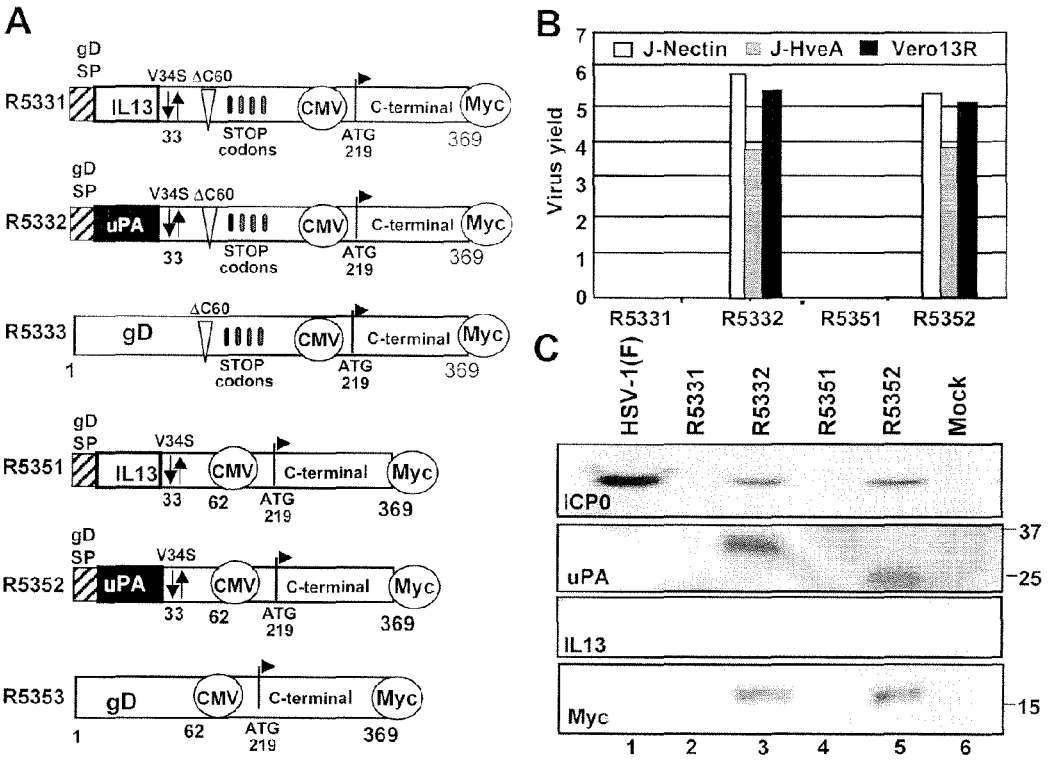
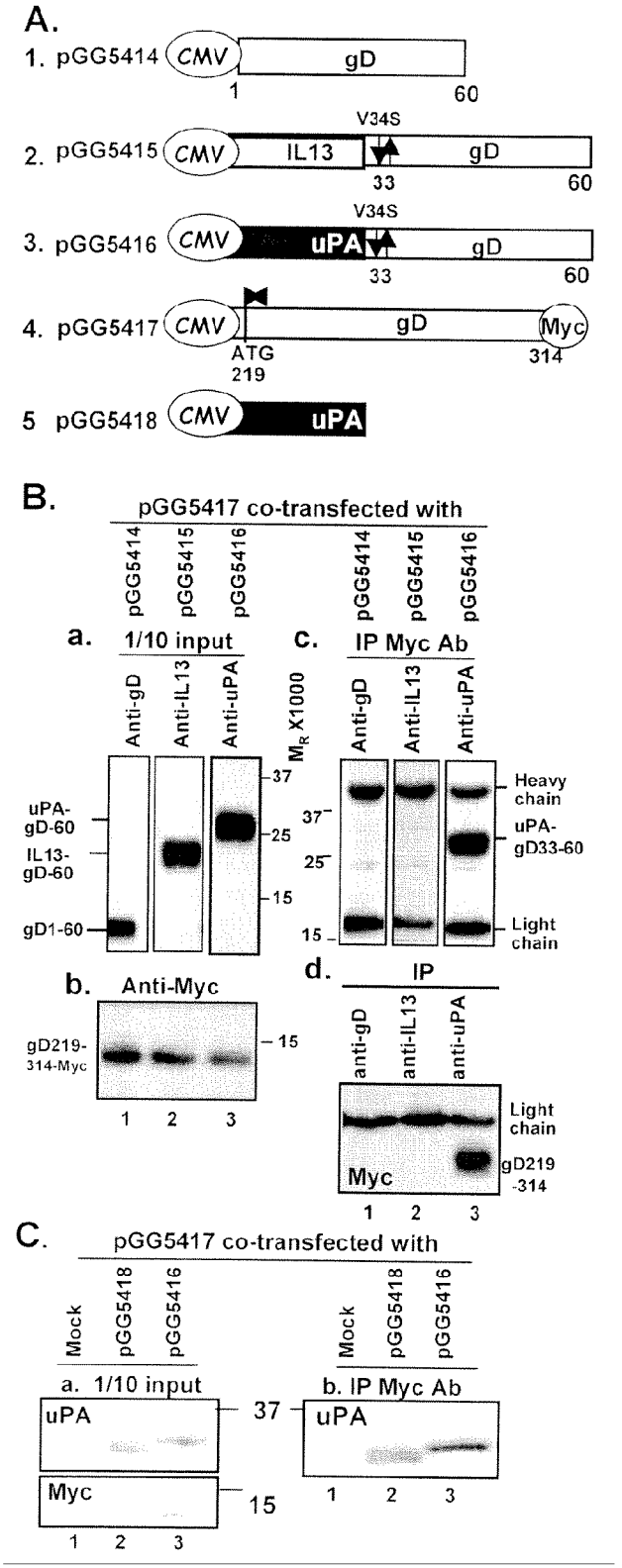

A
gD SP
V34S ΔC60
R5331
IL13
CMV
C-terminal
Myc
33
STOP codons
ATG 219
369
R5332
uPA
R5333
gD
ΔC60
1
R5351
V34S
62
R5352
R5353
B
J-Nectin
J-HveA
Vero13R
Virus yield
R5331
R5332
R5351
R5352
C
HSV-1(F)
Mock
ICP0
uPA
IL13
Myc
37
25
15
1 2 3 4 5 6

Figure 19


A.
1. pGG5414
CMV
gD
1
60
2. pGG5415
V34S
IL13
gD
33
60
3. pGG5416
V34S
uPA
gD
33
60
4. pGG5417
CMV
gD
Myc
ATG
219
314
5 pGG5418
CMV
uPA
B.
pGG5417 co-transfected with
pGG5414
pGG5415
pGG5416
a. 1/10 input
Anti-gD
Anti-IL13
Anti-uPA
uPA-gD-60
IL13-gD-60
gD1-60
b. Anti-Myc
gD219-314-Myc
1 2 3
c. IP Myc Ab
Heavy chain
uPA-gD33-60
Light chain
d. IP
anti-gD
anti-IL13
anti-uPA
Light chain
gD219-314
Myc
C.
pGG5417 co-transfected with
Mock
pGG5418
pGG5416
a. 1/10 input
b. IP Myc Ab
uPA
Myc
37
15
1 2 3

TARGETING OF HERPES SIMPLEX VIRUS TO SPECIFIC RECEPTORS

[0001] This application is a continuation-in-part application of U.S. Ser. No. 11/215,636, filed Aug. 30, 2005, which is a continuation-in-part of U.S. Ser. No. 10/530,774, filed Apr. 7, 2005, which is the US national phase of PCT/US03/31598, filed Oct. 6, 2003, which claims the priority benefit of U.S. Ser. No. 60/416,716, filed Oct. 7, 2002. This application also claims priority to PCT/US2006/032291, filed Aug. 18, 2006, which claims the priority benefit of U.S. Ser. No. 60/709,597, filed Aug. 19, 2005.

GOVERNMENT INTERESTS

[0002] This invention was made with U.S. government support under CA 115662, CA 83939, CA 71933, CA 78766 and CA 88860 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention

BACKGROUND OF THE INVENTION

[0003] A steady rate of healthcare advances has led to continuing improvement in the health and quality of life for humans and animals. Nevertheless, a variety of diseases, disorders, and conditions have largely eluded the best efforts at prevention or treatment. Chief among these maladies is the loss of cell-cycle control that frequently results in the undesirable cell proliferation characteristic of cancer in its many forms, such as malignant glioma. Malignant gliomas are devastating brain tumors that afflict animals such as humans. The average life span after diagnosis is less than one year and few patients have been reported to survive five years. Furthermore, none of the conventional anti-cancer therapies has been successful in significantly prolonging the lifespan of patients with this disease. In recent years there have been numerous attempts to use genetically engineered herpes simplex viruses (HSV) as oncolytic agents to treat malignant gliomas. Because wild-type viruses are highly virulent, the viruses used in preclinical evaluations and in phase-1 clinical studies have been thoroughly attenuated. While several deletion mutants have been tested, the mutants that reached clinical trials lacked the $\gamma_1$34.5 gene encoding infected cell protein 34.5 (ICP34.5) and optionally, the U_L39 gene encoding the large subunit of ribonucleotide reductase.

[0004] These attenuated HSV viruses, however, have been imperfectly engineered as oncolytic agents. One advantage of these mutant viruses is that they have a significantly reduced capacity to replicate in normal, non-dividing cells in vivo. Viral ribonucleotide reductase is an essential gene for viral replication in resting cells and, hence, the U_L39 mutant virus is dysfunctional in the normal environment of the central nervous system (Simard et al. 1995). The major function of ICP34.5 is to preclude the shutoff of protein synthesis caused by activation of protein kinase R in infected cells. Once activated, this enzyme phosphorylates the α subunit of translation initiation factor 2 (eIF2α), resulting in complete cessation of translation. Mutants lacking the $\gamma_1$34.5 genes are highly attenuated because the lytic life cycle is completely blocked in an interferon$^+$ cellular background. In contrast, $\gamma_1$34.5 mutants are nearly as virulent as wild-type virus in mice lacking interferon receptor. Although mutants deleted in both $\gamma_1$34.5 and U_L39 are not significantly more attenuated than those lacking the $\gamma_1$34.5 genes, such mutants do provide added insurance in the form of a reduced risk of reversion.

[0005] A significant disadvantage of these mutant HSV viruses is their poor replication, even in dividing cells. In experimental animal systems, the mutant viruses do not exhibit sustained lytic life cycles, with the loss of a potentially amplified response to a given therapeutic dose of the virus that would be expected upon re-infection of tumor cells by the multiplied viral progeny. Consequently, maximum killing of tumors cells requires high doses of virus. Given the poor growth of these mutant HSV viruses, even in dividing cells, production of virus pools large enough to yield efficacious inocula of $>10^9$ plaque forming units (PFU) has remained a major obstacle. Moreover, indiscriminate binding of virus to non-tumor cells further diminishes the effectiveness of HSV virus dosages because mis-targeted viral particles do not contribute to the desired beneficial therapeutic effect of tumor cell destruction. One approach to overcoming these obstacles is to achieve a more thorough understanding of the HSV lytic life cycle and thereby facilitate the development of HSV mutants tailored for use as targeted therapeutic agents, such as targeted oncolytic agents.

[0006] HSV enters host cells using a two-step mechanism. The first step of entry is HSV attachment to the cell surface. Common receptors for viral entry are nectin-1, HveA, and a specific O-linked sulfated proteoglycan. This step is initiated by glycoproteins B and C (gB and gC), which project from the viral envelope, attaching to heparan sulfate proteoglycans on host cell surfaces. The gB and gC domains interacting with heparan sulfate have been mapped at the sequence level (Laquerre et al. 1998). Following this initial attachment, viral glycoprotein D (gD, 369 amino acids) interacts with one of several receptors. Of these gD receptors, two are particularly important for entry (Spear et al, 2000). One receptor, designated HveA (formerly, HveM), is a member of the family of receptors for tumor necrosis proteins. A second receptor, designated Nectin-1 (HveC), is a member of the nectin family of proteins, structurally related to the immunoglobulin superfamily, which serve as intercellular connectors (Campadelli-Fiume et al. 2000). The second step of HSV entry into a cell is fusion of the viral envelope with the plasma membrane of the cell. To effect fusion, gD, when bound to its receptor, recruits glycoproteins B, H and L, which results in fusion of the envelope with the plasma membrane.

[0007] Additional understanding of HSV infection has come from recent studies that have lent significance to an old observation that gD interacts with the cation-independent mannose 6 phosphate receptor, contributing to the accumulation of HSV in endosomes. Endocytosis of viral particles results in particle degradation by lysosomal enzymes, but the cells succumb as a consequence of the degradation of cellular DNA by lysosomal DNase. HSV gD blocks this apoptotic pathway to cell death through its interaction with the mannose 6 phosphate receptor. In fact, transduction of cells with specific domains of gD, treatment with chloroquine or overexpression of cation-independent mannose 6-phosphate receptor prevents cell death (15-17). The domains required for blocking cell death have been mapped by insertional mutagenesis to several sites of gD (17). Thus, gD interacts with HveA, nectins, the mannose 6 phosphate

receptor, and at least one of the complex of viral glycoproteins involved in the fusion of HSV with the plasma membrane.

[0008] In an attempt to target HSV-1 infection to specific cells, a recombinant HSV having a chimeric protein comprising gC and erythropoietin (EPO) on its surface was constructed. Although the recombinant virus bound to cells expressing EPO receptor and endocytosis of the virus occurred, successful infection of these EPO-receptor expressing cells did not occur.

[0009] Accordingly, a need continues to exist in the art for viral therapeutic agents exhibiting improved targeting capacities while retaining sufficient capacity to infect to be therapeutically useful. Ideally, suitable viruses would be therapeutic agents, such as oncolytic agents, themselves as well as providing a targeting vehicle or vector for the controlled delivery of polynucleotide coding regions useful as therapeutic agents. Another need in the art is for targeted agents useful in diagnostic applications as, e.g., imaging agents or targeted vehicles for imaging agents.

SUMMARY

[0010] The invention satisfies the aforementioned need in the art by providing viral forms suitable for use as therapeutic and diagnostic agents themselves, as well as providing a ready vehicle for the delivery of therapeutic or diagnostic polynucleotide coding regions to cells. These viral forms are modified viruses of the Herpesviridae family of viruses, and are preferably derived from herpes simplex virus type 1 or type 2. The invention provides a method of making virus particles with a novel ligand (or binding pair member), and making said particles totally dependent on a receptor of the ligand (or binding pair member) for entry into targeted cells.

[0011] Disclosed herein are methods to modify the surface of, e.g., an HSV virus particle in a manner that targets the virus to a specific receptor present on the surface of a cell of choice, typically a cell in need of therapy or a cell whose presence provides information of diagnostic value. The invention provides viral particles, e.g., HSV particles, having a reduced affinity for their natural cell-surface receptor(s), and methods for producing and using such particles, which minimizes or eliminates the problem of reduced efficiency associated with the mis-targeting of therapeutic and diagnostic viruses. Additionally, the invention provides viral particles, e.g., HSV particles, that exhibit specific affinity for a cell surface component that is not a natural viral receptor and that is present solely or predominantly on a given target cell, as well as methods for producing and using such viruses. Modified viral particles (e.g., HSV) having increased affinity for a cell surface component associated with one or more target cells exhibit improved targeting capabilities relative to known viral particles. The modified HSV particles have reduced indiscriminate binding, thereby minimizing sequestration of viral dosages away from the target cells. The invention further provides modified viral particles, such as modified HSV particles, that have both a reduced affinity for natural viral receptors and an increased affinity for a cell surface component associated with a particular target cell(s), with the modified viral particle effectively recruiting that cell surface component for use as a viral receptor. Other benefits of the modified viruses are described herein and will be apparent to those of skill in the art upon review of this disclosure.

[0012] In one aspect, the invention provides a herpes simplex virus comprising a first polynucleotide encoding a gD polypeptide fragment comprising about amino acids 244-394 of SEQ ID NO:26 (preferably amino acids 244-394 of SEQ ID NO:26, corresponding to amino acids 219-369 of mature gD) and a second polynucleotide encoding a targeting peptide, wherein said targeting peptide specifically interacts with the gD polypeptide fragment in addition to specifically interacting with its binding partner, the target. The viruses according to the invention provide for a simplified modular approach to the construction of re-targeted HSV, requiring a coding region for any peptide capable of specifically interacting with a target of interest. Typically, the targeting peptide is a member of a binding pair or is an antibody product specifically recognizing a target of interest, which is typically a peptide target. In some embodiments, the targeting peptide is a urokinase plasminogen activator peptide that specifically interacts with urokinase plasminogen activator receptor.

[0013] A related aspect of the invention is drawn to the herpes simplex virus described above, wherein the second polynucleotide further encodes a second gD polypeptide fragment comprising at least about 28 (preferably 28) contiguous amino acids of SEQ ID NO:26 and having a C-terminus at about position 60 (preferably at position 60) of SEQ ID NO:26. In an embodiment, the second gD polypeptide fragment comprises, or consists essentially of, amino acids 33-60 of SEQ ID NO:26. In some embodiments, the herpes simplex virus comprises a second polynucleotide wherein the second polynucleotide further encodes a peptide linker interposed between said targeting peptide and said second gD polypeptide fragment. An exemplary peptide linker is $(Gly_4Ser)_n$, wherein n is an integer between 3 and 5, inclusive.

[0014] Another aspect of the invention is drawn to a herpes simplex virus comprising a polynucleotide encoding a gD polypeptide fragment comprising about amino acids 244-394 (preferably amino acids 244-394) of SEQ ID NO:26 (corresponding to residues 219-369 of mature gD) and a targeting peptide, wherein the targeting peptide is a member of a binding pair that specifically interacts with the other member of the binding pair. In some embodiments, the polynucleotide further encodes a peptide linker interposed between the targeting peptide and the gD polypeptide fragment. Again, an exemplary peptide linker is $(Gly_4Ser)_n$, wherein n is an integer between 3 and 5, inclusive. In some embodiments according to this aspect, the polynucleotide further encodes a second gD polypeptide fragment comprising at least about 28 (preferably 28) contiguous amino acids of SEQ ID NO:26 and having a C-terminus at about position 60 (preferably position 60) of SEQ ID NO:26. In some embodiments, the second gD polypeptide fragment comprises, or consists essentially of, amino acids 33-60 of SEQ ID NO:26. Consistent with other aspects of the invention, the polynucleotide may further encode a peptide linker interposed between the gD polypeptide fragment and the second gD polypeptide fragment, and/or the same or a different peptide linker may be interposed between the targeting peptide and the gD polypeptide fragment. In these various product aspects of the invention, exemplary peptide

linkers are linkers conforming to the pattern of $(Gly_4Ser)_n$, wherein n is an integer between 3 and 5, inclusive.

[0015] As noted above, the products according to the invention provide a simplified modular approach to re-targeted HSV in which a targeting peptide is encoded by a polynucleotide. Increasing the versatility of the invention are constructs that further provide an engineered pair of interacting domains/motifs to ensure that the N-terminally disposed interaction domain of gD (e.g., gD residues 33-60) interacts with the C-terminally disposed fusogenic domain of gD (e.g., gD residues 219-369, or 244-394 of SEQ ID NO:26). A wide variety of interacting domains/motifs are known in the art or can be generated (e.g., antibodies elicited to a particular peptide). One member of a pair of interacting domains/motifs is fused to the fusion peptide comprising a targeting peptide and the N-terminally disposed interaction domain of gD on a single polypeptide or polypeptide fragment (e.g., targeting peptide—interacting domain—gD interaction domain); the other member of the pair of interacting domains/motifs is translationally fused to the fusogenic domain of gD on a polypeptide or polypeptide fragment (e.g., interacting domain partner—gD fusogenic domain). The two fusion polypeptides or polypeptide fragments, moreover, can be present on a single protein chain or on distinct protein chains. Given the freedom from constraints placed upon the targeting peptide by the design of the invention, it is apparent that any polynucleotide encoding any targeting peptide would be suitable for use in the invention. In some embodiments, the targeting peptide is selected from the group consisting of a urokinase plasminogen activator peptide fragment and an interleukin 13 peptide fragment, wherein said peptide fragment specifically interacts with its binding partner. In other embodiments, the targeting peptide comprises an antibody variable domain, wherein the peptide specifically interacts with its binding partner.

[0016] Another aspect of the invention is a pharmaceutical composition comprising the herpes simplex virus described herein, and a pharmaceutically acceptable excipient, carrier or diluent. A related aspect of the invention is drawn to a kit comprising the herpes simplex virus described herein and a label providing instruction for administration of the virus.

[0017] Yet another aspect of the invention is a method of producing a herpes simplex virus as described herein comprising (a) contacting a permissive host cell with a herpes simplex virus as described herein; (b) incubating the host cell; and (c) recovering the herpes simplex virus.

[0018] Another aspect of the invention is a method of treating a condition in an organism characterized by the presence of a deleterious cell in the organism comprising administering a therapeutically effective amount of a herpes simplex virus as described herein to the organism. Conditions contemplated as amenable to treatment include cancer, autoimmune disease and any other hyperproliferative cell disorder. In some embodiments, the condition is cancer.

[0019] Still another aspect of the invention is a method of screening for a targeting peptide comprising (a) preparing a plurality of herpes simplex viruses as described herein wherein the plurality of viruses comprises a plurality of targeting peptide coding regions; (b) contacting the targeting peptides with a target peptide; and (c) measuring the interaction of the targeting peptides and the target peptide, thereby identifying a targeting peptide binding partner of the target peptide. In some embodiments, the target peptide is associated with a cell. In such embodiments, measurements of peptide interactions include measurements of HSV entry into the cell using any technique known in the art, such as viral titering, uptake of labeled virus and the like.

[0020] In another aspect, the invention provides a recombinant herpes simplex virus (HSV) particle having at least one protein on its surface, comprising: (a) an altered gD, wherein the alteration reduces binding of gD to one or more of its cellular receptors, said alteration comprising (i) a heterologous peptide ligand on the surface of the recombinant HSV particle forming a fusion protein with the altered gD; and (ii) an amino acid alteration; wherein said recombinant HSV particle preferentially binds to cells expressing a binding partner to said heterologous peptide ligand. In some embodiments, these particles preferentially bind to target cells (cell expressing a binding partner) in whole or part due to the greater frequency of the binding partner on the surface of the cell relative to any natural HSV binding proteins on the surface of that cell. In some embodiments, the recombinant HSV particle further comprises an altered viral surface protein, wherein the alteration reduces binding of the viral surface protein to a sulfated proteoglycan. Such recombinant herpes simplex virus (HSV) particles comprise a virus surface protein altered to reduce the wild-type level of binding of that protein to a sulfated proteoglycan on the surface of a cell and an altered gD. The altered gD exhibits a reduced binding to one or more of the natural cellular receptors for gD; the altered gD is also fused to a heterologous peptide ligand (or binding pair member) having a binding partner, e.g., a peptide ligand receptor, found on the surface of a cell. Stated in the alternative, this aspect of the invention provides a recombinant herpes simplex virus (HSV) particle having at least one protein on its surface, comprising: (a) an altered viral surface protein, wherein the alteration reduces binding of the viral surface protein to a sulfated proteoglycan; and (b) an altered gD, wherein the alteration reduces binding of gD to one or more of its cellular receptors, the alteration comprising (i) a heterologous peptide ligand (or binding pair member) on the surface of the recombinant HSV particle forming a fusion protein with the altered gD; and (ii) an amino acid alteration; wherein the recombinant HSV particle preferentially binds to cells expressing a binding partner to the heterologous peptide ligand (or binding pair member).

[0021] The invention comprehends a recombinant HSV particle wherein the amino acid alteration is selected from the group consisting of an amino acid deletion, an amino acid substitution and an amino acid insertion. A preferred site for the amino acid alteration is amino acid position 34 of gD. Exemplary recombinant HSV particles according to the invention include HSV R5141 and HSV R5161, each described below.

[0022] Contemplated amino acid alterations include insertions or deletions of 1-10 amino acids, such as insertions or deletions of 1-5 amino acids. Exemplary insertions occur immediately upstream (N-terminal) or downstream (C-terminal) to amino acid position 34 of gD. Exemplary deletions include amino acid position 34 of gD. For alterations comprising amino acid substitutions, 1-10 amino acids are substituted, such as substitutions of 1-5 amino acids. Non-contiguous (dispersed) or contiguous amino acid substitu-

tions are contemplated. In some embodiments, conservative amino acids known in the art are substituted. Exemplary amino acid substitutions include single amino acid substitutions for the valine at position 34 of mature gD (position 59 of holo-gD, see SEQ ID NO:26). Preferably, substitutions for Val34 will be V34S or a conservative substitution for the native Val at position 34 of mature gD.

[0023] The altered gD, moreover, reduces binding of the recombinant HSV particle to at least one HSV entry mediator (Hve) cell-surface protein, such as an Hve selected from the group consisting of HveA (formerly, HveM) and Nectin-1 (HveC). Further, the recombinant HSV particles of the invention include particles wherein the altered viral surface protein is selected from the group consisting of gB and gC. In some embodiments, the altered viral surface protein, preferably selected from the group of gB and gC, forms a fusion protein with a heterologous peptide ligand. In some embodiments, the binding partner is a cell surface receptor for the heterologous peptide ligand.

[0024] The preferential binding of the recombinant HSV particles of the invention results in a detectable variation in effective binding of the particle to the cells being compared. By "effective binding" is meant either sufficiently stable binding to permit detection of binding or binding sufficient to result in productive infection of the cell. In preferred embodiments, the preferential binding is such that the recombinant HSV particles bind only to one of the cell types being compared (e.g., cancer cells compared to healthy cells of the same type). Suitable cells include any hyperproliferative cell type, such as a cancer cell. A cancer cell, in turn, includes a tumor cell, e.g., a malignant gliomal cell.

[0025] The invention comprehends recombinant HSV particles wherein the heterologous peptide ligand (or binding pair member) is any ligand (or binding pair member) for which a cell surface binding partner exists. Preferably, heterologous peptide ligands have specific cell surface binding partners, e.g., ligand receptors, that are preferentially exhibited on the surface of a target cell. More preferably, the cell surface binding partner is only exhibited on the surface of a target cell, when compared to the cells in an organism containing the target cell. Exemplary heterologous peptide ligands that include cytokines, such as IL113, and fragments, variants and derivatives thereof, provided that the ligand retains the capacity of binding to a cell-surface binding partner. An exemplary binding pair member contemplated as suitable for each aspect of the invention is a single-chain antibody, for which a binding partner would include an antigen thereof, or a fragment, derivative or variant thereof that retains the capacity to bind to the single-chain antibody.

[0026] Another aspect of the invention is drawn to the recombinant HSV particle described above, wherein a polynucleotide encoding the fusion protein is joined to a heterologous coding region for a leader sequence. In this context, "heterologous" means that the leader sequence is not found naturally associated with the upstream or 5' coding region participating in the fusion. Exemplary leader sequences include HSV leader sequences, e.g., an HSV gD leader sequence. In a related aspect, the invention provides the recombinant HSV particle described above, wherein a polynucleotide encoding the fusion protein is joined to a heterologous expression control element, such as a heterologous promoter (a promoter not naturally found in association with the polynucleotide coding region fused upstream or 5' in the fusion), a heterologous enhancer, or expression factor binding site known in the art.

[0027] Another aspect of the invention provides a pharmaceutical composition comprising a recombinant HSV particle described above and a pharmaceutically acceptable carrier, diluent, or excipient. Any pharmaceutical carrier, diluent or excipient known in the art is contemplated. A related aspect of the invention provides a kit comprising the pharmaceutical composition and a set of instructions for administering the composition to a subject in need. In each of these aspects of the invention, i.e., the pharmaceutical compositions and the kits, the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described in the context of describing the recombinant HSV particles are contemplated.

[0028] Yet another aspect of the invention provides a method of targeting a recombinant HSV particle to a cell comprising (a) identifying a binding pair member, such as a ligand for a ligand binding partner, exhibited on the surface of a target cell; and (b) creating an HSV particle as described herein, wherein the ligand or, more generally, the binding pair member, binds to the binding partner exhibited on the surface of the target cell. In some preferred embodiments of this aspect of the invention, the altered viral surface protein is selected from the group consisting of gB and gC. In some embodiments, the alteration to gD reduces binding of gD to at least one cellular receptor for gD selected from the group consisting of HveA and Nectin-1. In preferred embodiments, the altered gD has a conservative substitution at position 34 of gD, such as a V34S substitution. A second fusion protein, joining the ligand (or binding pair member) and either of gB or gC, is also contemplated in some embodiments. Suitable cells for targeting include any hyperproliferative cell, such as a cancer cell, including tumor cells (e.g., malignant gliomal cells). Any of the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described above in the context of describing the recombinant HSV particles is suitable for use in the method.

[0029] Another aspect of the invention is drawn to a method of imaging a cell comprising: (a) contacting the cell with a recombinant HSV particle as described above, the recombinant HSV particle further comprising a coding region for a marker protein; and (b) detecting the presence of the marker protein. Any type of cell exhibiting a cell-surface binding partner for a ligand (or binding pair member) fusible to HSV gD is suitable for use in this aspect of the invention, such as a cancer cell. Using cancer cells as an example, the method is useful provided that the binding partner is present at a higher number on the cancer cell as compared to a non-cancerous cell of the same type. Any known marker protein is useful in this aspect of the invention, e.g., a marker protein selected from the group consisting of thymidine kinase, green fluorescent protein, and luciferase. In preferred embodiments, the altered gD exhibits an amino acid substitution of V34S. Any of the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described above in the context of describing the recombinant HSV particles is suitable for use in the method.

[0030] Another aspect of the invention provides a method of treating a cell-based disease comprising delivering a

therapeutically effective amount of a recombinant HSV particle as described herein to a subject in need. A related aspect is the use of a recombinant HSV particle as described above in the preparation of a medicament for the treatment of a cell-based disease. A therapeutically effective amount of a recombinant HSV particle is that amount that produces the desired therapeutic effect, as would be understood and readily determinable by those of skill in the art. Any cell-based disease known or reasonably suspected to be amenable to treatment with a specifically targeted HSV is contemplated, e.g., a cell hyperproliferation disease such as cancer. Any of the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described above in the context of describing the recombinant HSV particles is suitable for use in the method.

[0031] In a related aspect, the invention provides a method of ameliorating a symptom associated with a disease comprising administering a therapeutically effective amount of a recombinant HSV particle described above to a subject in need. Another aspect is drawn to the use of a recombinant HSV particle as described above in the preparation of a medicament for ameliorating a symptom associated with a disease in a subject in need. Again, any disease known or reasonably suspected to have a symptom amenable to application of a specifically targeted HSV is contemplated, including any disease characterized by hyperproliferative cells, such as cancer. Any of the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described above in the context of describing the recombinant HSV particles is suitable for use in the method.

[0032] Another aspect of the invention is directed to a method of delivering a therapeutically useful peptide to a cell comprising: (a) inserting a therapeutically useful polynucleotide, such as an expression control element, an rDNA, or a coding region for a therapeutically useful peptide, into the DNA of a recombinant HSV particle as described above, thereby producing a recombinant HSV clone; and (b) delivering a therapeutically effective amount of the recombinant HSV clone to the cell. In a related aspect, the invention provides for the use of a recombinant HSV clone comprising a recombinant HSV particle according to claim **1** in the preparation of a medicament for delivering a therapeutically useful peptide to a cell comprising inserting a coding region for a therapeutically useful peptide into the DNA of the recombinant HSV particle, thereby producing the recombinant HSV clone. Each of the method and use comprehends delivery of the recombinant HSV clone in vivo, ex vivo, or in vitro. Any of the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described above in the context of describing the recombinant HSV particles is suitable for use in the method or use.

[0033] Another aspect of the invention provides a method of killing a target cell, comprising contacting the target cell with a recombinant HSV particle as described above. A related aspect is the use of a recombinant HSV particle as described above in the preparation of a medicament for killing a target cell by contacting the target cell with the recombinant HSV particle. In preferred embodiments of either the method or the use, the recombinant HSV particle has an altered gD in which the V34S substitution is found. Any of the heterologous peptide ligands (or binding pair members) and cell-surface binding partners described above in the context of describing the recombinant HSV particles is suitable for use in the method or use.

[0034] In each of the above-described aspects of the invention, it is preferred that gD, or a portion thereof, maintains its membrane fusion properties, but has reduced capacity to bind HveA and/or Nectin-1.

[0035] Other features and advantages of the invention will be better understood by reference to the brief description of the drawing and the description of the illustrative embodiments that follow.

