US 20100221228A1

(19) United States(12) Patent Application Publication

Smith et al.

(10) Pub. No.: US 2010/0221228 A1
(43) Pub. Date: Sep. 2, 2010

(54) Y134.5 DEFICIENT HSV AND THE MAPK PATHWAY

 (75) Inventors: Kerrington D. Smith, Chicago, IL
 (US); James J. Mezhir, La Grange, IL (US); Ralph Weichselbaum, Chicago, IL (US); Bernard
 Roizman, Chicago, IL (US)

> Correspondence Address: MARSHALL, GERSTEIN & BORUN LLP 233 SOUTH WACKER DRIVE, 6300 WILLIS TOWER CHICAGO, IL 60606-6357 (US)

- (73) Assignee: **The University of Chicago**, Chicago, IL (US)
- (21) Appl. No.: 12/161,763
- (22) PCT Filed: Jan. 24, 2007
- (86) PCT No.: PCT/US07/61002
 - § 371 (c)(1), (2), (4) Date: Nov. 10, 2008

Related U.S. Application Data

(60) Provisional application No. 60/761,661, filed on Jan. 24, 2006.

Publication Classification

(51)	Int. Cl.	
	A61K 35/76	(2006.01)
	A61P 35/00	(2006.01)
	C12N 7/00	(2006.01)

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

(57) **ABSTRACT**

The invention provides materials and methods for the identification of cells exhibiting a cell proliferative disorder that are amenable to treatment with a herpes simplex virus that does not express an approximately wild-type level of ICP34.5. Also provided are methods of treating cell proliferative diseases, disorders or conditions, such as cancers, rheumatoid arthritis and macular degeneration, using these HSVs. Further provided are methods for preventing such cell proliferative disorders by administering the HSVs as well as methods for ameliorating a symptom associated with a cell proliferative disorder by administering such HSVs.



























5 days post IP injection 9 x 10⁸ PFU













FIG. 14









Y134.5 DEFICIENT HSV AND THE MAPK PATHWAY

GOVERNMENT INTEREST

[0001] The U.S. Government may own rights in the invention pursuant to grant no. CA7193307-07 from the National Institutes of Health.

BACKGROUND

[0002] In the general field of human health and animal welfare, a variety of diseases, disorders, and conditions have largely eluded the best efforts at prevention or treatment. Chief among such maladies is the loss of cell-cycle control that frequently results in the undesirable cell proliferation characteristic of cancer in its many forms. Malignant gliomas, for example, 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. Many of the more devastating forms of cancer, such as malignant gliomas and metastasized forms of a variety of cancers, are inoperable, further reducing the likelihood of receiving effective treatment with conventional therapies.

[0003] One approach to the development of new and effective anti-cancer therapies has been directed at engineered viral therapeutics. Chief among the viruses being explored for use as oncolytic agents are genetically engineered forms of herpes simplex viruses (HSV). 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 a functional γ_1 34.5 gene encoding infected cell protein 34.5 (ICP34.5).

[0004] In principle, use of an avirulent mutant of herpes simplex viruses 1 (HSV-1) to destroy cancer cells in situ, e.g., in inoperable human tumors, is a sound approach to treating such disease conditions. As noted above, the most promising HSV candidate is an HSV mutant lacking a functional $\gamma_1 34.5$ gene. The product of the γ_1 34.5 gene of HSV, ICP34.5, is a multifunctional protein that blocks a major host response to infection. In brief, after the onset of viral DNA synthesis, infected cells accumulate large amounts of complementary viral RNA transcripts. The consequence of this accumulation is the activation of double-stranded RNA-dependent protein kinase R(PKR). In infected cells, activated PKR phosphorylates the α subunit of the eukaryotic translation initiation factor 2 (eIF-2 α), resulting in loss of protein synthesis. In the case of HSV-1, ICP34.5 acts as a phosphatase accessory factor to recruit protein phosphatase 1a to dephosphorylate eIF-2a. As a consequence, protein synthesis continues unimpeded. Mutants derived from $\Delta \gamma_1 34.5$ viruses lack the capacity to counteract PKR-induced loss of protein synthesis and cell apoptosis. Another significant property of $\gamma_1 34.5$ mutant HSV is that they are highly attenuated in animal model systems and phase I clinical studies have demonstrated that $\Delta \gamma_1 34.5$ mutants can be administered safely at escalating doses in patients with malignancy. A major impediment to the widespread use of these mutants for cancer therapy is the observation that in animal model systems, human tumor cells differ widely with respect to their ability to support the replication of γ_1 34.5 mutant HSV. In cancer cells that do support replication of γ_1 34.5-deficient HSV, these viral constructs exhibit lytic cytotoxicity specific to the cancer cells, and are able to act on such cells regardless of body location and distribution. Thus, a need exists in the art for effective and safe viral-based therapies to treat cell proliferation disorders such as cancers.

[0005] Investigations of eukaryotic cell physiology have revealed a variety of signal transduction pathways involved in the coordinate regulation of complex physiological processes such as cell proliferation. For example, mitogen-activated protein kinases (MAPKs) have been implicated as elements of regulatory pathways controlling cell proliferation in all eukaryotes. The MAPK pathway is organized in modules, of which there are six different modules presently known. This pathway typically contains an "upstream" (i.e., early step in the pathway) G-protein and a core module containing three kinase enzymes: a MAPK kinase kinase (i.e., MAPKKK) that phosphorylates and thereby activates a MAPK Kinase (i.e., MAPKK), which in turn phosphorylates and activates a MAPK. In one example, the ERK (extracellular-signal-regulated) pathway, Ras is a G-protein, Raf is a MAPKKK, MEK (i.e., MAPK/ERK Kinase) is a MAPKK and ERK is a MAPK. Complicating even this one example of a MAPK signal transduction pathway regulating cell proliferation is the existence of a number of isoforms for the particular kinases. For example, there are three mammalian Raf isoforms, i.e., Raf-1, A-Raf and B-Raf; two MEK isoforms, i.e., MEK1 and MEK2; and two ERK isoforms, i.e., ERK1 and ERK2. Moreover, other kinase enzymes can be substituted for the prototypes listed above. For example, in addition to Raf kinases, MEKK-1, (i.e., MEK Kinase-1), mos or Tpl-2 can activate MEK isoforms.

[0006] Complicating the regulatory picture even further, the MAPK pathway also embraces a variety of accessory proteins such as exchange factors, modulators, scaffolding molecules, adapter proteins, and chaperones, collectively providing capacities to localize elements of the pathway, to translocate elements, to finely control the activation/inhibition of elements of the pathway and to ensure that signal propagation is achieved in an efficient and directed manner. An illustrative exchange factor is the Ras GTP/GDP exchange factor known as Son of Sevenless (SOS), a protein that promotes the exchange of GTP for GDP on Ras, thereby activating cell membrane-bound Ras. An example of a modulator involved in the MAPK pathway is SUR-8 (i.e., Suppressor of Ras-8), which binds to Raf-1 and Ras-GTP, forming a ternary complex that enhances Raf-1 activation. Two exemplary scaffolding proteins are the mammalian Kinase Suppressor of Ras (i.e., KSR) and the yeast PBS2 protein (i.e., polymyxin B sensitivity). KSR has been shown to associate with elements of the above-described module of the MAPK pathway, i.e., Raf, MEK and ERK. Consistent with its role as a scaffolding protein for elements of the pathway, KSR has been shown to either activate or inhibit the MAPK pathway, depending on the stoichiometric ratios of KSR to the elements of the pathway (e.g., Raf, MEK, and ERK). In terms of non-binding theory, either an insufficiency or an excess of KSR relative to the pathway components or elements would be expected to lead to an unorganized or poorly organized pathway impeding the capacity of the elements to cooperatively propagate a signal, e.g., a signal modulating cell proliferation. An example of an adapter protein is the mammalian 14-3-3 protein, which modulates a variety of signaling proteins, for example by changing the subcellular location of target proteins or by altering protein associations. As a consequence, 14-3-3 plays a role in regulating cell-cycle checkpoints, cell proliferation, cell differentiation and cell apoptosis. Finally, the MAPK pathway comprehends chaperones such as Hsp90, Hsp50/Cdc37, FKBP65 and Bag-1. Loss of functional chaperone activity results in reduced kinase activity and may be due to a chaperone's stabilization of kinase tertiary structure and/or a role for the chaperone in recruiting kinase, e.g., Raf-1, activators.

[0007] The preceding discussion of MAPK pathways illustrates the classes of proteins involved in these complex pathways of regulating such physiological processes as cell proliferation and cell apoptosis. Additional elements of the pathways are known in the art, as illustrated by the disclosures in Kolch, W., J. Biochem. 351:289-305 (2000) and English et al., Exp. Cell Res. 253:255-270 (1999), both of which are incorporated herein by reference in their entireties.