BRIEF DESCRIPTION OF THE DRAWING

[0036] FIG. **1**. Schematic representation of the HSV-1 (F) genome and gene manipulations in glycoprotein C (gC) (FIG. **1**A), glycoprotein B (gB) (FIG. **1**B), and glycoprotein D (gD) (FIG. **1**C). Line 1, sequence arrangement of the HSV-1 genome. The rectangular boxes represent the inverted repeat sequences ab and b'a' flanking the unique long (U_L) sequence, and inverted repeat c'a' and ca flanking the unique short (U_S) sequence. Line 2, sequence arrangement of domains of the glycoprotein C; the signal peptide (SP) domain and heparan sulfate (HS)-binding domain of gC are highlighted. Line 3, human IL13 with signal peptide that replaced the N-terminal segment of 148 amino acids of gC. Line 4, sequence arrangement of the poly-lysine domain of gB. Line 5, schematic representation of a recombinant HSV-1(F) genome, in which the N-terminal domain of gC was replaced with IL13 and the polylysine domain (from codon 68 to codon 77) of gB was deleted. Line 6, sequence arrangement of glycoprotein J (gJ), glycoprotein D (gD), and glycoprotein I (gI) in U_S. Line 7, replacement of gD with the immediate early promoter of CMV in order to enable the expression of gI. Line 8, schematic representation of recombinant HSV-1 (F) genome, in which the N-terminal domain of gC was replaced with IL13, the poly-lysine domain of gB was deleted, and IL13 was inserted after amino acid 24 of gD (FIG. **1**D). Line 9, a polylinker XhoI-BglII-EcoRI-KpnI was inserted after amino acid 24 of gD, with IL13 inserted into the XhoI and KpnI sites of gD.

[0037] FIG. **2**. Amino acid sequence alignment of IL13-gC, IL13-gD junction sequence, and the HS binding domain of gB. FIG. **2**A. The amino-terminal sequence of IL13-gC chimeric protein (SEQ ID NO:22). The sequences upstream and downstream of the HS binding site portion are shown. IL13 was inserted between the two restriction enzyme sites that are underlined. FIG. **2**B. The domain of the gB open reading frame (i.e., ORF) from which the poly lysine [poly(K)] sequence was deleted (SEQ ID NO:23). The underlined sequences (codons 68-77) were not present in gB amplified from R5107. FIG. **2**C. The amino-terminal sequence of IL13-gD (SEQ ID NO:24). The first underlined sequence identifies the gD signal peptide. IL13 (bracketed by arrows) was inserted between residues 24 and 25 (underlined) of gD, between the XhoI and KpnI restriction enzyme sites.

[0038] FIG. **3**. Verification of R5111 viral DNA by PCR. Photographs of electrophoretically separated PCR products amplified directly from the plaques picked from Vero (FIG. **3**A) and HEp-2 (FIG. **3**B) cells. Viral DNAs were extracted as described in Example 1 and subjected to PCR with "IL13" primers from the IL13 ORF and IL13-gD primers, which bracketed IL13 and the gD ectodomain.

[0039] FIG. 4. Photograph of electrophoretically separated proteins from lysates of cells infected with R5111 reacted with antibody to gC, gD or IL13. HEp-2 cells grown in 25 cm^2 flasks were exposed to 10 PFU of HSV-1 or R5111 per cell. The cells were harvested 24 hours after infection, solubilized, subjected to electrophoresis in 10% denaturing polyacrylamide gels, electrically transferred onto a nitrocellulose sheet, and exposed to a monoclonal antibody against gD (FIG. 4A), gC (FIG. 4B) or IL13 (FIG. 4C), respectively. The protein bands corresponding to the gC, IL13-gC fusion protein, gD and the IL13-gD fusion protein are indicated. IL13-gC was the same size as native gC, as expected.

[0040] FIG. 5. HA-tagged IL13Rα2 expression from individual clones of stable transfectants of the J1.1 cell line. The individual clones were amplified as described in Example 1. Cells were harvested from 25 cm^2 flasks, solubilized, and subjected to electrophoresis in 12% denaturing polyacrylamide gels, electrically transferred onto a nitrocellulose sheet, and exposed to a polyclonal antibody to HA tag.

[0041] FIG. 6. Diagram of the pgD− vector.

[0042] FIG. 7. Schematic representation of the HSV-1 (F) genome and genetic structure of R5141 and R5144.

[0043] FIG. 8. Schematic representation of the construction of the ATF-uPA-gD and BD-uPA-gD recombinant viruses. (FIG. 1A) Schematic representation of the uPA constructs. (Line 1) Full length of uPA. (Line 2) ATF-uPA. (Line 3) BD-uPA. (FIG. 1B) Schematic representation of recombinant HSV 5181 and 5182. (Line 1) Sequence arrangement of HSV-1 genome where rectangular boxes represent the inverted repeat sequences ab and b'a' flanking the unique long (UL) sequence and inverted repeat c'a' and ca flanking the unique short (US) sequence. (Line 2) Schematic representation of recombinant HSV-1 (F) genome, in which the N-terminal domain of gC was replaced with IL-13, and the polylysine domain (codons 68-77) of gB was deleted. The domain of gD was replaced with the immediately early promoter of cytomegalovirus to enable the expression of gI. (Line 3) Sequence arrangements of the glycoprotein D are highlighted. IL13 was replaced with ATF-uPA (Line 4) or BD-uPA (Line 5).

[0044] FIG. 9. Amino acid sequence alignment of ATF-uPA-gD junction and BD-uPA-gD junction. (FIG. 2A) The amino-terminal sequence of ATF-uPA-gD (SEQ ID NO:45). The first underlined sequence identifies the gD signal peptide. ATF-uPA (bracketed by arrows) was inserted between residues 24 and 25 (underlined) of gD, between the XhoI and KpnI restriction enzyme sites. (FIG. 2B) The amino-terminal sequence of BD-uPA-gD (SEQ ID NO:46). BD-uPA (bracketed by arrows) was inserted between residues 24 and 25 (underlined) of gD, between the XhoI and KpnI restriction enzyme sites.

[0045] FIG. 10. Photograph of electrophoretically separated proteins from lysates of cells infected with ATF-uPA-gD or BD-uPA-gD virus reacted with antibody to ICP0, gD, US11 or ATF-uPA. Vero cells grown in 25-cm^2 flasks were exposed to 10 pfu of HSV-1, ATF-uPA-gD or BD-uPA-gD virus per cell. The cells were harvested 24 hours after infection, solubilized, subjected to electrophoresis in 10% denaturing polyacrylamide gels, electrically transferred onto a nitrocellulose sheet, and reacted with a monoclonal antibody against ICP0, gD, US11 or ATF-uPA, respectively. The protein bands corresponding to the ICP0, gD, ATF-uPA-gD fusion protein, BD-uPA-gD fusion protein and US11 are indicated.

[0046] FIG. 11. Human uPAR expression from the individual clones of stable transfectants of the J1.1 cell line. The individual clones were amplified as below. The cells were harvested from 25-cm^2 flasks, solubilized, and subjected to electrophoresis in 10% denaturing polyacrylamide gels, electrically transferred onto a nitrocellulose sheet, and reacted with a monoclonal antibody to human uPAR.

[0047] FIG. 12. Replication of R5182 and R5182 virus and HSV-1 (F) in J-uPAR, J1.1, J-HveA and J-Nectin cells. Cells grown in 25-cm^2 flasks were exposed to 0.1 pfu of the recombinant virus or wild-type HSV per cell and harvested 24 hours after infection. Progeny virus was titered on Vero cells.

[0048] FIG. 13. Photographs of DNA bands derived by reverse transcription and PCR amplification of RNAs extracted from J1.1 or J-uPAR cells using primers as described below. The PCR was performed with primers specific for the hamster (A) and human uPAR (B) ORFs indicated.

[0049] FIG. 14. scuPA competition assay on R5181 virus infectivity in J-uPAR and J1.1. J-uPAR and J1.1 were exposed 1 hour to either 10 nM or 100 nM of scuPA, respectively. The cells were then infected with 0.1 pfu of R5181 virus per cell and harvested 24 hours post-infection. Progeny virus was titrated on Vero cells.

[0050] FIG. 15. The Effect of NH_4Cl on R5181 virus infectivity in J-uPAR and J1.1. Cells grown in 25-cm^2 flasks were exposed to increasing concentrations of NH_4Cl for 30 minutes, infected with R5181 virus at 0.1 pfu/cell for 120 minutes in the same medium, and harvested 24 hours after infection. Progeny virus was titered on Vero cells.

[0051] FIG. 16. Amino acid sequence of wild type and R5322 gD

[0052] The 155 amino-terminal residues of uPA were inserted between the gD signal peptide and residue 33 of mature gD. (Other positions identified use the numbering of the mature gD.) In addition, valine 34 was substituted with serine. The frameshifts resulting from deletion of a cytosine after codon 60 and insertion of a cytosine after codon 201 introduced in frame new codons including 4 stop codons at the positions shown. The wild-type gD sequences resume at codon 202. Residue 219 is the first methionine of the carboxyl terminal portion of gD.

[0053] FIG. 17. Structure of chimeric gDs present in R5322 or designed for construction of recombinants R5321 or R5323 viruses and the properties of the recombinant viruses Panel A, the sequence arrangement of gD present in R5322 or constructed for other recombinant viruses. Panel B, the replication of recombinant viruses in J-Nectin, J-HveA or Vero13R cell lines. Cells grown in 25-cm2 flasks were exposed to 0.1 PFU of the recombinant virus per cell and harvested 24 h after infection. Progeny virus was titrated on Vero-13R cells. Panel C, photograph of electrophoretically separated proteins from lysates of cells infected with R5321, R5322, and R5323 recombinant viruses. Vero-13R cells grown in 25-cm2 flasks were exposed to 1.0 PFU of R5321, R5322, and R5323 virus per cell. The cells were

harvested 24 h after infection, solubilized, subjected to electrophoresis in 10% denaturing polyacrylamide gels, electrically transferred onto a nitrocellulose sheet, and reacted with a monoclonal antibody against ICP0, gD(ZC25), uPA and IL13 respectively.

[0054] FIG. **18**. Structure of chimeric gD and the properties of the recombinant viruses incorporating these glycoproteins. Panel A. sequence arrangement of the chimeric gD designed for construction of recombinant viruses. Panel B. the yields of R5332 and R5352 from cells infected with 0.1 PFU of virus/cell. The recombinant viruses R5331 and R5351 did not infect these cells or produce plaques in VeroIL13 cells. Panel C. accumulation of ICP0. the N-terminal polypeptide of gD identified by the antibody against uPA or the C-terminal polypeptide of gD identified by its reactivity with the anti-Myc antibody.

[0055] FIG. **19**. Co-precipitation of the polypeptides consisting of the N-terminal uPA-gD33-60 and the C-terminal gD219-314 domains (numbering of mature gD; add 25 for the numbering of SEQ ID NO:26). Panel A: the structure of the plasmids transfected in a pair-wise manner into HEK293 cells. The transfected cells were harvested, 40 hours after transfection. Portions comprising 10% of the total cell lysates were solubilized, subjected to electrophoresis in a denaturing gel and probed with antibody to uPA (Panel B-a, Panel C-a), IL13 (Panel B-a) or gD (Panel B-a) or with antibody to Myc (Panel B-b, Panel C-b). Other aliquots of the lysates were reacted with antibodies to gD, uPA or IL13. The precipitates were collected, solubilized subjected to electrophoresis in denaturing gels and reacted with antibody to uPA (Panel B-c) or Myc (Panel B-d).

DETAILED DESCRIPTION

[0056] The invention provides benefits that will improve the health and well-being of animals such as man by providing a targeted approach to the treatment of a variety of conditions and diseases that currently impair health, resulting in significant economic burdens using conventional treatments. In providing modified viral particles having controllable targeting capacities, the diagnostic and therapeutic benefit of the viruses themselves can be delivered with greater precision to particular cells. Additionally, these viral particles can be used as targeting vehicles for the delivery of a wide variety of therapeutic and diagnostic biomolecules, such as polynucleotides encoding therapeutic or diagnostic peptides.

[0057] Beyond the modified viral particles, the invention provides methods for making such therapeutic and diagnostic agents as well as methods for using the agents to diagnose or treat a variety of diseases and conditions, such as tumorigenic disease (e.g., gliomas). To facilitate an understanding of the invention and all of its aspects, illustrative embodiments are described below. The descriptions of these illustrative embodiments are not meant to limit the invention to the embodiments disclosed herein. In light of the description, one of skill in the art will understand that many changes and modifications can be made to the illustrative embodiments and still remain within the invention. The illustrative embodiments are disclosed using as an exemplary virus a member of the Herpesviridae family of viruses, herpes simplex virus (HSV).

[0058] As noted above, HSV-1 and HSV-2 are members of the family of viruses known as the Herpesviridae, whose structures are well known in the art. The targeting methods of the invention are applicable to any member of the Herpesviridae and are not limited to the exemplary embodiments described in the examples. Furthermore, a large number of recombinant HSV viruses are known in the art. Such viruses may contain one or more heterologous genes. Also, such viruses may contain one or more mutated HSV genes, for example, mutations that render the virus replication-deficient or affect the virulence of the virus in one or more cell types.

[0059] Examples of recombinant HSV containing a heterologous gene and methods of making and using such viruses are described in U.S. Pat. No. 5,599,691 (incorporated herein by reference in its entirety). Preferred heterologous genes include genes encoding marker proteins. Marker proteins, such as green fluorescent protein, luciferase, and beta-galactosidase, allow detection of cells expressing the protein. In other embodiments, the heterologous gene encodes an enzyme that activates a prodrug thereby killing adjacent uninfected cells. In yet other embodiments, the heterologous gene encodes a protein that affects the immune response, such as interleukin 12 (IL-12). Such proteins that activate the immune response against a tumor are particularly useful.

[0060] The 369-residue glycoprotein D (gD) is a receptor-binding protein of herpes simplex virus 1 involved in cellular entry; the protein appears to function as a dimer (9). The common receptors for viral entry are Nectin-1, HveA, and a specific O-linked sulfated proteoglycan. The major receptor-binding sites of gD are at the N-terminus whereas the domain required for fusion of the viral envelope with the plasma membrane is at the C-terminus of the ectodomain (residues 260-310). These become actively engaged in membrane fusion following the interaction of the N-terminal domain with one of its cognate receptors (6-8, 10-14).

[0061] The domains of gD critical for its functions may be summarized as follows: The interaction of gD with HveA is abolished by deletion of the N-terminal 32 residues of gD. The interactions of gD with Nectin-1 are abolished by mutations at gD residues 34, 38, 215, 222, and 223. A pro-fusion domain, essential for activation of fusogenic glycoproteins gB, gH and gL has been mapped to residues 260 to 310 in close proximity to the transmembrane domain beginning at residue 314 (6). gD suppresses apoptosis induced by mutants lacking gD (17). Linker insertion analyses have identified several sites that abolish this function and some are located between residues 61 to 218 (17). Although failure of many linker insertion mutants to inactivate infectivity was attributed to the flexibility of the gD structure, in view of the disclosure herein, the data can be understood as showing that at least a portion of gD plays no sequence-specific role in virus entry other than to hold the receptor binding domain linked to the pro-fusion domain.

[0062] Efforts to re-target gD to the urokinase plasminogen activator receptor for therapeutic applications led to a genetically engineered infectious virus in which the receptor-binding domain, consisting of the N-terminal domain of urokinase plasminogen activator (uPA) fused to residues 33 to 60 of gD, was separated from an independently expressed C-terminal domain of gD containing residues 219 to 369 (numbering of mature gD; add 25 to each number for the numbering used in SEQ ID NO:26). The intervening

sequence (residues 62 to 218 of mature gD) was replaced by a stop codon and a promoter for the C-terminal domain of gD. The physical interaction of the two components was reconstructed by co-immune precipitation of the N-terminal domain of uPA with the C-terminal domain of gD. These results indicate that codons 61 to 218 of mature gD do not encode executable functions required for viral entry into cells and suggest that the receptor-binding ligand must interact with, but need not alter the structure of, the residual portion of gD to effect virus entry. This finding opens the way for development of a family of recombinant viruses in which the pro-fusion domain of gD is independently furnished, with receptor-binding domains interacting with the pro-fusion domain to effect entry of the virus via a range of receptors not involved in wild-type HSV entry.

[0063] The data disclosed herein establish that there are two domains of gD involved in specific viral entry. An N-terminal domain recognizes specific targets used by wild-type HSV to effect cell entry. That target domain, however, also provides for interaction with a second domain relevant to entry, i.e., a C-terminal fusogenic domain that interacts with fusogenic gB, gH and gL, thereby coordinating the entry process. Constructs disclosed herein provide the N-terminal sub-domain of gD responsible for interaction with the C-terminal fusogenic domain of gD, with and without the N-terminal sub-domain of gD conferring specific target recognition. Thus, constructs provide for an N-terminal target domain of gD that both specifically recognizes a target and that interacts with the C-terminal fusogenic domain of gD. Other constructs provide for an N-terminal sub-domain of gD providing the capacity to interact with the C-terminal fusogenic domain but lack the specific targeting capacity of full-length gD. The invention comprehends the latter constructs as cassettes useful for screening for specific targeting sequences, such as a sequence encoding uPA, as well as products useful in the simplified construction of HSVs designed to specifically recognize any desired target amenable to specific interaction with a peptide.

[0064] Consistent with the foregoing principles, the invention comprehends a coding region for a peptide forming a binding pair with a target of choice, that coding region optionally, although typically, being translationally fused to a coding region specifying the N-terminal interaction sub-domain (e.g., amino acids 33-60 of mature gD). In embodiments in which the targeting peptide (e.g., uPA) is capable of directly interacting with a C-terminal fusogenic peptide (e.g., amino acids 219-369 of mature gD), (see the Examples below), the targeting peptide may be encoded by a distinct polynucleotide. The targeting peptide or targeting peptide fusion may be further fused to a peptide comprising the C-terminal fusogenic peptide (e.g., mature gD amino acids 219-369), such as by direct translational fusion to a coding region for residues 219-369 or by translational fusion to a coding region for amino acids 61-369 of mature gD. In additional embodiments, the coding region encodes a portion of the region of mature gD (amino acids 61-218) not essential to viral entry. Additionally, constructs are contemplated that translationally fuse a linker peptide between the targeting peptide fusion coding sequence and the fusogenic peptide coding sequence. Coding regions for any of the peptide linkers known in the art may be used in this context, such as the $(Gly_4Ser)_n$ (n=3-5) series of linkers used in engineered antibody products. Use of a linker is expected to provide the spacing and flexibility compatible with effective interaction of the targeting fusion peptide and the fusogenic peptide. Further, constructs embraced by the invention provide a linker interposed between a targeting peptide and a peptide providing the N-terminal sub-domain for interaction with the fusogenic peptide. Any linker known in the art may be used in such constructs to provide the spacing and flexibility compatible with effective target interaction by the targeting peptide with effective interaction between the fusogenic peptide and the N-terminal fusogenic interaction sub-domain of gD.

[0065] The targeting peptides contemplated by the invention include any peptide capable of specifically interacting with a target of choice. Typically, the chosen targets will be peptides presented on the surface of a host cell targeted for HSV entry. Non-peptide targets are also comprehended, as are targets not presented on the surface of a host cell, provided that the specific interaction between the target and the HSV targeting fusion leads to viral entry into the host cell (e.g., the targeting fusion may specifically bind a binding pair partner that isn't naturally associated with a host cell but that is capable of interacting specifically with the host cell of interest, effectively "marking" that host cell for viral entry). Beyond naturally occurring members of a binding pair, and fragments and variants thereof retaining the capacity for specific interaction with the binding pair partner, the invention contemplates engineered targeting peptides, such as antibody products, and functional fragments and variants thereof. For example, a single-chain variable fragment of an antibody specifically interacting with a peptide preferentially displayed on the surface of a target host cell, such as a cancer cell, may be fused to the N-terminal sub-domain of gD capable of interaction with the fusogenic domain of gD. In other embodiments, the minimal complementarity determining regions (CDRs) of an antibody are linked in a peptide fused to the interaction sub-domain of gD. Another alternative embodiment places the single variable region, or minimal CDR determinants thereof, of a camelid antibody into a fusion peptide with the interaction sub-domain of gD. In fact, any engineered antibody product, or non-antibody binding peptide, that is amenable to peptide fusion, with or without a linker, is suitable for fusion to the interaction sub-domain of gD in the constructs according to the invention.

[0066] The preceding constructs are preferred constructs in that these constructs are re-targeted insofar as the natural targeting provided by the complete N-terminal targeting domain of gD is not present to compete with the targeting provided by the peptide member of a binding pair. In some embodiments of the invention, however, retention of the wild-type HSV targeting provided by the N-terminal domain of gD is acceptable and, in such embodiments, the invention embraces targeting peptide fusions, with or without intervening linkers, to an N-terminal domain of gD extending from a point between amino acids 1-32, inclusive, to amino acid 60 of gD.

[0067] The invention also contemplates screens or assays for identifying suitable targeting peptides. In this aspect of the invention, a wide variety of coding regions may be ultimately linked by any manner described above to the coding region for the fusogenic domain of gD. Thus, the set of coding regions may be linked directly to the coding region for the fusogenic domain, or may be linked to the complete or partial intervening sequence of gD that is, in turn, fused

to the fusogenic domain, or may be fused to a linker that is in turn fused to the fusogenic domain. The collection of constructs (e.g., library) may then be subjected to any of a variety of well-known assays to identify particular constructs that re-target HSV entry. For example, a functional assay may be employed in which a target host cell is monitored for either cell death attending successful infection or for HSV replication associated with successful infection. Alternative assays are available to detect specific interaction of an HSV fusion peptide (targeting peptide ultimately fused to the interaction sub-domain of gD) with a given target, such as a cell-surface peptide, for example using an immunoassay technique such as ELISA.

[0068] In the following examples, it is revealed that residues 61 to 218 of gD do not perform functions required for entry of virus into cells. Two series of recombinant viruses were constructed that target receptors uniquely present on the surface of tumor cells (18-20). In one recombinant (R5111) designed to target the IL13α2 receptor enriched in malignant glioma cells, 140 residues encoding IL13 were inserted between residues 24 and 25 of gD. This recombinant virus can infect cells expressing the IL13α2 receptor but it also retained the capacity to interact with Nectin-1 and HveA receptors (18). In the second recombinant virus (R5141), the IL13 ligand was fused to residue 33 of gD. In addition, valine 34 was replaced with serine. This recombinant entered cells solely via the IL13α2 receptor. IL13 added to the medium blocked entry of the recombinant virus expressing the IL13α2 receptor (20). The second target selected for these studies was urokinase plasminogen activator receptor (uPAR), commonly found on the surface of most cells but highly enriched on the surface of cancer cells (19).

[0069] In the first of this series of recombinant viruses, R5181, the N-terminal 155 residues of urokinase plasminogen activator (uPA) were inserted after residue 24 of mature gD. This recombinant virus infected cells expressing Nectin-1, HveA, or even hamster cells that lacked known HSV receptors but were subsequently shown to express hamster-specific uPAR mRNA. In this instance, removal of uPAR by digestion of the glucosyl-inositol anchor with phosphatidyl inositol-specific phospholipase C blocked virus entry (19). The results reported below emerged from an attempt to construct a chimeric gD modeled after the recombinant virus R5141 in that uPA sequences were fused to residue 33 of mature gD and valine 34 was replaced with serine. Although the genetically engineered virus designated R5322 replicated as expected in all cell lines tested, sequencing of the chimeric gD present in the recombinant virus revealed that it contained two frame-shift mutations, one each after residues 60 and 201 (mature gD numbering). The frame shifts introduced 4 stop codons between residues 60 and 201. The first available potential initiator methionine codon was at position 219 of mature gD.

[0070] In one aspect, the invention relates to altering the surface of an HSV particle to target the virus to a specific receptor. By creating a fusion protein comprising a portion of gD and a ligand (or binding pair member), the virus is targeted to a cell having a cell surface receptor that binds the ligand (or binding pair member). In preferred embodiments, one or more HSV surface proteins, such as gB (SEQ ID NOs:27 and 28), gC (SEQ ID NOs:29 and 30), or gD (SEQ ID NOs:25 and 26), are altered to reduce binding to natural HSV receptors.

[0071] "Alterations" of the surface of an HSV particle or HSV surface protein include insertions, deletions, and/or substitutions of one or more amino acid residues. One type of alteration is an insertion, which involves the incorporation of one or more amino acids into a known peptide, polypeptide or protein structure. For ease of exposition, alterations will be further described using a protein exemplar. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of known proteins, which yield proteins such as fusion proteins and proteins having amino acid tags or labels.

[0072] Another type of alteration is a deletion, wherein one or more amino acid residues in a protein are removed. Deletions can be effected at one or both termini of the protein, or with removal of one or more residues within the amino acid sequence. Deletion alterations, therefore, include all fragments of a protein described herein.

[0073] Yet another type of alteration is a substitution, which includes proteins wherein one or more amino acid residues are removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature; however, the invention embraces substitutions that are also non-conservative. Conservative substitutions for this purpose may be defined as set out in Tables A or B, below.

[0074] Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table A as described in Lehninger, [Biochemistry, 2nd Edition; Worth Publishers, Inc. New York (1975), pp. 71-77] and set out immediately below.

TABLE A

Conservative Substitutions I

SIDE CHAIN CHARACTERISTIC	AMINO ACID
Non-polar (hydrophobic):	
A. Aliphatic	A L I V P
B. Aromatic	F W
C. Sulfur-containing	M
D. Borderline	G
Uncharged-polar:	
A. Hydroxyl	S T Y
B. Amides	N Q
C. Sulfhydryl	C
D. Borderline	G
Positively charged (basic)	K R H
Negatively charged (acidic)	D E

[0075] Alternative, exemplary conservative substitutions are set out in Table B, immediately below.

TABLE B

Conservative Substitutions II

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTION
Ala (A)	Val, Leu, Ile
Arg (R)	Lys, Gln, Asn
Asn (N)	Gln, His, Lys, Arg
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
His (H)	Asn, Gln, Lys, Arg
Ile (I)	Leu, Val, Met, Ala, Phe,
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, Gln, Asn
Met (M)	Leu, Phe, Ile
Phe (F)	Leu, Val, Ile, Ala
Pro (P)	Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser
Val (V)	Ile, Leu, Met, Phe, Ala

[0076] The binding site of HveA has been reported to be at the amino terminal domain of gD (Carfi A., et al., 2001). The precise binding sites of gD for Nectin 1 are not known, although it has previously been reported to involve gD amino acids 38 and 221 (Manoj S., et al., 2004; Zago A., et al., 2004; Connolly S A., 2005). Accordingly, in one aspect the invention relates to amino acid alterations in the N-terminal region of gD such that the ability of gD to bind HveA or Nectin1 is reduced or eliminated. A "natural receptor" as used herein is a cell surface molecule that interacts with wild-type HSV in the absence of human intervention. For example, gB and gC of HSV-1 interact with heparan sulfate proteoglycans in a natural infection. In preferred embodiments, gB and/or gC are altered to reduce or eliminate binding to heparan sulfate proteoglycans. As another example, gD is known to bind to several receptors, including HveA and Nectin-1, in a natural infection. In preferred embodiments, gD is altered to reduce or eliminate binding to HveA and/or Nectin-1.

[0077] Receptor-Ligands

[0078] As used herein, "receptor" and "ligand" refer to two members of a specific binding pair and, hence, are binding partners. A receptor is that member of the pair that is found localized on the surface of the cell; the ligand is the member of the pair that is found on the surface of HSV. Thus, in certain embodiments, the "ligand" may actually be what the art recognizes as a receptor outside the context of the invention and the "receptor" may be its respective ligand. More generally, the invention comprehends an HSV exhibiting a member of a binding pair, or a fragment thereof that retains the capacity to specifically bind the other member of the binding pair, on its surface and the other member of that binding pair, or a fragment thereof that retains the capacity to specifically bind its partner, is present on the surface of a target cell.

[0079] One advantage of the invention is the ability to tailor HSV to target a specific receptor while maintaining infectivity of the virus. In an exemplary embodiment, an HSV particle contains a fusion protein comprising a portion of gD and the cytokine IL-13. Such a virus is able to infect cells expressing the receptor IL-13Rα2. Because IL-13Rα2 is expressed on the surface of cells of malignant gliomas, HSV containing the gD/IL-13 fusion protein are effectively targeted to such cells. Ligands that bind to receptors which are overexpressed or differentially expressed on either tumor cells or cells associated with tumor growth (e.g., neovasculature) are particularly preferred. Examples include the $\alpha_v\beta_3$-$\alpha_v\beta_5$ integrins, which are overexpressed in tumor neovasculature; epidermal growth factor receptor (EGFR), which is overexpressed in head, neck, lung, colon, breast, and brain cancer cells; HER-2/Neu, which is overexpressed in breast cancer cells; MUC-1, which is overexpressed in breast, lung, and pancreas cancer cells; and prostate-specific membrane antigen, which is overexpressed in prostate cancer cells. In certain embodiments, the ligand is a single-chain antibody that binds to its cognate specific binding pair member, herein referred to as a receptor. Single-chain antibodies have been shown to be effective in targeting applications, as evidenced by their ability to target retroviruses to specific receptors.

[0080] Essentially any two binding pair members or partners may be used as receptor-ligands in the invention. However, it is contemplated that certain factors, such as the distance from the binding site on the receptor to the membrane, or the conformation of the ligand when fused to gD, may affect the efficiency of recombinant HSV fusion to the cell membrane. Therefore, screens for effective receptor-ligand pairs are contemplated, using no more than routine procedures known in the art. Additional screens, conventional in nature, may be used to optimize constructs. One routine method of screening is to follow the protocol provided in the example for candidate receptor/ligand pairs, using IL-13R/IL-13 as a control receptor/ligand pair.

[0081] Alternatively, one may use a membrane fusion assay as described in Turner et al., 1998, incorporated herein by reference in its entirety. In the Turner assay, cells transfected with construct(s) encoding gB, gH, gL, and the gD/ligand fusion protein, and cells expressing the receptor, are co-cultured and the cells are examined for membrane fusion. Membrane fusion between gD/ligand-expressing cells and receptor-expressing cells indicates that the candidate receptor-ligand pair (the ligand being a gD/ligand fusion protein) is functional. Constructs encoding functional gD/ligand proteins can then be used to create recombinant HSV that are targeted to cells expressing the receptor.

[0082] Cell Targeting

[0083] Evident from the preceding discussion, another aspect of the invention is the targeting of a recombinant HSV to a cell having a specific receptor on its surface. In preferred embodiments, a recombinant HSV is designed to comprise a ligand that interacts with a receptor known to be expressed on a cell of interest. The cell of interest is then infected with recombinant HSV. Such targeting methods may be used for a variety of purposes.

[0084] In one aspect, a recombinant HSV is used to introduce a heterologous gene into a cell that expresses the receptor. In preferred embodiments, the cell is not infected by, or is poorly infected by, wild-type HSV. Thus, in certain embodiments, the invention provides a vector for transforming a cell of interest with a heterologous gene.

[0085] Further, a cell can be rendered a target of a recombinant HSV of the invention. The cell can be rendered a target by transforming the cell to express one member of a binding pair, e.g., a receptor capable of specifically binding a ligand expressed on a recombinant HSV. For example, as described in Example 2, the J1.1 cell line, which was resistant to infection by a recombinant HSV expressing an IL-13 ligand, was rendered susceptible to infection by transforming the cell line with a vector encoding IL12Rα2 to produce the cell line J13R.

[0086] Generally, the targeted HSV according to the invention exhibit one member of a binding pair, with the other member of that pair found on the surface of a target cell. In some embodiments of the invention, targeting is achieved with a ligand-receptor binding pair, with the ligand exhibited on the targeted HSV and the cognate receptor found on the surface of the target cell, as described above. Although the invention comprehends embodiments involving binding pairs that do not exhibit a ligand-receptor relationship (e.g., biotin-avidin) and embodiments in which the receptor is exhibited by the targeted HSV (the "receptor" defined above as a "ligand" using an alternative definition of "ligand") while the cognate ligand is found on the target cell (the "ligand" defined above as a "receptor" using an alternative definition of "receptor"), embodiments in which the targeted HSV exhibits a ligand and the target cell presents the cognate receptor on its surface is used as an illustrative embodiment to reveal the versatility of the invention and to disclose the full scope thereof. For example, several ligands have been used for receptor-mediated polynucleotide transfer. Some ligands that have been characterized are asialoorosomucoid (ASOR) and transferrin (Wagner et al., Proc. Natl. Acad. Sci. USA, 87(9):3410-3414, 1990). A synthetic neoglycoprotein, which recognizes the same receptor as ASOR, has also been used in a polynucleotide delivery vehicle (Ferkol et al., FASEB J., 7:1081-1091, 1993; Perales et al., Proc. Natl. Acad. Sci., USA 91:4086-4090, 1994) and epidermal growth factor (EGF) has further been used to deliver polynucleotides to squamous carcinoma cells (Myers, EPO 0273085). Each of these specific approaches, and other approaches known in the art to achieve some selectivity in DNA delivery, or targeting, are amenable to use in the compositions and methods of the invention and are contemplated as embodiments of the invention.

[0087] For embodiments in which a targeted HSV harboring a coding region, e.g., a therapeutic coding region or gene, is delivered to a target cell, the nucleic acid encoding the therapeutic gene product may ultimately be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the therapeutic polynucleotide may be stably integrated into the genome of the cell. This integration may place the gene in its native location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or epitomes encode functions sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed, as would be understood in the art.

[0088] It is envisioned that promoters subject to cell cycle regulation will be useful in the present invention. For example, in a bicistronic HSV vector designed to treat a disease, disorder or condition by killing a target cell, use of a strong CMV promoter to drive expression of a first gene, such as p16, that arrests a cell in the GI phase is accompanied by expression of a second gene, such as p53, under the control of a promoter that is active in the GI phase of the cell cycle, thus providing a dual-gene approach to ensure that the target cell undergoes apoptosis. Other promoters, such as those of various cyclins, PCNA, galectin-3, E2F1, p53, BRCA1, and, indeed, any suitable promoter or expression element known in the art, could be used.

[0089] In embodiments of the invention designed to treat diseases, disorders, or conditions associated with unwanted or excessive cell proliferation, such as cancer or restenosis, HSV is targeted to proliferating cells thereby killing the cells. Because HSV is lethal to infected cells, expression of a heterologous gene is not required. However, in embodiments wherein the lethality of HSV is attenuated, an HSV harboring a gene that is lethal to the infected cell or that prevents proliferation of the infected cell may be used to target a cell.

[0090] Alternatively, HSV targeted to specific surface markers can be used to visualize the distribution of tumor cells in tissues. This diagnostic tool had been unavailable because of the indiscriminate binding of HSV to cells. Modification of HSV by eliminating (ablating) or reducing the indiscriminate binding of HSV to heparan sulfate proteoglycans without deleteriously affecting the capacity of such HSV to replicate in both dividing and non-dividing cells makes possible the use of these modified viral forms to visualize the distribution of tumor cells.

[0091] In one preferred method for visualizing the distribution of tumor cells, radioactive visualization is achieved by viral thymidine kinase (TK)-dependent incorporation of a radioactive precursor. Methods of molecular imaging of gene expression are well known in the art. Methods often use highly sensitive detection techniques such as positron emission tomography (PET) or single-photon emission-computed tomography (SPECT). In one embodiment, TK expression is measured using a gancyclovir analog, such as 9-(3-[^{18}F]fluoro-1-hydroxy-2-propoxy)methylguanine, as the tracer or marker (Vries et al., 2002). For a review of imaging TK gene expression using PET or SPECT, see Sharma et al., 2002 or Vries et al., 2002.

[0092] A second preferred imaging method is to fuse a non-critical tegument protein (e.g. U_S11, which is present in nearly 2000 copies per virus particle) to a marker protein, such as green fluorescent protein, which is capable of being visualized in vivo. Alternatively, a non-critical protein can be fused to a luciferase and the presence of the luciferase visualized with a luminescent or chromatic luciferase substrate. Although a marker protein can be fused to essentially any viral structural protein, preferred viral proteins include gC, gE, gI, gG, gJ, gK, gN, U_L11, U_L13, U_L14, U_L21, U_L41, U_L35, U_L45, U_L46, U_L47, U_L51, U_L55, U_L56, U_S10, and U_S11. The marker protein also may be fused to thymidine kinase (Soling et al., 2002).

[0093] Library Screening

[0094] As noted above, HSV comprising a gD/ligand fusion protein can bind and infect cells expressing a receptor

to the ligand. In one embodiment, a cell line expressing a receptor is used in screening for ligands of the receptor. cDNA from a cDNA library is cloned into a vector encoding a portion of gD to produce a gD/cDNA-encoded fusion protein. The resulting vectors are then screened for membrane fusion using the assay of Turner et al. described above or by creating recombinant HSV expressing the gD/cDNA-encoded fusion protein and screening the viruses for the ability to infect receptor-expressing cells. Such methods may be used, e.g., to identify a ligand to an orphan receptor.

[0095] In other embodiments, mutations in, or variants of, the receptor or ligand are screened to determine whether the mutants or variants maintain the ability to interact with the respective partner. Such methods may be useful in determining the specific residues important in receptor-ligand interaction.

[0096] Therapeutic Methods

[0097] Another aspect of the invention is the use of the targeted HSV in therapeutic methods. By altering the cell-binding and infectivity properties of the virus, many routes and methods of administration become viable. For example, non-targeted HSV will bind indiscriminately to a variety of cells. Because of this property, large virus numbers are used and intravenous administration is generally not effective. However, by targeting the virus, one may lower the viral load (i.e., quantity of virus), yet maintain or increase efficacy. Furthermore, the targeted HSV can be administered intravenously and produce therapeutic effects.

[0098] Therapeutic methods of the invention include those methods wherein an HSV is targeted to a receptor of a cell that contributes to, or is the basis of, a disease or disorder. These targeted HSV can either exploit the therapeutic properties of HSV itself (e.g., the lethality of HSV to infected cells) or the targeted HSV can serve as a vector for the targeted delivery of at least one therapeutic polynucleotide, such as an expressible polynucleotide comprising a coding region. For example, in methods wherein the targeted HSV contains one or more gene products that render the virus toxic to the cell or that prevent or inhibit cell proliferation, a preferred receptor is overexpressed or selectively expressed on harmful or undesirable cells, such as cancer cells. In other embodiments, the targeted HSV encodes a gene product that provides a desired function or activity in the targeted cell, e.g., when a cell has one or more genetic defects preventing the cell from functioning properly.

[0099] Additionally, it is contemplated that a therapeutic polynucleotide (e.g., gene or coding region) of a targeted HSV may be engineered to be under the expression control of a cell- or tissue-specific expression control element, e.g., a promoter. In such embodiments, the targeted HSV provide a further enhancement to the selective treatment of a suitable disorder, disease or condition. The targeted HSV is specific for a binding partner located on the surface of those cells for which treatment is intended, and expression of the therapeutic coding region or gene borne by the targeted HSV is limited to particular cells or tissues.

[0100] As HSV has been engineered to overcome the barriers to vector-based therapies, the choice of recombinant polynucleotide to be inserted into the vector has widened to the point where a wide variety of diseases, disorders and conditions are amenable to treatment with targeted HSV. A number of diseases are amenable to polynucleotide-based therapy using HSV (see, e.g., Kennedy, et al. *Brain.* 120, 1245-1259, 1997, incorporated by reference herein in its entirety). Though most attention has focused on cancers, there has been success in treating Parkinson's disease by expressing tyrosine hydroxylase in striatal cells, thus restoring L-dopa-induced nerve repair following axotomy of the superior cervical ganglion. Injection of a vector expressing nerve growth factor resulted in restored levels of tyrosine hydroxylase. More generally, HSV can now be used in polynucleotide-based therapy to replace missing or defective coding regions in the target cells. In the event of an inherited single-geNe disorder (such as Lesch-Nyhan syndrome) where the complete DNA sequence, cause, and effect of the disorder are known, a single polynucleotide replacement mediated by targeted HSV is appropriate and contemplated. Another strategy amenable to the use of targeted HSV is the enhancement of endogenous expression levels of a gene product, e.g., a growth factor or enzyme. Yet another strategy for using targeted HSV is HSV-directed enzyme pro-drug therapy. The delivery of a drug-sensitivity gene would be beneficial in the treatment of, e.g., a malignant brain tumor, making the tumor more susceptible to conventional anti-cancer agents.

[0101] In other embodiments, the targeted HSV of the invention provide for vector-mediated delivery of anti-sense oligodeoxyribonucleotides (oligonucleotides). The oligonucleotides, short segments of DNA (e.g., 2-100 nucleotides in length), are delivered to target cells and therein bind to complementary mRNA, thus blocking the expression of specific genes within the target cells. The encoded protein fail to be synthesized, as the mRNA is not be recognized by the translational components of the cell. In preferred embodiments, a deleterious gene is targeted.

[0102] In yet other embodiments, targeted HSV are used to deliver polynucleotides, e.g., DNAs encoding gene products, that can recruit or enhance an immune system response, thereby bringing a subject's or patient's own immune system to bear in the treatment of a disease, disorder or condition known in the art to be amenable to immune system activity. For example, an increase in cellular antigen expression of tumor cells, mediated by delivery of an expressible coding region for the antigen by a targeted HSV, would enhance the immune response and increase the susceptibility of such tumor cells to host cytotoxic immunity.

[0103] In some embodiments, a targeted HSV composition of the invention is delivered to a patient at or around the site of a tumor, which is a very efficient method for counteracting clinical disease. Alternatively, systemic delivery of targeted HSV compositions may be appropriate in other circumstances, for example, where extensive metastasis has occurred, or where inaccessible tumors are encountered.

[0104] It is contemplated that in certain embodiments of the invention a protein that acts as an angiogenesis inhibitor is targeted to a tumor. Also, an angiogenesis inhibitor agent may be administered in combination with a targeted HSV of the invention. These agents include, for example, Marimastat (British Biotech, Annapolis Md.; indicated for non-small cell lung, small cell lung and breast cancers); AG3340 (Agouron, LaJolla, Calif.; for glioblastoma multiforme); COL-3 (Collagenex, Newtown Pa.; for brain tumors); Neov-

astat (Aeterna, Quebec, Canada; for kidney and non-small cell lung cancer) BMS-275291 (Bristol-Myers Squibb, Wallingford Conn.; for metastatic non-small cell lung cancer); Thalidomide (Celgen; for melanoma, head and neck cancer, ovarian, and metastatic prostate cancers; Kaposi's sarcoma; recurrent or metastatic colorectal cancer (with adjuvants); gynecologic sarcomas, liver cancer; multiple myeloma; CLL, recurrent or progressive brain cancer, multiple myeloma, and non-small cell lung, nonmetastatic prostate, refractory multiple myeloma, and renal cancer); Squalamine (Magainin Pharmaceuticals Plymouth Meeting, Pa.; non-small cell lung cancer and ovarian cancer); Endostatin (EntreMEd, Rockville, Md.; for solid tumors); SU5416 (Sugen, San Francisco, Calif.; recurrent head and neck, advanced solid tumors, stage IIIB or IV breast cancer; recurrent or progressive brain (pediatric) cancer; ovarian cancer, AML (acute myeloid leukemia); glioma, advanced malignancies, advanced colorectal cancer, von-Hippel Lindau disease, advanced soft tissue cancer; prostate cancer, colorectal cancer, metastatic melanoma, multiple myeloma, malignant mesothelioma: metastatic renal, advanced or recurrent head and neck cancer, metastatic colorectal cancer); SU6668 (Sugen San Francisco, Calif.; advanced tumors); interferon-α; Anti-VEGF antibody (National Cancer Institute, Bethesda Md.; Genentech San Franscisco, Calif., for refractory solid tumors; metastatic renal cell cancer, in untreated advanced colorectal cancer; EMD121974 (Merck KCgaA, Darmstadt, Germany, for HIV-related Kaposi's sarcoma, and progressive or recurrent Anaplastic Glioma); Interleukin 12 (Genetics Institute, Cambridge, Mass., for Kaposi's sarcoma) and IM862 (Cytran, Kirkland, Wash., for ovarian cancer, untreated metastatic cancers of colon and rectal origin, and Kaposi's sarcoma). The parenthetical information following the agents indicates the cancers against which the agents are being used in these trials. It is contemplated that any of these disorders may be treated with the targeted HSV compositions of the invention, either alone or in combination with the agents listed.

[0105] In order to prepare a therapeutic composition for clinical use, it will be necessary to prepare the therapeutic composition as a pharmaceutical composition, i.e., in a form appropriate for in vivo applications. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or other vertebrates.

[0106] Generally, appropriate salts and buffers are included to render delivery vectors stable and to allow for uptake by target cells. Aqueous compositions of the invention comprise an effective amount of the targeted HSV, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carriers" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Unless a conventional medium or agent is incompatible with either the vectors of the invention or the intended subject receiving treatment, its use in therapeutic compositions is contemplated. Supplementary active or inert ingredients also can be incorporated into the compositions.

[0107] The active compositions of the invention include standard pharmaceutical preparations. Administration of these compositions according to the invention is by any known route, provided that the target tissue is accessible via that route. The pharmaceutical compositions may be introduced into the subject by any conventional method, e.g., by intravenous, intradermal, intramusclar, intramammary, intraperitoneal, intrathecal, retrobulbar, intravesicular, intrapulmonary (e.g., term release); sublingual, nasal, anal, vaginal, or transdermal delivery, or by surgical implantation at a particular site. The treatment may consist of a single dose or a plurality of doses over a period of time.

[0108] Upon formulation, solutions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. Appropriate dosages may be ascertained through the use of established routine assays. As studies are conducted, further information will emerge regarding optimal dosage levels and duration of treatment for specific diseases, disorders, and conditions.

[0109] In preferred embodiments, the unit dose may be calculated in terms of the dose of viral particles being administered. Viral doses are defined as a particular number of virus particles or plaque forming units (pfu). Particular unit doses include 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13} or 10^{14} pfu. Particle doses may be somewhat higher (10- to 100-fold) due to the presence of infection-defective particles, which is determinable by routine assays known in the art.

[0110] The pharmaceutical compositions and treatment methods of the invention are useful in the fields of human medicine and veterinary medicine. Thus, the subject to be treated may be a vertebrate, e.g., a mammal, preferably human. For veterinary purposes, subjects include, for example, farm animals such as cows, sheep, pigs, horses and goats, companion animals such as dogs and cats, exotic and/or zoo animals, laboratory animals including mice, rats, rabbits, guinea pigs and hamsters; and poultry such as chickens, turkey, ducks and geese.

[0111] In some embodiments of the invention, it is contemplated that the targeted HSV is administered in conjunction with chemo- or radiotherapeutic intervention, immunotherapy, or with any other therapy conventionally employed in the treatment of cancer.

[0112] To kill cells, inhibit cell growth, inhibit metastasis, inhibit angiogenesis or otherwise reverse or reduce malignant phenotypes using the methods and compositions of the invention, one contacts a "target" cell, a tumor, or its vasculature with a targeted HSV composition and at least one other agent. The components of these compositions are provided in a combined amount effective to kill or inhibit proliferation of cancer cells. This process may involve contacting the cells with the targeted HSV composition and the agent(s) or factor(s) at the same time. This may be achieved by contacting the subject organism, or cell of interest, with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations, at the same or different times, wherein one composition includes a

targeted HSV composition of the invention and the other composition includes the second agent.

[0113] Another aspect of the invention provides diagnostic methods that involve imaging a tumor or diseased tissue using a targeted HSV. Such methods are useful in diagnosing a patient with a disease, disorder, or condition that is indicated by the presence of a receptor on the surface of a cell. Diagnostic imaging methods are discussed above.

[0114] Kits

[0115] Kits according to the invention may include recombinant viruses of the invention or may include vectors for producing such recombinant viruses. A vector for producing a recombinant virus of the invention may encode the gD/ligand fusion protein or may be designed to facilitate cloning of a ligand to produce a gD/ligand fusion protein (e.g., a vector containing a multiple cloning site within the gD coding region that facilitates in-frame insertions).

[0116] Other components that can be included in a kit of the invention include a receptor-expressing cell line (useful as a control), a nucleic acid molecule for expressing the receptor in a particular cell type, and instructions for using the kit to effect diagnostic analyses or therapeutic treatments. In certain embodiments, a therapeutic kit will further contain a component for bringing about a therapeutic effect, such as a prodrug or a toxic compound. In other embodiments, a diagnostic kit will contain a compound useful in imaging methods, such as a chromophore or fluorophore, or an antibody for detecting infected cells.

[0117] Having provided a general description of the various aspects of the invention, the following disclosure provides examples illustrative of the invention. In these Examples, the numbering of gD residues is the numbering for mature gD unless indicated otherwise. The complete amino acid sequence of gD provided in SEQ ID NO:26 also includes the N-terminal signal peptide. To convert the numbering for mature gD to SEQ ID NO:26 numbering, add 25, the size of the signal peptide.

EXAMPLE 1

Construction of HSV Targeting Vector R5111

[0118] A targeted HSV was constructed to target the recombinant virus to cells of malignant gliomas. The target for entry of the virus into such cells is the IL13Rα2 receptor known to be present in malignant gliomas. Unlike the more prevalent IL13αR1 receptor, the IL13Rα2 receptor has a shorter cytoplasmic domain and does not interact with IL4, of which IL13 is a close relative. In general, the construction of the targeted HSV involved mutagenizing gB and gC to preclude their interaction with heparan sulfate. Also, IL13 was inserted into gD at amino acid 24 thereby disrupting the gD binding site for HveA. The resulting IL13-gD chimeric virus can use IL13Rα2 for entry into cells carrying that receptor.

[0119] More specifically, the targeted HSV R5111 was constructed in several steps depicted in the four panels of FIG. **1** and detailed below.

[0120] (i) Substitution of Amino Terminal Domain of gC with IL13 Fused to the Signal Sequence of gC.

[0121] FIG. **1**A, lines 1-3 schematically depicts a cDNA consisting of the IL13 coding sequence fused at its amino terminus to its signal sequence. The complete cDNA of IL13, with the N-terminal signal peptide coding region, was amplified using the PCR primer elongation method. The primers were as follows:

```
pIL13F1,
                                                (SEQ ID NO: 1)
CATTGCTCTCACTTGCCTTGGCGGCTTTGCCTCCCCAGGCCCTGTGC-
CTCCCTCTACAGC;

pIL13F2,
                                                (SEQ ID NO: 2)
GCAGCTAGCCTCATGGCGCTTTTGTTGACCACG-
GTCATTGCTCTCACTTGCCTTGGCGGC;
and

pIL13REcoRI,
                                                (SEQ ID NO: 3)
GAGCTCGGATCCTGAATTCAACCGTCCCTC.
```

[0122] First-round PCR used pIL13F1 and pIL13REcoRI as primers, with pRB5830 (containing the IL13 coding region) as the template. The PCR reaction mixture was then diluted 10-fold and 1 μl of the diluted reaction mixture was used as template for the second round of PCR amplifications with pIL13F2 and pIL13REcoRI as the primer set. The PCR product was gel-purified, digested with NheI/EcoRI, and ligated into pBluescript II KS(+) at XbaI/EcoRI sites to generate pRB5832. To construct the transfer plasmid pRB5835, a 4.8-kbp HindIII/Sac fragment containing the HSV-1 gC coding region was released from cosmid pBC1007 and inserted into pBluescript II KS(+) to generate pRB5833. pRB5833 was cleaved with NheI and EcoRI and the N-terminal 148 residues of gC were replaced with the gC-signal/IL13 chimeric sequence (pRB5834). The insert in pRB5834 was released by XhoI/SacI digestion and ligated into pKO5Y at the same sites to generate pRB5835.

[0123] The recombinant virus R5107 (FIG. **1**A, line 1) carrying the IL13-gC chimera was generated with the aid of the BAC-HSV system. RR1 competent cells that harbored bacterial artificial chromosome (BAC)-HSV bacmids were transformed with the transfer plasmid pRB5835 by electroporation. After incubation for 1 hour at 30° C. in LB broth, the transformed bacteria were plated on pre-warmed Zeocine (Zeo) plus chloramphenicol (Cm) (20 μg/ml of each) plates and incubated overnight at 43° C. for integration. The next day, six colonies were picked and each was separately diluted in 1 ml LB. Five μl of the diluted bacteria were then plated on Cm/10% sucrose (Suc) plates, and incubated at 30° C. overnight. To further confirm the loss of the replacement vector, 24 Cm/Suc-resistant colonies (four colonies from each plate) were restreaked in duplicate on Cm LB and Zeo LB plates, respectively. The $Suc^r/Cm^r/Zeo^r$ colonies were further screened by PCR (95° C., 4 minutes for cycle 1; then 35 cycles of 94° C., 1 minute; 60° C., 1 minute; and 72° C., 1 minute). The primers were:

```
pgC-F,
GACACGGGCTACCCTCACTATCGAGGGC    (SEQ ID NO: 4; from
                                nt 96158 to 96185
                                in HSV-1 strain
                                17),
```

-continued

and

pgC-R,
GGTGATGTTCGTCAGGACCTCCTCTAGGTC (SEQ ID NO: 5; from nt 96859 to 96830 in HSV-1 strain 17).

[0124] The DNA fragment amplified from PCR-positive clones (FIG. 2B) was sequenced to further confirm the integration of IL13 in the correct open reading frame (ORF) of gC. To verify the viability of the recombinant (R5607), the recombinant BAC-HSV DNA was prepared as described elsewhere (Ye et al., 2000) and transfected into rabbit skin cells by Lipofectamine reagent (Life Technologies, Grand Island, N.Y.). The resultant virus, R5607, was stored at −80° C.

[0125] (ii) Deletion of the polylysine track from gB, FIG. 1 panel B. To make a transfer plasmid for the deletion of the gB heparan sulfate binding domain (polylysine), a 4.76 kbp BstEII fragment (from nt 53164 to 57923 of HSV-1) containing the U_L27 (gB) ORF released from cosmid BC1014 was blunt-ended and cloned into pBluescript II KS (+) at a SmaI site to generate pRB5846. To construct pRB5847, from which the 10-amino-acid polylysine domain of gB was deleted, two fragments flanking the polylysine domain were amplified by PCR from pRB5846. The primer sets were:

pgB1BamHI:
GTTCTTCTTCGGTTTCGGATCCCCCG; (SEQ ID NO: 6)

pgB2BspEI:
CGGCATTTCCGGAATAACGCCCACTC; (SEQ ID NO: 7)
and

pgB3BamHI:
CAGAAAACCGGATCCCCCAAAGCCGCC; (SEQ ID NO: 8)

pgB4BsiWI:
GCCAACACAAACTCGTCGTACGGGTAC. (SEQ ID NO: 9)

[0126] PCR amplified fragments were then cut with BspEI/BamHI, or BsiWI/BamHI and ligated into pRB5846, which had the 1.2 kbp BsiWI/BspEI fragment already deleted. To generate the transfer plasmid pRB5848, the 4.76 kbp insert in pRB5847 was released by XbaI/EcoRV digestion and ligated into pKO5Y at the sites of XbaI and ScaI. Recombinant HSV-1 virus R5108 is based on R5107 with the additional deletion of the gB heparan sulfate binding domain. It was made by the same procedure as BAC-R5607, except that the transfer plasmid pRB5848 was used instead of BAC-HSV wild-type and pRB5835. The sequence of the mutant gB was verified by sequencing the entire ORF.

[0127] (iii) Deletion of gD (FIG. 1 panel C, lines 6 and 7). The coding sequence of gD was replaced with the human cytomagolovirus immediate early promoter to enable the expression of glycoprotein I. A 0.65 kbp fragment containing the promoter was released from pRB5836 by ClaI digestion and inserted into pgD^- (FIG. 6), a plasmid obtained from G. Campadelli-Fiume. This plasmid, containing the flanking sequences of gD but lacking the gD ORF, had been cut with ClaI to generate pRB5849. pRB5849 was then cut with NotI and PmeI and ligated into pKO5Y at the NotI and ScaI sites to generate the transfer plasmid pRB5850. Recombinant HSV-1 virus R5110 is based on R5608 with the additional deletion of gD. It was made by the same procedure as BAC-R5607 except that transfer plasmid pRB5850 was used instead of BAC-HSV wild-type and pRB5835. The recombinant BAC-HSV DNA was prepared as described in (Ye et al., 2000). The mutant virus was designated R5110.

[0128] (iv) Construction of the R5111 mutant carrying the IL-13-gD chimeric gene (FIG. 1 panel D). Plasmid pRB123 carries a 6,584 bp BamHI J fragment containing the gD coding region and flanking sequences in the BamHI site of pBR322. To construct the IL 13-gD chimeric plasmid, pRB123 was digested with AflII and HpaI to release two fragments of 7.6 kb and 3.2 kb. The 3.2 kb fragment was further digested with FspI to release 2.5 kb and 0.7 kb fragments that contain the amino-terminal 661 bp of the gD ORF. A polylinker sequence containing the restriction sites XhoI-BglII-EcoRI-KpnI was inserted into the 0.7 kb fragment downstream of the 24th codon of gD by two PCR reactions using a first forward primer,

(SEQ ID NO: 10)
5'-CAGTTATCCTTAAGGTCTCTTTTGTGTGGTG-3',
and a first reverse primer,

(SEQ ID NO: 11)
5'-CCGGAATTCCGGAGATCTTCCCTCGAGGACCGGAAGGTCTTTGCCGC GAAAG-3',
and a second forward primer,

(SEQ ID NO: 12)
5'CCGGAATTCCGGGGTACCCTGGACCAGCTGACCGACCCTCCGG-3',
and a second reverse primer,

(SEQ ID NO: 13)
5'-CGGGGGGATGCGCAGCGGGAGGGCGTACTTAC-3',

[0129] respectively. After digestion of the two PCR products by EcoRI, they were ligated and amplified by PCR again to obtain the desired DNA fragment containing the polylinker insertion.

IL13 was amplified by PCR with the forward primer,
(SEQ ID NO: 14)
5'-CCGCTCGAGATGGCGCTTTTGTTGACCACGG-3',
and the reverse primer,

(SEQ ID NO: 15)
5'-GGGGTACCGTTGAACCGTCCCTCGCGAAA-3',

[0130] and then inserted into the XhoI and KpnI sites of the 0.7 kb fragment described above. This new fragment with the IL13 insertion was then ligated with the 2.5 kb and 7.6 kb fragments (see above) to generate the IL13-gD chimeric transfer plasmid, pRB13-24.

[0131] R5111 was generated by co-transfection of transfer plasmid pRB13-24 and the R5110 viral DNA into U87 glioma cells. The progeny of the transfection was plated at a high dilution on Vero and HEp-2 cell cultures to yield individual, well-spaced plaques. From each of the infected cell cultures, six single plaques were picked, frozen-thawed, sonicated, and then replated on fresh cultures of Vero or HEp-2 cells (depending on the origin of the plaque) for preparation of virus stocks and to prepare viral DNA for sequencing.

[0132] Viral DNA extraction. Infected cells were removed from each of the cm^2 flasks exposed to individual plaque isolates, rinsed, and resuspended in 500 μl of Lyse-O-Lot (150 mM NaCl, 10 mM Tris, 1.5 mM $MgCl_2$ in the presence of 0.1% of NP40). Nuclei were removed by low-speed centrifugation. To the supernatant fluid were added sodium dodecyl sulfate (SDS) to 0.2%, EDTA to 5 mM and β-ME to 50 mM. The solution was then extracted twice with phenol/chloroform. Viral DNA was finally precipitated by ethanol, resuspended, and the IL13 ORF and IL13-gD chimeric reading frame were amplified by PCR with two sets of primers. The first set, designed to amplify IL13, consisted of: a forward primer, 5'-CCGCTCGAGATG-GCGCTTTTGTTGACCACGG-3' (SEQ ID NO:16), and a reverse primer, 5'-GGGGTACCGTTGAACCGTC-CCTCGCGAAA-3' (SEQ ID NO:17), which will amplify the IL13 ORF. The second set, designed to amplify the IL13-gD junction, consisted of a forward junction primer,

[0133] 5'-CCGCTCGAGATGGCGCTTTTGTTGAC-CACGG-3' (SEQ ID NO:18), and a reverse junction primer, 5'-AACTGCAGGTTGTTCGGGGTGGCCGGGGG-3' (SEQ ID NO:19). All 12 IL13-gD PCR products were sequenced to determine whether the gD sequence contained deletions or substitutions.

[0134] Verification of the structure of R5111 The construction of the R5111 virus is depicted in FIG. **1**. The design involved replacement of the HveA binding site with the IL13 ligand to enable the recombinant virus to bind the IL13α2 receptor on cell surfaces and to delete the sequences reported to bind to heparan sulfate. Verification of the structure of R5111 was done as follows:

[0135] (i) The replacement of the amino-terminal domain of gC with IL13 and the disruption of the heparan sulfate binding site was initially verified by sequencing gC from recombinant R5107 (FIG. **2** A).

[0136] (ii) The deletion of codons 68-77 of gB was verified by sequencing the gB ORF amplified by PCR from recombinant R5108 (FIG. **2** B). The nucleotide and amino acid sequences of gB with the polylysine track deleted are set out in SEQ ID NOs.:35 and 36, respectively.

[0137] (iii) The presence of chimeric IL13-gD in R5111 was verified by PCR, as illustrated in FIG. **3**, and by sequencing the entire IL13-gD coding region, amplified by PCR, as shown in FIG. **2**. The nucleotide and amino acid sequences of gD with the IL13 integration are set out in SEQ ID NOs.:39 and 40, respectively. The R5111 recombinant was initially isolated from transfected U87 cells and grown in parallel in Vero cells and HEp-2 cells. To determine whether the virus grown in Vero cells or HEp-2 cells differed with respect to amino acid sequence, six plaques each from Vero or HEp-2 cultures containing well-separated plaques were picked. In this series of verification experiments, two sets of primers were used to confirm the presence of the IL13 insert in gD and to verify the presence of a junction between IL13 and gD. In a second round of verifications, the 12 clones of gD were sequenced to determine whether the isolates obtained from the viruses passaged in Vero cells or in HEp-2 cells differed in amino acid sequence. No differences were found. Furthermore, except for the inserted IL13 sequence, no differences were found between the sequence of HSV-1 (F) gD and those of the cloned IL-13-gD chimeric genes (FIG. **2** C).

[0138] (iv) In denaturing polyacrylamide gels, IL13 migrated as a protein with an apparent Mr of 15-17,000. In the recombinant R5111, IL13 replaced 148 amino acids of gC. FIG. **4**B shows an immunoblot of electrophoretically separated proteins from a lysate of R5111 mutant-infected cells exposed to an antibody to gC. As illustrated in that figure, the anti-gC antibody reacted with proteins present in lysates of HSV-1 (F) and with proteins from R5111 lysates, exhibiting similar electrophoretic mobilities. In contrast, an antibody to IL13 reacted with a band of similar mobility in R5111 lysates (FIG. **4**C, lane 3) but not in lysates of HSV-1(F) (FIG. **4** C, lane 2). The IL13-gD fusion protein in the R5111 mutant virus was verified by reacting the cell lysates with gD and IL13 antibody. Comparison of wild-type gD and the chimeric IL13-gD chimeric protein (FIG. **4** A, lane 3), showed that, as expected, IL13-gD migrated more slowly than the wild-type gD (FIG. **4** A, lane 2). The faster migrating band of gD did not react with the antibody to IL13 (FIG. **4**C, lane 2).

EXAMPLE 2

Construction of a Cell Line Expressing the IL13 Receptor (IL13Rα2)

[0139] A rigorous test of the ability of R5111 to utilize the IL13Rα2 protein as a receptor for entry required construction of a cell line expressing IL13Rα2 (nucleotides 126-1265 of SEQ ID NO.:33; SEQ ID NO:34) in the absence of other HSV-1 entry receptors. The J1.1 cell line was selected for this construction. In essence, this cell line lacks the receptors necessary for the entry of virus into cells and the cell line is not susceptible to infection by wild-type virus. The construction of a plasmid encoding a IL13Rα2 protein fused at its carboxyl terminus to a HA tag, transfection of J1.1 cells with the plasmid encoding the tagged IL13Rα2 protein, and selection of the cell line expressing the protein is described below.

[0140] To test for the production of IL113Rα2 protein, five clones of the selected cells were harvested, solubilized, subjected to electrophoresis in denaturing polyacrylamide gels and tested for expression of the protein.

[0141] Construction of J13R, a cell line stably expressing IL13Rα2 receptor. The IL13α2 coding region was tagged with an HA tag at its 3' end (the carboxyl terminus of the encoded polypeptide) by PCR with forward primer, 5'-AA-GATTTGGGC-TAGCATGGCTTTCGTTTGC-3' (SEQ ID NO:20), and reverse primer,

[0142] 5'-TCCCTCGAAGCTTCAAGCATAATCTG-GCACATCATATGTATCACAGAA-AAA-3' (SEQ ID NO:21). NheI and HindIII restriction digests were used to create compatible ends. The DNA fragment was then inserted into pcDNA 3.1 (zeo) vector (Invitrogen; Carlsbad, Calif.) to generate transfer plasmid pRB 13-R2. All of the constructs were sequenced to insure fidelity.

[0143] J1.1, a derivative of BHK thymidine kinase$^-$ cells which lack both HveA and nectin 1 receptors, was obtained from Dr. G. Campadelli-Fiume, University of Bologna, Italy. J1.1 cells, stably transfected with pRB 13-R2 using a Lipofectamine kit (Gibco-BRL), were selected on the basis of their resistance to zeocin (Invitrogen). Zeocin-resistant clones were amplified and screened for IL13Rα2 expression by immunoblotting with anti-HA polyclonal antibody.

Lysates of parental and transformed cells formed by solubilized in SDS were each electrophoretically separated in a denaturing gel (50 μg/lane), transferred to a nitrocellulose sheet, and probed with antibody against HA (Santa Cruz Biotechnology). The protein bands were visualized by an enhanced chemiluminescent detection (ECL) system (Pierce, Rockford, Ill.) according to the instructions of the manufacturer. One (J13R-2) was selected for testing the ability of R5111 to use the IL13Rα2 receptor.

[0144] As shown in FIG. **5**, all clones expressed a protein band reactive with the anti-HA antibody. The apparent size of the protein was consistent with the reported size of IL13Rα2. Of 5 J13R-positive clones, J13R-2 (FIG. **5**, lane 3) was selected and designated J13R.

EXAMPLE 3

Infection by the HSV Targeting Vector R5111

[0145] SK-N-SH, HEp-2, Vero, and U87 cells were obtained from American Type Culture Collection (Rockville, Md.) and maintained in Dulbecco's modification of Eagle's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum. Replicate cultures of SK-N-SH, HEp-2, Vero, U87, J1.1, and J13R were exposed to 0.01 PFU of R5111 virus per cell. After 24 hours of incubation, the cells were harvested and viral yields were titered on Vero cells.