[0008] Applications of HSV-1 oncolytic therapy have principally utilized local injection of virus directly into the tumor. For this reason, HSV-1 vectors have been clinically tested primarily in malignant gliomas which remain confined to the CNS. In the context of developing HSV-1 as a broader anticancer agent, it would be valuable to be able to administer HSV-1 systemically (intravenously or intraperitoneally) to effectively treat disseminated metastases in addition to the primary tumor. Moreover, a variety of human tumor types, such as melanomas, sarcomas, and carcinomas of the colon, ovary, liver, breast, esophagus, stomach, pancreas, and lung have been reported to overexpress MEK activity.

[0009] Thus, a need continues to exist in the art for virusbased cancer therapeutics and corresponding methods for use in treating a variety of target cancer cells amenable to such virus-based treatment. Accordingly, a need also exists for identifying amenable target cancer cells suitable for virusbased anti-cancer treatment.

SUMMARY

[0010] The invention disclosed herein satisfies at least one of the aforementioned needs in the art by providing therapeutic agents in the form of herpes simplex viruses that do not elaborate wild-type levels of active ICP34.5, the $\gamma_134.5$ gene product. These therapeutic agents are useful in treating target cells exhibiting a cell proliferative disorder, such as a cancer (including a solid-tumor cancer), rheumatoid arthritis, macular degeneration and other diseases, disorders and conditions known in the art to be associated with abnormal, preferably elevated, cell proliferation. Further, such HSVs are shown herein to exhibit improved replication, and hence cytotoxicity due to lytic cell cycle completion, in target cells having an active MAPK pathway, e.g., an active Ras/Rak/MEK/ERK pathway. Delivery of γ_1 34.5 deficient HSV, such as R3616, selectively targets and destroys human xenograft tumors that overexpress MEK activity as compared to tumors that express lower MEK activity. In addition, effective delivery can be achieved by a variety of routes, including systemic administration. The results reported herein indicate that systemic delivery of γ_1 34.5 deficient HSV is effective in the treatment of human tumors. The invention also provides a method for identifying or diagnosing a cell proliferative disorder amenable to treatment with the above-described HSVs by determining the status of a MAPK pathway in a candidate target cell exhibiting a cell proliferative disorder. Those candidate target cells that have an active MAPK pathway are preferred target cells for administration of the above-described HSVs. In providing methods for advantageously using viral-based therapy for the treatment of cell proliferation diseases, disorders or conditions, the invention provides the benefit of effective treatment for those diseases, disorders or conditions that have proven refractory to conventional treatment, such as inoperable tumors and metastasized cancers.

[0011] One aspect of the invention is drawn to a method of treating a cell proliferation (or cell proliferative) disorder comprising administration of an effective amount of a $\gamma_1 34.5$ deficient herpes simplex virus, such as a $\gamma_1 34.5$ deficient herpes simplex virus-1, comprising at least one expressible coding region of the MAPK pathway to a subject in need. In some embodiments, the method comprises administration of a γ_1 34.5 deficient herpes simplex virus-1 that comprises a coding region for MEK. In exemplary embodiments, the MEK is selected from the group consisting of MEK1 and MEK2. In some embodiments, the $\gamma_1 34.5$ deficient herpes simplex virus-1 comprises a coding region for ERK, such as ERK1 or ERK2. In some embodiments, the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for Raf. In exemplary embodiments, the Raf is selected from the group consisting of Raf-1, A-Raf and B-Raf. In some embodiments, γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for a protein selected from the group consisting of MEK Kinase-1, mos and Tpl-2. Embodiments of the method according to this aspect of the invention may comprise administration of a γ_1 34.5 deficient herpes simplex virus-1 that comprises a coding region for Ras. In other embodiments of the method according to the invention, the coding region for the MAPK pathway encodes a variant of a member of the pathway. In particular embodiments, the variant is selected from the group consisting of K-Ras V12, K-Ras D12, H-Ras V12, K-Ras D13, N-Ras V12, Raf S338A, Raf S339A, B-Raf V600E, Raf-CAAX, Raf BXB, AN3MKK1 S218E/S222D, ΔN3MKK2 S218E/S222D, ERK2 E58Q, ERK2 D122A, ERK2 S151A, ERK2 S221A, ERK2 S151D ERK L73P and a full-length MEK-ERK fusion. Other embodiments comprise administration of an effective amount of a $\gamma_1 34.5$ deficient herpes simplex virus-1 comprising at least one expressible coding region encoding a protein selected from the group consisting of a catalytically inactive mutant of PKR, a catalytically inactive mutant of eIF-2 α , a growth factor and an active mutant of a tyrosine kinase receptor, wherein the protein and encoding nucleic acid are known in the art.

[0012] In embodiments of this aspect of the invention, the $\gamma_134.5$ deficient herpes simplex virus-1 lacks any $\gamma_134.5$ gene. In some embodiments, the $\gamma_134.5$ deficient herpes simplex virus-1 comprises a $\gamma_134.5$ gene with a point mutation. Also contemplated are HSV that are $\gamma_134.5$ deficient due to an inability to effectively express an otherwise intact $\gamma_134.5$ gene. Additionally contemplated are HSV combining the various mechanisms for rendering the virus $\gamma_134.5$ deficient, such as by deletion of one $\gamma_134.5$ gene and mutation of a second $\gamma_134.5$ gene, for example by insertional inactivation, partial deletion, or non-silent point mutation.

[0013] The methods according to this aspect of the invention extend to methods wherein the treating ameliorates at least one symptom associated with the cell proliferation disorder. Exemplary symptoms include pain, swelling, or loss of physiological function due to cell proliferation, or a tumor mass impinging on one or more tissues or organs. **[0014]** A variety of cell proliferation, or cell proliferative, disorders are comprehended by the invention, including cancer, macular degeneration, and autoimmune disease.

[0015] Another aspect of the invention is use of a $\gamma_1 34.5$ deficient HSV comprising at least one expressible coding region of the MAPK pathway in the preparation of a medicament for the treatment of a patient with a cell proliferation disorder. Comprehended in various embodiments of the use are the MAPK pathway coding regions identified above in the context of describing the treatment methods according to the invention, i.e., MEK (e.g., MEK1 and/or MEK2), ERK (e.g., ERK1 and/or ERK2), Raf (e.g., Raf-1, A-Raf, B-Raf), Ras, MEK Kinase-1, mos, Tpl-2, variants of each of the members of the MAPK pathway, such as K-Ras V12, K-Ras D12, H-Ras V12, K-Ras D13, N-Ras V12, Raf S338A, Raf S339A, B-Raf V600E, Raf-CAAX, Raf BXB, AN3MKK1 S218E/ S222D, AN3MKK2 S218E/S222D, ERK2 E58Q, ERK2 D122A, ERK2 S151A, ERK2 S221A, ERK2 S151D ERK L73P and a full-length MEK-ERK fusion, and a catalytically inactive mutant of PKR, a catalytically inactive mutant of eIF-2 α , a growth factor and an active mutant of a tyrosine kinase receptor. Additionally, the use may comprise any of a variety of γ_1 34.5 deficient HSV, as described herein.

[0016] Yet another aspect of the invention is a $\gamma_1 34.5$ deficient HSV comprising at least one expressible coding region of the MAPK pathway. As noted for the aspects of the invention described above, the expressible MAPK pathway coding region may be a region encoding MEK (e.g., MEK1 and/or MEK2), ERK (e.g., ERK1 and/or ERK2), Raf (e.g., Raf-1, A-Raf, B-Raf), Ras, MEK Kinase-1, mos, Tpl-2, variants of each of the members of the MAPK pathway, such as K-Ras V12, K-Ras D12, H-Ras V12, K-Ras D13, N-Ras V12, Raf S338A, Raf S339A, B-Raf V600E, Raf-CAAX, Raf BXB, ΔN3MKK1 S218E/S222D, ΔN3MKK2 S218E/S222D, ERK2 E58Q, ERK2 D122A, ERK2 S151A, ERK2 S221A, ERK2 S151D ERK L73P and a full-length MEK-ERK fusion, and a catalytically inactive mutant of PKR, a catalytically inactive mutant of eIF-2 α , a growth factor and an active mutant of a tyrosine kinase receptor. This aspect of the invention comprehends a variety of HSV that are $\gamma_1 34.5$ deficient HSV, such as a γ_1 34.5 deficient herpes simplex virus-1 that lacks any γ_1 34.5 gene (i.e., an HSV containing a deletion of each of the two γ_1 34.5 genes found in wild-type HSV). Further comprehended is a $\gamma_1 34.5$ deficient herpes simplex virus-1 that comprises a γ_1 34.5 gene with a point mutation. Also contemplated are HSV that are $\gamma_1 34.5$ deficient due to an inability to effectively express an otherwise intact $\gamma_1 34.5$ gene. Additionally contemplated are HSV combining the various mechanisms for rendering the virus $\gamma_1 34.5$ deficient, such as by deletion of one $\gamma_1 34.5$ gene and mutation of a second γ_1 34.5 gene, for example by insertional inactivation, partial deletion, or non-silent point mutation.