[0146] Immunoblotting electrophoretically separated proteins. The indicated cells were mock-infected or exposed to 10 PFU of recombinant or wild-type HSV-1(F) per cell. The cells were harvested at 24 hours after infection, disrupted in SDS disruption buffer, boiled, cleared by centrifugation and electrophoretically separated on a 10% denaturing polyacrylamide gel. After transfer to a nitrocellulose membrane, the isolated proteins were reacted with antibodies as indicated using known and conventional techniques. Monoclonal antibodies against gD– (clone H170), gC- and HA-specific polyclonal antisera were purchased from the Goodwin Institute, Plantation, Fla. Polyclonal antibodies against IL13 were purchased from Santa Cruz Biotechnology.

[0147] The results shown in Table 1 were as follows: R5111 replicated to within a 10-fold range in HEp-2, Vero, U87, and J13R cells. The titer obtained from J1.1 cells was approximately 10^5-fold lower than that obtained from all other cell lines. To test whether the J13R cell line had acquired a receptor for wild-type HSV-1 (HSV-1(F)), J1.1 and J13R cells were also exposed to the wild-type virus. The results, also shown in Table 1, indicate that the cells remain resistant to the wild-type virus. It was known that HEp-2 cells express the nectin receptor but not the HveA receptor. The results show that the targeted HSV containing an IL13-gD fusion can target (i.e., bind and infect) cells expressing a particular receptor (IL13Rα2) approximately as well as wild-type HSV targets cells expressing the HveA receptor. The results indicate that R5111 can use IL13Rα2 as a receptor for entry in a cell line lacking all other HSV-1 receptors.

TABLE 1

Replication of R5111 in various cell lines

Virus	Cell Line*	Yield**
R5111	Vero	11×10^7
	HEp-2	1.2×10^7
	SK-N-SH	17×10^7
	U87	27×10^7
	J1.1	2×10^2
	J13R	11×10^7
HSV-1 (F)	J1.1	6×10^3
	J13R	4×10^3

*cell lines derived from human brain tumors.
**The cells were exposed to 0.01 PFU of R5111 or HSV-1 (F) per cell and harvested 24 hours after infection. Progeny virus were titered on Vero cells.

[0148] This disclosure contains an exemplary description of the construction and properties of a recombinant HSV virus, R5111. In R5111, the heparan sulfate binding sites on the surface of the viral particle were ablated to preclude or at least reduce the attachment of virus to non-targeted cells. Attachment even in the absence of fusogenic activity may lead to endocytosis, degradation of the virus particle, and to potential damage to the cell by lysosomal enzymes (Zhou et al. 2002; Zhou et al. 2000). At the same time, a copy of IL13 was inserted into gC to enhance binding of virus particles to the IL13Rα2 receptor. The major restructuring of the viral genome consisted of insertion of IL13 at amino acid 24 of gD. Available data indicate that this modification ablates the gD binding site for the HveA receptor (Carfi et al. 2001). The data obtained using R5111 indicate that the virus retains the capacity to interact with the Nectin receptor. Nonetheless, the R5111-targeted HSV was able to infect and replicate in J13R cells but not in the parental, J1.1, cells.

EXAMPLE 4

Construction of HSV Targeting Vector R5141 and R5144

[0149] A therapeutic herpes simplex virus 1 (HSV-1) capable of infecting and replicating solely in cells harboring the IL13Rα2 receptor was constructed using recombinant DNA techniques. As disclosed above, construction of R5111, which expresses IL13 on its surface and lacks the binding sites for heparin sulfate, allowed R5111 to infect J-13R cells as well as cells exhibiting the natural receptors for HSV-1. Thus, the involvement of fusogenic glycoproteins of R5111— a key step in viral entry—is independent of the receptor with which gD interacts.

[0150] The binding site of HveA has been reported to be at the amino terminal domain of gD (Carfi A., et al., 2001). The precise binding sites of gD for Nectin 1 are not known, although it has previously been reported to involve gD amino acids 38 and 221 (Manoj S., et al., 2004; Zago A., et al., 2004; Connolly S A., 2005). The general assumption within the field is that the HveA and Nectin 1 sites do not overlap and that each independently promotes the same structural alteration of gD to enable entry of the virus into cells. However, it is possible that the surface structure of HveA and Nectin 1 at their binding sites may be similar even

though the Hve1 and Nectin1 amino acid sequences are not identical.

[0151] Based in part on the foregoing information, viruses capable of productive replication solely in targeted cells were designed as shown in FIG. 7. Using standard molecular biological cloning techniques known in the art, recombinant virus R5141 was constructed by inserting IL13 in the place of gD residues 1-32. In addition, the valine residue at position 34 was substituted with serine ("V34S") (SEQ ID NOs.:41 and 42, respectively). Similarly, recombinant virus R5144 was constructed by inserting IL13 in the place of gD residues 1-32, and the valine at position 37 was substituted with serine ("V37S) (SEQ ID NOs.:43 and 44, respectively).

[0152] One of skill would appreciate that a variety of re-targeted SHV, both HSV-1 and HSV-2, could be constructed and assessed using routine techniques in view of the disclosures herein. In particular, substituting a binding domain of a binding partner for the N-terminal region of gD, e.g., for amino acids 1-32 of gD, would be within the skill in the art. Further alteration of the gD fusion, e.g., by amino acid substitution, whether conservative substitution or not, would also be within the skill in the art. Of course, additions or deletions to gD fusions would also be within the skill in the art and assessing the targeting capacities of such constructs would involve routine experimentation in view of the teachings herein.

EXAMPLE 5

Infection by the HSV Targeting Vectors R5141 and R5144

[0153] The capacities of the recombinant viruses described in Example 4 to productively replicate solely in targeted cells were assessed using the cell lines which express either HveA (J-HveA) alone (relative to the group of HveA, Nectin1, and IL13R α2), Nectin1 (J-Nectin1) alone, or IL13Rα2 (J-13R) alone. For cell infection, the procedure set forth in Example 3 was repeated using recombinant virus R5141 and recombinant virus R5144.

[0154] The replication of R5141 and R5144 in J-Nectin-, J-HveA-, and J-13R-specific cells are summarized in Table 2. R5141 and R5144 do not productively interact with either native gD receptors, HveA or Nectin1. Significantly, however, R5141 interacts with and replicates in IL13Rα2 for productive entry into cells.

TABLE 2

Replication of R5141 and R5144 in J-Nectin, J-HveA and J-13R cells.

	HSV-1 (F)	R5141	R5144
J-Nectin	4×10^8	7×10^1	5×10^1
J-HveA	3×10^8	4×10^1	3×10^2
J-13R	3×10^1	5×10^6	7×10^2

[0155] Thus, recombinant virus R5141 is capable of productive replication solely in targeted cells and this result opens the way for development of therapeutic viruses targeting cells exhibiting the IL13Rα2 receptor, such as malignant gliomas and other human tumors exhibiting IL13Rα2. It is expected that other mutations (i.e., those that abolish binding of Nectin and those that have a similar effect on HveA) will yield viruses that enter solely via non-natural HSV receptors.

EXAMPLE 6

HSV Targeting Vector R5161

[0156] An HSV targeting vector designated HSV R5161 has a structure analogous to the structure of HSV R5141, and HSV R5161 was constructed in the manner described in Example 4, above, with the exception that HSV R5161 contains the sequence encoding the HSV gD leader sequence, whereas HSV R5141 contains the sequence encoding the IL-13 leader sequence. In particular, HSV R5161 encodes an IL-13-gD fusion protein in which IL-13 sequence replaces the sequence encoding gD amino acids 1-32, with a V34S substitution in the gD moiety of the fusion protein, as described above in the context of describing HSV R5141.

[0157] The relative capacities of recombinant viruses HSV R5141 and HSV R5161 to productively replicate in targeted cells were measured in the J-13R cell line, which expresses IL13Rα2, but not HveA or Nectin1. HSV R5161 was expressed at a level 10-fold higher than the expression level of HSV R5141 in J-13R cells.

[0158] It is expected that in the majority of embodiments of the invention, relatively high levels of expression will be advantageous in ensuring that targeted cells are efficiently contacted by the re-targeted HSV. In re-targeting the HSV, the invention provides an approach to controlling the virulence of the virus in a manner that minimizes undesirable pathogenicity, i.e., pathogenicity towards non-targeted cells. In addition, the virulence of any re-targeted HSV can be further attenuated using known approaches to virulence control that do not interfere with the re-targeting, such as by mutating the $\gamma_1$34.5 gene(s). In those embodiments in which it is desirable to have relatively high expression levels of the re-targeted HSV, it is preferred that the leader sequence of gD be used. Alternative leader sequences, such as leaders from other HSV genes, are contemplated. Moreover, expression control elements (e.g., promoters, enhancers, expression factor binding sites) can be engineered to achieve desired expression levels of the fusion protein using ordinary levels of skill and techniques known in the art.

EXAMPLE 7

Construction of HSV Recombinant Viruses R5181 (ATF-uPA-gD) and R5182 (BD-uPA-gD)

[0159] I. Construction of the ATF-uPA-gD Plasmids.

[0160] The complete cDNA of amino-terminal fragment (ATF) of uPA without the N-terminal signal peptide was amplified from pULscuPA plasmid using the following primers:

```
ATF-uPA-XhoI:
5'-CCGCTCGAAGCAATGAACTTCATCAAGT-  (SEQ ID NO: 47)
TCCATC-3'

ATF-uPA-KpnI:
5'-GGGGTACCTTTTCCATCTGCGCAGTCAT-  (SEQ ID NO: 48)
GCACC-3'
```

[0161] The PCR product was gel purified and ligated into pGEM-T Easy Vector (Promega, Madison, Wis.). The sequence of ATF-uPA was verified by sequencing the entire ATF-uPA. Plasmid pGG5112 carries a 3648 bp fragment

containing gD, mutant IL-13 (E13Y) and flanking sequences in the EcoRI/XbaI sites of pBR322 (Reuning U, et al., 1998). To construct the ATF-uPA-gD chimeric plasmid, pGG5112 was digested with XhoI and KpnI to release two fragments, 6.2 and 0.4 kb, respectively. Plasmid ATF-uPA was digested with XhoI and KpnI and then inserted into the XhoI and KpnI sites of the 6.2-kb fragment to generate the ATF-uPA-gD chimeric transfer plasmid.

[0162] II. Construction of the R5181 (ATF-uPA-gD) and R5182 (BD-uPA-gD) Recombinant Viruses.

[0163] The R5181 and R5182 viruses were generated by co-transfection of transfer plasmid ATF-uPA-gD or BD-uPA-gD and the R5110 viral DNA into rabbit skin cells by Lipofectamine reagent (Life Technologies, Grand Island, N.Y.). The R5110 vector was described in WO 2004/033639 A3, incorporated by reference herein; R5110 contains a deletion of gD, a deletion of the heparan sulfate binding domain of gB, and a substitution of the amino terminal domain of gC with IL13 (FIG. **1**B).

[0164] The progeny of the transfection was plated at a high dilution on Vero cell cultures so as to yield individual, well-spaced plaques. From each of the infected cell cultures, four single plaques were picked, frozen-thawed, sonicated, and then replated on fresh cultures of Vero cells for preparation of virus stocks and viral DNA for sequencing. Vero cells were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in Dulbecco's modification of Eagles minimal essential medium (DMEM) supplemented with 5% newborn calf serum (NBCS). Rabbit skin cells were maintained in DMEM supplemented with 5% NBCS.

[0165] III. Viral DNA Extraction.

[0166] Infected cells were removed from each of the 25-cm^2 flasks exposed to the individual plaque isolates, rinsed, and resuspended in 500 μl of Lyse-O-Lot (150 mM NaCl/10 mM Tris/1.5 mM $MgCl_2$) in the presence of 0.1% of Nonidet P-40 (Zhou et al., 2002). The nuclei were removed by low-speed centrifugation. SDS to 0.2%, EDTA to 5 mM, and β-ME to 50 mM were added to the supernatant fluid, which was then extracted twice with phenol/chloroform. Viral DNA was finally precipitated by ethanol and resuspended, and the ATF-uPA-gD or BD-uPA-gD chimeric reading frames were amplified by PCR with the following primers:

(SEQ ID NO: 49)
5'-CGGAATTCGATGGGGGGGGCTGCCGCCAG-3'

(SEQ ID NO: 50)
5'-AACTGCAGCTAGTAAAACAAGGGCTGGTGCG-3'

[0167] The gD PCR products were sequenced to determine whether the gD and ATF-uPA, or BD-uPA sequences contained deletions or substitutions. The R5181 virus encodes an uPA peptide insert of 135 residues in length (residues 20-155 of uPA of SEQ ID NO:52, encoded, e.g., by SEQ ID NO:51) between residues 24 and 25 of the HSV-1 gD of SEQ ID NO:26. The complete polynucleotide and amino acid sequences of ATF-uPA-gD are set out in SEQ ID NOs:53 and 54. R5182 contains a much smaller peptide insert of 23 residues (residues 34-57 of uPA of SEQ ID NO:52) and consisting of the binding site of uPA for uPAR. The complete polynucleotide and amino acid sequences of BD-uPA-gD are set out in SEQ ID NOs:55 and 56. The structures of R5181 and R5182 viruses were verified as follows:

[0168] (i) The presence of a chimeric ATF-uPA-gD gene in the R5181 virus and of a BD-uPA-gD gene in R5182 were verified by sequencing of the entire open reading frames amplified by PCR (FIG. **2**A and FIG. **2**B, respectively).

[0169] (ii) As expected, anti-gD antibody reacted with the ATF-uPA-gD band in lysates of cells infected with the R5181 mutant virus but not with the gD band in lysates of wild-type virus (FIG. **3**). Moreover, as expected, the chimeric ATF-uPA-gD protein of the R5181 virus (FIG. **3**, lanes 3-5), migrated more slowly than wild-type gD (FIG. **3**, lane 1).

EXAMPLE 8

Construction of a Cell Line Expressing uPAR

[0170] I. Construction of J-uPAR, a Cell Line Stably Expressing Human uPAR.

[0171] To determine whether R5181 and R5182 viruses were able to use the human uPAR protein as a receptor for entry, we constructed, in parallel, a cell line expressing this protein in the absence of other HSV-1 entry receptors. J1.1 cells stably transfected with the human uPAR expression plasmids by using Lipofectamine kit (GIBCO/BRL) were selected on the basis of their resistance to hygromycin B (Invitrogen, Carlsbad, Calif.). Hygromycin B-resistant clones were amplified and screened for uPAR expression by immunoblotting with monoclonal anti-human uPAR antibody (R&D Systems, Inc., Minneapolis, Minn.).

[0172] Parental and transformed cells were solubilized in SDS, electrophoretically separated in a denaturing gel (100 μg/lane), transferred to a nitrocellulose sheet, and probed with antibody against uPAR followed by the appropriate secondary antibody conjugated to alkaline phosphatase (Bio-Rad). The protein bands were visualized with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (Denville Scientific, Metuchen, N.J.). As shown in FIG. **4**, all clones expressed a protein reactive with the anti-human uPAR antibody but differed in the level of expression. The apparent size of the protein was consistent with the reported size of uPAR. Of 14 J-uPAR-positive clones, J-uPAR-7 (FIG. **4**, lane 8) was selected for further studies because it provided the highest expression level of uPAR.

[0173] II. Cell surfaces Immunostaining.

[0174] Hygromycin B-resistant cells (J-uPAR) in 25-cm^2 flasks were removed from the dish by gentle scraping and reacted with human uPAR antibodies (5 μg/ml) for 15 minutes on ice. The cells were then rinsed with cold PBS and reacted for 15 minutes with a 1:64 dilution of a goat anti-mouse immunoglobulin G conjugated to fluorescein isothiocyanate (FITC) (Sigma, St, Louis, Mo.) in ice-cold PBS. After rinsing with cold PBS, the cells were suspended in 100 μl of ice-cold PBS for further immunofluorescence analyses. 10 μl of the surface-stained cells were plated onto four-well glass slides and mounted in 90% glycerol. Slides were analyzed with the aid of a Zeiss confocal microscope. A total of 200 cells in adjacent fields were examined for surface fluorescence.

[0175] As noted above, uPAR is anchored to the plasma membrane via glycosylphosphatidylinositol and lacks transmembrane and cytosolic domains. The expression of uPAR was investigated in J-uPAR-7 cells using immunofluorescence as the detection method for uPAR. In a first series of experiments, 70% of the J-uPAR-7 cells stained positive for uPAR. (FIG. **4**, lane 8) in cultures passaged by gentle scraping of the cells. The number of positive cells after single detachment of cells with versene and totally disappeared after detachment with trypsin. These results indicated that treatment with trypsin or versene irreversibly altered the properties of uPAR.

[0176] In a second series of experiments, the effect of fetal bovine serum (FBS) on the expression of uPAR was investigated because FBS could internalize the cell surface receptor. J-uPAR cells were grown in serum-free and specialty medium (AIM-V Medium, Invitrogen, Carlsbad, Calif.) containing different concentrations of FBS. FBS affected the signal intensity but not the percentage of positive cells. Cells grown in AIM-V medium in the absence of serum grew poorly. Moreover, the cells could not be passaged. The results indicated that although FBS negatively affected the presence of uPAR on the cell surface, at least a low concentration of FBS was needed to maintain J-uPAR cells. It is expected that culturing J-uPAR cells, or equivalent cells, in one of the relatively new media formulations known in the art to reduce cell dependence on sera for growth will allow greater expression and cell-surface presentation of uPAR.

EXAMPLE 9

Infection by the HSV Targeting Vector R5181 and R5182

[0177] I. Virus Titration.

[0178] Replicate cultures of J-uPAR, J1.1, J-HveA (expresses HveA alone) and J-Nectin-1 (expressed Nectin-1 alone) were exposed to 0.1 pfu of ATF-uPA-gD, BD-uPA-gD, or HSV-1 (F) virus per cell. After 24 hours of incubation, the cells were harvested, sonicated and titrated on Vero cells.

[0179] For controls, the indicated cells were mock-infected or exposed to 10 pfu of recombinant or wild-type HSV-1(F) per cell. The cells were harvested 24 hours after infection, disrupted in SDS disruption buffer, boiled, cleared by centrifugation, electrophoretically separated on a 10% denaturing polyacrylamide gel, transferred onto a nitrocellulose membrane, and exposed to appropriate antibodies under standard conditions. The results shown in FIG. **5** are as follows:

[0180] (i) HSV-1(F) replicated poorly in J-uPAR or J1.1. The amounts recovered from infected cells may represent, in large part, attached unpenetrated virus.

[0181] (ii) The titer obtained from cells infected with R5181 virus from infected J-uPAR cells was about 10^2 to 10^3-fold higher than that obtained from HSV-1 (F)-infected J-uPAR cells.

[0182] (iii) The concentration of FBS did not affect the replication of R5181 virus in J-uPAR cells.

[0183] (iv) The titer obtained from infected J-uPAR cells infected with R5181 mutant virus was 10- to 100-fold lower than that obtained from J-uPAR cells infected with R5182 mutant virus.

[0184] (v) Unexpectedly, the titer obtained from ATF-uPA-gD virus-infected J1.1 cells was of the same order as that obtained from ATF-uPA-gD virus-infected J-uPAR cells.

[0185] II. Isolation and Reverse Transcription of RNA Extracted from J1.1 and J-uPAR Cells.

[0186] The experiments described above indicated that R5181 virus replicated in J1.1 cells. One possible explanation for these results is that J1-1 cells express an endogenous hamster uPAR. Consistent with this hypothesis is the evidence reported elsewhere that human uPA binds to hamster uPAR with high affinity (Fowler, B., et al., 1998). To test this hypothesis, two series of experiments were performed. In the first, J1.1 cells were tested to determine if they express hamster uPAR mRNA.

[0187] Total RNA was extracted with the aid of TRIzol reagent according to the manufacturer's instructions (Life Technologies, Rockville, Md.). DNase I treatment (Life Technologies), phenol/chloroform extraction, and ethanol precipitation (Fisher Scientific, Houston, Tex.) were carried out to remove possible DNA contamination. Total RNA (3 μl) was reverse-transcribed to yield single-stranded cDNA with 20 U of AMV reverse transcriptase (Promega) in a total reaction volume of 30 μl. The reverse transcription was primed with the following primers:

```
Human uPAR reverse:
5'-GGCAGTCATTAGCAGGGTGATGGTG-     (SEQ ID NO: 57)
3'

Hamster uPAR reverse
5'-GTTGCCCTCGCAGCTGTAACACTGG-     (SEQ ID NO: 58)
3'
```

[0188] Reverse transcription was performed using a pool of nucleotides consisting of 10 mM concentrations (each) of dGTP, dATP, dTTP, and dCTP (Promega). Forty units of RNasin (Promega) were added to each reaction mixture. The mixture containing only the RNA template, and the primer was first heated at 70° C. for 10 minutes, chilled on ice, and after the addition of the other components, incubated at 42° C. for 30 minutes, shifted to 52° C. for 30 minutes, and then heat-inactivated at 95° C. for 5 minutes. cDNAs obtained from reverse transcription of RNA extracted from J1.1 and J-uPAR cells were amplified by PCR under the following conditions: 1 minute at 94° C., 1 minute at 60° C., and 75 seconds at 72° C. The following primers were used for PCR:

```
uPAR-start forward:
5'-ATGGGTCACCCGCCGCTGCTGCCGC-3'   (SEQ ID NO: 59)
```

[0189] Human uPAR reverse primer or hamster uPAR reverse primer used in PCR were as described above. As shown in FIG. **6**, the J1.1 cell line contained detectable levels of hamster uPAR mRNA but not human uPAR mRNA.

[0190] In the second series of experiments, human single-chain uPA was used to compete with the virus for the putative hamster uPAR receptor on J1.1 cells. Human single chain uPA (scuPA) was purchased from American Diagnostics Inc. (Stamford, Conn.). Cells were exposed to increasing concentrations of the human scuPA for 60 minutes at 37° C.

and then exposed to 0.1 pfu of R5181 for 120 minutes at 37° C. in the presence of human scuPA. After 24 hours of incubation, the cells were harvested, sonicated and titrated on Vero cells.

[0191] Previous studies showed that human scuPA bound to human uPAR with high affinity (Kd of about 1 nM; Roldan, A. L., et al., 1990; Barnathan, E. S., et al., 1990) and human scuPA binds to hamster uPAR with the same affinity as for the human receptor (Kd=11.1 nM) (Fowler, et al., 1998). The J1.1 cells exposed to the highest concentration of scuPA produced 10-fold less virus than control untreated cells (FIG. **7**). The results (FIG. **7**) showed that scuPA competed with the virus more successfully in J1-1 cells than in J-uPAR cells, consistent with the expectation that J-uPAR cells would have more receptors than J1.1 cells.

[0192] III. Treatment with Endosome Inhibitors.

[0193] Cells were exposed to freshly prepared NH_4Cl at the concentrations indicated in FIG. **8** for 30 minutes at 37° C. and then exposed to 0.01 pfu of R5181 virus or HSV-1(F) per cell in the presence of the NH_4Cl. After 24 hours of incubation, the cells were harvested, sonicated and titrated on Vero cells. The entry of R5181 virus into J-uPAR or J1.1 cells was not inhibited by NH_4Cl even at the highest concentration tested (FIG. **8**). The results indicated that the R5181 virus does not depend on the endocytic pathway as a primary mechanism for entry into J-uPAR cells.

[0194] The preceding Examples describe the construction and properties of recombinant viruses designed to target cells expressing uPAR, with the results summarized below.

[0195] (i) R5181 virus infected and replicated in cells exhibiting uPAR. R5182 virus was unable to infect cells via uPAR. It is unknown whether the R5182 virus failed to infect cells via uPAR because the secondary structure of the chimeric gD blocked the binding site from interacting with uPAR or whether the insert was incompatible with the predicted modification of gD following its interaction with a receptor.

[0196] (ii) Two lines of evidence indicated that R5181 entered J1.1 cells by interacting with uPAR. Foremost, scuPA competed with R5181 virus for entry into J1.1 cells. The second line of evidence is based on studies that indicated that virions may undergo endocytosis in the absence of a cell-surface receptor. The results herein (e.g., FIG. **8**) show that endocytosis was not the major mechanism whereby R5181 entered J1.1 or J-uPAR cells inasmuch as it was unaffected by NH_4Cl. In contrast, NH_4Cl reduced the already low yield of HSV-1 (F) by approximately 10-fold in both J1.1 and J-uPAR cells which is consistent with the expectation that a small amount of virus may enter cells by endocytosis in the absence of a specific receptor.

[0197] (iii) Cells transduced with uPAR required special care in the maintenance of the receptor on serial passage. The stability of the receptor was dependent on cell type, serum concentration, and, as in the studies described herein, the method by which the cells were dislodged for serial passage. Although this study began with the premise that BHK TK^- cells would not exhibit an uPA receptor, BHK TK^- cells that were passaged numerous times by a variety of means still expressed the endogenous receptor, albeit at levels significantly lower than that that of transduced cells. Thus, receptors may not always be present in tumor cells passaged serially in culture or they may be unstable in transduced cells.

[0198] (iv) Ligands or other binding pair members may be inserted into the HSV-1 virion to provide a mode of entry into cells. Additionally, binding pair members may be targeted to receptors that are not anchored via their own transmembrane domain.

EXAMPLE 10

Supplemental Materials and Methods

[0199] The experimental work described in Examples 11-16, below, were conducted in general conformity with the materials and methods described above. In addition, the following materials and methods were also used in conducting these experiments.

[0200] Cells. Vero and HEK293 were obtained from the American Type Culture Collection (Manassas, Va.). R6 cells were derived by transduction of rabbit skin cells with plasmid pEA102 containing the HSV-1 gD coding sequence under the U_L26.5 promoter (Zhou 2000). R6 cells expresses gD and enable ΔgD viruses to replicate and spread from cell to cell by complementing the virions with gD made ectopically. The thymidine kinase minus J1.1 cell line lacking all receptors for wild-type virus, J-HveA and J-nectin cell lines the kind gifts of G. Campadelli-Fiume, Univ. of Bologna, Italy (Zhou 2002). The properties of the Vero13R cell line expressing the IL13α2 receptor were described elsewhere (Zhou 2005).

[0201] Viruses. HSV-1(F) is the prototype wild-type virus used in this laboratory. The recombinant viruses described in this report were generated by co-transfection of R6 cells with a plasmid containing the desired construct of chimeric glycoprotein D and intact DNA of the ΔgD virus R5110 as described elsewhere (Zhou 2002). The progeny of transfection was then plated on R6 cells, plaque purified and the progeny virus. The gD contained in the virion was then analyzed to insure that it contained the sequence of the desired gD construct. The plasmids encoding gD constructs and used for production of recombinant viruses are described in result and were made by standard procedures described elsewhere (Zhou 2002).

[0202] Virus replication in cell lines. Replicate cultures of J-HveA, J-Nectin or Vero13R cells were exposed to 0.1 PFU of recombinant viruses or HSV-1 (F) per cell. After 24 hours at 36° C., the cells were harvested, disrupted by sonication. Viral progeny was titered on Vero13R cells.

[0203] Transfection plasmids. Transfer vector pAc-CMV, which contains the CMV-IE promoter-enhancer sequences in the XhoI-BamHI sites of pAc-SG2, was described elsewhere (Zhou and Galvan 2000). To construct pGG5414, pGG5415, pGG5416, an EcoRI-PstI fragment was derived by PCR from R5321, R5322 and R5323 respectively with primers 5'-CGGAATTCATGGGGGGGGC TGCCGCCAG-3' (for 5414; SEQ ID NO:60), 5'-CGGAATTCATG-GCGCTTTTG T TGACCACGG-3' (for 5415; SEQ ID NO:61), 5'-CGGAATT CATGAGAGCC CTGCTG-GCGCGCC-3' (for 5416; SEQ ID NO:62), along with 5'-AACTGCAGCTAG GCGTAGTAAACCGTGATCGGG-3' (SEQ ID NO:63) and then inserted into the EcoRI-PstI sites of pAc-CMV transfer vector. To construct the transfer

vector expressing residues 219-314 of mature gD with Myc tag, an EcoRI-PstI fragment was amplified by PCR from gD-A (Zhou and Roizman 2001) with primers 5'-CGGAATTCATGCTGCCCCGCTTCATCCCCGAG-3' (SEQ ID NO:64) and 5'-AACTGCAGTTACAGGTC CTCCTCTGAGATCAGCTTCTGCATTGATGCGTTGTTCGGGGGTGGCCGGGGGG-3' (SEQ ID NO:65) and then inserted into the EcoRI-PstI sites of pAc-CMV. Transfer vector pGG5418 was constructed the uPA ATF fragment into the EcoRI-PstI sites of pAc-CMV transfer vector by PCR amplification using the primers 5'-CGGAATT CATGAGAGCC CTGCTGGCGCGCC-3' (SEQ ID NO:66) along with 5'-AACTGCAGCTATTTTCCATCTGCGCAGTCATGC-3' (SEQ ID NO:67).

[0204] Antibodies. The ZC25 antibody to the C-terminal domain of gD was the kind gift of G. H. Cohen and R. J. Eisenberg (Zhou 2006). Monoclonal antibodies against gD (clone H170) and ICP0 (clone H1083) were from the Goodwin Institute, Plantation, Fla. Monoclonal antibodies against IL13 protein and described previously (Zhou 2002). Anti Myc antibody were from and Santa Cruz Biotechnology. Monoclonal antibodies against human ATF of uPA (Kamiyama 2006) were purchased from American Diagnostica Inc. (Stamford, Conn.).

[0205] Communoprecipitation assays. Subconfluent cultures of HEK293 cells in 25-cm^2 flasks were co-transfected with 1 μg of pGG5417 ands 1 μg of pGG5414, pGG5415 or pGG5416. The cells were harvested 40 hours after transfection, collected by centrifugation, rinsed twice with 5 ml of PBS, resuspended in 200 μl of lysis buffer (20 mM Tris [pH 8.0], 1 mM EDTA, 1% NP-40, 400 mM NaCl, 2 mM dithiothreitol [DTT], 0.1 mM $NaVO_4$, 10 mM NaF, 1× protease inhibitor cocktail [Sigma, St. Luis, Mo.]), and chilled on ice for 40 minutes. The supernatant fluid (150 μl) collected after centrifugation at 1,000 rpm for 2 min (Eppendorf, 5415C) was diluted with 150 μl of low-salt lysis buffer (20 mM Tris [pH 8.0], 1 mM EDTA, 1% NP-40, 16 mM NaCl, 2 mM DTT) and incubated with 5% rabbit preimmune serum at 4° C. for 1 hour, followed by incubation with 50 μl of protein A-Sepharose for 1 hour at 4° C. and centrifugation at 3,000 rpm for 3 minutes to remove nonspecifically bound proteins. The supernatants fluids were reacted with ant-Myc monoclonal antibody at 4° C. for 16 hours, and then reacted with 20 μl of protein A-Sepharose at 4° C. for 1 hour. Immune complexes bound to protein A-Sepharose were rinsed three times with rinse buffer (50 mM Tris [pH 7.4], 10 mM $MgCl_2$, 5 mM DTT), collected by centrifugation disrupted by boiling in sample buffer for 5 minutes, subjected to electrophoresis on denaturing gels, transferred to a nitrocellulose sheet and probed with monoclonal antibodies to gD(H170), IL13 or uPA. In parallel, aliquots of the supernatant fluids were reacted with antibodies to gD(H170), IL13 or uPA. The collected immune precipitates were subjected to electrophoresis in denaturing gels, transferred to a nitrocellulose sheet and probed with antibody to the Myc epitope.

EXAMPLE 11

Construction and Infection

[0206] Studies conducted with the HSV constructs described herein have shown that residues 61 through 218 (all numbering herein is the numbering for mature gD; for the nascent gD sequence of SEQ ID NO:26, add 25) of the wild-type gD glycopolypeptide are dispensable for viral entry. The design of the R5322 recombinant virus involved replacement of residues 1-32 with the N-terminal 155 residues of uPA and substitution of valine 34 with serine but retention of the signal sequence of wild-type gD. R5322 was generated by co-transfecting R6 cells with intact R5110 DNA which lacks the gD gene and a plasmid encoding the desired chimeric gD. R6 cells were derived by transduction of rabbit skin cells with plasmid pEA102 containing the HSV-1 gD coding sequence under the $U_L26.5$ promoter. These R6 cells express gD ectopically and enable viruses lacking gD to replicate and spread (15). The infectious virus was plaque purified and initial stocks were produced in R6 cells. To verify the presence of the N-terminal domain of uPA, the recombinant gD was amplified by PCR and sequenced. Repeated sequencing concurrently with appropriate wild-type controls revealed that in gD of recombinant R5322 one cytosine was deleted after codon 60 and one was inserted after codon 201 (FIG. **16**). The frameshifts resulting from the deletion and insertion of the cytosines introduced stop codons at residues 139, 148, 159, and 190. The first potential initiator methionine is at position 219 of mature gD. Further tests showed that the virus infected and replicated in 4 test cell lines, i.e., in J1.1, a hamster cell line that lacks receptors for wild-type virus; J-HveA, a hamster cell line that expresses the HveA receptor; J-Nectin, a hamster cell line that expresses nectin 1; and Vero13R, a Vero derived cell line that expresses IL13α2 receptor in addition to the natural receptors for HSV-1. The observation that R5322 replicated in J1.1 cells is consistent with earlier results showing that these cells accumulate uPAR mRNA (19).

EXAMPLE 12

Fusion Variants

[0207] The results disclosed in Example 10 raised the question whether residues 60-218 of gD are dispensable for entry just of this virus or whether they play no essential role in wild-type virus as well. To answer this question, two additional viruses were constructed and designated R5321 and R5323. To produce R5321, R6 cells were transfected with R5110 DNA and a chimeric gD construct that was identical to that of R5322 except that the N-terminal domain of uPA was replaced with that of IL13 (19). In the chimeric plasmid designed to generate R5323 the residues uPA-gD_{33-60} of R5322 were replaced with residues 1-60 of wild-type gD. Schematic representations of the chimeric gDs are shown in FIG. **17**, Panel A. Viral stocks were made and characterized as described herein and tested by exposing replicate 25 cm^2 cultures of J-HveA, J-Nectin, or Vero13R cells to 0.1 PFU/cell. The progeny viruses were titered in Vero13R cells. gD ectopically expressed in R6 cells enabled the amplification of recombinants produced in these cells. The spread and replication of recombinant viruses in J-HveA, J-Nectin or Vero13R cells were dependent on the presence of an appropriate receptor binding domain in the chimeric gD. As shown in FIG. **17**, Panel B, R5322 replicated in all cell lines to approximately the same extent as the R5181 recombinant virus in which the chimeric gD consisted of the uPA sequences inserted after codon 24 of gD (19). The recombinant R5321 replicated to a very low level

and only in Vero13R cells (FIG. **17**, panel B). The products of transfection used to generate R5323 did not recombine to form an infectious virus.

EXAMPLE 13

Expression

[**0208**] To determine whether the recombinant viruses expressed the appropriate protein, Vero13R cells were exposed to 1.0 PFU of wild-type HSV-1 (F), R5321, R5322 or R5323 viruses. As shown in FIG. **17**, Panel C, the only protein detected in lysates of R5323-infected cells was a truncated form of gD. Cells infected with either wild-type virus or the R5322 mutant produced ICP0, a major α (immediate early) regulatory protein of the virus. As could be expected from the presence of in-frame stop codons, the major product of R5322 virus reacting with either gD or uPA antibody was significantly smaller than the wild-type gD made in wild-type virus-infected cells, consistent with the hypothesis that the N-terminal and C-terminal domains of gD were produced independently.

EXAMPLE 14

Constructs with Internal P_{CMV}

[**0209**] HSV-1 infected cells can suppress one stop codon at a very low level (21). To make a single protein based on the sequence of the chimeric gD in R5322, all 4 stop codons would have to be suppressed. An alternative explanation is that a promoter domain within the gD ORF enables the synthesis of mRNA that encodes a functional protein starting with methionine 219 (mature gD). Implicit in this hypothesis is the notion that residues 61 to 218 simply link the N-terminus of gD to the C-terminal domain but that the sequence itself is important only in the sense that it maintains the two ends in the proper relationship. To investigate these observations, two sets of viruses were constructed (FIG. **18**).

[**0210**] The viruses predicted to be formed by recombinant gD constructs designated R5331, R5332 and R5333 differed from the corresponding recombinant gD constructs in R5321, R5322, and R5323, in that a human cytomegalovirus immediate early (CMV-IE) promoter was inserted upstream of residue 219 (mature gD). In recombinant gD constructs designed to make R5351, R5352 and R5353, residues 63-218 were deleted, and a stop codon and the CMV-IE promoter were inserted between residues 62 and 219 (mature gD). In addition, a Myc epitope tag was inserted at the C-terminus of gD to enable identification of the protein made from the corresponding domain of chimeric gD.

[**0211**] Recombinant viruses R5332 and R5352 that were produced and titered in R6 cells were used to infect other cell lines at a ratio of 0.1 PFU/cell. As shown in FIG. **18**, Panel B, these recombinant viruses replicated in J-Nectin, J-HveA and Vero13R cell lines to the same extent as R5322 (see Panel B of FIG. **17**). R6 cells transfected with chimeric gD constructs designed to produce the recombinant viruses R5331 or R5351 exhibited extensive cytopathic effects. However, the lysates of these cells did not replicate in the 3 test lines (J-HveA, J-Nectin, or Vero 13R). No infectious virus progeny of the transfection of R6 cells with R5333 or R5353 were detected and these constructs were not tested further.

EXAMPLE 15

Western Analyses

[**0212**] Lastly, analyses of key proteins made by the recombinant viruses constructed in this series of experiments are shown in FIG. **18**, Panel C. Vero13R cells infected with either R5332 or R3552 accumulated two sets of co-migrating bands, one that reacted with antibody to ICP0 and one that reacted with anti-Myc antibody. The anti-uPA antibody reacted with a protein band in R5332-mutant virus-infected cells that migrated more slowly than the protein detected by the anti-uPA antibody in R5352 mutant virus-infected cells. We conclude that (i) the tagged gD proteins containing the residues 219 to 369 (mature gD) were expressed as separate entities from the proteins reactive with the anti-uPA antibody and (b) the differences in electrophoretic mobility of proteins reactive with the anti-uPA antibody reflect their sizes. Thus, in R5352 mutant virus-infected cells, the N-terminal polypeptide comprises uPA and gD residues 33 to 60. In R5332 mutant virus-infected cells, translation of the mRNA proceeds beyond codon 60 to at least the first stop codon at position 139.

[**0213**] We conclude that (i) the residues 61 to 218 of gD are not required for entry into cells, (ii) the essential N-terminal and C-terminal domains of gD can be produced independently of each other, and (iii) infectivity depends on the nature of the ligand attached to the N-terminal domain of gD.

EXAMPLE 16

Peptide Interactions

[**0214**] The N-terminal domain of gD linked to uPA co-precipitated with the C-terminal domain of gD. The results shown in FIG. **18** indicated that in R5332 or R5352 infected cells the amino acid stretch comprising the N-terminal domain of uPA linked to residues 33-60 of gD were in a different polypeptide (uPA-gD_{33-60}) than the C-terminal domain of gD consisting of residues 219 to 369 ($gD_{219-369}$, numbering of mature gD). To infect cells, however, the two functional domains of the chimeric gD had to interact. To determine whether the two polypeptides interact, we constructed 4 plasmids schematically illustrated in FIG. **19**, Panel A. Plasmid pGG5414 encoded residues 1-60 of gD (SEQ ID NO:26) driven by the CMV promoter. Plasmids pGG5415 and pGG5416 consisted of uPA-gD_{33-60} and IL13-gD_{33-60} driven by the CMV promoter, respectively. In each of the latter two plasmids, the valine codon 34 was substituted with that of serine. Finally, plasmid R5417 contained mature gD residues 219-314, i.e., gD without the transmembrane and cytoplasmic domains tagged at the C-terminus with the Myc epitope and driven by the CMV promoter. The plasmids were transfected into HEK293 cells in pair-wise fashion, i.e., pGG5417 with either pGG5414, pGG5415 or pGG5416. The cells were harvested 40 hours after transfection, lysed and reacted with antibodies to either gD (transfection of pGG5417+pGG5414), IL13 (pGG5417+pGG5415), uPA (pGG5417+pGG5416) or Myc. As shown in Panel Bc of FIG. **19**, anti-Myc antibody precipitated a protein reactive with the anti-uPA antibody. Conversely, as shown in panel Bd, the anti-uPA antibody precipitated a protein reactive with Myc. Anti-Myc antibody did not pull down proteins reactive with the mature $gD_{219-314}$ polypeptide from cells transfected with pGG5417+pGG5414, or

pGG5417+pGG5415. The results are described using the numbering of mature gD and show that the uPA-gD_{33-60} polypeptide physically interacts with the $gD_{219-314}$ polypeptide. No evidence has been found that either IL13-gD_{33-60} or wild-type gD1-60 interact physically with the $gD_{219-360}$ polypeptide. One explanation for the failure to detect infectious R5333 or R5353 virus is the failure of the two key components of gD to interact with each other.

[0215] One explanation of our results is that the co-precipitation of uPA-gD_{33-60} (mature gD numbering) with $gD_{219-369}$ (mature gD numbering) reflects the interaction of a Kringle domain (residues 50 to 132) in the N-terminal domain of uPA with lysines in the C-terminal domain of gD. To test this explanation, HEK293 cells were transfected with mixtures of pGG5417 and a plasmid (pGG5418) encoding the N-terminal domain of uPA or pGG5417 and pGG5416. The lysates of the transfected cells were reacted with antibody to Myc. The immune precipitates were collected, electrophoretically separated in a denaturing gel, transferred to a nitrocellulose membrane and reacted with anti-uPA antibody. As shown in FIG. **19**, panel Cb, anti-Myc antibody co-precipitated uPA from lysates of cells co-transfected with pGG5417 and pGG5418 (lane 2) and uPA 119-314 from lysates of cells co-transfected with pGG5417 and pGG5416 (lane 3).

[0216] An infectious virus with two frameshifts, one after codon 60 and the second after codon 201, was constructed. The frameshifts replaced the codons 61 through 201 with a sequence that contained 4 stop codons. The salient feature of the recombinant virus that emerged from these studies is that a virus with a gD consisting of polypeptide A containing the N-terminus of uPA fused to residues 33-60 of gD and a polypeptide B containing the C-terminal domain of gD was infectious. Attempts to construct recombinant viruses in which the A polypeptides consisted solely of gD residues 1-60 or of the 132 residues encoding IL13 fused to gD residues 33 to 60 were not infectious. These results led to the following conclusions, stated using the numbering of mature gD:

[0217] (i) Residues 61 to 218 of gD do not execute a function required for HSV-1 entry into cells. They appear to serve as a linker between the N-terminal targeting domain and the C-terminal pro-fusion domains of gD. It is of interest to note that residues 61-218 coincide almost entirely with the immunoglobulin-like core of gD located between residues 56 and 184 (7). In light of these results, the primary function of the gD core appears to be to hold the key N-terminal and C-terminal domains in proper orientation. This domain may also be required to block cell death as a consequence of a discharge of lysosomal enzymes (17).

[0218] (ii). The fundamental difference between the constructs which yielded an infectious virus and those that failed is that polypeptide A, consisting of uPA_{33-60}, interacted with the C-terminal domain of gD, whereas those containing gD1-60 or IL13-gD_{33-60} did not. The evidence supports the conclusion that uPA itself can interact with $gD_{219-314}$. The results support the conclusion that physical interaction of the domain capable of binding a cell surface receptor with the C-terminal domain of gD may lead to successful virus entry into cells whereas lack of physical interaction may lead to failure. The results reinforce the conclusion that entry requires the physical rapprochement of gD to the cell surface receptor.

[0219] (iii) Current studies indicate that interaction of gD with its receptors alters its conformation and exposes the C-terminus of gD to fusogenic glycoproteins gB, gH and gL (23, 24, 25). It is unlikely that each of the three ligands selected by chance alters the conformation of the remaining portions of gD in an identical manner to enable virus entry. One hypothesis that remains to be explored is that gD is "armed" by a conformational change resulting from replacement of residues 1-32 by any of the 3 ligands, and that cell membrane-envelope fusion is executed when the ligands interact with their cognate receptors. A corollary of this hypothesis is that while the interaction of the receptor binding domain with its natural cell surface receptor may alter the conformation of the distal portions of gD, such changes are not obligatory consequences of the interaction of chimeric gD molecules with novel cognate receptors.

[0220] Apparent from the experimental results disclosed above, the invention comprehends a herpes simplex virus providing an expressible coding region in which a coding sequence for a targeting peptide of interest is translationally fused to a peptide fusion containing a sequence encoding mature gD 33-60 fused to a peptide interaction domain capable of specific interaction with mature gD 219-369. An exemplary peptide interaction domain is a Kringle domain, such as the Kringle domain of uPA residues 50-132, shown to interact with the C-terminal fusogenic domain of gD. In fusing a coding region for a targeting peptide of interest to a coding sequence for the peptide fusion, the targeting peptide is freed from a role in the interaction of gD domains to effect HSV entry. Thus, the only role for such a targeting peptide is to specifically recognize its binding partner, thereby providing maximal flexibility in the design of a viral vector amenable to customized targeting to a cell of interest.

[0221] Expanding upon the aspect of the invention described immediately above, the uPA ligand described above might be replaced by any of a number of alternative ligands in trans, provided that they can associate with the pro-fusogenic domain, to extend the host range of recombinant viruses in useful ways. More generally, the invention comprehends an engineered interaction between the fused targeting peptide-gD interaction domain and the gD fusogenic domain. Based on the disclosure herein that the N-terminally disposed gD interaction domain interacts with the C-terminally disposed fusogenic domain in effecting HSV entry, constructs according to the invention can rely on naturally occurring domains or motifs, such as the Kringle domain and the polylysine region, to ensure interaction of these two gD domains or reliance can be placed on any interacting domains/motifs that are engineered as part of fusion polypeptides. One fusion polypeptide comprises a targeting peptide, the N-terminally disposed gD interaction domain (e.g., mature gD residues 33-60), and an interacting domain/motif, the other polypeptide comprises a fusogenic domain of mature gD (e.g., residues 219-369) and the cognate member of an interacting pair of domains/motifs. The two polypeptides may be part of a single protein chain or they may be separate chains. As with the other inventive constructs described herein, linkers may be interposed between any and all elements of the polypeptides to provide spacing and flexibility, using routine procedures known in the art.

[0222] From the foregoing it will be appreciated that, although specific embodiments of the invention have been

described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

REFERENCES

[0223] Davis F G, Freels S, Grutsch J, Barlas S, Brem S. (1998) *J. Neurosurg.* 88:1-10.

[0224] Pyles R B, Warnick R E, Chalk C L, Szanti B E, Parysek L M. (1997) *Hum Gene Ther.* 8(5):533-44.

[0225] Rampling R, Cruickshank G, Papanastassiou V, Nicoll J, Hadley D, Brennan D, Petty R, MacLean A, Harland J, McKie E, Mabbs R, Brown M. (2000) *Gene Ther.* 7(10):859-66.

[0226] McKie E A, Brown S M, MacLean A R, Graham D I. (1998) *Neuropathol Appl Neurobiol.* 24(5):367-72.

[0227] Markert J M, Medlock M D, Rabkin S D, Gillespie G Y, Todo T, Hunter W D, Palmer C A, Feigenbaum F, Tornatore C, Tufaro F, Martuza R L. (2000) *Gene Ther.* 7(10):867-74.

[0228] Mineta T, Rabkin S D, Yazaki T, Hunter W D, Martuza R L. (1995) *Nat Med.* 1(9):938-43.

[0229] Simard C, Langlois I, Styger D, Vogt B, Vlcek C, Chalifour A, Trudel M, Schwyer M. (1995) *Virology.* 212(2):734-40.

[0230] Chou J, Chen J J, Gross M, Roizman B. (1995) *Proc Natl Acad Sci USA.* 92(23):10516-20.

[0231] He B, Chou J, Brandimarti R, Mohr I, Gluzman Y, Roizman B. (1997) *J Virol.* 71(8):6049-54

[0232] Cassady K A, Gross M, Roizman B. (1998) *J Virol.* 72(9):7005-11.

[0233] Leib, D. A., Harrison, T. E., Laslo, K. M., Machalek, M. A., Moorman N. J. and Virgin, H. A W. (1999) *J. Exp. Med.* 189:663-672.

[0234] Laquerre S, Argnani R, Anderson D B, Zucchini S, Manservigi R, Glorioso J C. (1998). *J Virol.* 72(7):6119-30.

[0235] Spear, P. G., R. J. Eisenberg, and G. H. Cohen. (2000) *Virology* 275: 1-9.

[0236] Montgomery, R. I., M. S. Warner, B. J. Lum, and P. G. Spear. (1996) *Cell* 87:427-436.

[0237] Campadelli-Fiume, G., F. Cocchi, L. Menotti, and M. Lopez. (2000) *Reviews in Medical Virology.* 10:305-319.

[0238] Zhou G, Roizman B. (2002) *J Virol.* 76(12):6197-204.

[0239] Debinski W, Gibo D M, Hulet S W, Connor J R, Gillespie G Y. (1999) *Cancer Res.* 5:985-990.

[0240] Mintz A, Gibo D M, Slagle-Webb B, Christensen N D, Debinski W. (2002) *Neoplasia* 4:388-399.

[0241] Debinski W. (1998) *Crit. Rev. Oncogen.* 9:255-268.

[0242] Debinski W, Gibo D M. (2000) *Mol. Med.* 6:440-449.

[0243] Zhou G, Roizman B. (2001) *J Virol.* 75(13):6166-72.

[0244] Arsenakis M, Tomasi L F, Speziali V, Roizman B, Campadelli-Fiume G. (1986) *J Virol.* 58(2):367-76.

[0245] Ye G J, Roizman B. (2000) *Proc Natl Acad Sci USA.* 97(20):11002-7.

[0246] Zhou G, Galvan V, Campadelli-Fiume G, Roizman B. (2000) *J Virol.* 74(24):11782-91.

[0247] Carfi A, Willis S H, Whitbeck J C, Kirummenacher C, Cohen G H, Eisenberg R J, Wiley D C. (2001) *Mol Cell.* 8(1):169-79.

[0248] Cocchi, F., Menotti, L., Mirandola, P and Campadelli-0Fiume, G. (1998) J. Virol. 72:9992-10002.

[0249] Debinski W, Thompson J P. 1999. *Clin Cancer Res.* 5(10 Suppl):3143s-3147s.

[0250] Brooks, P. C., Clark, R. A. F., and Cheresh, D. A. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 264:569-571, 1994.

[0251] Brooks, P. C., Montgomery, A. M. P., Rosenfeld, M., Reisfeld, R. A., HU, T., Klier, G., and Cheresh, D. A. Integrin $\alpha_v\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 79:1157-1164, 1994.

[0252] Burger, M. J., Tebay, M. A., Keith, P. A., Samaratunga, H. M., Clements, J., Lavin, M. F., and Gardiner, R. A. Expression analysis of δ-Catenin and prostate-specific membrane antigen: Their potential as diagnostic markers for prostate cancer. Int. J. Cancer 100:228-237, 2002.

[0253] Ellerman, T. C., Domagala, T., McKern N. T. et al, Identification of the determinant of Epidermal growth factor receptor ligand-binding specificity using truncated, high affinity form of the ectodomain. 2001 Biochemistry 40 8930-8939.

[0254] Genbitsky, D. S., Bozso, Z., O'Flaharty, M. et al., 2001 A specific binding site for a fragment of the B-loop of epidermal growth factor and related peptides. Peptides 23:97-102 A.

[0255] Urbanelli, L., Ronchini, C., Fontana, L. et al., Tergeted gene transduction of mammalian cells expressing the HER2/neu receptor by filamentous phage. *J Mol Biol.* 2001 Nov. 9; 313(5):965-76.

[0256] Hayashi, T., Takahashi, T., Motoya, S., et al. MUC1 Mucin core protein binds to the dfomasin of ICAM-1 2001 Digestion 63:87-92.

[0257] Fracasso, G., Bellisola, G., Cingarlini, S., Castelletti, D., Prayer-Galletti, T., Pagano, F., Tridente, G., and Colombatti, M. Anti-tumor effects of toxins targeted to the prostate specific membrane antigen. Prostate 53:9-23, 2002.

[0258] Mabjeesh, N. J., Zhong, H., and Simons, J. W. Gene therapy of prostate cancer: current and future directions. Endo. Related Cancer 9:115-139, 2002.

[0259] Ross, S., Spencer, S. D., Holcomb, I., Tan, C., Hongo, J., Devaux, B., Rangell, L., Keller, G. A., Schow, P., Steeves, R. M., Lutz, R. J., Frantz, G., Hillan, K., Peale, F., Tobin, P., Eberhard, D., Rubin, M. A., Lasky, L. A., and Koeppen, H. Prostate stem cell antigen as therapy target: Tissue expression and in Vivo efficacy of an immunoconjugate. Cancer Res. 62:2546-2553, 2002.

[0260] Ruoslahti, E. RGD and other recognition sequences for integrins. Annu. Rev. Cell Dev. Biol. 12:697-715, 1996.

[0261] Thomas, J., Gupta, M., Grasso, Y., Reddy, C. A., Heston, W. D., Zippe, C., Dreicer, R., Kupelian, P. A., Brainard, J., Levin, H. S., and Klein, E. A. Preoperative combined nested reverse transcriptase polymerase chain reaction for prostate-specific antigen and prostate-specific membrane antigen does not correlate with pathologic stage or biochemical failure in patients with localized prostate cancer undergoing radical prostatectomy. *J. Clin. Oncol.* 20:3213-3218, 2002.

[0262] Lorimer and Lavictoire, Targeting retrovirus to cancer cells expressing a mutant EGF receptor by insertion of a single chain antibody variable domain in the envelope glycoprotein receptor binding lobe, *J Immunol Methods* 237(1-2):147-57, 2000.

[0263] Turner et al., Glycoproteins gB, gD, and gHgL of Herpes Simplex Virus Type 1 are Necessary and Sufficient t Mediate membrane fusion in a Cos cell transfection system, *J of Virol,* 72(1): 873-75, 1998.

[0264] Brunetti et al., Herpes Simplex Virus gD and Virions Accumulate in Endosomes by Mannose 6-Phosphate-Dependent and -Independent Mechanisms, *J of Virol,* 72(4):3330-3339, 1998.

[0265] Sharma et al., Molecular imaging of gene expression and protein function in vivo with PET and SPECT, *J Magn Reson Imaging,* 16(4):336-51, 2002.

[0266] Vries et al., Scintgraphic Imaging of HSVtk Gene Therapy, *Curr Pharm Des,* 8(16):1435-50, 2002.

[0267] Vries et al., Positron emission tomography: measurement of transgene expression, *Methods,* 27(3):234, 2002.

[0268] Soling et al., Intracellular localization of Herpes simplex virus type 1 thymidine kinase fused to different fluorescent proteins depends on choice of fluorescent tag, *FEBS Lett,* 527(1-3):153, 2002.

[0269] Zhou G., and Roizman B., *J Virol,* 9:5272-5277 (2005)

[0270] Manoj S., et al., *Proc. Natl. Acad. Sci. USA.,* 101: 12414-12421 (2004);

[0271] Zago A., et al., *Proc. Natl. Acad. Sci. USA.,* 101:17498-17503 (2004);

[0272] Connolly S A., *J. Virol.,* 79:1282-1295 (2005)

[0273] 2. Spear, P. G. (2004) *Cell Microbiol* 6, 401-410.

[0274] 3. Spear, P. G. & Longnecker, R. (2003) *J. Virol* 77, 10179-10185.

[0275] 6. Fusco, D., Forghieri, C. & Campadelli-Fiume, G. (2005) *Proc. Natl. Acad. Sci. USA* 102, 9323-9328.

[0276] 7. Kirummenacher, C., Supekar, V. M., Whitneck, J. C., Connolly, S. A., Eisenberg, R. J., Cohen, G., Wiley, D. C. & Carfi, A. (2005) *EMBO J* 24, 4144-4153.

[0277] 8. Cocchi F, Fusco D, Menotti L, Gianni T, Eisenberg R J, Cohen G H, Campadelli-Fiume G. (2004). *Proc. Natl. Acad. Sci. USA* 101, 7445-7450.

[0278] 9. Willis S H, Rux A H, Peng C, Whitbeck J C, Nicola A V, Lou H, Hou W, Salvador L, Eisenberg R J, Cohen G H. (1998). J. Virol. 72, 5937-47.

[0279] 10. Connolly S A, Landsburg D J, Carfi A, Wiley D C, Eisenberg R J, Cohen G H. (2002) J Virol. 76, 10894-904.

[0280] 11. Connolly S A, Landsburg D J, Carfi A, Wiley D C, Cohen G H, Eisenberg R J. (2003). J Virol. 77, 8127-40.

[0281] 12. Yoon, M. & Spear, P. G. (2004) *Proc. Natl. Acad. Sci. USA* 101, 17252-17257.

[0282] 17. Zhou, G., Avitabile, E., Campadelli-Fiume, G. & Roizman, B. (2003) *J. Virol* 77, 3759-3767.

[0283] 18. Zhou, G., Ye, G. J., Debinski, W. & Roizman, B. (2002) *Proc. Natl. Acad. Sci. USA* 99, 15124-15129.

[0284] 19. Kamiyama H, Zhou G, Roizman B. (2006) Gene Ther. 13, 621-9.

[0285] 20. Zhou G, Roizman B. (2006). Proc Natl Acad Sci USA. 103, 5508-13.

[0286] 21. Chou, J., Poon A. P. W., Johnson, J., and Roizman. (1994). J. Virol, 68, 8.304-8.311.

[0287] 23. Gianni T, Menotti L, Campadelli-Fiume G. (2005) J Virol. 79, 7042-9.

[0288] 25. Martinez W M, Spear P G. (2002). J Virol. 76, 7255-62.

[0289] Zhou G, et al., Proc. Natl. Acad. Sci. USA, 99(23):15124-9 (2002)

[0290] Ploug M, et al., J. Biol. Chem., 266:1926-33 (1991)

[0291] Moller J V, et al., J. Biol. Chem., 268:18659-72 (1993)

[0292] Cantero D, et al. Br. J. Cancer, 75:388-95 (1997)

[0293] Reuning U, et al., Int. J. Oncol., 13:893-906 (1998)

[0294] Wei Y, et al., Biol. Chem., 269: 32380-32388 (1994)

[0295] Fowler B, et al., Thromb. Haemost., 80: 148-154 (1998)

[0296] Roldan A L, et al., EMBO J., 9: 467-474 (1990)

[0297] Barnathan E S, et al., J. Biol. Chem., 265:2865-2872 (1990)

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 67

<210> SEQ ID NO 1
<211> LENGTH: 60
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pIL13F1 primer

<400> SEQUENCE: 1

cattgctctc acttgccttg gcggctttgc ctccccaggc cctgtgcctc cctctacagc 60

<210> SEQ ID NO 2
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pIL13F2 primer

<400> SEQUENCE: 2

gcagctagcc tcatggcgct tttgttgacc acggtcattg ctctcacttg ccttggcggc 60

<210> SEQ ID NO 3
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pIL13REcoRI primer

<400> SEQUENCE: 3

gagctcggat cctgaattca accgtccctc 30

<210> SEQ ID NO 4
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pgC-F

<400> SEQUENCE: 4

gacacgggct accctcacta tcgagggc 28

<210> SEQ ID NO 5
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pgC-R

<400> SEQUENCE: 5

ggtgatgttc gtcaggacct cctctaggtc 30

<210> SEQ ID NO 6
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pgB1BamHI primer

<400> SEQUENCE: 6

gttcttcttc ggtttcggat cccccg 26

<210> SEQ ID NO 7
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pgB2BspEI primer

<400> SEQUENCE: 7

-continued

cggcatttcc ggaataacgc ccactc 26

<210> SEQ ID NO 8
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pgB3BamHI primer

<400> SEQUENCE: 8

cagaaaaccg gatcccccaa agccgcc 27

<210> SEQ ID NO 9
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pgB4BsiWI primer

<400> SEQUENCE: 9

gccaacacaa actcgtcgta cgggtac 27

<210> SEQ ID NO 10
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: first forward primer

<400> SEQUENCE: 10

cagttatcct taaggtctct tttgtgtggt g 31

<210> SEQ ID NO 11
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: first reverse primer

<400> SEQUENCE: 11

ccggaattcc ggagatcttc cctcgaggac cggaaggtct ttgccgcgaa ag 52

<210> SEQ ID NO 12
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: second forward primer

<400> SEQUENCE: 12

ccggaattcc ggggtaccct ggaccagctg accgaccctc cgg 43

<210> SEQ ID NO 13
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: second reverse primer

<400> SEQUENCE: 13

cgggggggatg cgcagcggga gggcgtactt ac 32

<210> SEQ ID NO 14

-continued

<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13 forward primer

<400> SEQUENCE: 14

ccgctcgaga tggcgctttt gttgaccacg g 31

<210> SEQ ID NO 15
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13 reverse primer

<400> SEQUENCE: 15

ggggtaccgt tgaaccgtcc ctcgcgaaa 29

<210> SEQ ID NO 16
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL 13 ORF forward primer

<400> SEQUENCE: 16

ccgctcgaga tggcgctttt gttgaccacg g 31

<210> SEQ ID NO 17
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13 ORF reverse primer

<400> SEQUENCE: 17

ggggtaccgt tgaaccgtcc ctcgcgaaa 29

<210> SEQ ID NO 18
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gD forward junction primer

<400> SEQUENCE: 18

ccgctcgaga tggcgctttt gttgaccacg g 31

<210> SEQ ID NO 19
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gD reverse junction primer

<400> SEQUENCE: 19

aactgcaggt tgttcggggt ggccggggg 29

<210> SEQ ID NO 20
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13 2 forward primer

-continued

<400> SEQUENCE: 20

aagatttggg ctagcatggc tttcgtttgc 30

<210> SEQ ID NO 21
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13 2 reverse primer

<400> SEQUENCE: 21

tccctcgaag cttcaagcat aatctggcac atcatatgta tcacagaaaa a 51

<210> SEQ ID NO 22
<211> LENGTH: 568
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gC chimeric protein

<400> SEQUENCE: 22

gcttggtcgg gaggccgcat cgaacgcaca cccccatccg gtggtccgtg tggaggtcgt 60

ttttcagtgc ccggtctcgc tttgccggga acgctagcct catggcgctt ttgttgacca 120

cggtcattgc tctcacttgc cttggcggct ttgcctcccc aggccctgtg cctccctcta 180

cagccctcag gtacctcatt gaggagctgg tcaacatcac ccagaaccag aaggctccgc 240

tctgcaatgg cagcatggta tggagcatca acctgacagc tggcatgtac tgtgcagccc 300

tggaatccct gatcaacgtg tcaggctgca gtgccatcga gaagacccag aggatgctga 360

gcggattctg cccgcacaag gtctcagctg ggcagttttc cagcttgcat gtccgagaca 420

ccaaaatcga ggtggcccag tttgtaaaag atctgctctt acatttaaag aaacttttc 480

gcgagggacg gttgaattcc acccgcatgg agttccgcct ccagatatgg cgttactcca 540

tgggtccgtc ccccccaatc gctccggc 568

<210> SEQ ID NO 23
<211> LENGTH: 350
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: domain of the gB open reading frame
(i.e., ORF) from which the poly lysine [poly(K)] sequence was
deleted

<400> SEQUENCE: 23

gggtcctggt ggcgtcggcg gctccgagtt cccccggcac gcctggggtc gcggccgcga 60

cccaggcggc gaacgggggga cctgccactc cggcgccgcc cgcccctggc cccgccccaa 120

cgggggatcc gaaaccgaag aagaacagaa aaccgaaacc cccaaagcgc cgcgccccgc 180

cggcgacaac gcgaccgtcg ccgcgggcca cgccaccctg cgcgagcacc tgcgggacat 240

caaggcggag aacaccgatg caaactttta cgtgtgccca ccccccacgg gcgccacggt 300

ggtgcagttc gagcagccgc gccgctgccc gacccggccc gagggtcaga 350

<210> SEQ ID NO 24
<211> LENGTH: 655
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: amino-terminal sequence of IL13-gD

<400> SEQUENCE: 24

atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtcggcctc 60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagctggc cgaccccaat 120

cgctttcgcc gcaaagacct tccggtcctc gagatggcgc ttttgttgac cacggtcatt 180

gctctcactt gccttggcgg ctttgcctcc ccaggccctg tgcctcccct ctacagccct 240

cagggagctc attgaggagc tggtcaacat cacccagaac cagaaggctc cgctctgcaa 300

tggcagcatg gtttggagca tcaacctgac agctggcatg tactgtgcag ccctggaatc 360

cctgatcaac gtgtcaggct gcagtgccat cgagaagacc cagaggatgc tgggcggatt 420

ctgcccgcac aaggtctcag ctgggcagtt ttccagcttg catgtccgag acaccaaaat 480

cgaggtggcc cagtttgtaa aggacctgct cttacattta aagaaacttt ttcgcgaggg 540

acggttcaac tgaaacggta ccctggacca gctgaccgac cctccggggg tccggcgcgt 600

gtaccacatc caggcgggcc taccggaccc gttccagccc cccagcctcc cgatc 655

<210> SEQ ID NO 25
<211> LENGTH: 1185
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gD polynucleotide

<400> SEQUENCE: 25

atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc 60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagatggc cgaccccaat 120

cgctttcgcg gcaaagacct tccggtcccg gaccggctga ccgaccctcc gggggtccgg 180

cgcgtgtacc acatccaggc gggcctaccg gacccgttcc agccccccag cctcccgatc 240

acggtttact acgccgtgtt ggagcgcgcc tgccgcagcg tgctcctaaa cgcaccgtcg 300

gaggcccccc agattgtccg cggggcctcc gaagacgtcc ggaaacaacc ctacaacctg 360

accatcgctt ggtttcggat gggaggcaac tgtgctatcc ccatcacggt catggagtac 420

accgaatgct cctacaacaa gtctctgggg gcctgtccca tccgaacgca gccccgctgg 480

aactactatg acagcttcag cgccgtcagc gaggataacc tggggttcct gatgcacgcc 540

cccgcgtttg agaccgccgg cacgtacctg cggctcgtga agataaacga ctggacggag 600

attacacagt ttatcctgga gcaccgagcc aagggctcct gtaagtacgc ccttccgctg 660

cgcatccccc cgtcagcctg cctctccccc caggcctacc agcagggggt gacggtggac 720

agcatcggga tgctgccccg cttcatcccc gagaaccagc gcaccgtcgc cgtatacagc 780

ttgaagatcg ccgggtggca cgggcccaag gccccataca cgagcaccct gctgcccccg 840

gagctgtccg agacccccaa cgccacgcag ccagaactcg ccccggaaga ccccgaggat 900

tcggccctct tggaggaccc cgtggggacg gtggtgccgc aaatcccacc aaactggcac 960

ataccgtcga tccaggacgc cgcgacgcct taccatcccc cggccacccc gaacaacatg 1020

ggcctgatcg ccggcgcggt gggcggcagt ctcctggtag ccctggtcat ttgcggaatt 1080

gtgtactgga tgcgccgccg cactcaaaaa gccccaaagc gcatacgcct cccccacatc 1140

cgggaagacg accagccgtc ctcgcaccag cccttgtttt actag 1185

-continued

<210> SEQ ID NO 26
<211> LENGTH: 394
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gD amino acid