[0017] A related aspect of the invention is drawn to a composition comprising the γ_1 34.5 deficient HSV as described above in combination with a pharmaceutically acceptable adjuvant, carrier, or diluent.

[0018] Another aspect of the invention provides a method of determining the susceptibility of a cell exhibiting a proliferative disorder to γ_1 34.5 deficient herpes simplex virus-1 cytotoxicity comprising measuring the activity of the MEK signaling pathway in the cell, wherein an active MEK signaling pathway is indicative of the susceptibility of the cell to γ_1 34.5 deficient HSV cytotoxicity. In some embodiments, the activity of the MEK signaling pathway in the cell is measured

by determining the level of a phosphorylated form of a protein selected from the group consisting of MEK1, MEK2, ERK 1, and ERK 2, and preferably selected from either MEK1 or MEK2. Some embodiments of this aspect of the invention involve the above-described method wherein the phosphorylated form of the protein is measured using an antibody specifically recognizing the phosphorylated form of the protein. The method described above may also involve measuring the activity of MEK signaling by determining the MEK haplotype, or partial genotype, of the cell, wherein a non-deficient MEK haplotype is indicative of an active MEK signaling pathway. In certain embodiments, the non-deficient MEK haplotype is homozygous wild-type MEK. Also in some embodiments, the method may involve a cell exhibiting a proliferative disorder that is a cancer cell. Also, the method described above may involve a γ_1 34.5 deficient HSV that is an HSV lacking the capacity to express a full-length ICP34.5 at about a wild-type level of expression.

[0019] Another aspect of the invention provides a method of identifying a patient with a cell proliferative disorder that is amenable to treatment with a $\gamma_1 34.5$ deficient HSV comprising obtaining a cell sample from the patient; and measuring the activity of the MEK signaling pathway in the cell, wherein an active MEK signaling pathway is indicative of a patient with a cell proliferative disorder that is amenable to treatment with a $\gamma_1 34.5$ deficient HSV. In some embodiments, the activity being measured is the level of a phosphorylated form of a protein selected from the group consisting of MEK1, MEK2, ERK1 and ERK2, preferably MEK1 or MEK2. In some embodiments of this aspect of the invention the activity of the MEK signaling pathway is measured by determining the MEK genotype of the cell, wherein a non-deficient MEK genotype is indicative of an active MEK signaling pathway. In some embodiments, the $\gamma_1 34.5$ deficient HSV is an HSV lacking the capacity to express a full-length ICP34.5 at about a wild-type level of expression. This aspect of the invention comprehends embodiments in which the cell proliferative disorder is a cancer, a rheumatoid arthritis or a macular degeneration, and preferably a cancer such as a solid tumor cancer or a metastasized cancer.

[0020] Yet another aspect of the invention is a use of a $\gamma_1 34.5$ deficient HSV in the preparation of a medicament for the treatment of a patient with a cell proliferative disorder comprising combining the $\gamma_1 34.5$ deficient HSV with a pharmaceutically acceptable adjuvant, carrier, or diluent.

[0021] Yet another aspect of the invention is a method of treating an MEK⁺ cell exhibiting a proliferative disorder comprising contacting the cell with a therapeutically effective amount of a γ_1 34.5 deficient HSV. In some embodiments of this aspect of the invention, the activity being measured is the level of a phosphorylated form of a protein selected from the group consisting of MEK1, MEK2, ERK 1 and ERK 2, preferably MEK1 or MEK2. Some embodiments of this aspect involve practice of the above-described method wherein the activity of the MEK signaling pathway is measured by determining the MEK haplotype of the cell, wherein a non-deficient MEK haplotype is indicative of an active MEK signaling pathway. In some embodiments of the method, the $\gamma_1 34.5$ deficient HSV is an HSV lacking the capacity to express a full-length ICP34.5 at about a wild-type level of expression. In some embodiments, the cell proliferative disorder is a cancer.

[0022] In yet another aspect, the invention provides a use of a γ_1 34.5 deficient HSV in the preparation of a medicament for

the treatment of a cell exhibiting a proliferative disorder comprising combining the γ_1 34.5 deficient HSV with a pharmaceutically acceptable adjuvant, carrier, diluent or excipient. Pharmaceutically acceptable adjuvants, carrier, diluents, and excipients are known in the art.

[0023] Other features and advantages of the invention will be better understood by reference to the brief description of the drawing and the detailed description of the invention that follow.

BRIEF DESCRIPTION OF THE DRAWING

[0024] FIG. 1. RSV R3616 viral yields in a variety of cells characteristic of a variety of tumors. Cells were exposed to 1 PFU/cell of R3616 in serum free medium for 2 hours, after which medium containing virus was removed and fresh medium containing 1% calf serum was added. At 36 hours post-infection, R3616 viral recovery was determined by standard plaque assay.

[0025] FIG. 2. Differential protein synthesis and activation of protein kinase R (PKR) in R3616 infected cancer cell lines inversely correlates with constitutive MEK activation in uninfected cancer cell lines A. Cell lines were infected with 10 PFU/cell of HSV R3616. At 11 hours post-infection, the cells were rinsed, starved of methionine for one hour, and then incubated in methionine-free medium supplemented with 100 μ Ci of [³⁵S] methionine per nil for two additional hours. At 14 hours post-infection, 20 µg of equilibrated protein lysates were electrophoretically separated in denaturing polyacrylamide gels, transferred to a PVDF membrane, and exposed to autoradiography film. B Cells were infected with 10 PFU/cell of R3616 and whole-cell lysates, harvested at 12 hours post-infection, were resolved by SDS-PAGE and immunoblotted with an antibody that recognizes the autophosphorylated form of PKR on Threonine 446. In the lower panel, after overnight serum starvation, uninfected total whole-cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the total and phosphorylated forms of ERK on threonine 202 and tyrosine 204.

[0026] FIG. **3**. Deletion of mutant N-ras in human fibrosarcoma cells restricts viral replication Replicate cultures of HT1080 and MCH603 cells were infected with 1 PFU of R3616 or HSV-1(F) viruses per cell in serum free medium for 2 hours, after which medium containing virus was removed and replaced with fresh medium containing 1% calf serum. At 36 hours post-infection, viral recovery was determined by standard plaque assay.

[0027] FIG. **4**. Diminished [35 S]-methionine metabolic labeling in virus infected human fibrosarcoma cells deleted for mutant N-ras. Replicate cultures of HT1080 and MCH603 cells were infected with 10 PFU of R3616 or HSV-1(F) viruses per cell. At 11 hours post-infection, the cells were rinsed, starved of methionine for one hour, and then incubated in methionine-free medium supplemented with 100 μ Ci of [35 S] methionine per ml for two additional hours. At 14 hours post-infection, 20 μ g of equilibrated protein lysates were electrophoretically separated in denaturing polyacrylamide gels, transferred to a PVDF membrane and exposed to autoradiography film.

[0028] FIG. 5. Increased PKR and eIF- 2α phosphorylation in human fibrosarcoma cells deleted for mutant N-ras during R3616 infection. Replicate cultures of HT1080 and MCH603 cells were exposed to 10 PFU of R3616 or HSV-1(F) viruses per cell. Cells were harvested at 14 hours post-infection and processed as described in Example 1. The electrophoretically separated proteins were immunoblotted with antibodies recognizing the phosphorylated form of PKR on threonine 446 and the phosphorylated form of eIF-2 α on serine 51, as well as for total PKR and eIF-2 α .

[0029] FIG. **6**. Inhibition of MEK by the addition of PD98059 increases PKR autophosphorylation and suppresses the accumulation of a $\gamma 2$ viral protein (gC) in HT1080 cells infected with R3616. Replicate cultures of serumstarved HT1080 cells were infected with 10 PFU of R3616 viruses per cell in the presence or absence of 40 μ M PD98059, as described in Example 1. Cells were harvested at 12 hours post-infection and the electrophoretically separated proteins were immunoblotted with antibodies recognizing either immediate-early [α (ICP27)], early [β (UL42)] or late [γ (gC)] viral proteins. The same lysates were immunoblotted to determine the total and phosphorylated forms of ERK1 and ERK2 (phosphorylated on threonine 202/tyrosine 204) and PKR (phosphorylated on threonine 446).