<400> SEQUENCE: 26

Met Gly Gly Ala Ala Ala Arg Leu Gly Ala Val Ile Leu Phe Val Val
1 5 10 15

Ile Val Gly Leu His Gly Val Arg Gly Lys Tyr Ala Leu Ala Asp Ala
20 25 30

Ser Leu Lys Met Ala Asp Pro Asn Arg Phe Arg Gly Lys Asp Leu Pro
35 40 45

Val Pro Asp Arg Leu Thr Asp Pro Pro Gly Val Arg Arg Val Tyr His
50 55 60

Ile Gln Ala Gly Leu Pro Asp Pro Phe Gln Pro Pro Ser Leu Pro Ile
65 70 75 80

Thr Val Tyr Tyr Ala Val Leu Glu Arg Ala Cys Arg Ser Val Leu Leu
85 90 95

Asn Ala Pro Ser Glu Ala Pro Gln Ile Val Arg Gly Ala Ser Glu Asp
100 105 110

Val Arg Lys Gln Pro Tyr Asn Leu Thr Ile Ala Trp Phe Arg Met Gly
115 120 125

Gly Asn Cys Ala Ile Pro Ile Thr Val Met Glu Tyr Thr Glu Cys Ser
130 135 140

Tyr Asn Lys Ser Leu Gly Ala Cys Pro Ile Arg Thr Gln Pro Arg Trp
145 150 155 160

Asn Tyr Tyr Asp Ser Phe Ser Ala Val Ser Glu Asp Asn Leu Gly Phe
165 170 175

Leu Met His Ala Pro Ala Phe Glu Thr Ala Gly Thr Tyr Leu Arg Leu
180 185 190

Val Lys Ile Asn Asp Trp Thr Glu Ile Thr Gln Phe Ile Leu Glu His
195 200 205

Arg Ala Lys Gly Ser Cys Lys Tyr Ala Leu Pro Leu Arg Ile Pro Pro
210 215 220

Ser Ala Cys Leu Ser Pro Gln Ala Tyr Gln Gln Gly Val Thr Val Asp
225 230 235 240

Ser Ile Gly Met Leu Pro Arg Phe Ile Pro Glu Asn Gln Arg Thr Val
245 250 255

Ala Val Tyr Ser Leu Lys Ile Ala Gly Trp His Gly Pro Lys Ala Pro
260 265 270

Tyr Thr Ser Thr Leu Leu Pro Pro Glu Leu Ser Glu Thr Pro Asn Ala
275 280 285

Thr Gln Pro Glu Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu
290 295 300

Glu Asp Pro Val Gly Thr Val Val Pro Gln Ile Pro Pro Asn Trp His
305 310 315 320

Ile Pro Ser Ile Gln Asp Ala Ala Thr Pro Tyr His Pro Pro Ala Thr
325 330 335

Pro Asn Asn Met Gly Leu Ile Ala Gly Ala Val Gly Gly Ser Leu Leu
340 345 350

Val Ala Leu Val Ile Cys Gly Ile Val Tyr Trp Met Arg Arg Arg Thr
355 360 365

-continued

Gln Lys Ala Pro Lys Arg Ile Arg Leu Pro His Ile Arg Glu Asp Asp
370 375 380

Gln Pro Ser Ser His Gln Pro Leu Phe Tyr
385 390

<210> SEQ ID NO 27
<211> LENGTH: 2715
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gB polynucleotide

<400> SEQUENCE: 27

atgcgccagg gcgcccccgc gcggggcgc cggtggttcg tcgtatgggc gctcttgggg 60

ttgacgctgg gggtcctggt ggcgtcggcg gctccgagtt cccccggcac gcctggggtc 120

gcggccgcga cccaggcggc gaacgggggc cctgccactc cggcgccgcc cgcccctggc 180

gccccccaa cgggggaccc gaaaccgaag aagaacagaa aaccgaaacc cccaaagccg 240

ccgcgccccg ccggcgacaa cgcgaccgtc gccgcgggcc acgccaccct gcgcgagcac 300

ctgcgggaca tcaaggcgga gaacaccgat gcaaactttt acgtgtgccc acccccacg 360

ggcgccacgg tggtgcagtt cgagcagccg cgccgctgcc cgacccggcc cgagggtcag 420

aactacacgg agggcatcgc ggtggtcttc aaggagaaca tcgccccgta caagttcaag 480

gccaccatgt actacaaaga cgtcaccgtt tcgcaggtgt ggttcggcca ccgctactcc 540

cagtttatgg ggatctttga ggaccgcgcc cccgtcccct tcgaggaggt gatcgacaag 600

atcaacgcca agggggtctg tcggtccacg gccaagtacg tgcgcaacaa cctggagacc 660

accgcgtttc accgggacga ccacgagacc gacatggagc tgaaaccggc caacgccgcg 720

acccgcacga gccggggctg gcacaccacc gacctcaagt acaacccctc gcgggtggag 780

gcgttccacc ggtacgggac gacggtaaac tgcatcgtcg aggaggtgga cgcgcgctcg 840

gtgtacccgt acgacgagtt tgtgttggcg actggcgact ttgtgtacat gtccccgttt 900

tacggctacc gggaggggtc gcacaccgaa cacaccagct acgccgccga ccgcttcaag 960

caggtcgacg gcttctacgc gcgcgacctc accaccaagg cccgggccac ggcgccgacc 1020

acccggaacc tgctcacgac ccccaagttc accgtggcct gggactgggt gccaaagcgc 1080

ccgtcggtct gcaccatgac caagtggcag gaggtggacg agatgctgcg ctccgagtac 1140

ggcggctcct tccgattctc ttccgacgcc atatccacca ccttcaccac caacctgacc 1200

gagtacccgc tctcgcgcgt ggacctgggg gactgcatcg gcaaggacgc ccgcgacgcc 1260

atggaccgca tcttcgcccg caggtacaac gcgacgcaca tcaaggtggg ccagccgcag 1320

tactacctgg ccaatggggg ctttctgatc gcgtaccagc cccttctcag caacacgctc 1380

gcggagctgt acgtgcggga acacctccgc gagcagagcc gcaagccccc aaaccccacg 1440

cccccgccgc ccggggccag cgccaacgcg tccgtggagc gcatcaagac cacctcctcc 1500

atcgagttcg ccaggctgca gtttacgtac aaccacatac agcgccatgt caacgatatg 1560

ttgggccgcg ttgccatcgc gtggtgcgag ctgcagaatc acgagctgac cctgtggaac 1620

gaggcccgca agctgaaccc caacgccatc gcctcggcca ccgtgggccg gcgggtgagc 1680

gcgcggatgc tcggcgacgt gatggccgtc tccacgtgcg tgccggtcgc cgcggacaac 1740

gtgatcgtcc aaaactcgat gcgcatcagc tcgcggcccg gggcctgcta cagccgcccc 1800

-continued

```
ctggtcagct ttcggtacga agaccagggc ccgttggtcg aggggcagct gggggagaac   1860

aacgagctgc ggctgacgcg cgatgcgatc gagccgtgca ccgtgggaca ccggcgctac   1920

ttcaccttcg gtgggggcta cgtgtacttc gaggagtacg cgtactccca ccagctgagc   1980

cgcgccgaca tcaccaccgt cagcaccttc atcgacctca acatcaccat gctggaggat   2040

cacgagtttg tccccctgga ggtgtacacc cgccacgaga tcaaggacag cggcctgctg   2100

gactacacgg aggtccagcg ccgcaaccag ctgcacgacc tgcgcttcgc cgacatcgac   2160

acggtcatcc acgccgacgc caacgccgcc atgtttgcgg gcctgggcgc gttcttcgag   2220

gggatgggcg acctggggcg cgcggtcggc aaggtggtga tgggcatcgt gggcggcgtg   2280

gtatcggccg tgtcgggcgt gtcctccttc atgtccaacc cctttggggc gctggccgtg   2340

ggtctgttgg tcctggccgg cctggcggcg gccttcttcg cctttcgcta cgtcatgcgg   2400

ctgcagagca accccatgaa ggccctgtac ccgctaacca ccaaggagct caagaacccc   2460

accaacccgg acgcgtccgg ggagggcgag gagggcggcg actttgacga ggccaagcta   2520

gccgaggccc gggagatgat acggtacatg gccctggtgt ctgccatgga gcgcacggaa   2580

cacaaggcca agaagaaggg cacgagcgcg ctgctcagcg ccaaggtcac cgacatggtc   2640

atgcgcaagc gccgcaacac caactacacc caagttccca acaaagacgg tgacgccgac   2700

gaggacgacc tgtga                                                    2715
```

<210> SEQ ID NO 28
<211> LENGTH: 904
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gB amino acid

<400> SEQUENCE: 28

```
Met Arg Gln Gly Ala Pro Ala Arg Gly Arg Arg Trp Phe Val Val Trp
1               5                   10                  15

Ala Leu Leu Gly Leu Thr Leu Gly Val Leu Val Ala Ser Ala Ala Pro
            20                  25                  30

Ser Ser Pro Gly Thr Pro Gly Val Ala Ala Ala Thr Gln Ala Ala Asn
        35                  40                  45

Gly Gly Pro Ala Thr Pro Ala Pro Pro Ala Pro Gly Ala Pro Pro Thr
    50                  55                  60

Gly Asp Pro Lys Pro Lys Lys Asn Arg Lys Pro Lys Pro Pro Lys Pro
65                  70                  75                  80

Pro Arg Pro Ala Gly Asp Asn Ala Thr Val Ala Ala Gly His Ala Thr
                85                  90                  95

Leu Arg Glu His Leu Arg Asp Ile Lys Ala Glu Asn Thr Asp Ala Asn
            100                 105                 110

Phe Tyr Val Cys Pro Pro Pro Thr Gly Ala Thr Val Val Gln Phe Glu
        115                 120                 125

Gln Pro Arg Arg Cys Pro Thr Arg Pro Glu Gly Gln Asn Tyr Thr Glu
    130                 135                 140

Gly Ile Ala Val Val Phe Lys Glu Asn Ile Ala Pro Tyr Lys Phe Lys
145                 150                 155                 160

Ala Thr Met Tyr Tyr Lys Asp Val Thr Val Ser Gln Val Trp Phe Gly
                165                 170                 175

His Arg Tyr Ser Gln Phe Met Gly Ile Phe Glu Asp Arg Ala Pro Val
            180                 185                 190
```

-continued

```
Pro Phe Glu Glu Val Ile Asp Lys Ile Asn Ala Lys Gly Val Cys Arg
        195                 200                 205

Ser Thr Ala Lys Tyr Val Arg Asn Asn Leu Glu Thr Thr Ala Phe His
    210                 215                 220

Arg Asp Asp His Glu Thr Asp Met Glu Leu Lys Pro Ala Asn Ala Ala
225                 230                 235                 240

Thr Arg Thr Ser Arg Gly Trp His Thr Thr Asp Leu Lys Tyr Asn Pro
                245                 250                 255

Ser Arg Val Glu Ala Phe His Arg Tyr Gly Thr Thr Val Asn Cys Ile
            260                 265                 270

Val Glu Glu Val Asp Ala Arg Ser Val Tyr Pro Tyr Asp Glu Phe Val
        275                 280                 285

Leu Ala Thr Gly Asp Phe Val Tyr Met Ser Pro Phe Tyr Gly Tyr Arg
    290                 295                 300

Glu Gly Ser His Thr Glu His Thr Ser Tyr Ala Ala Asp Arg Phe Lys
305                 310                 315                 320

Gln Val Asp Gly Phe Tyr Ala Arg Asp Leu Thr Thr Lys Ala Arg Ala
                325                 330                 335

Thr Ala Pro Thr Thr Arg Asn Leu Leu Thr Thr Pro Lys Phe Thr Val
            340                 345                 350

Ala Trp Asp Trp Val Pro Lys Arg Pro Ser Val Cys Thr Met Thr Lys
        355                 360                 365

Trp Gln Glu Val Asp Glu Met Leu Arg Ser Glu Tyr Gly Gly Ser Phe
    370                 375                 380

Arg Phe Ser Ser Asp Ala Ile Ser Thr Thr Phe Thr Thr Asn Leu Thr
385                 390                 395                 400

Glu Tyr Pro Leu Ser Arg Val Asp Leu Gly Asp Cys Ile Gly Lys Asp
                405                 410                 415

Ala Arg Asp Ala Met Asp Arg Ile Phe Ala Arg Arg Tyr Asn Ala Thr
            420                 425                 430

His Ile Lys Val Gly Gln Pro Gln Tyr Tyr Leu Ala Asn Gly Gly Phe
        435                 440                 445

Leu Ile Ala Tyr Gln Pro Leu Leu Ser Asn Thr Leu Ala Glu Leu Tyr
    450                 455                 460

Val Arg Glu His Leu Arg Glu Gln Ser Arg Lys Pro Pro Asn Pro Thr
465                 470                 475                 480

Pro Pro Pro Pro Gly Ala Ser Ala Asn Ala Ser Val Glu Arg Ile Lys
                485                 490                 495

Thr Thr Ser Ser Ile Glu Phe Ala Arg Leu Gln Phe Thr Tyr Asn His
            500                 505                 510

Ile Gln Arg His Val Asn Asp Met Leu Gly Arg Val Ala Ile Ala Trp
        515                 520                 525

Cys Glu Leu Gln Asn His Glu Leu Thr Leu Trp Asn Glu Ala Arg Lys
    530                 535                 540

Leu Asn Pro Asn Ala Ile Ala Ser Ala Thr Val Gly Arg Arg Val Ser
545                 550                 555                 560

Ala Arg Met Leu Gly Asp Val Met Ala Val Ser Thr Cys Val Pro Val
                565                 570                 575

Ala Ala Asp Asn Val Ile Val Gln Asn Ser Met Arg Ile Ser Ser Arg
            580                 585                 590

Pro Gly Ala Cys Tyr Ser Arg Pro Leu Val Ser Phe Arg Tyr Glu Asp
```

-continued

```
        595                 600                 605

Gln Gly Pro Leu Val Glu Gly Gln Leu Gly Glu Asn Asn Glu Leu Arg
    610                 615                 620

Leu Thr Arg Asp Ala Ile Glu Pro Cys Thr Val Gly His Arg Arg Tyr
625                 630                 635                 640

Phe Thr Phe Gly Gly Gly Tyr Val Tyr Phe Glu Glu Tyr Ala Tyr Ser
                645                 650                 655

His Gln Leu Ser Arg Ala Asp Ile Thr Thr Val Ser Thr Phe Ile Asp
            660                 665                 670

Leu Asn Ile Thr Met Leu Glu Asp His Glu Phe Val Pro Leu Glu Val
        675                 680                 685

Tyr Thr Arg His Glu Ile Lys Asp Ser Gly Leu Leu Asp Tyr Thr Glu
    690                 695                 700

Val Gln Arg Arg Asn Gln Leu His Asp Leu Arg Phe Ala Asp Ile Asp
705                 710                 715                 720

Thr Val Ile His Ala Asp Ala Asn Ala Ala Met Phe Ala Gly Leu Gly
                725                 730                 735

Ala Phe Phe Glu Gly Met Gly Asp Leu Gly Arg Ala Val Gly Lys Val
            740                 745                 750

Val Met Gly Ile Val Gly Gly Val Val Ser Ala Val Ser Gly Val Ser
        755                 760                 765

Ser Phe Met Ser Asn Pro Phe Gly Ala Leu Ala Val Gly Leu Leu Val
    770                 775                 780

Leu Ala Gly Leu Ala Ala Ala Phe Phe Ala Phe Arg Tyr Val Met Arg
785                 790                 795                 800

Leu Gln Ser Asn Pro Met Lys Ala Leu Tyr Pro Leu Thr Thr Lys Glu
                805                 810                 815

Leu Lys Asn Pro Thr Asn Pro Asp Ala Ser Gly Glu Gly Glu Glu Gly
            820                 825                 830

Gly Asp Phe Asp Glu Ala Lys Leu Ala Glu Ala Arg Glu Met Ile Arg
        835                 840                 845

Tyr Met Ala Leu Val Ser Ala Met Glu Arg Thr Glu His Lys Ala Lys
    850                 855                 860

Lys Lys Gly Thr Ser Ala Leu Leu Ser Ala Lys Val Thr Asp Met Val
865                 870                 875                 880

Met Arg Lys Arg Arg Asn Thr Asn Tyr Thr Gln Val Pro Asn Lys Asp
                885                 890                 895

Gly Asp Ala Asp Glu Asp Asp Leu
            900


<210> SEQ ID NO 29
<211> LENGTH: 1800
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gC polynucleotide

<400> SEQUENCE: 29

agggcgcttg gtcgggaggc cgcatcgaac gcacaccccc atccggtggt ccgtgtggag     60

gtcgttttca tgcccggtct cgctttgtcg ggaacgctag ccggtccctc gcgaggggga    120

ggcgtcgggc atggccccgg ggcgggtggg ccttgccgtg gtcctgtgga gcctgttgtg    180

gctcggggcg ggggtggccg ggggctcgga aactgcctcc accgggccca cgatcaccgc    240
```

-continued

gggagcggtg acgaacgcga gcgaggcccc cacatcgggg tcccccgggt cagccgccag 300

cccggaagtc acccccacat cgaccccaaa ccccaacaat gtcacacaaa acaaaaccac 360

ccccaccgag ccggccagcc ccccaacaac ccccaagccc acctccacgc ccaaaagccc 420

ccccacgtcc acccccgacc ccaaacccaa gaacaacacc acccccgcca agtcgggccg 480

ccccactaaa ccccccgggc ccgtgtggtg cgaccgccgc gacccattgg cccggtacgg 540

ctcgtgggtg cagatccgat gccggttttg gaattccacc cgcatggagt tccgcctcca 600

gatatggcgt tactccatgg gtccgtcccc cccaatcgct ccggctcccg acctagagga 660

ggtcctgacg aacatcaccg ccccacccgg gggactcctg gtgtacgaca gcgcccccaa 720

cctgacggac ccccacgtgc tctgggcgga gggggccggc ccgggcgccg accctccgtt 780

gtattctgtc accgggccgc tgccgaccca gcggctgatt atcggcgagg tgacgcccgc 840

gacccaggga atgtattact tggcctgggg ccggatggac agcccgcacg agtacgggac 900

gtgggtgcgc gtccgcatgt tccgcccccc gtctctgacc ctccagcccc acgcggtgat 960

ggagggtcag ccgttcaagg cgacgtgcac ggccgccgcc tactacccgc gtaaccccgt 1020

ggagtttgtc tggttcgagg acgaccgcca ggtgtttaac ccgggccaga tcgacacgca 1080

gacgcacgag caccccgacg ggttcaccac agtctctacc gtgacctccg aggctgtcgg 1140

cggccaggtc cccccgcgga ccttcacctg ccagatgacg tggcaccgcg actccgtgac 1200

gttctcgcga cgcaatgcca ccgggctggc cctggtgctg ccgcggccaa ccatcaccat 1260

ggaatttggg gtccggcatg tggtctgcac ggccggctgc gtccccgagg gcgtgacgtt 1320

tgcctggttc ctgggggacg acccctcacc ggcggctaag tcggccgtta cggcccagga 1380

gtcgtgcgac caccccgggc tggctacggt ccggtccacc ctgcccattt cgtacgacta 1440

cagcgagtac atctgtcggt tgaccggata tccggccggg attcccgttc tagagcacca 1500

cggcagtcac cagcccccac ccagggaccc caccgagcgg caggtgatcg aggcgatcga 1560

gtgggtgggg attggaatcg gggttctcgc ggcgggggtc ctggtcgtaa cggcaatcgt 1620

gtacgtcgtc cgcacatcac agtcgcggca gcgtcatcgg cggtaacgcg agacccccccc 1680

gttacctttt taatatctat atagtttggt ccccccctcta tcccgcccac cgctgggcgc 1740

tataaagccg ccaccctctc ttccctcagg tcatccttgg tcgatcccga acgacacacg 1800

<210> SEQ ID NO 30
<211> LENGTH: 511
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gC amino acid

<400> SEQUENCE: 30

Met Ala Pro Gly Arg Val Gly Leu Ala Val Val Leu Trp Gly Leu Leu
1 5 10 15

Trp Leu Gly Ala Gly Val Ala Gly Gly Ser Glu Thr Ala Ser Thr Gly
20 25 30

Pro Thr Ile Thr Ala Gly Ala Val Thr Asn Ala Ser Glu Ala Pro Thr
35 40 45

Ser Gly Ser Pro Gly Ser Ala Ala Ser Pro Glu Val Thr Pro Thr Ser
50 55 60

Thr Pro Asn Pro Asn Asn Val Thr Gln Asn Lys Thr Thr Pro Thr Glu
65 70 75 80

-continued

```
Pro Ala Ser Pro Pro Thr Thr Pro Lys Pro Thr Ser Thr Pro Lys Ser
                85                  90                  95

Pro Pro Thr Ser Thr Pro Asp Pro Lys Pro Lys Asn Asn Thr Thr Pro
            100                 105                 110

Ala Lys Ser Gly Arg Pro Thr Lys Pro Pro Gly Pro Val Trp Cys Asp
        115                 120                 125

Arg Arg Asp Pro Leu Ala Arg Tyr Gly Ser Arg Val Gln Ile Arg Cys
    130                 135                 140

Arg Phe Arg Asn Ser Thr Arg Met Glu Phe Arg Leu Gln Ile Trp Arg
145                 150                 155                 160

Tyr Ser Met Gly Pro Ser Pro Pro Ile Ala Pro Ala Pro Asp Leu Glu
                165                 170                 175

Glu Val Leu Thr Asn Ile Thr Ala Pro Pro Gly Gly Leu Leu Val Tyr
            180                 185                 190

Asp Ser Ala Pro Asn Leu Thr Asp Pro His Val Leu Trp Ala Glu Gly
        195                 200                 205

Ala Gly Pro Gly Ala Asp Pro Pro Leu Tyr Ser Val Thr Gly Pro Leu
    210                 215                 220

Pro Thr Gln Arg Leu Ile Ile Gly Glu Val Thr Pro Ala Thr Gln Gly
225                 230                 235                 240

Met Tyr Tyr Leu Ala Trp Gly Arg Met Asp Ser Pro His Glu Tyr Gly
                245                 250                 255

Thr Trp Val Arg Val Arg Met Phe Arg Pro Pro Ser Leu Thr Leu Gln
            260                 265                 270

Pro His Ala Val Met Glu Gly Gln Pro Phe Lys Ala Thr Cys Thr Ala
        275                 280                 285

Ala Ala Tyr Tyr Pro Arg Asn Pro Val Glu Phe Asp Trp Phe Glu Asp
    290                 295                 300

Asp Arg Gln Val Phe Asn Pro Gly Gln Ile Asp Thr Gln Thr His Glu
305                 310                 315                 320

His Pro Asp Gly Phe Thr Thr Val Ser Thr Val Thr Ser Glu Ala Val
                325                 330                 335

Gly Gly Gln Val Pro Pro Arg Thr Phe Thr Cys Gln Met Thr Trp His
            340                 345                 350

Arg Asp Ser Val Thr Phe Ser Arg Arg Asn Ala Thr Gly Leu Ala Leu
        355                 360                 365

Val Leu Pro Arg Pro Thr Ile Thr Met Glu Phe Gly Val Arg His Val
    370                 375                 380

Val Cys Thr Ala Gly Cys Val Pro Glu Gly Val Thr Phe Ala Trp Phe
385                 390                 395                 400

Leu Gly Asp Asp Pro Ser Pro Ala Ala Lys Ser Ala Val Thr Ala Gln
                405                 410                 415

Glu Ser Cys Asp His Pro Gly Leu Ala Thr Val Arg Ser Thr Leu Pro
            420                 425                 430

Ile Ser Tyr Asp Tyr Ser Glu Tyr Ile Cys Arg Leu Thr Gly Tyr Pro
        435                 440                 445

Ala Gly Ile Pro Val Leu Glu His His Gly Ser His Gln Pro Pro Pro
    450                 455                 460

Arg Asp Pro Thr Glu Arg Gln Val Ile Glu Ala Ile Glu Trp Val Gly
465                 470                 475                 480

Ile Gly Ile Gly Val Leu Ala Ala Gly Val Leu Val Val Thr Ala Ile
                485                 490                 495
```

-continued

```
Val Tyr Val Val Arg Thr Ser Gln Ser Arg Gln Arg His Arg Arg
            500                 505                 510
```

<210> SEQ ID NO 31
<211> LENGTH: 1282
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

```
aagccaccca gcctatgcat ccgctcctca atcctctcct gttggcactg ggcctcatgg     60

cgcttttgtt gaccacggtc attgctctca cttgccttgg cggctttgcc tccccaggcc    120

ctgtgcctcc ctctacagcc ctcagggagc tcattgagga gctggtcaac atcacccaga    180

accagaaggc tccgctctgc aatggcagca tggtatggag catcaacctg acagctggca    240

tgtactgtgc agccctggaa tccctgatca acgtgtcagg ctgcagtgcc atcgagaaga    300

cccagaggat gctgagcgga ttctgcccgc acaaggtctc agctgggcag ttttccagct    360

tgcatgtccg agacaccaaa atcgaggtgg cccagtttgt aaaggacctg ctcttacatt    420

taaagaaact ttttcgcgag ggacagttca actgaaactt cgaaagcatc attatttgca    480

gagacaggac ctgactattg aagttgcaga ttcatttttc tttctgatgt caaaaatgtc    540

ttgggtaggc gggaaggagg gttagggagg ggtaaaattc cttagcttag acctcagcct    600

gtgctgcccg tcttcagcct agccgacctc agccttcccc ttgcccaggg ctcagcctgg    660

tgggcctcct ctgtccaggg ccctgagctc ggtggaccca gggatgacat gtccctacac    720

ccctcccctg ccctagagca cactgtagca ttacagtggg tgccccccct gccagacatg    780

tggtgggaca gggacccact tcacacacag gcaactgagg cagacagcag ctcaggcaca    840

cttcttcttg gtcttattta ttattgtgtg ttatttaaat gagtgtgttt gtcaccgttg    900

gggattgggg aagactgtgg ctgctagcac ttggagccaa gggttcagag actcagggcc    960

ccagcactaa agcagtggac accaggagtc cctggtaata agtactgtgt acagaattct   1020

gctacctcac tggggtcctg gggcctcgga gcctcatccg aggcagggtc aggagagggg   1080

cagaacagcc gctcctgtct gccagccagc agccagctct cagccaacga gtaatttatt   1140

gtttttcctt gtatttaaat attaaatatg ttagcaaaga gttaatatat agaagggtac   1200

cttgaacact gggggagggg acattgaaca agttgtttca ttgactatca aactgaagcc   1260

agaaataaag ttggtgacag at                                            1282
```

<210> SEQ ID NO 32
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

```
Met His Pro Leu Leu Asn Pro Leu Leu Leu Ala Leu Gly Leu Met Ala
1               5                   10                  15

Leu Leu Leu Thr Thr Val Ile Ala Leu Thr Cys Leu Gly Gly Phe Ala
            20                  25                  30

Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu
        35                  40                  45

Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
    50                  55                  60

Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
```

-continued

65 70 75 80

Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
85 90 95

Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
100 105 110

Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
115 120 125

Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Gln
130 135 140

Phe Asn
145

<210> SEQ ID NO 33
<211> LENGTH: 1376
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13R 2 polynucleotide

<400> SEQUENCE: 33

gtaagaacac tctcgtgagt ctaacggtct tccggatgaa ggctatttga agtcgccata 60

acctggtcag aagtgtgcct gtcggcgggg agagaggcaa tatcaaggtt ttaaatctcg 120

gagaaatggc tttcgtttgc ttggctatcg gatgcttata tacctttctg ataagcacaa 180

catttggctg tacttcatct tcagacaccg agataaaagt taaccctcct caggattttg 240

agatagtgga tcccggatac ttaggttatc tctatttgca atggcaaccc ccactgtctc 300

tggatcattt taaggaatgc acagtggaat atgaactaaa ataccgaaac attggtagtg 360

aaacatggaa gaccatcatt actaagaatc tacattacaa agatgggttt gatcttaaca 420

agggcattga agcgaagata cacacgcttt taccatggca atgcacaaat ggatcagaag 480

ttcaaagttc ctgggcagaa actacttatt ggatatcacc acaaggaatt ccagaaacta 540

aagttcagga tatggattgc gtatattaca attggcaata tttactctgt tcttggaaac 600

ctggcatagg tgtacttctt gataccaatt acaacttgtt ttactggtat gagggcttgg 660

atcatgcatt acagtgtgtt gattacatca aggctgatgg acaaaatata ggatgcagat 720

ttccctattt ggaggcatca gactataaag atttctatat ttgtgttaat ggatcatcag 780

agaacaagcc tatcagatcc agttatttca cttttcagct tcaaaatata gttaaacctt 840

tgccgccagt ctatcttact tttactcggg agagttcatg tgaaattaag ctgaaatgga 900

gcataccttt gggacctatt ccagcaaggt gttttgatta tgaaattgag atcagagaag 960

atgatactac cttggtgact gctacagttg aaaatgaaac atacaccttg aaaacaacaa 1020

atgaaacccg acaattatgc tttgtagtaa gaagcaaagt gaatatttat tgctcagatg 1080

acggaatttg gagtgagtgg agtgataaac aatgctggga aggtgaagac ctatcgaaga 1140

aaactttgct acgtttctgg ctaccatttg gtttcatctt aatattagtt atatttgtaa 1200

ccggtctgct tttgcgtaag ccaaacacct acccaaaaat gattccagaa tttttctgtg 1260

atacatgaag actttccata tcaagagaca tggtattgac tcaacagttt ccagtcatgg 1320

ccaaatgttc aatatgagtc tcaataaact gaatttttct tgcgaatgtt gaaaaa 1376

<210> SEQ ID NO 34
<211> LENGTH: 380
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13R 2 amino acid

<400> SEQUENCE: 34

```
Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
1               5                   10                  15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
            20                  25                  30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
        35                  40                  45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
    50                  55                  60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65                  70                  75                  80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
                85                  90                  95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
            100                 105                 110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
        115                 120                 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
    130                 135                 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145                 150                 155                 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
                165                 170                 175

Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
            180                 185                 190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
        195                 200                 205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
    210                 215                 220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225                 230                 235                 240

Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
                245                 250                 255

Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
            260                 265                 270

Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
        275                 280                 285

Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
    290                 295                 300

Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305                 310                 315                 320

Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
                325                 330                 335

Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
            340                 345                 350

Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr
        355                 360                 365

Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
    370                 375                 380
```

-continued

<210> SEQ ID NO 35
<211> LENGTH: 2685
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gB lacking polylysine tract, polynucleotide