[0030] FIG. 7. Differences in cytopathic effects in virus infected caMEK (constitutively active MEK) and dnMEK (dominant negative MEK) stable cell lines. Replicate cultures of HT-caMEK and HT-dnMEK cells were infected with 10 PFU of Mock, R3616 or HSV-1(F) viruses per cell. Photos were taken at 12 hours post-infection.

[0031] FIG. 8. The effect of dnMEK and caMEK overexpression on R3616 viral recovery and PKR function during R3616 infection. A. Replicate cultures of HT-dnMEK, HT1080, and HT-caMEK cells were exposed to one PFU of R3616 virus per cell in serum-free medium for 2 hours, after which medium containing virus was removed and fresh medium containing 1% calf serum was added. At 36 hours post-infection, R3616 viral recovery was determined by standard plaque assay B. To determine the influence of mutant MEK expression on PKR activation, replicate cultures of HT-dnMEK, HT1080 and HT-caMEK cells were exposed to 10 PFU of R3616 virus per cell. Cells were harvested at 12 hours post-infection and processed as described in Example 1. Electrophoretically separated proteins were immunoblotted with antibodies recognizing the total and phosphorylated form of the following proteins: ERK1 and ERK2 (phosphorylated on threonine 202 and tyrosine-204), PKR (phosphorylated on threonine 446), and eIF-2 α (phosphorylated on serine 51). The same lysates were immunoblotted with antibodies recognizing immediate-early $[\alpha(ICP27)]$ and late [y(gC)] viral proteins. C. R3616 viral recovery from replicate cultures of Mia-dnMEK, MiaPaCa2 and Mia-caMEK at 36 hours post-infection. D. Immunoblotting was performed on replicate lysates of the Mia-dnMEK, MiaPaCa2 and MiacaMEK cells described in Section B, above.

[0032] FIG. 9. Diminished [35 S]-methionine metabolic labeling in R3616 infected human fibrosarcoma cells expressing dnMEK. Replicate cultures of HT-caMEK and HTdnMEKcells were infected with 10 PFU of R3616 or HSV-1(F) viruses per cell. At 11 hours post-infection, Mock and virus infected cells were rinsed, starved of methionine for one hour, and then incubated in methionine-free medium supplemented with 100 µCi of [35 S] methionine per ml for two additional hours. At 14 hours post-infection, 20 µg of equilibrated protein lysates were electrophoretically separated in denaturing polyacrylamide gels, transferred to a PVDF membrane and exposed to autoradiography film.

[0033] FIG. **10**. Bioluminescence of systemically delivered R2636 in mice growing bilateral dnMEK- and caMEK-expressing tumor xenografts. HT-dnMEK and HT-caMEK

tumors were established in the left and right hind limbs of athymic nude mice. Once tumors reached an average volume of 350 mm^3 animals were given a single intraperitoneal injection of 9×10^8 PFU of R2636 virus. Bioluminescence imaging was performed 5 days after intraperitoneal injection.

[0034] FIG. 11. A model for the interaction between activated MEK and the suppression of PKR function during viral infection of tumor cells by $\Delta \gamma_1 34.5$ mutant viruses. Activation of the extracellular signal-regulated kinase (ERK)-kinase (MEK)/ERK pathway (i.e., the MAPK pathway) by either oncogenic activating mutations of Ras isoforms, point mutations within B-Raf alleles, or receptor tyrosine kinase activation/overexpression have been shown to be involved in transformation and tumor progression. In addition, Rasindependent activation of Raf/MEK/ERK signaling is celland tumor type-specific. This Figure schematically illustrates that activated MEK suppresses PKR auto-phosphorylation and effectively blocks PKR-mediated eIF-2a phosphorylation. Tumor cells with activated MEK/ERK signaling, therefore, are exquisitely permissive to $\Delta \gamma_1 34.5$ mutant viral replication and oncolysis.

[0035] FIG. 12. In tumor regrowth studies, systemic delivery of R3616 by intraperitoneal injection resulted in oncolysis of xenografts dependent on tumor MEK activity. Tumor xenografts were established in the hindlimbs of nude mice by injection of 5×10^6 cells per animal. Tumor volume was determined by direct caliper measurement. Once tumors reached a mean volume of $115-150 \text{ mm}^3$, animals were treated on day 0 and day 5 with 2×10^6 , 2×10^7 , or 2×10^8 PFU intraperitoneal or 10^8 PFU intratumoral R3616. Tumor growth was measured by calculating the ratio of tumor volume V to initial tumor volume V₀. A) HT-caMEK B) HT-dnMEK C) Hep3B (high MEK activity) D) PC-3 (low MEK activity)

[0036] FIG. 13. In vivo luciferase imaging of R2636 replication shows that HT-caMEK tumors permitted increasing viral replication and HT-dnMEK tumors restricted viral replication. Intraperitoneal administration of R2636 in HT-caMEK tumor bearing mice allowed viral localization to the hindlimb xenograft and subsequent replication. Tumor xenografts were established as described previously. Mice were injected with intratumoral (5×10^7 PFU) or intraperitoneal (10⁸ PFU) R2636. On days 1, 3, 8, 12, and 22 following R2636 treatment, imaging of luciferase activity was performed on a charge-coupled device camera 15 minutes following IP injection of D-luciferin at 15 mg/kg body weight. A) HT-caMEK, intratumoral B) HT-dnMEK, intratumoral C) HT-caMEK, intraperitoneal D) FIT-dnMEK, intraperitoneal. [0037] FIG. 14. Quantified luciferase activity from HTcaMEK and HT-dnMEK tumor-bearing mice treated with 5×10^7 PFU intratumoral or 10^8 PFU intraperitoneal R2636. Using image analysis software to process images generated from R2636-treated mice bearing HT-caMEK and HT-dn-MEK xenografts, luminescence was quantified as total photon flux, calculated using an area-under-the-curve analysis (MetaMorph). The baseline luminescence in the untreated HT-caMEK tumors was 1.8×10⁵±5.9×10³ photons. In HTcaMEK tumors injected intratumorally with 5×10^7 PFU of R2636, the measured photon activity was $1.8 \times 10^6 \pm 6.6 \times 10^5$, $1.1 \times 10 \pm 3.9 \times 10^6$, $2.7 \times 10^6 \pm 1.2 \times 10^6$, $4.3 \times 10^6 \pm 3.1 \times 10^6$, and $1.6 \times 10^7 \pm 6.7 \times 10^6$ on days 1, 3, 8, 12, and 22 respectively (p=0.042, 0.0208, 0.0726, 0.2149, and 0.0477, respectively, with reference to baseline luminescence in untreated control mice bearing HT-caMEK tumors). HT-caMEK xenografts treated with 10⁸ PFU of intraperitoneal R2636 resulted in

measured photon emission of $6.6 \times 10^5 \pm 1.1 \times 10^5$, $2.4 \times 10^6 \pm 1$. 1×10^6 , $8.4 \times 10^6 \pm 2.7 \times 10^6$, $1.1 \times 10^7 \pm 5.0 \times 10^6$, and $4.8 \times 10^7 \pm 2$. 1×10^7 on days 1, 3, 8, 12, and 22, respectively (p=0.0019, 0.064, 0.0163, 0.0557, and 0.0499, respectively, with reference to untreated control tumor-bearing mice). In untreated control mice bearing HT-dnMEK tumors, baseline luminescence was $9.9 \times 10^4 \pm 1.3 \times 10^4$ photons. HT-dnMEK xenografts injected intratumorally with 5×10^7 PFU R2636 resulted in measured photon activity of $4.0 \times 10^6 1.6 \times 10^6$, $6.8 \times 10^5 \pm 2.3 \times$ 10^5 , $6.9 \times 10^5 \pm 5.0 \times 10^5$, $9.4 \times 10^5 \pm 7.9 \times 10^5$, and $3.2 \times 10^6 \pm 2.8 \times$ 10^6 on days 1, 3, 8, 12, and 22, respectively. HT-dnMEK xenografts treated with 10^8 PFU intraperitoneal R2636 resulted in measured photon activity of $5.0 \times 10^5 \pm 1.4 \times 10^5$, $2.6 \times 10^5 \pm 7.3 \times 10^4$, $2.0 \times 10^5 \pm 1.5 \times 10^5$, $4.2 \times 10^4 \pm 4.1 \times 10^3$, and $4.4 \times 10^4 \pm 1.9 \times 10^3$ on days 1, 3, 8, 12, and 22, respectively.

[0038] FIG. **15**. Immunohistochemistry of HT-caMEK tumor for HSV-1 antigen 5 days following R3616 treatment demonstrated a different pattern of viral spread with intratumoral versus intraperitoneal injection. HT-caMEK xenografts were harvested 5 days following intratumoral $(5\times10^7 \text{ PFU})$ or intraperitoneal (10^8 PFU) injection of R3616. Tumors were formalin-fixed, paraffin-embedded, and probed with anti-HSV-1 antibody. A) Intratumoral injection (low and high power) showed viral spread outward from the needle track. B) Intraperitoneal injection showed a more diffuse pattern with multiple foci of replication.

[0039] FIG. **16**. Viral recovery from HT-caMEK tumors 5 days following intratumoral injection with 5×10^7 PFU R3616 or 10^8 PFU R3616 was comparable. HT-caMEK xenografts were harvested 5 days post-treatment with either intratumoral 5×10^7 PFU or intraperitoneal 10^8 PFU of R3616. Viral titers from homogenized samples were determined by standard plaque formation assays on Vero cell monolayers.

DETAILED DESCRIPTION

[0040] The present invention provides materials and methods for identifying target cells exhibiting a cell proliferation disease, disorder or condition that are amenable to herpes simplex virus-based therapy. The HSV useful in methods of the invention do not express wild-type levels of ICP34.5 and, for that reason, are relatively safe, as exhibited by the attenuated virulence of such HSV. In identifying those cells that not only exhibit a cell proliferative disease, disorder or condition, but also have an active MAPK pathway, e.g., are MEK⁺, the methods of the invention facilitate the identification or diagnosis of those diseases, disorder or conditions amenable to treatment with such HSV. Methods of treating such diseases, disorders or conditions, as well as methods of ameliorating a symptom of such a disease, disorder or condition and methods of preventing such diseases, disorders or conditions, are other beneficial aspects of the invention. In combining HSVs having cytotoxic effects that are relatively specific to cells exhibiting cell proliferative disorders with target cells having an active MAPK pathway, e.g., Ras/Raf/MEK/ERK pathway, the invention provides methods for identifying or diagnosing cell diseases, disorders or conditions best suited to treatment with the modified HSV, as well as methods of preventing, treating, or ameliorating at least one symptom associated with such disease, disorder or condition.

[0041] Studies described herein demonstrate that transduction of a cell line with a constitutively active mitogen-activated protein kinase (MAPK) kinase (MEK) coding region conferred susceptibility to a γ_1 34.5 deficient HSV, such as the HSV R3616 virus, whereas cells transduced with a dominant

negative MEK coding region became more resistant to the recombinant virus (Smith et al., J. Virol. 80:1110-1120 (2006)). MEK is a key regulator in the MAPK pathway and is activated by MAPK kinase kinases (A-RAF, B-RAF, and C-RAF) which are downstream of RAS. MEK, in turn, phosphorylates its only known substrates, the MAPKs (ERK1 and ERK2). MEK is constitutively activated in a wide variety of tumors, and functions to promote cell survival (Ballif et al., Cell Growth Differ. 12:397-408 (2001), Von Gise et al., Mol. Cell. Biol. 21:2324-2336 (2001), and Xia et al., Science 270: 1326-1331 (2001)) and to protect tumor cells from multiple apoptotic stimuli. Extensive analyses of the phenotype of the parent and transduced tumor cells exposed to the $\gamma_1 34.5$ mutant virus indicated that in cells transduced with the constitutively active MEK, PKR is not activated, in contrast to cells transduced with the dominant negative MEK. Further consideration of the disclosure of the invention will be facilitated by a consideration of the following express definitions of terms used herein.

[0042] An "abnormal condition" is broadly defined to include mammalian diseases, mammalian disorders and any abnormal state of mammalian health (i.e., a mammalian condition) that is characterized by abnormal cell proliferation in an animal, such as man, relative to a healthy individual of that species. Preferably, the abnormal cell proliferation involves excess cell proliferation. Exemplary conditions include any of the wide variety of cancers afflicting humans or other animal species (e.g., mammalian species), including solid tumors and metastasized cancers, as well as rheumatoid arthritis, macular degeneration, and the like.

[0043] "Administering" is given its ordinary and accustomed meaning of delivery by any suitable means recognized in the art. Exemplary forms of administering include oral delivery, anal delivery, direct puncture or injection, including intravenous, intraperitoneal, intramuscular, subcutaneous, intratumoral, and other forms of injection, spray (e.g., nebulizing spray), gel or fluid application to an eye, ear, nose, mouth, anus or urethral opening, and cannulation.

[0044] An "effective dose" is that amount of a substance that provides a beneficial effect on the organism receiving the dose and may vary depending upon the purpose of administering the dose, the size and condition of the organism receiving the dose, and other variables recognized in the art as relevant to a determination of an effective dose. The process of determining an effective dose involves routine optimization procedures that are within the skill in the art.

[0045] An "animal" is given its conventional meaning of a non-plant, non-protist living being. A preferred animal is a mammal, such as a human.

[0046] "Ameliorating" means reducing the degree or severity of, consistent with its ordinary and accustomed meaning. **[0047]** "Pharmaceutical composition" means a formulation of compounds suitable for therapeutic administration, to a living animal, such as a human patient. Typical pharmaceutical compositions comprise a therapeutic agent such as an HSV virus not elaborating a wild-type level of active ICP34. 5, in combination with an adjuvant, excipient, carrier, or diluent recognized in the art as compatible with delivery or administration to an animal, e.g., a human.

[0048] "Adjuvants," "excipients," "carriers," and "diluents" are each given the meanings those terms have acquired in the art. An adjuvant is one or more substances that serve to prolong the immunogenicity of a co-administered immunogen. An excipient is an inert substance that serves as a vehicle,

and/or diluent, for a therapeutic agent. A carrier is one or more substances that facilitates manipulation of a substance (e.g., a therapeutic), such as by translocation of a substance being carried. A diluent is one or more substances that reduce the concentration of, or dilute, a given substance exposed to the diluent.

[0049] "Media" and "medium" are used to refer to cell culture medium and to cell culture media throughout the application. As used herein, "media" and "medium" may be used interchangeably with respect to number, with the singular or plural number of the nouns becoming apparent upon consideration of the context of each usage.

[0050] Mindful of the preceding definitions, it is noted that herpes simplex virus mutants lacking the $\gamma_1 34.5$ gene, or lacking the capacity to express active ICP34.5, are not destructive to normal tissues but are potent cytolytic agents in human tumor cells in which the activation of protein kinase R (PKR) is suppressed. Thus, replication of a $\Delta \gamma_1 34.5$ mutant (R3616) in 12 genetically defined cancer cell lines correlated with suppression of PKR but not with the haplotype of Ras (i.e., the Ras-specific genotype). Extensive analyses of two cell lines transduced with either dominant negative MEK (dnMEK) or constitutively active MEK (caMEK) indicated that in R3616 mutant infected cells, dnMEK enabled PKR activation and decreased virus yields, whereas caMEK suppressed PKR and enabled better viral replication and cell destruction in transduced cells in vitro or in mouse xenografts. The results indicated that activated MEK mediated the suppression of PKR and that the status of MEK predicts the ability of $\Delta \gamma_1 34.5$ mutant viruses to replicate and destroy tumor cells. In addition, $\Delta \gamma_1 34.5$ mutant HSV comprising one or more coding regions for the expression of heterologous gene product(s) are useful in effectively converting or ensuring that a tumor cell exhibits a suppressed PKR phenotype, thereby rendering such a cell susceptible to destruction by the $\Delta \gamma_1 34.5$ mutant HSV.

[0051] PKR appears to play a key role in conferring resistance to $\Delta\gamma_1 34.5$ mutants. The importance of PKR to a cell's innate antiviral response to viral infection is underscored by the observation that $\Delta\gamma_1 34.5$ mutants replicate to near wild-type levels in murine embryonal fibroblast (MEF) cells derived from mice lacking PKR. Moreover, $\Delta\gamma_1 34.5$ HSV mutants are virulent in PKR^{-/-} mice, but not in wild-type mice. In addition, exogenous α interferon (INF- α) effectively suppresses $\Delta\gamma_1 34.5$ mutant replication in PKR^{+/+} MEFs, but has no effect in PKR^{-/-} MEFs, while wild-type HSV-1 was reported to be resistant to the anti-viral effects of IFN in these cells. Therefore, replication of mutants lacking $\gamma_1 34.5$ is largely dependent on the ability of cells to activate PKR-dependent pathways of host cell defense.