<400> SEQUENCE: 35

atgcgccagg gcgcccccgc gcgggggcgc cggtggttcg tcgtatgggc gctcttgggg 60

ttgacgctgg gggtcctggt ggcgtcggcg gctccgagtt cccccggcac gcctggggtc 120

gcggccgcga cccaggcggc gaacgggggc cctgccactc cggcgccgcc cgcccctggc 180

gcccccccaa cgggggaccc gccaaagccg ccgcgccccg ccggcgacaa cgcgaccgtc 240

gccgcgggcc acgccaccct gcgcgagcac ctgcgggaca tcaaggcgga gaacaccgat 300

gcaaactttt acgtgtgccc acccccacg ggcgccacgg tggtgcagtt cgagcagccg 360

cgccgctgcc cgacccggcc cgagggtcag aactacacgg agggcatcgc ggtggtcttc 420

aaggagaaca tcgccccgta caagttcaag gccaccatgt actacaaaga cgtcaccgtt 480

tcgcaggtgt ggttcggcca ccgctactcc cagtttatgg ggatctttga ggaccgcgcc 540

cccgtcccct tcgaggaggt gatcgacaag atcaacgcca agggggtctg tcggtccacg 600

gccaagtacg tgcgcaacaa cctggagacc accgcgtttc accgggacga ccacgagacc 660

gacatggagc tgaaaccggc caacgccgcg acccgcacga gccggggctg gcacaccacc 720

gacctcaagt acaacccctc gcgggtggag gcgttccacc ggtacgggac gacggtaaac 780

tgcatcgtcg aggaggtgga cgcgcgctcg gtgtacccgt acgacgagtt tgtgttggcg 840

actggcgact ttgtgtacat gtccccgttt tacggctacc gggaggggtc gcacaccgaa 900

cacaccagct acgccgccga ccgcttcaag caggtcgacg gcttctacgc gcgcgacctc 960

accaccaagg cccgggccac ggcgccgacc acccggaacc tgctcacgac ccccaagttc 1020

accgtggcct gggactgggt gccaaagcgc ccgtcggtct gcaccatgac caagtggcag 1080

gaggtggacg agatgctgcg ctccgagtac ggcggctcct tccgattctc ttccgacgcc 1140

atatccacca ccttcaccac caacctgacc gagtacccgc tctcgcgcgt ggacctgggg 1200

gactgcatcg gcaaggacgc ccgcgacgcc atggaccgca tcttcgcccg caggtacaac 1260

gcgacgcaca tcaaggtggg ccagccgcag tactacctgg ccaatggggg ctttctgatc 1320

gcgtaccagc cccttctcag caacacgctc gcggagctgt acgtgcggga acacctccgc 1380

gagcagagcc gcaagccccc aaaccccacg cccccgccgc ccggggccag cgccaacgcg 1440

tccgtggagc gcatcaagac cacctcctcc atcgagttcg ccaggctgca gtttacgtac 1500

aaccacatac agcgccatgt caacgatatg ttgggccgcg ttgccatcgc gtggtgcgag 1560

ctgcagaatc acgagctgac cctgtggaac gaggcccgca agctgaaccc caacgccatc 1620

gcctcggcca ccgtgggccg gcgggtgagc gcgcggatgc tcggcgacgt gatggccgtc 1680

tccacgtgcg tgccggtcgc cgcggacaac gtgatcgtcc aaaactcgat gcgcatcagc 1740

tcgcggcccg gggcctgcta cagccgcccc ctggtcagct ttcggtacga agaccagggc 1800

ccgttggtcg aggggcagct gggggagaac aacgagctgc ggctgacgcg cgatgcgatc 1860

gagccgtgca ccgtgggaca ccggcgctac ttcaccttcg gtgggggcta cgtgtacttc 1920

gaggagtacg cgtactccca ccagctgagc cgcgccgaca tcaccaccgt cagcaccttc 1980

-continued

atcgacctca acatcaccat gctggaggat cacgagtttg tccccctgga ggtgtacacc 2040

cgccacgaga tcaaggacag cggcctgctg gactacacgg aggtccagcg ccgcaaccag 2100

ctgcacgacc tgcgcttcgc cgacatcgac acggtcatcc acgccgacgc caacgccgcc 2160

atgtttgcgg gcctgggcgc gttcttcgag gggatgggcg acctggggcg cgcggtcggc 2220

aaggtggtga tgggcatcgt gggcggcgtg gtatcggccg tgtcgggcgt gtcctccttc 2280

atgtccaacc cctttggggc gctggccgtg ggtctgttgg tcctggccgg cctggcggcg 2340

gccttcttcg cctttcgcta cgtcatgcgg ctgcagagca accccatgaa ggccctgtac 2400

ccgctaacca ccaaggagct caagaacccc accaacccgg acgcgtccgg ggagggcgag 2460

gagggcggcg actttgacga ggccaagcta gccgaggccc gggagatgat acggtacatg 2520

gccctggtgt ctgccatgga gcgcacggaa cacaaggcca agaagaaggg cacgagcgcg 2580

ctgctcagcg ccaaggtcac cgacatggtc atgcgcaagc gccgcaacac caactacacc 2640

caagttccca acaaagacgg tgacgccgac gaggacgacc tgtga 2685

<210> SEQ ID NO 36
<211> LENGTH: 894
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gB lacking polylysine tract, amino acid

<400> SEQUENCE: 36

Met Arg Gln Gly Ala Pro Ala Arg Gly Arg Arg Trp Phe Val Val Trp
1 5 10 15

Ala Leu Leu Gly Leu Thr Leu Gly Val Leu Val Ala Ser Ala Ala Pro
20 25 30

Ser Ser Pro Gly Thr Pro Gly Val Ala Ala Ala Thr Gln Ala Ala Asn
35 40 45

Gly Gly Pro Ala Thr Pro Ala Pro Pro Ala Pro Gly Ala Pro Pro Thr
50 55 60

Gly Asp Pro Pro Lys Pro Pro Arg Pro Ala Gly Asp Asn Ala Thr Val
65 70 75 80

Ala Ala Gly His Ala Thr Leu Arg Glu His Leu Arg Asp Ile Lys Ala
85 90 95

Glu Asn Thr Asp Ala Asn Phe Tyr Val Cys Pro Pro Pro Thr Gly Ala
100 105 110

Thr Val Val Gln Phe Glu Gln Pro Arg Arg Cys Pro Thr Arg Pro Glu
115 120 125

Gly Gln Asn Tyr Thr Glu Gly Ile Ala Val Val Phe Lys Glu Asn Ile
130 135 140

Ala Pro Tyr Lys Phe Lys Ala Thr Met Tyr Tyr Lys Asp Val Thr Val
145 150 155 160

Ser Gln Val Trp Phe Gly His Arg Tyr Ser Gln Phe Met Gly Ile Phe
165 170 175

Glu Asp Arg Ala Pro Val Pro Phe Glu Glu Val Ile Asp Lys Ile Asn
180 185 190

Ala Lys Gly Val Cys Arg Ser Thr Ala Lys Tyr Val Arg Asn Asn Leu
195 200 205

Glu Thr Thr Ala Phe His Arg Asp Asp His Glu Thr Asp Met Glu Leu
210 215 220

Lys Pro Ala Asn Ala Ala Thr Arg Thr Ser Arg Gly Trp His Thr Thr

-continued

```
225                 230                 235                 240

Asp Leu Lys Tyr Asn Pro Ser Arg Val Glu Ala Phe His Arg Tyr Gly
                245                 250                 255

Thr Thr Val Asn Cys Ile Val Glu Glu Val Asp Ala Arg Ser Val Tyr
            260                 265                 270

Pro Tyr Asp Glu Phe Val Leu Ala Thr Gly Asp Phe Val Tyr Met Ser
        275                 280                 285

Pro Phe Tyr Gly Tyr Arg Glu Gly Ser His Thr Glu His Thr Ser Tyr
    290                 295                 300

Ala Ala Asp Arg Phe Lys Gln Val Asp Gly Phe Tyr Ala Arg Asp Leu
305                 310                 315                 320

Thr Thr Lys Ala Arg Ala Thr Ala Pro Thr Thr Arg Asn Leu Leu Thr
                325                 330                 335

Thr Pro Lys Phe Thr Val Ala Trp Asp Trp Val Pro Lys Arg Pro Ser
            340                 345                 350

Val Cys Thr Met Thr Lys Trp Gln Glu Val Asp Glu Met Leu Arg Ser
        355                 360                 365

Glu Tyr Gly Gly Ser Phe Arg Phe Ser Ser Asp Ala Ile Ser Thr Thr
    370                 375                 380

Phe Thr Thr Asn Leu Thr Glu Tyr Pro Leu Ser Arg Val Asp Leu Gly
385                 390                 395                 400

Asp Cys Ile Gly Lys Asp Ala Arg Asp Ala Met Asp Arg Ile Phe Ala
                405                 410                 415

Arg Arg Tyr Asn Ala Thr His Ile Lys Val Gly Gln Pro Gln Tyr Tyr
            420                 425                 430

Leu Ala Asn Gly Gly Phe Leu Ile Ala Tyr Gln Pro Leu Leu Ser Asn
        435                 440                 445

Thr Leu Ala Glu Leu Tyr Val Arg Glu His Leu Arg Glu Gln Ser Arg
    450                 455                 460

Lys Pro Pro Asn Pro Thr Pro Pro Pro Pro Gly Ala Ser Ala Asn Ala
465                 470                 475                 480

Ser Val Glu Arg Ile Lys Thr Thr Ser Ser Ile Glu Phe Ala Arg Leu
                485                 490                 495

Gln Phe Thr Tyr Asn His Ile Gln Arg His Val Asn Asp Met Leu Gly
            500                 505                 510

Arg Val Ala Ile Ala Trp Cys Glu Leu Gln Asn His Glu Leu Thr Leu
        515                 520                 525

Trp Asn Glu Ala Arg Lys Leu Asn Pro Asn Ala Ile Ala Ser Ala Thr
    530                 535                 540

Val Gly Arg Arg Val Ser Ala Arg Met Leu Gly Asp Val Met Ala Val
545                 550                 555                 560

Ser Thr Cys Val Pro Val Ala Ala Asp Asn Val Ile Val Gln Asn Ser
                565                 570                 575

Met Arg Ile Ser Ser Arg Pro Gly Ala Cys Tyr Ser Arg Pro Leu Val
            580                 585                 590

Ser Phe Arg Tyr Glu Asp Gln Gly Pro Leu Val Glu Gly Gln Leu Gly
        595                 600                 605

Glu Asn Asn Glu Leu Arg Leu Thr Arg Asp Ala Ile Glu Pro Cys Thr
    610                 615                 620

Val Gly His Arg Arg Tyr Phe Thr Phe Gly Gly Gly Tyr Val Tyr Phe
625                 630                 635                 640
```

-continued

```
Glu Glu Tyr Ala Tyr Ser His Gln Leu Ser Arg Ala Asp Ile Thr Thr
                645                 650                 655

Val Ser Thr Phe Ile Asp Leu Asn Ile Thr Met Leu Glu Asp His Glu
            660                 665                 670

Phe Val Pro Leu Glu Val Tyr Thr Arg His Glu Ile Lys Asp Ser Gly
        675                 680                 685

Leu Leu Asp Tyr Thr Glu Val Gln Arg Arg Asn Gln Leu His Asp Leu
    690                 695                 700

Arg Phe Ala Asp Ile Asp Thr Val Ile His Ala Asp Ala Asn Ala Ala
705                 710                 715                 720

Met Phe Ala Gly Leu Gly Ala Phe Phe Glu Gly Met Gly Asp Leu Gly
                725                 730                 735

Arg Ala Val Gly Lys Val Val Met Gly Ile Val Gly Gly Val Val Ser
            740                 745                 750

Ala Val Ser Gly Val Ser Ser Phe Met Ser Asn Pro Phe Gly Ala Leu
        755                 760                 765

Ala Val Gly Leu Leu Val Leu Ala Gly Leu Ala Ala Ala Phe Phe Ala
    770                 775                 780

Phe Arg Tyr Val Met Arg Leu Gln Ser Asn Pro Met Lys Ala Leu Tyr
785                 790                 795                 800

Pro Leu Thr Thr Lys Glu Leu Lys Asn Pro Thr Asn Pro Asp Ala Ser
                805                 810                 815

Gly Glu Gly Glu Glu Gly Gly Asp Phe Asp Glu Ala Lys Leu Ala Glu
            820                 825                 830

Ala Arg Glu Met Ile Arg Tyr Met Ala Leu Val Ser Ala Met Glu Arg
        835                 840                 845

Thr Glu His Lys Ala Lys Lys Lys Gly Thr Ser Ala Leu Leu Ser Ala
    850                 855                 860

Lys Val Thr Asp Met Val Met Arg Lys Arg Arg Asn Thr Asn Tyr Thr
865                 870                 875                 880

Gln Val Pro Asn Lys Asp Gly Asp Ala Asp Glu Asp Asp Leu
                    885                 890
```

<210> SEQ ID NO 37
<211> LENGTH: 1626
<212> TYPE: DNA
<213> ORGANISM: Herpes Simplex Virus-1

<400> SEQUENCE: 37

```
atggcgcttt tgttgaccac ggtcattgct ctcacttgcc ttggcggctt tgcctcccca     60

ggccctgtgc ctccctctac agccctcagg gagctcattg aggagctggt caacatcacc    120

cagaaccaga aggctccgct ctgcaatggc agcatggtat ggagcatcaa cctgacagct    180

ggcatgtact gtgcagccct ggaatccctg atcaacgtgt caggctgcag tgccatcgag    240

aagacccaga ggatgctgag cggattctgc ccgcacaagg tctcagctgg gcagttttcc    300

agcttgcatg tccgagacac caaaatcgag gtggcccagt ttgtaaagga cctgctctta    360

catttaaaga aacttttcg cgagggacag ttcaacgaat tccacccgca tggagttccg    420

cctccagata tggcgttact ccatgggtcc gtccccccca atcgctccgg ctcccgacct    480

agaggaggtc ctgacgaaca tcaccgcccc acccgggggа ctcctggtgt acgacagcgc    540

ccccaacctg acggaccccc acgtgctctg ggcggagggg gccggcccgg gcgccgaccc    600

tccgttgtat tctgtcaccg ggccgctgcc gacccagcgg ctgattatcg gcgaggtgac    660
```

-continued

gcccgcgacc cagggaatgt attacttggc ctggggccgg atggacagcc cgcacgagta 720

cgggacgtgg gtgcgcgtcc gcatgttccg ccccccgtct ctgaccctcc agccccacgc 780

ggtgatggag ggtcagccgt tcaaggcgac gtgcacggcc gccgcctact acccgcgtaa 840

ccccgtggag tttgtctggt tcgaggacga ccgccaggtg tttaacccgg gccagatcga 900

cacgcagacg cacgagcacc ccgacgggtt caccacagtc tctaccgtga cctccgaggc 960

tgtcggcggc caggtccccc cgcggacctt cacctgccag atgacgtggc accgcgactc 1020

cgtgacgttc tcgcgacgca atgccaccgg gctggccctg gtgctgccgc ggccaaccat 1080

caccatggaa tttggggtcc ggcatgtggt ctgcacggcc ggctgcgtcc ccgagggcgt 1140

gacgtttgcc tggttcctgg gggacgaccc ctcaccggcg gctaagtcgg ccgttacggc 1200

ccaggagtcg tgcgaccacc ccgggctggc tacggtccgg tccaccctgc ccatttcgta 1260

cgactacagc gagtacatct gtcggttgac cggatatccg gccgggattc ccgttctaga 1320

gcaccacggc agtcaccagc ccccacccag ggaccccacc gagcggcagg tgatcgaggc 1380

gatcgagtgg gtggggattg gaatcggggt tctcgcggcg ggggtcctgg tcgtaacggc 1440

aatcgtgtac gtcgtccgca catcacagtc gcggcagcgt catcggcggt aacgcgagac 1500

ccccccgtta ccttttaat atctatatag tttggtcccc cctctatccc gcccaccgct 1560

gggcgctata aagccgccac cctctcttcc ctcaggtcat ccttggtcga tcccgaacga 1620

cacacg 1626

<210> SEQ ID NO 38
<211> LENGTH: 497
<212> TYPE: PRT
<213> ORGANISM: Herpes Simplex Virus-1

<400> SEQUENCE: 38

Met Ala Leu Leu Leu Thr Thr Val Ile Ala Leu Thr Cys Leu Gly Gly
1 5 10 15

Phe Ala Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu
20 25 30

Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys
35 40 45

Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys
50 55 60

Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu
65 70 75 80

Lys Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala
85 90 95

Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala
100 105 110

Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu
115 120 125

Gly Gln Phe Asn Glu Phe Ser Thr Arg Met Glu Phe Arg Leu Gln Ile
130 135 140

Trp Arg Tyr Ser Met Gly Pro Ser Pro Pro Ile Ala Pro Ala Pro Asp
145 150 155 160

Leu Glu Glu Val Leu Thr Asn Ile Thr Ala Pro Pro Gly Gly Leu Leu
165 170 175

Val Tyr Asp Ser Ala Pro Asn Leu Thr Asp Pro His Val Leu Trp Ala

-continued

```
            180                 185                 190

Glu Gly Ala Gly Pro Gly Ala Asp Pro Pro Leu Tyr Ser Val Thr Gly
        195                 200                 205

Pro Leu Pro Thr Gln Arg Leu Ile Ile Gly Glu Val Thr Pro Ala Thr
    210                 215                 220

Gln Gly Met Tyr Tyr Leu Ala Trp Gly Arg Met Asp Ser Pro His Glu
225                 230                 235                 240

Tyr Gly Thr Trp Val Arg Val Arg Met Phe Arg Pro Pro Ser Leu Thr
                245                 250                 255

Leu Gln Pro His Ala Val Met Glu Gly Gln Pro Phe Lys Ala Thr Cys
            260                 265                 270

Thr Ala Ala Ala Tyr Tyr Pro Arg Asn Pro Val Glu Phe Asp Trp Phe
        275                 280                 285

Glu Asp Asp Arg Gln Val Phe Asn Pro Gly Gln Ile Asp Thr Gln Thr
    290                 295                 300

His Glu His Pro Asp Gly Phe Thr Thr Val Ser Thr Val Thr Ser Glu
305                 310                 315                 320

Ala Val Gly Gly Gln Val Pro Pro Arg Thr Phe Thr Cys Gln Met Thr
                325                 330                 335

Trp His Arg Asp Ser Val Thr Phe Ser Arg Arg Asn Ala Thr Gly Leu
            340                 345                 350

Ala Leu Val Leu Pro Arg Pro Thr Ile Thr Met Glu Phe Gly Val Arg
        355                 360                 365

His Val Val Cys Thr Ala Gly Cys Val Pro Glu Gly Val Thr Phe Ala
    370                 375                 380

Trp Phe Leu Gly Asp Asp Pro Ser Pro Ala Ala Lys Ser Ala Val Thr
385                 390                 395                 400

Ala Gln Glu Ser Cys Asp His Pro Gly Leu Ala Thr Val Arg Ser Thr
                405                 410                 415

Leu Pro Ile Ser Tyr Asp Tyr Ser Glu Tyr Ile Cys Arg Leu Thr Gly
            420                 425                 430

Tyr Pro Ala Gly Ile Pro Val Leu Glu His His Gly Ser His Gln Pro
        435                 440                 445

Pro Pro Arg Asp Pro Thr Glu Arg Gln Val Ile Glu Ala Ile Glu Trp
    450                 455                 460

Val Gly Ile Gly Ile Gly Val Leu Ala Ala Gly Val Leu Val Val Thr
465                 470                 475                 480

Ala Ile Val Tyr Val Val Arg Thr Ser Gln Ser Arg Gln Arg His Arg
                485                 490                 495

Arg
```

<210> SEQ ID NO 39
<211> LENGTH: 1593
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gD-IL13 integration polynucleotide

<400> SEQUENCE: 39

```
atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc     60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagatggc cgaccccaat    120

cgctttcgcg gcaaagacct tccggtcctc gagatggcgc ttttgttgac cacggtcatt    180
```

-continued

```
gctctcactt gccttggcgg ctttgcctcc ccaggccctg tgcctccctc tacagccctc    240

agggagctca ttgaggagct ggtcaacatc acccagaacc agaaggctcc gctctgcaat    300

ggcagcatgg tatggagcat caacctgaca gctggcatgt actgtgcagc cctggaatcc    360

ctgatcaacg tgtcaggctg cagtgccatc gagaagaccc agaggatgct gagcggattc    420

tgcccgcaca aggtctcagc tgggcagttt tccagcttgc atgtccgaga caccaaaatc    480

gaggtggccc agtttgtaaa ggacctgctc ttacatttaa agaaactttt tcgcgaggga    540

cagttcaacg gtaccctgga ccggctgacc gaccctccgg gggtccggcg cgtgtaccac    600

atccaggcgg gcctaccgga cccgttccag ccccccagcc tcccgatcac ggtttactac    660

gccgtgttgg agcgcgcctg ccgcagcgtg ctcctaaacg caccgtcgga ggccccccag    720

attgtccgcg gggcctccga agacgtccgg aaacaaccct acaacctgac catcgcttgg    780

tttcggatgg gaggcaactg tgctatcccc atcacggtca tggagtacac cgaatgctcc    840

tacaacaagt ctctgggggc ctgtcccatc cgaacgcagc cccgctggaa ctactatgac    900

agcttcagcg ccgtcagcga ggataacctg gggttcctga tgcacgcccc cgcgtttgag    960

accgccggca cgtacctgcg gctcgtgaag ataaacgact ggacggagat tacacagttt   1020

atcctggagc accgagccaa gggctcctgt aagtacgccc ttccgctgcg catccccccg   1080

tcagcctgcc tctcccccca ggcctaccag caggggggtga cggtggacag catcgggatg   1140

ctgccccgct tcatccccga gaaccagcgc accgtcgccg tatacagctt gaagatcgcc   1200

gggtggcacg ggcccaaggc cccatacacg agcaccctgc tgcccccgga gctgtccgag   1260

acccccaacg ccacgcagcc agaactcgcc ccggaagacc ccgaggattc ggccctcttg   1320

gaggaccccg tggggacggt ggtgccgcaa atcccaccaa actggcacat accgtcgatc   1380

caggacgccg cgacgcctta ccatcccccg gccaccccga acaacatggg cctgatcgcc   1440

ggcgcggtgg gcggcagtct cctggtagcc ctggtcattt gcggaattgt gtactggatg   1500

cgccgccgca ctcaaaaagc cccaaagcgc atacgcctcc cccacatccg ggaagacgac   1560

cagccgtcct cgcaccagcc cttgttttac tag                                1593
```

<210> SEQ ID NO 40
<211> LENGTH: 530
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gD-IL13 integration amino acid

<400> SEQUENCE: 40

```
Met Gly Gly Ala Ala Ala Arg Leu Gly Ala Val Ile Leu Phe Val Val
1               5                   10                  15

Ile Val Gly Leu His Gly Val Arg Gly Lys Tyr Ala Leu Ala Asp Ala
            20                  25                  30

Ser Leu Lys Met Ala Asp Pro Asn Arg Phe Arg Gly Lys Asp Leu Pro
        35                  40                  45

Val Leu Glu Met Ala Leu Leu Leu Thr Thr Val Ile Ala Leu Thr Cys
    50                  55                  60

Leu Gly Gly Phe Ala Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu
65                  70                  75                  80

Arg Glu Leu Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala
                85                  90                  95

Pro Leu Cys Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly
```

-continued

```
           100                 105                 110

Met Tyr Cys Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser
        115                 120                 125

Ala Ile Glu Lys Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys
    130                 135                 140

Val Ser Ala Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile
145                 150                 155                 160

Glu Val Ala Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu
                165                 170                 175

Phe Arg Glu Gly Gln Phe Asn Gly Thr Pro Asp Arg Leu Thr Asp Pro
            180                 185                 190

Pro Gly Val Arg Arg Val Tyr His Ile Gln Ala Gly Leu Pro Asp Pro
        195                 200                 205

Phe Gln Pro Pro Ser Leu Pro Ile Thr Val Tyr Tyr Ala Val Leu Glu
    210                 215                 220

Arg Ala Cys Arg Ser Val Leu Leu Asn Ala Pro Ser Glu Ala Pro Gln
225                 230                 235                 240

Ile Val Arg Gly Ala Ser Glu Asp Val Arg Lys Gln Pro Tyr Asn Leu
                245                 250                 255

Thr Ile Ala Trp Phe Arg Met Gly Gly Asn Cys Ala Ile Pro Ile Thr
            260                 265                 270

Val Met Glu Tyr Thr Glu Cys Ser Tyr Asn Lys Ser Leu Gly Ala Cys
        275                 280                 285

Pro Ile Arg Thr Gln Pro Arg Trp Asn Tyr Tyr Asp Ser Phe Ser Ala
    290                 295                 300

Val Ser Glu Asp Asn Leu Gly Phe Leu Met His Ala Pro Ala Phe Glu
305                 310                 315                 320

Thr Ala Gly Thr Tyr Leu Arg Leu Val Lys Ile Asn Asp Trp Thr Glu
                325                 330                 335

Ile Thr Gln Phe Ile Leu Glu His Arg Ala Lys Gly Ser Cys Lys Tyr
            340                 345                 350

Ala Leu Pro Leu Arg Ile Pro Pro Ser Ala Cys Leu Ser Pro Gln Ala
        355                 360                 365

Tyr Gln Gln Gly Val Thr Val Asp Ser Ile Gly Met Leu Pro Arg Phe
    370                 375                 380

Ile Pro Glu Asn Gln Arg Thr Val Ala Val Tyr Ser Leu Lys Ile Ala
385                 390                 395                 400

Gly Trp His Gly Pro Lys Ala Pro Tyr Thr Ser Thr Leu Leu Pro Pro
                405                 410                 415

Glu Leu Ser Glu Thr Pro Asn Ala Thr Gln Pro Glu Leu Ala Pro Glu
            420                 425                 430

Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro Val Gly Thr Val Val
        435                 440                 445

Pro Gln Ile Pro Pro Asn Trp His Ile Pro Ser Ile Gln Asp Ala Ala
    450                 455                 460

Thr Pro Tyr His Pro Pro Ala Thr Pro Asn Asn Met Gly Leu Ile Ala
465                 470                 475                 480

Gly Ala Val Gly Gly Ser Leu Leu Val Ala Leu Val Ile Cys Gly Ile
                485                 490                 495

Val Tyr Trp Met Arg Arg Arg Thr Gln Lys Ala Pro Lys Arg Ile Arg
            500                 505                 510
```

-continued

Leu Pro His Ile Arg Glu Asp Asp Gln Pro Ser Ser His Gln Pro Leu
515 520 525

Phe Tyr
530

<210> SEQ ID NO 41
<211> LENGTH: 1416
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gD V34S polynucleotide

<400> SEQUENCE: 41

atggcgcttt tgttgaccac ggtcattgct ctcacttgcc ttggcggctt tgcctcccca 60

ggccctgtgc ctccctctac agccctcagg gagctcattg aggagctggt caacatcacc 120

cagaaccaga aggctccgct ctgcaatggc agcatggtat ggagcatcaa cctgacagct 180

ggcatgtact gtgcagccct ggaatccctg atcaacgtgt caggctgcag tgccatcgag 240

aagacccaga ggatgctgag cggattctgc ccgcacaagg tctcagctgg gcagttttcc 300

agcttgcatg tccgagacac caaaatcgag gtggcccagt ttgtaaagga cctgctctta 360

catttaaaga aacttttttcg cgagggacag ttcaacggta ccgggtcccg gcgcgtgtac 420

cacatccagg cgggcctacc ggacccgttc cagcccccca gcctcccgat cacggtttac 480

tacgccgtgt tggagcgcgc ctgccgcagc gtgctcctaa acgcaccgtc ggaggccccc 540

cagattgtcc gcggggcctc cgaagacgtc cggaaacaac cctacaacct gaccatcgct 600

tggtttcgga tgggaggcaa ctgtgctatc cccatcacgg tcatggagta caccgaatgc 660

tcctacaaca agtctctggg ggcctgtccc atccgaacgc agccccgctg gaactactat 720

gacagcttca gcgccgtcag cgaggataac ctggggttcc tgatgcacgc ccccgcgttt 780

gagaccgccg gcacgtacct gcggctcgtg aagataaacg actggacgga gattacacag 840

tttatcctgg agcaccgagc caagggctcc tgtaagtacg cccttccgct gcgcatcccc 900

ccgtcagcct gcctctcccc ccaggcctac cagcaggggg tgacggtgga cagcatcggg 960

atgctgcccc gcttcatccc cgagaaccag cgcaccgtcg ccgtatacag cttgaagatc 1020

gccgggtggc acgggcccaa ggccccatac acgagcaccc tgctgccccc ggagctgtcc 1080

gagaccccca acgccacgca gccagaactc gccccggaag accccgagga ttcggccctc 1140

ttggaggacc ccgtggggac ggtggtgccg caaatcccac caaactggca cataccgtcg 1200

atccaggacg ccgcgacgcc ttaccatccc ccggccaccc cgaacaacat gggcctgatc 1260

gccggcgcgg tgggcggcag tctcctggta gccctggtca tttgcggaat tgtgtactgg 1320

atgcgccgcc gcactcaaaa agccccaaag cgcatacgcc tcccccacat ccgggaagac 1380

gaccagccgt cctcgcacca gcccttgttt tactag 1416

<210> SEQ ID NO 42
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gD V34S amino acid

<400> SEQUENCE: 42

Met Ala Leu Leu Leu Thr Thr Val Ile Ala Leu Thr Cys Leu Gly Gly
1 5 10 15

-continued

```
Phe Ala Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu
            20                  25                  30

Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys
        35                  40                  45

Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys
    50                  55                  60

Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu
65                  70                  75                  80

Lys Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala
                85                  90                  95

Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala
            100                 105                 110

Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu
        115                 120                 125

Gly Gln Phe Asn Gly Thr Gly Ser Arg Arg Val Tyr His Ile Gln Ala
    130                 135                 140

Gly Leu Pro Asp Pro Phe Gln Pro Pro Ser Leu Pro Ile Thr Val Tyr
145                 150                 155                 160

Tyr Ala Val Leu Glu Arg Ala Cys Arg Ser Val Leu Leu Asn Ala Pro
                165                 170                 175

Ser Glu Ala Pro Gln Ile Val Arg Gly Ala Ser Glu Asp Val Arg Lys
            180                 185                 190

Gln Pro Tyr Asn Leu Thr Ile Ala Trp Phe Arg Met Gly Gly Asn Cys
        195                 200                 205

Ala Ile Pro Ile Thr Val Met Glu Tyr Thr Glu Cys Ser Tyr Asn Lys
    210                 215                 220

Ser Leu Gly Ala Cys Pro Ile Arg Thr Gln Pro Arg Trp Asn Tyr Tyr
225                 230                 235                 240

Asp Ser Phe Ser Ala Val Ser Glu Asp Asn Leu Gly Phe Leu Met His
                245                 250                 255

Ala Pro Ala Phe Glu Thr Ala Gly Thr Tyr Leu Arg Leu Val Lys Ile
            260                 265                 270

Asn Asp Trp Thr Glu Ile Thr Gln Phe Ile Leu Glu His Arg Ala Lys
        275                 280                 285

Gly Ser Cys Lys Tyr Ala Leu Pro Leu Arg Ile Pro Pro Ser Ala Cys
    290                 295                 300

Leu Ser Pro Gln Ala Tyr Gln Gln Gly Val Thr Val Asp Ser Ile Gly
305                 310                 315                 320

Met Leu Pro Arg Phe Ile Pro Glu Asn Gln Arg Thr Val Ala Val Tyr
                325                 330                 335

Ser Leu Lys Ile Ala Gly Trp His Gly Pro Lys Ala Pro Tyr Thr Ser
            340                 345                 350

Thr Leu Leu Pro Pro Glu Leu Ser Glu Thr Pro Asn Ala Thr Gln Pro
        355                 360                 365

Glu Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro
    370                 375                 380

Val Gly Thr Val Val Pro Gln Ile Pro Pro Asn Trp His Ile Pro Ser
385                 390                 395                 400

Ile Gln Asp Ala Ala Thr Pro Tyr His Pro Pro Ala Thr Pro Asn Asn
                405                 410                 415

Met Gly Leu Ile Ala Gly Ala Val Gly Gly Ser Leu Leu Val Ala Leu
            420                 425                 430
```

-continued

```
Val Ile Cys Gly Ile Val Tyr Trp Met Arg Arg Arg Thr Gln Lys Ala
        435                 440                 445

Pro Lys Arg Ile Arg Leu Pro His Ile Arg Glu Asp Asp Gln Pro Ser
    450                 455                 460

Ser His Gln Pro Leu Phe Tyr
465                 470
```

<210> SEQ ID NO 43
<211> LENGTH: 1416
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gD V37S polynucleotide

<400> SEQUENCE: 43

```
atggcgcttt tgttgaccac ggtcattgct ctcacttgcc ttggcggctt tgcctcccca     60

ggccctgtgc ctccctctac agccctcagg gagctcattg aggagctggt caacatcacc    120

cagaaccaga aggctccgct ctgcaatggc agcatggtat ggagcatcaa cctgacagct    180

ggcatgtact gtgcagccct ggaatccctg atcaacgtgt caggctgcag tgccatcgag    240

aagacccaga ggatgctgag cggattctgc ccgcacaagg tctcagctgg gcagttttcc    300

agcttgcatg tccgagacac caaaatcgag gtggcccagt ttgtaaagga cctgctctta    360

catttaaaga aacttttcg cgagggacag ttcaacggta ccggggtccg gcgctcgtac    420

cacatccagg cgggcctacc ggacccgttc cagcccccca gcctcccgat cacggtttac    480

tacgccgtgt tggagcgcgc ctgccgcagc gtgctcctaa acgcaccgtc ggaggccccc    540

cagattgtcc gcggggcctc cgaagacgtc cggaaacaac cctacaacct gaccatcgct    600

tggtttcgga tgggaggcaa ctgtgctatc cccatcacgg tcatggagta caccgaatgc    660

tcctacaaca agtctctggg ggcctgtccc atccgaacgc agccccgctg gaactactat    720

gacagcttca gcgccgtcag cgaggataac ctggggttcc tgatgcacgc ccccgcgttt    780

gagaccgccg gcacgtacct gcggctcgtg aagataaacg actggacgga gattacacag    840

tttatcctgg agcaccgagc caagggctcc tgtaagtacg cccttccgct gcgcatcccc    900

ccgtcagcct gcctctcccc ccaggcctac cagcaggggg tgacggtgga cagcatcggg    960

atgctgcccc gcttcatccc cgagaaccag cgcaccgtcg ccgtatacag cttgaagatc   1020

gccgggtggc acgggcccaa ggccccatac acgagcaccc tgctgccccc ggagctgtcc   1080

gagacccccа acgccacgca gccagaactc gccccggaag accccgagga ttcggccctc   1140

ttggaggacc ccgtggggac ggtggtgccg caaatcccac caaactggca cataccgtcg   1200

atccaggacg ccgcgacgcc ttaccatccc ccggccaccc cgaacaacat gggcctgatc   1260

gccggcgcgg tgggcggcag tctcctggta gccctggtca tttgcggaat tgtgtactgg   1320

atgcgccgcc gcactcaaaa agccccaaag cgcatacgcc tcccccacat ccgggaagac   1380

gaccagccgt cctcgcacca gcccttgttt tactag                              1416
```