[0052] PKR also exerts potent growth suppressive effects and apoptotic cell death effects induced by multiple stimuli. Alternatively, inhibition of PKR function by over-expression of catalytically inactive mutants of PKR α and eIF-2 α , transformed NIH 3T3 cells and primary human cells when coexpressed with large T antigen and human telomerase reverse transcriptase (hTERT) in a manner similar to the necessary mitogenic signal transmitted by activated Ras.

[0053] Growth factor withdrawal also induces PKR activation, eIF- 2α phosphorylation and apoptosis in several growth factor-dependent hematopoietic cell lines. Growth factor withdrawal also downregulated the activity of MEK, a critical downstream Ras effector kinase, while overexpression of constitutively active MEK mutants protected growth factordependent cell lines from multiple apoptotic stimuli, including growth factor withdrawal. MEK is a key regulatory kinase activated by MAPK-kinase-kinases (A-Raf, B-Raf, C-Raf) that functions to promote cell survival. Accordingly, MEK and its only known substrate, MAPKs (ERK1 and ERK 2) are constitutively activated in a large percentage of tumors as a consequence of dysregulated growth factor secretion, tyrosine kinase receptor activation, activating mutations in Ras isoforms and somatic activating missense mutations of B-Raf.

[0054] The data disclosed herein establish that PKR activation is suppressed in a subset of cancer cells, thereby rendering them susceptible to viral replication and cytolysis by a $\Delta\gamma_1 34.5$ mutant HSV, e.g., HSV R3616. Using pharmacologic inhibitors of MEK and catalytically active and inactive mutants of MEK, constitutive MEK activity was shown to suppress the viral activation of PKR. The status of MEK correlates with the ability of tumor cells to support the replication of $\Delta\gamma_1 34.5$ mutant HSV viruses and that replication ultimately destroys the host tumor cells. Accordingly, the status of MEK is predictive of those cancer cells most susceptible to destruction by HSV viruses not elaborating wild-type levels of active ICP34.5.

[0055] The invention contemplates any herpes simplex virus, including HSV-1, HSV-2 and hybrids thereof, that does not express a wild-type level of ICP34.5, although it is preferred that the HSV is an HSV-1. Derivatives of these viruses are also contemplated by the invention, provided such derivatives both retain the capacity to exert a cytotoxic or cytopathic effect (i.e., lytically infect) at least one tumor cell type and do not express a wild-type level of ICP34.5. Suitable viral derivatives include HSV having at least one mutation, silent or not, in addition to any mutation associated with the failure to express a wild-type level of ICP34.5, as well as viral fragments. Preferably, such viral derivatives retain the ability to form infectious virion, eliminating the need for engineered forms of delivering the viral agent.

[0056] The invention also comprehends HSV having any known mechanism of reducing the level of expressed, active ICP34.5 below wild-type levels including, but not limited to, $\gamma_1 34.5$ deletion mutants (i.e., $\Delta \gamma_1 34.5$ mutants) that either express a truncated gene product of reduced or undetectable activity or that do not express any gene product. Alternatively, or in conjunction with a deletion mutant, the invention contemplates an insertion mutant that reduces or eliminates the ICP34.5 activity of any expressed gene product, missense or nonsense mutations that eliminate or reduce expressed ICP34.5 activity in terms of either the level or stability of such activity, second-site mutations such as the insertion of an anti-sense coding region in the HSV genome, non-coding region mutations affecting the expression control of $\gamma_1 34.5$ such as a down-regulating mutation in a promoter affecting γ_1 34.5 expression, or any other HSV modification known in the art to reduce the level of expressed ICP34.5 activity below wild-type levels. Preferably, the modification of HSV, e.g., the mutation, is present in each copy of the relevant genetic element (e.g., a mutation in the coding region of $\gamma_1 34.5$ is preferably found in both copies of $\gamma_1 34.5$ found in the HSV genome). The invention also embraces singular modifications of HSV where the genetic element is naturally present as a single copy in HSV or where an HSV derivative has been rendered hemizygous for the relevant genetic element. Preferably, the level of expressed ICP34.5 is reduced below detectable levels.

[0057] With respect to heterologous coding regions, the invention contemplates a variety of coding regions useful in effectively suppressing PKR when expressed. Suitable heterologous coding regions include the coding region for a functional member of the MAPK (Ras/Raf/MEK/ERK) pathway, and preferably a constitutively active member of the pathway. Exemplary Ras coding regions encode any of wild-type N-Ras (SEQ ID NO:7 encoding SEQ ID NO:8), K-Ras (SEQ ID NO:9 encoding SEQ ID NO:10) or H-Ras (SEQ ID NO:11 encoding SEQ ID NO:12), as well as mutant active Ras isoform variants. For compact yet complete disclosure, wildtype sequences of members of the MAPK pathway are provided and the sequence differences from wild type are indicated for the variants. The most common mutations are at residues C/G12, G13 and Q61. There are numerous examples of active mutant Ras isoforms known in the art including, but not limited to, K-RasV12, K-RasD12, K-RasG12, H-RasV12, K-RasD13, and N-RasV12 (Bos, 49(17):4682-9, 1989, incorporated herein by reference).

[0058] Exemplary Raf coding regions encode any one of the wild-type forms of Raf (SEQ ID NO:13 encoding SEQ ID NO:14 for B-Raf), Raf-CAAX (Leevers et al. Nature 369 (6479):411-4, 1994, incorporated herein by reference), RafS338A (Diaz et al. Molecular and Cellular Biology 17(8): 4509, 1997; incorporated herein by reference), RafS339A (id.), or Raf BXB (Bruder et al., Genes & Dev. 6:545-556, 1992, incorporated herein by reference. Further, the invention embraces V600E B-Raf (Andersen et al., Cancer Res. 1 64:5456-60, 2004, incorporated herein by reference notwithstanding the identification therein to V599E due to a sequence error in the publication). The variations from the wild-type Raf sequence found in any of Raf-CAAX, RafS338A, RafS339A, Raf BXB, and V600E B-Raf can be present in any combination. Two isoforms of MEK are found in humans, i.e., MEK1 and MEK2. The invention comprehends wildtype MEK1 (SEQ ID NO:1, encoding SEQ ID NO:2) and wild-type MEK2 (SEQ ID NO:5 encoding SEQ ID NO:6). Also contemplated are active mutant MEKs, including constitutively active MEKs. Examples of active mutants known in the art and embraced by the invention include $\Delta N3MKK1$ S218E/S222D, an N-terminal truncation mutant of MEK1 that also includes missense mutations at residues 218 and 222; an analogous variant (N-terminal truncation and amino acid substitutions at the equivalent of positions 218 and 222 of MEK1) of MEK2 is also contemplated (Mansour, et al., Science 265(5174):966-70, 1994, incorporated herein by reference). Further, full-length MEK1 and MEK2 proteins containing a missense mutation yielding S281E or S222D, and preferably both mutations, are contemplated.

[0059] The ERK component of the MAPK pathway is present in two isoforms, ERK1 and ERK2, in humans. Contemplated by the invention are HSV comprising coding regions for wild-type ERK, including wild-type human ERK1 (SEQ ID NOS:15 and 17 encode SEQ ID NOS:16 and 18, respectively, with SEQ ID NOS:15 and 16 relating to transcript variant 1 and SEQ ID NOS:17 and 18 relating to transcript variant 2) and/or ERK2 (SEQ ID NO:3 encodes SEQ ID NO:4 of ERK2) (Emrick, et al., J. Biol. Chem. 276:46469-46479, 2001, incorporated herein by reference). Exemplary variants of ERK2 include, but are not limited to, variants known in the art such as variants containing an amino acid substitution at E58Q, D122A, S151A, or S221A (Zhang,

et al., J. Biol. Chem. 278: 29901-29912, 2003, incorporated herein by reference), as well as S151D or L73P (Emrick et al., supra).