<210> SEQ ID NO 44
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IL13-gD V37S amino acid

<400> SEQUENCE: 44

-continued

```
Met Ala Leu Leu Leu Thr Thr Val Ile Ala Leu Thr Cys Leu Gly Gly
1               5                   10                  15

Phe Ala Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu
            20                  25                  30

Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys
        35                  40                  45

Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys
    50                  55                  60

Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu
65                  70                  75                  80

Lys Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala
                85                  90                  95

Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala
            100                 105                 110

Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu
        115                 120                 125

Gly Gln Phe Asn Gly Thr Gly Val Arg Arg Ser Tyr His Ile Gln Ala
    130                 135                 140

Gly Leu Pro Asp Pro Phe Gln Pro Pro Ser Leu Pro Ile Thr Val Tyr
145                 150                 155                 160

Tyr Ala Val Leu Glu Arg Ala Cys Arg Ser Val Leu Leu Asn Ala Pro
                165                 170                 175

Ser Glu Ala Pro Gln Ile Val Arg Gly Ala Ser Glu Asp Val Arg Lys
            180                 185                 190

Gln Pro Tyr Asn Leu Thr Ile Ala Trp Phe Arg Met Gly Gly Asn Cys
        195                 200                 205

Ala Ile Pro Ile Thr Val Met Glu Tyr Thr Glu Cys Ser Tyr Asn Lys
    210                 215                 220

Ser Leu Gly Ala Cys Pro Ile Arg Thr Gln Pro Arg Trp Asn Tyr Tyr
225                 230                 235                 240

Asp Ser Phe Ser Ala Val Ser Glu Asp Asn Leu Gly Phe Leu Met His
                245                 250                 255

Ala Pro Ala Phe Glu Thr Ala Gly Thr Tyr Leu Arg Leu Val Lys Ile
            260                 265                 270

Asn Asp Trp Thr Glu Ile Thr Gln Phe Ile Leu Glu His Arg Ala Lys
        275                 280                 285

Gly Ser Cys Lys Tyr Ala Leu Pro Leu Arg Ile Pro Pro Ser Ala Cys
    290                 295                 300

Leu Ser Pro Gln Ala Tyr Gln Gln Gly Val Thr Val Asp Ser Ile Gly
305                 310                 315                 320

Met Leu Pro Arg Phe Ile Pro Glu Asn Gln Arg Thr Val Ala Val Tyr
                325                 330                 335

Ser Leu Lys Ile Ala Gly Trp His Gly Pro Lys Ala Pro Tyr Thr Ser
            340                 345                 350

Thr Leu Leu Pro Pro Glu Leu Ser Glu Thr Pro Asn Ala Thr Gln Pro
        355                 360                 365

Glu Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro
    370                 375                 380

Val Gly Thr Val Val Pro Gln Ile Pro Pro Asn Trp His Ile Pro Ser
385                 390                 395                 400

Ile Gln Asp Ala Ala Thr Pro Tyr His Pro Pro Ala Thr Pro Asn Asn
```

-continued

```
               405                 410                 415

Met Gly Leu Ile Ala Gly Ala Val Gly Gly Ser Leu Leu Val Ala Leu
            420                 425                 430

Val Ile Cys Gly Ile Val Tyr Trp Met Arg Arg Arg Thr Gln Lys Ala
        435                 440                 445

Pro Lys Arg Ile Arg Leu Pro His Ile Arg Glu Asp Asp Gln Pro Ser
    450                 455                 460

Ser His Gln Pro Leu Phe Tyr
465                 470
```

<210> SEQ ID NO 45
<211> LENGTH: 773
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: ATF-uPA-gD

<400> SEQUENCE: 45

```
atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc     60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagatggc cgaccccaat    120

cgctttcgcg gcaaagacct tccggtcctc gagagcaatg aacttcatca agttccatcc    180

aactgtgact gtctaaatgg aggaacatgt gtgtccaaca agtacttctc caacattcac    240

tggtgcaact gcccaaagaa attcggaggg cagcactgtg aaatagataa gtcaaaaacc    300

tgctatgagg ggaatggtca cttttaccga ggaaaggcca gcactgacac catgggccgg    360

ccctgcctgc cctggaactc tgccactgtc cttcagcaaa cgtaccatgc ccacagatct    420

gatgctcttc agctgggcct ggggaaacat aattactgca ggaacccaga caaccggagg    480

cgaccctggt gctatgtgca ggtgggccta aagccgcttg tccaagagtg catggtgcat    540

gactgcgcag atggaaaagg taccctggac cggctgaccg accctccggg ggtccggcgc    600

gtgtaccaca tccaggcggg cctaccggac ccgttccagc cccccagcct cccgatcacg    660

gtttactacg ccgtgttgga gcgcgcctgc cgcagcgtgc tcctaaacgc accgtcggag    720

gccccccaga ttgtccgcgg ggcctccgaa gacgtccgga aacaacccta caa           773
```

<210> SEQ ID NO 46
<211> LENGTH: 440
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BD-uPA-gD

<400> SEQUENCE: 46

```
atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc     60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagatggc cgaccccaat    120

cgctttcgcg gcaaagacct tccggtcctc gagctaaatg gaggaacatg tgtgtccaac    180

aagtacttct ccaacattca ctggtgcaac tgcccaaaga aattcggtac cctggaccgg    240

ctgaccgacc ctccgggggt ccggcgcgtg taccacatcc aggcgggcct accggacccg    300

ttccagcccc ccagcctccc gatcacggtt tactacgccg tgttggagcg cgcctgccgc    360

agcgtgctcc taaacgcacc gtcggaggcc ccccagattg tccgcggggc ctccgaagac    420

gtccggaaac aaccctacaa                                               440
```

-continued

<210> SEQ ID NO 47
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: ATF-uPA-XhoI

<400> SEQUENCE: 47

ccgctcgaag caatgaactt catcaagttc catc 34

<210> SEQ ID NO 48
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: ATF-uPA-KpnI

<400> SEQUENCE: 48

ggggtacctt ttccatctgc gcagtcatgc acc 33

<210> SEQ ID NO 49
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for ATF-uPA-gD or BD-uPA-gD

<400> SEQUENCE: 49

cggaattcga tgggggggc tgccgccag 29

<210> SEQ ID NO 50
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for ATF-uPA-gD or BD-uPA-gD

<400> SEQUENCE: 50

aactgcagct agtaaaacaa gggctggtgc g 31

<210> SEQ ID NO 51
<211> LENGTH: 2333
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: uPA polynucleotide

<400> SEQUENCE: 51

cccgggccag ggtccacctg tccccgcagc gccggctcgc gccctcctgc cgcagccacc 60

gagccgccgt ctagcgcccc gacctcgcca ccatgagagc cctgctggcg cgcctgcttc 120

tctgcgtcct ggtcgtgagc gactccaaag gcagcaatga acttcatcaa gttccatcga 180

actgtgactg tctaaatgga ggaacatgtg tgtccaacaa gtacttctcc aacattcact 240

ggtgcaactg cccaaagaaa ttcggagggc agcactgtga aatagataag tcaaaaacct 300

gctatgaggg gaatggtcac ttttaccgag gaaaggccag cactgacacc atgggccggc 360

cctgcctgcc ctggaactct gccactgtcc ttcagcaaac gtaccatgcc cacagatctg 420

atgctcttca gctgggcctg gggaaacata attactgcag gaacccagac aaccggaggc 480

gaccctggtg ctatgtgcag gtgggcctaa agccgcttgt ccaagagtgc atggtgcatg 540

actgcgcaga tggaaaaaag ccctcctctc ctccagaaga attaaaattt cagtgtggcc 600

-continued

aaaagactct gaggccccgc tttaagatta ttgggggaga attcaccacc atcgagaacc 660

agccctggtt tgcggccatc tacaggaggc accgggggggg ctctgtcacc tacgtgtgtg 720

gaggcagcct catcagccct tgctgggtga tcagcgccac acactgcttc attgattacc 780

caaagaagga ggactacatc gtctacctgg gtcgctcaag gcttaactcc aacacgcaag 840

gggagatgaa gtttgaggtg gaaaacctca tcctacacaa ggactacagc gctgacacgc 900

ttgctcacca caacgacatt gccttgctga agatccgttc caaggagggc aggtgtgcgc 960

agccatcccg gactatacag accatctgcc tgccctcgat gtataacgat ccccagtttg 1020

gcacaagctg tgagatcact ggctttggaa aagagaattc taccgactat ctctatccgg 1080

agcagctgaa aatgactgtt gtgaagctga tttcccaccg ggagtgtcag cagccccact 1140

actacggctc tgaagtcacc accaaaatgc tgtgtgctgc tgacccacag tggaaaacag 1200

attcctgcca gggagactca gggggacccc tcgtctgttc cctccaaggc cgcatgactt 1260

tgactggaat tgtgagctgg ggccgtggat gtgccctgaa ggacaagcca ggcgtctaca 1320

cgagagtctc acacttctta ccctggatcc gcagtcacac caaggaagag aatggcctgg 1380

ccctctgagg gtccccaggg aggaaacggg caccacccgc tttcttgctg gttgtcattt 1440

ttgcagtaga gtcatctcca tcagctgtaa gaagagactg ggaagatagg ctctgcacag 1500

atggatttgc ctgtgccacc caccagggtg aacgacaata gctttaccct caggcatagg 1560

cctgggtgct ggctgcccag acccctctgg ccaggatgga ggggtggtcc tgactcaaca 1620

tgttactgac cagcaacttg tctttttctg gactgaagcc tgcaggagtt aaaaagggca 1680

gggcatctcc tgtgcatggg tgaagggaga gccagctccc ccgacggtgg gcatttgtga 1740

ggcccatggt tgagaaatga ataatttccc aattaggaag tgtaacagct gaggtctctt 1800

gagggagctt agccaatgtg ggagcagcgg tttggggagc agagacacta acgacttcag 1860

ggcagggctc tgatattcca tgaatgtatc aggaaatata tatgtgtgtg tatgtttgca 1920

cacttgtgtg tgggctgtga gtgtaagtgt gagtaagagc tggtgtctga ttgttaagtc 1980

taaatatttc cttaaactgt gtggactgtg atgccacaca gagtggtctt tctggagagg 2040

ttataggtca ctcctggggc ctcttgggtc ccccacgtga cagtgcctgg gaatgtatta 2100

ttctgcagca tgacctgtga ccagcactgt ctcagtttca ctttcacata gatgtccctt 2160

tcttggccag ttatcccttc cttttagcct agttcatcca atcctcactg ggtggggtga 2220

ggaccactcc ttacactgaa tatttatatt tcactatttt tatttatatt tttgtaattt 2280

taaataaaag tgatcaataa aatgtgattt ttctgatgaa aaaaaaaaaa aaa 2333

<210> SEQ ID NO 52
<211> LENGTH: 431
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: uPA amino acid

<400> SEQUENCE: 52

Met Arg Ala Leu Leu Ala Arg Leu Leu Leu Cys Val Leu Val Val Ser
1 5 10 15

Asp Ser Lys Gly Ser Asn Glu Leu His Gln Val Pro Ser Asn Cys Asp
20 25 30

Cys Leu Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile
35 40 45

-continued

```
His Trp Cys Asn Cys Pro Lys Lys Phe Gly Gly Gln His Cys Glu Ile
    50                  55                  60

Asp Lys Ser Lys Thr Cys Tyr Glu Gly Asn Gly His Phe Tyr Arg Gly
65                  70                  75                  80

Lys Ala Ser Thr Asp Thr Met Gly Arg Pro Cys Leu Pro Trp Asn Ser
                85                  90                  95

Ala Thr Val Leu Gln Gln Thr Tyr His Ala His Arg Ser Asp Ala Leu
            100                 105                 110

Gln Leu Gly Leu Gly Lys His Asn Tyr Cys Arg Asn Pro Asp Asn Arg
        115                 120                 125

Arg Arg Pro Trp Cys Tyr Val Gln Val Gly Leu Lys Pro Leu Val Gln
    130                 135                 140

Glu Cys Met Val His Asp Cys Ala Asp Gly Lys Lys Pro Ser Ser Pro
145                 150                 155                 160

Pro Glu Glu Leu Lys Phe Gln Cys Gly Gln Lys Thr Leu Arg Pro Arg
                165                 170                 175

Phe Lys Ile Ile Gly Gly Glu Phe Thr Thr Ile Glu Asn Gln Pro Trp
            180                 185                 190

Phe Ala Ala Ile Tyr Arg Arg His Arg Gly Gly Ser Val Thr Tyr Val
        195                 200                 205

Cys Gly Gly Ser Leu Ile Ser Pro Cys Trp Val Ile Ser Ala Thr His
    210                 215                 220

Cys Phe Ile Asp Tyr Pro Lys Lys Glu Asp Tyr Ile Val Tyr Leu Gly
225                 230                 235                 240

Arg Ser Arg Leu Asn Ser Asn Thr Gln Gly Glu Met Lys Phe Glu Val
                245                 250                 255

Glu Asn Leu Ile Leu His Lys Asp Tyr Ser Ala Asp Thr Leu Ala His
            260                 265                 270

His Asn Asp Ile Ala Leu Leu Lys Ile Arg Ser Lys Glu Gly Arg Cys
        275                 280                 285

Ala Gln Pro Ser Arg Thr Ile Gln Thr Ile Cys Leu Pro Ser Met Tyr
    290                 295                 300

Asn Asp Pro Gln Phe Gly Thr Ser Cys Glu Ile Thr Gly Phe Gly Lys
305                 310                 315                 320

Glu Asn Ser Thr Asp Tyr Leu Tyr Pro Glu Gln Leu Lys Met Thr Val
                325                 330                 335

Val Lys Leu Ile Ser His Arg Glu Cys Gln Gln Pro His Tyr Tyr Gly
            340                 345                 350

Ser Glu Val Thr Thr Lys Met Leu Cys Ala Ala Asp Pro Gln Trp Lys
        355                 360                 365

Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Ser Leu
    370                 375                 380

Gln Gly Arg Met Thr Leu Thr Gly Ile Val Ser Trp Gly Arg Gly Cys
385                 390                 395                 400

Ala Leu Lys Asp Lys Pro Gly Val Tyr Thr Arg Val Ser His Phe Leu
                405                 410                 415

Pro Trp Ile Arg Ser His Thr Lys Glu Glu Asn Gly Leu Ala Leu
            420                 425                 430
```

<210> SEQ ID NO 53
<211> LENGTH: 1602
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: ATF-uPA-gD polynucleotide

<400> SEQUENCE: 53

atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc 60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagatggc cgaccccaat 120

cgctttcgcg gcaaagacct tccggtcctc gagagcaatg aacttcatca agttccatcc 180

aactgtgact gtctaaatgg aggaacatgt gtgtccaaca agtacttctc caacattcac 240

tggtgcaact gcccaaagaa attcggaggg cagcactgtg aaatagataa gtcaaaaacc 300

tgctatgagg ggaatggtca cttttaccga ggaaaggcca gcactgacac catgggccgg 360

ccctgcctgc cctggaactc tgccactgtc cttcagcaaa cgtaccatgc ccacagatct 420

gatgctcttc agctgggcct ggggaaacat aattactgca ggaacccaga caaccggagg 480

cgaccctggt gctatgtgca ggtgggccta aagccgcttg tccaagagtg catggtgcat 540

gactgcgcag atggaaaagg taccctggac cggctgaccg accctccggg ggtccggcgc 600

gtgtaccaca tccaggcggg cctaccggac ccgttccagc cccccagcct cccgatcacg 660

gtttactacg ccgtgttgga gcgcgcctgc cgcagcgtgc tcctaaacgc accgtcggag 720

gcccccaga ttgtccgcgg ggcctccgaa gacgtccgga aacaacccta caacctgacc 780

atcgcttggt ttcggatggg aggcaactgt gctatcccca tcacggtcat ggagtacacc 840

gaatgctcct acaacaagtc tctgggggcc tgtcccatcc gaacgcagcc ccgctggaac 900

tactatgaca gcttcagcgc cgtcagcgag gataacctgg ggttcctgat gcacgccccc 960

gcgtttgaga ccgccggcac gtacctgcgg ctcgtgaaga taaacgactg gacggagatt 1020

acacagttta tcctggagca ccgagccaag ggctcctgta agtacgccct tccgctgcgc 1080

atccccccgt cagcctgcct ctcccccag gcctaccagc agggggtgac ggtggacagc 1140

atcgggatgc tgccccgctt catccccgag aaccagcgca ccgtcgccgt atacagcttg 1200

aagatcgccg ggtggcacgg gcccaaggcc ccatacacga gcaccctgct gcccccggag 1260

ctgtccgaga cccccaacgc cacgcagcca gaactcgccc cggaagaccc cgaggattcg 1320

gccctcttgg aggaccccgt ggggacggtg gtgccgcaaa tcccaccaaa ctggcacata 1380

ccgtcgatcc aggacgccgc gacgccttac catcccccgg ccaccccgaa caacatgggc 1440

ctgatcgccg gcgcggtggg cggcagtctc ctggtagccc tggtcatttg cggaattgtg 1500

tactggatgc gccgccgcac tcaaaaagcc ccaaagcgca tacgcctccc ccacatccgg 1560

gaagacgacc agccgtcctc gcaccagccc ttgttttact ag 1602

<210> SEQ ID NO 54
<211> LENGTH: 534
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: ATF-uPA-gD amino acid

<400> SEQUENCE: 54

Met Gly Gly Ala Ala Ala Arg Leu Gly Ala Val Ile Leu Phe Val Val
1 5 10 15

Ile Val Gly Leu His Gly Val Arg Gly Lys Tyr Ala Leu Ala Asp Ala
20 25 30

Ser Leu Lys Met Ala Asp Pro Asn Arg Phe Arg Gly Lys Asp Leu Pro
35 40 45

-continued

```
Val Leu Glu Gly Ser Asn Glu Leu His Gln Val Pro Ser Asn Cys Asp
    50                  55                  60

Cys Leu Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile
65                  70                  75                  80

His Trp Cys Asn Cys Pro Lys Lys Phe Gly Gly Gln His Cys Glu Ile
                85                  90                  95

Asp Lys Ser Lys Thr Cys Tyr Glu Gly Asn Gly His Phe Tyr Arg Gly
            100                 105                 110

Lys Ala Ser Thr Asp Thr Met Gly Arg Pro Cys Leu Pro Trp Asn Ser
        115                 120                 125

Ala Thr Val Leu Gln Gln Thr Tyr His Ala His Arg Ser Asp Ala Leu
    130                 135                 140

Gln Leu Gly Leu Gly Lys His Asn Tyr Cys Arg Asn Pro Asp Asn Arg
145                 150                 155                 160

Arg Arg Pro Trp Cys Tyr Val Gln Val Gly Leu Lys Pro Leu Val Gln
                165                 170                 175

Glu Cys Met Val His Asp Cys Ala Asp Gly Lys Gly Thr Leu Asp Gln
            180                 185                 190

Leu Thr Asp Pro Pro Gly Val Arg Arg Val Tyr His Ile Gln Ala Gly
        195                 200                 205

Leu Pro Asp Pro Phe Gln Pro Pro Ser Leu Pro Ile Thr Val Tyr Tyr
    210                 215                 220

Ala Val Leu Glu Arg Ala Cys Arg Ser Val Leu Leu Asn Ala Pro Ser
225                 230                 235                 240

Glu Ala Pro Gln Ile Val Arg Gly Ala Ser Glu Asp Val Arg Lys Gln
                245                 250                 255

Pro Tyr Asn Leu Thr Ile Ala Trp Phe Arg Met Gly Gly Asn Cys Ala
            260                 265                 270

Ile Pro Ile Thr Val Met Glu Tyr Thr Glu Cys Ser Tyr Asn Lys Ser
        275                 280                 285

Leu Gly Ala Cys Pro Ile Arg Thr Gln Pro Arg Trp Asn Tyr Tyr Asp
    290                 295                 300

Ser Phe Ser Ala Val Ser Glu Asp Asn Leu Gly Phe Leu Met His Ala
305                 310                 315                 320

Pro Ala Phe Glu Thr Ala Gly Thr Tyr Leu Arg Leu Val Lys Ile Asn
                325                 330                 335

Asp Trp Thr Glu Ile Thr Gln Phe Ile Leu Glu His Arg Ala Lys Gly
            340                 345                 350

Ser Cys Lys Tyr Ala Leu Pro Leu Arg Ile Pro Pro Ser Ala Cys Leu
        355                 360                 365

Ser Pro Gln Ala Tyr Gln Gln Gly Val Thr Val Asp Ser Ile Gly Met
    370                 375                 380

Leu Pro Arg Phe Ile Pro Glu Asn Gln Arg Thr Val Ala Val Tyr Ser
385                 390                 395                 400

Leu Lys Ile Ala Gly Trp His Gly Pro Lys Ala Pro Tyr Thr Ser Thr
                405                 410                 415

Leu Leu Pro Pro Glu Leu Ser Glu Thr Pro Asn Ala Thr Gln Pro Glu
            420                 425                 430

Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro Val
        435                 440                 445

Gly Thr Val Val Pro Gln Ile Pro Pro Asn Trp His Ile Pro Ser Ile
```

-continued

```
    450                 455                 460

Gln Asp Ala Ala Thr Pro Tyr His Pro Pro Ala Thr Pro Asn Asn Met
465                 470                 475                 480

Gly Leu Ile Ala Gly Ala Val Gly Gly Ser Leu Leu Val Ala Leu Val
                485                 490                 495

Ile Cys Gly Ile Val Tyr Trp Met Arg Arg Arg Thr Gln Lys Ala Pro
            500                 505                 510

Lys Arg Ile Arg Leu Pro His Ile Arg Glu Asp Asp Gln Pro Ser Ser
        515                 520                 525

His Gln Pro Leu Phe Tyr
    530
```

<210> SEQ ID NO 55
<211> LENGTH: 1269
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BD-uPA-gD polynucleotide

<400> SEQUENCE: 55

```
atgggggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc     60

catggggtcc gcggcaaata tgccttggcg gatgcctctc tcaagatggc cgaccccaat    120

cgctttcgcg gcaaagacct tccggtcctc gagctaaatg gaggaacatg tgtgtccaac    180

aagtacttct ccaacattca ctggtgcaac tgcccaaaga aattcggtac cctggaccgg    240

ctgaccgacc ctccgggggt ccggcgcgtg taccacatcc aggcgggcct accggacccg    300

ttccagcccc ccagcctccc gatcacggtt tactacgccg tgttggagcg cgcctgccgc    360

agcgtgctcc taaacgcacc gtcggaggcc ccccagattg tccgcggggc ctccgaagac    420

gtccggaaac aaccctacaa cctgaccatc gcttggtttc ggatgggagg caactgtgct    480

atccccatca cggtcatgga gtacaccgaa tgctcctaca acaagtctct gggggcctgt    540

cccatccgaa cgcagccccg ctggaactac tatgacagct tcagcgccgt cagcgaggat    600

aacctggggt tcctgatgca cgcccccgcg tttgagaccg ccggcacgta cctgcggctc    660

gtgaagataa acgactggac ggagattaca cagtttatcc tggagcaccg agccaagggc    720

tcctgtaagt acgcccttcc gctgcgcatc cccccgtcag cctgcctctc cccccaggcc    780

taccagcagg gggtgacggt ggacagcatc gggatgctgc cccgcttcat ccccgagaac    840

cagcgcaccg tcgccgtata cagcttgaag atcgccgggt ggcacgggcc caaggcccca    900

tacacgagca ccctgctgcc cccggagctg tccgagaccc ccaacgccac gcagccagaa    960

ctcgccccgg aagaccccga ggattcggcc ctcttggagg accccgtggg gacggtggtg   1020

ccgcaaatcc caccaaactg gcacataccg tcgatccagg acgccgcgac gccttaccat   1080

cccccggcca ccccgaacaa catgggcctg atcgccggcg cggtgggcgg cagtctcctg   1140

gtagccctgg tcatttgcgg aattgtgtac tggatgcgcc gccgcactca aaaagcccca   1200

aagcgcatac gcctcccccа catccgggaa gacgaccagc cgtcctcgca ccagcccttg   1260

ttttactag                                                           1269
```

<210> SEQ ID NO 56
<211> LENGTH: 422
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: BD-uPA-gD amino acid

<400> SEQUENCE: 56

```
Met Gly Gly Ala Ala Ala Arg Leu Gly Ala Val Ile Leu Phe Val Val
1               5                  10                  15

Ile Val Gly Leu His Gly Val Arg Gly Lys Tyr Ala Leu Ala Asp Ala
            20                  25                  30

Ser Leu Lys Met Ala Asp Pro Asn Arg Phe Arg Gly Lys Asp Leu Pro
        35                  40                  45

Val Leu Glu Leu Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser
    50                  55                  60

Asn Ile His Trp Cys Asn Cys Pro Lys Lys Phe Gly Thr Leu Asp Gln
65                  70                  75                  80

Leu Thr Asp Pro Pro Gly Val Arg Arg Val Tyr His Ile Gln Ala Gly
                85                  90                  95

Leu Pro Asp Pro Phe Gln Pro Pro Ser Leu Pro Ile Thr Val Tyr Tyr
            100                 105                 110

Ala Val Leu Glu Arg Ala Cys Arg Ser Val Leu Leu Asn Ala Pro Ser
        115                 120                 125

Glu Ala Pro Gln Ile Val Arg Gly Ala Ser Glu Asp Val Arg Lys Gln
    130                 135                 140

Pro Tyr Asn Leu Thr Ile Ala Trp Phe Arg Met Gly Gly Asn Cys Ala
145                 150                 155                 160

Ile Pro Ile Thr Val Met Glu Tyr Thr Glu Cys Ser Tyr Asn Lys Ser
                165                 170                 175

Leu Gly Ala Cys Pro Ile Arg Thr Gln Pro Arg Trp Asn Tyr Tyr Asp
            180                 185                 190

Ser Phe Ser Ala Val Ser Glu Asp Asn Leu Gly Phe Leu Met His Ala
        195                 200                 205

Pro Ala Phe Glu Thr Ala Gly Thr Tyr Leu Arg Leu Val Lys Ile Asn
    210                 215                 220

Asp Trp Thr Glu Ile Thr Gln Phe Ile Leu Glu His Arg Ala Lys Gly
225                 230                 235                 240

Ser Cys Lys Tyr Ala Leu Pro Leu Arg Ile Pro Pro Ser Ala Cys Leu
                245                 250                 255

Ser Pro Gln Ala Tyr Gln Gln Gly Val Thr Val Asp Ser Ile Gly Met
            260                 265                 270

Leu Pro Arg Phe Ile Pro Glu Asn Gln Arg Thr Val Ala Val Tyr Ser
        275                 280                 285

Leu Lys Ile Ala Gly Trp His Gly Pro Lys Ala Pro Tyr Thr Ser Thr
    290                 295                 300

Leu Leu Pro Pro Glu Leu Ser Glu Thr Pro Asn Ala Thr Gln Pro Glu
305                 310                 315                 320

Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro Val
                325                 330                 335

Gly Thr Val Val Pro Gln Ile Pro Pro Asn Trp His Ile Pro Ser Ile
            340                 345                 350

Gln Asp Ala Ala Thr Pro Tyr His Pro Pro Ala Thr Pro Asn Asn Met
        355                 360                 365

Gly Leu Ile Ala Gly Ala Val Gly Gly Ser Leu Leu Val Ala Leu Val
    370                 375                 380

Ile Cys Gly Ile Val Tyr Trp Met Arg Arg Arg Thr Gln Lys Ala Pro
```

-continued

385 390 395 400

Lys Arg Ile Arg Leu Pro His Ile Arg Glu Asp Asp Gln Pro Ser Ser
405 410 415

His Gln Pro Leu Phe Tyr
420

<210> SEQ ID NO 57
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human uPAR reverse

<400> SEQUENCE: 57

ggcagtcatt agcagggtga tggtg 25

<210> SEQ ID NO 58
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hamster uPAR reverse

<400> SEQUENCE: 58

gttgccctcg cagctgtaac actgg 25

<210> SEQ ID NO 59
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: uPAR-start forward

<400> SEQUENCE: 59

atgggtcacc cgccgctgct gccgc 25

<210> SEQ ID NO 60
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: R5321 (pGG5414) forward primer

<400> SEQUENCE: 60

cggaattcat gggggggct gccgccag 28

<210> SEQ ID NO 61
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: R5322 (pGG5415) forward primer

<400> SEQUENCE: 61

cggaattcat ggcgcttttg ttgaccacgg 30

<210> SEQ ID NO 62
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: R5323 (pGG5416) forward primer

<400> SEQUENCE: 62

-continued

cggaattcat gagagccctg ctggcgcgcc 30

<210> SEQ ID NO 63
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: EcoRI-PstI fragment reverse primer

<400> SEQUENCE: 63

aactgcagct aggcgtagta aaccgtgatc ggg 33

<210> SEQ ID NO 64
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gD-A forward primer

<400> SEQUENCE: 64

cggaattcat gctgccccgc ttcatccccg ag 32

<210> SEQ ID NO 65
<211> LENGTH: 68
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: gD-A reverse primer

<400> SEQUENCE: 65

aactgcagtt acaggtcctc ctctgagatc agcttctgca ttgatgcgtt gttcggggtg 60

gccggggg 68

<210> SEQ ID NO 66
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: uPA ATF insertion forward primer

<400> SEQUENCE: 66

cggaattcat gagagccctg ctggcgcgcc 30

<210> SEQ ID NO 67
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: uPA ATF insertion reverse primer

<400> SEQUENCE: 67

aactgcagct attttccatc tgcgcagtca tgc 33

1. A herpes simplex virus comprising a first polynucleotide encoding a gD polypeptide fragment comprising amino acids 219-369 of SEQ ID NO:26 and a second polynucleotide encoding a targeting peptide, wherein said targeting peptide specifically interacts with the gD polypeptide fragment.

2. The herpes simplex virus according to claim 1 wherein the targeting peptide is a urokinase plasminogen activator peptide that specifically interacts with urokinase plasminogen activator receptor.

3. The herpes simplex virus according to claim 1 wherein the second polynucleotide further encodes a second gD polypeptide fragment comprising at least 28 contiguous amino acids of SEQ ID NO:26 and having a C-terminus at position 60 of SEQ ID NO:26.

4. (canceled)

5. (canceled)

6. (canceled)

7. A herpes simplex virus comprising a polynucleotide encoding a gD polypeptide fragment comprising amino acids 219-369 of SEQ ID NO:26 and a targeting peptide, wherein said targeting peptide is a member of a binding pair that specifically interacts with the other member of said binding pair.

8. (canceled)

9. (canceled)

10. The herpes simplex virus according to claim 7 wherein said polynucleotide further encodes a second gD polypeptide fragment comprising at least 28 contiguous amino acids of SEQ ID NO:26 and having a C-terminus at position 60 of SEQ ID NO:26.

11. (canceled)

12. (canceled)

13. (canceled)

14. (canceled)

15. The herpes simplex virus according to claim 7 wherein the targeting peptide is selected from the group consisting of a urokinase plasminogen activator peptide fragment and an interleukin 13 peptide fragment, wherein said peptide fragment specifically interacts with its binding partner.

16. The herpes simplex virus according to claim 1 wherein the targeting peptide comprises an antibody variable domain, wherein said peptide specifically interacts with its binding partner.

17. A pharmaceutical composition comprising the herpes simplex virus according to claim 1 and a pharmaceutically acceptable excipient, carrier or diluent.

18. A kit comprising the herpes simplex virus according to claim 1 and a label providing instruction for administration of said virus.

19. A method of producing a herpes simplex virus according to claim 1 comprising:

(a) contacting a permissive host cell with the herpes simplex virus;

(b) incubating said host cell; and

(c) recovering said herpes simplex virus.

20. A method of treating a condition in an organism characterized by the presence of a deleterious cell in said organism comprising administering a therapeutically effective amount of a herpes simplex virus according to claim 1 to said organism.

21. The method according to claim 20 wherein said condition is cancer.

22. (canceled)

23. (canceled)

24. (canceled)

25. The herpes simplex virus according to claim 7 wherein the targeting peptide comprises an antibody variable domain, wherein said peptide specifically interacts with its binding partner.

26. A pharmaceutical composition comprising the herpes simplex virus according to claim 7 and a pharmaceutically acceptable excipient, carrier or diluent.

27. A kit comprising the herpes simplex virus according to claim 7 and a label providing instruction for administration of said virus.

28. A method of producing a herpes simplex virus according to claim 7 comprising:

(a) contacting a permissive host cell with the herpes simplex virus;

(b) incubating said host cell; and

(c) recovering said herpes simplex virus.

29. A method of treating a condition in an organism characterized by the presence of a deleterious cell in said organism comprising administering a therapeutically effective amount of a herpes simplex virus according to claim 7 to said organism.

30. The method according to claim 29 wherein said condition is cancer.

* * * * *