[0060] In addition to the foregoing wild-type and variant members of the MAPK pathway, the HSV according to the invention may comprise fusion proteins, such as a MEK2-ERK1 fusion as described in Robinson, et al., Curr. Biol. 8:1141-1150, 1998, incorporated herein by reference. The MEK2-ERK1 fusion of Robinson et al. encodes a full length MEK2 (SEQ ID NO:6 encoded, e.g., by SEQ ID NO:5) fused to a coding region for a linker, such as a ten-amino acid linker (Glu-Gly), in turn fused to a full-length ERK1 (SEQ ID NO:16 or 18 encoded, e.g., by SEQ ID NO:15 or 17, respectively). The linker can vary in length and/or sequence, provided that it is compatible with secondary and tertiary structure formation required for activity as an ultimate suppressor of PKR activity. Also contemplated are full-length fusions of MEK1-ERK1, MEK2-ERK2, MEK1-ERK2 and fusions in which the orientation of the two proteins are reversed, along with a linker conforming to the requirements provided above. Collectively, each of the MEK1/2-ERK1/2 and ERK1/2-MEK1/2 fusions is referred to herein as a MEK-ERK fusion. Further, N-terminally deleted MEK1 or MEK2, particularly N-terminal deletions of the four leucine residues contributing to the nuclear export signal, as described in Robinson et al., supra, incorporated herein by reference, are contemplated as elements of MEK-ERK fusions. In addition, conservative coding regions specifying amino acids that are conservative substitutions for the above-identified wild-type variants are envisaged (e.g., any conservative substitution for the serine residues as positions 218 and 222 in the above-described upregulated MEK variants is contemplated). In the present context, a conservative substitution preferably conforms to conventional understanding and more preferably conserves the functional characteristic (contribution to activity level) of the amino acid being substituted, such as the like susceptibility to phosphorylation of S, T, Y and other phosphorylatable amino acids (D, E, H). Non-conservative substitutions, deletions and insertions (relative to wild-type counterparts rather than the upregulated variants described above) that result in upregulated activity of the MAPK pathway are also comprehended, such as those non-conservative substitutions, deletions and insertions of coding regions of the MAPK pathway known in the art.

[0061] Beyond the various coding regions of the MAPK pathway, HSVs according to the invention may comprise a heterologous (foreign to wild-type HSV) coding region for a catalytically inactive mutant of PKR or for a catalytically inactive mutant of eIF- 2α , as known in the art. Further, HSV comprising a coding region for a growth factor, the overexpression of which is known in the art to result in upregulated activity of the MAPK pathway is suitable, as is an active mutant of a tyrosine kinase receptor that is known in the art to regulate the activity of the MAPK pathway.

[0062] The methods of the invention comprehend any process or assay known in the art for detecting or measuring a protein indicative of the status of a MAPK pathway in a cell. Suitable proteins include, but are not limited to, members of the Ras/Raf/MEK/ERK module of the MAPK pathway, e.g., any form of Ras, a G-protein specifically interacting with any such form of Ras, Raf (A-Raf, B-Raf, Raf-1; also referred to as Raf-A, Raf-B, and Raf-C, respectively), MEK1 (MKK1), MEK2 (MKK2), ERK1, and ERK2. Any known isoform of a protein involved in a MAPK pathway may be the sole com-

ponent detected or measured, or may be one of a plurality of elements detected or measured, for example in the context of assays measuring a plurality of isoforms of a given protein or assays collectively measuring one or more isoforms of at least two proteins in a MAPK pathway. In preferred embodiments, the proteins being detected or measured are phosphorylated derivatives of the proteins, wherein the phosphorylation is known in the art to be associated with activation of that protein. Further, it is expected that accessory proteins in a MAPK pathway, e.g., exchange factors, modulators, scaffolding molecules, adapter proteins, and/or chaperones, that are known to vary in activity (whether that variance is attributable to changes in specific activity or active protein level) in a manner predictive of MAPK pathway activation, may also serve alone or in combination with other suitable proteins as the basis for detecting and/or measuring MAPK pathway status. Exemplary accessory proteins include, but are not limited to, MEKK-1, mos, Tpl-2, SOS, SUR-8, KSR, PBS2, 14-3-3, Hsp90, Hsp50/Cdc37, FKBP65, Bag-1, Rsk-1, and proteins identified in Kolch, W., Nat. Rev. Cell Biol. 6:827-837 (2005), incorporated herein by reference. Preferred accessory proteins are human proteins identified above and human orthologs of non-human proteins identified above. In other processes of the invention, comparative measures of one or more isoforms of one or more MAPK pathway proteins is obtained to provide a comparative measure indicative of MAPK pathway status. Preferred proteins for use in any of these processes include MEK1, MEK2, ERK1 and ERK2.

[0063] Yet other processes according to the invention involve haplotyping a target cell, by which is meant the partial or complete characterization of at least one genetic element involved in the expression of at least one isoform of a MAPK pathway protein indicative of MAPK pathway status. The characterizations will typically provide partial or complete sequence information for at least one genetic element, which may be obtained by any method known in the art, including but not limited to chemical or enzymatic sequencing techniques, whether automated or not. Also contemplated are hybridization-based technologies using one or more probes of any suitable length and under any suitable hybridization conditions that are compatible with the reliable identification of a particular genetic element predictive, alone or in combination with additional information, of MAPK pathway status. Preferably, the probe is an oligonucleotide of 8-50 nucleotides and stringent hybridization conditions are employed to facilitate the inferential determination of at least a partial sequence diagnostic of MAPK pathway status. Also included in the haplotyping processes of the invention are genetic complementation studies in which distinct naturally existing, or engineered, phenotype are associated with the relevant haplotypes. Any other process known in the art for determining the absolute or relative level of activity of at least one isoform of a protein in a MAPK pathway that is predictive of MAPK pathway status is also embraced by the invention.

[0064] The invention also provides methods of treating diseases, disorders or conditions characterized by abnormal cell proliferation, typically hyperproliferation, provided that the abnormally proliferating cells have a MAPK pathway of active status. Diseases, disorders or conditions suitable for treatment include any form of cancer, including solid-tumor cancers such as inoperably located tumors or metastasized cancers, as well as rheumatoid arthritis, macular degeneration, and any disease, disorder or condition characterized by abnormal cell proliferation, as would be understood in the art, provided the cells have an active MAPK pathway. A related aspect of the invention provides methods for ameliorating at least one symptom associated with such disease, disorder or condition. For example, the invention contemplates administering an effective dose of an HSV that does not express a wild-type level of active ICP34.5 to an organism suffering from a cancerous condition due to MAPK-active cancer cells, wherein the dose is sufficient to reduce the pain, swelling, or other physiological symptom attending tumor growth. A benefit provided by these methods of the invention is that the HSV therapeutic is effective in embodiments of the disease, disorder or condition that have proven refractory to treatment with conventional therapies, such as inoperable tumors of the brain or other inaccessible regions of a body as well as metastasized cancers.

[0065] The invention further contemplates prophylactic methods wherein a dose of an HSV, as described above, that is known to be effective in ameliorating a symptom or treating a disease, disorder or condition characterized by abnormal cell proliferation is administered to an organism at risk of developing such a disease, disorder or condition.

[0066] Administration of the above-described HSV compositions according to the invention is by any known route, provided that the target cell or tissue is accessible via that route. Notably, the experimental results disclosed herein establish that two isogenic tumor cell lines differing in susceptibility to the $\Delta \gamma_1 34.5$ mutant R3616 were used to study the distribution and persistence of virus delivered by different routes. As expected, the virus replicated better and persisted longer in the susceptible (high MEK activity) tumors in mouse xenografts. A significant finding was that systemic administration to the tumor-bearing mouse was as effective as intratumoral delivery with regard to tumor oncolysis. Accordingly, the pharmaceutical compositions may be introduced into the subject by any conventional method, e.g., by intravenous, intradermal, intramuscular, 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.

[0067] 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.

[0068] 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} , 1011, 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.

[0069] The pharmaceutical compositions and methods of the invention are useful in the fields of human medicine and veterinary medicine. Thus, the subject to be treated (whether to treat or prevent a disease, disorder or condition, or to ameliorate a symptom thereof) 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.

[0070] Having provided a general description of the various aspects of the invention, the following disclosure provides examples illustrative of the invention, wherein Example 1 describes the materials and methods used in conducting the studies reported herein, Example 2 discloses data establishing the correlation of $\gamma_1 34.5$ deficient HSV replication and the MAPK (e.g., MEK) phenotype of host cells, Example 3 reveals that an N-Ras mutation enables efficient replication of R3616 mutant HSV virus in human fibrosarcoma cells; Example 4 discloses that the inhibition of MEK by PD98059 (a known MEK inhibitor) resulted in increased levels of PKR phosphorylation, decreased viral protein accumulation, and diminished replication of mutant HSV virus R3616; Example 5 discloses data showing that viral activation of PKR by mutant HSV R3616 is suppressed in tumor cell lines that overexpressed constitutively active MEK, while expression of dominant negative MEK increased PKR activation and restricted R3616 viral replication; Example 6 establishes that intratumoral inoculation of R3616 mutant HSV virus resulted in tumor regression in tumors expressing caMEK, but not in tumors expressing dnMEK; Example 7 shows that the systemic administration of a recombinant HSV virus R2636, expressing the gC-Luc construct, targeted tumor tissue overexpressing constitutively active MEK; and Example 8 reveals that various routes of administration of mutant HSV, including systemic delivery, are suitable for the treatment of MEKoverexpressing tumors.

Example 1

Materials and Methods

[0071] Molecular Constructs—Constitutively active MEK-1-encoding (caMEK) and dominant negative MEKlencoding (dnMEK) plasmids, designated pNC84 and pNC92, respectively, were provided by J. Charron (Quebec, Canada). Their constructions are detailed in Ref. 34, incorporated herein by reference. Briefly, coding sequences for serine residues 218 and 222 of human wild-type MEK-1 were mutated either to aspartic acid residues (D218S and D222S), creating a constitutively active, phosphomimetic mutant, or to alanine residues (A218S and A222S) to create a dominant negative-functioning kinase mutant. The mutant MEK-1 cDNAs contain an in-frame FLAG epitope at the N-terminus under the transcriptional control of a CMV promoter in the pCMV-Tag2b mammalian expression vector (Qiagen Inc. Valencia, Calif.). Orientation and cDNA insert sequence were confirmed by DNA sequencing.

[0072] Cell Culture—PC-3 and DU145 (human prostate cancer), Panc-1, BxPc3, and MiaPaCa2 (human pancreatic cancer) MCF7 and MDA-MB-231 (human breast cancer), DLD-1 and WiDr (Colorectal cancer), Hep3B (human hepatoma), Vero (Green Monkey Kidney) cell lines were originally obtained from the American Type Culture Collection (Manassas, Va.). The Huh7 hepatoma cell line was originally obtained from J. R. Wands (Harvard Medical School, Boston, Mass., USA). The HT1080 (human fibrosarcoma) cell line containing one wild-type and one oncogenic (Q61K) N-ras allele (1, 40) was also obtained from the American Type Culture Collection. HT1080 cells having lost the activated mutant N-ras allele were obtained from EJ, Stanbridge (Irv-

ine, Calif.) and have been described previously and published as MCH603 (40). HT-caMEK and HT-dnMEK are clonal cell lines constructed from the parental cell line HT1080, a human fibrosarcoma. The methods of transfection with genetic constructs pNC84 and pNC92, which express constitutively active and dominant negative MEK respectively, are described in Smith et al., J. Virol. 80:1110-1120 (2006) and Mansour et al., Biochem. 35:15529-15536 (1996), both incorporated herein by reference. The above cell lines were grown in DMEM (GIBCO/Invitrogen Corporation, Grand Island, N.Y.)/10% FCS (Intergen, Purchase, N.Y.)/1% penicillin-streptomycin at 37° C. and 7% CO₂. HT-caMEK and HT-dnMEK were grown in medium supplemented with 500 μ g/ml of G418 (Geneticin, Gibco BRL).

[0073] Viruses—HSV-1(F) is the prototype wild-type HSV-1 strain (18). The derivation and properties of the recombinant virus R3616, which lacks both copies of the γ_1 34.5 gene (11), and recombinant R2636 carrying the luciferase gene driven by the glycoprotein C (gC) promoter (gC-luc) in place of the γ_1 34.5 gene, were reported in Nakamura et al. (ref. 37), and that description is incorporated herein by reference.

[0074] Construction of stable cell lines—Mutant FLAGtagged caMEK-1- or dnMEK-containing plasmids were transfected into replicate cultures of HT1080 or MiaPaCa2 cells on 60 mm dishes using Superfect Reagent (Qiagen Inc. Valencia, Calif.). Briefly, 5 µg of plasmid DNA was diluted in 300 µl of serum and antibiotic free DMEM, complexed with Superfect (20 µl) reagent for 10 minutes at room temperature and added to cells at 37° C. for 6 hours, after which medium was removed and replaced with DMEM containing 10% calf serum. After 24 hours of incubation, the cells were harvested, suspended in 5 ml of DMEM medium containing 10% FCS and 1 ml of this cellular suspension was grown on separate 100 mm dishes in a total volume of 10 ml of DMEM containing 10% calf serum supplemented with antibiotics (e.g., penicillin and streptomycin, each at conventional concentrations well-known in the art) and 800 µg/ml of G418 (Geneticin [Gibco BRL]). Medium containing G418 was replaced every four days until approximately 2 weeks after culture initiation, when cell colonies were visible and could be selected for clonal expansion using sterile cloning cylinders, as described in Gupta et al. (ref. 22), which is incorporated herein by reference. The level of FLAG-MEK expression was assessed by immunoblotting 20 µg of equilibrated lysates from isolated clones using a monoclonal antibody to the FLAG epitope (Sigma Co. St. Louis, Mo.). Clonal transfectants derived from the HT1080 parent cell line, designated HTcaMEK and HT-dnMEK, and from the MiaPaCa2 parent cell line, designated Mia-caMEK and Mia-dnMEK, with equivalent levels of FLAG-MEK expression, were chosen for further analysis.

[0075] Viral Infection—Cells were seeded onto 60 mm dishes at 1×10^6 cells per dish. The next day cells were generally exposed to the viruses (1 or 10 plaque forming units per cell (PFU/cell)) for 2 hours at 37° C. and then removed and replaced with medium containing 1% calf serum. The infection continued at 37° C. for the length of time indicated for each experiment. Cells were either labeled for de novo protein synthesis, harvested for immunoblotting, or collected for assaying viral recovery on Vero cell monolayers as previously described in Chou et al. (ref. 11), incorporated herein by reference.

[0076] [³⁵S] Methionine Labeling—For metabolic labeling experiments, at 11 hours post-infection cells were washed once in warm medium 199V containing 1% calf serum lacking methionine (Sigma Chemical Co., St. Louis, Mo.) and incubated for an additional hour in 199V methionine-free medium after which cells were overlaid with medium 199V lacking methionine but supplemented with 100 μ Ci of [³⁵S] methionine (specific activity, >1000 Ci/mmol; Amersham Pharmacia Biotech) per ml and incubated for an additional two hours. The cells were then harvested at 14 hours postinfection, solubilized in lysis buffer [20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 100 µM sodium orthovanidate, 1 µg leupeptin per ml and 1 mM PMSF], sonicated for 10 seconds, and insoluble material was removed by centrifugation. Total protein from the supernatant was quantified by the Bradford method (BioRad Laboratories, Hercules, Calif.) and 20 µg of equilibrated protein was subjected to electrophoresis in denaturing 12% (vol/vol) polyacrylamide gels, transferred to Polyvinylidene Difluoride membranes (PVDF; Millipore Corporation, Bedford, Mass.) and subjected to autoradiography.

[0077] Immunoblotting—Experiments to analyze the accumulation of viral proteins and phosphorylation of ERK, PKR and eIF- 2α were performed on whole-cell lysates harvested on ice at either 12 or 14 hours post-infection with lysis buffer, sonicated for 10 seconds, and clarified by centrifugation. Total protein from the supernatant was quantified by the Bradford method and 20 µg of equilibrated protein was subjected to electrophoresis in 12% or 7.5% (vol/vol) denaturing polyacrylamide gels, transferred to PVDF membranes (Millipore Corporation), blocked, and reacted with primary antibody followed by appropriate secondary antibody.

[0078] Antibodies—Polyclonal antibodies to the total and phosphorylated forms of PKR (Thr446), eIF- 2α (Ser51), and ERK (Thr202/Tyr204) were purchased from Cell Signaling Technology (Beverly, Mass.). Polyclonal antibody to ICP27 was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif.). Monoclonal antibody to Glycoprotein C was purchased from Fitzgerald Industries International, Inc. (Concord, Mass.). Antibodies to Us11 and UL42 were described in refs. 43 and 45, each incorporated herein by reference for the relevant description. Secondary antibodies (Cell Signaling Technology, Beverly, Mass.) were conjugated to horseradish peroxidase. Protein bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, III.).

[0079] Inhibitor studies—For experiments employing the known MEK inhibitor, PD98059, HT1080 cells were starved over night in serum-free medium and then exposed to $40 \,\mu$ M of PD98059 (EMD Biosciences, San Diego, Calif.), or DMSO (1:1000 dilution) 6 hours prior to, and during, infection. At 12 hours post-infection, whole cell lysates were created as described above for immunoblotting.

[0080] In vitro viral recovery—Cells were exposed to viruses (1 plaque forming unit per cell (PFU/cell)) for 2 hours in serum-free medium at 37° C., after which the supernatant was aspirated and cells were overlaid with 2 ml of DMEM containing 1% calf serum and incubated at 37° C. At 36 hours post-infection, 2 ml of sterile skimmed milk was added to triplicate samples and plates were frozen at -80° C. Frozen cell suspensions were thawed and sonicated three times for 15 seconds each and titered on Vero cells.

[0081] HTcaMEK, HTdnMEK xenograft regression studies—HT-dnMEK and HT-caMEK tumor xenografts were established in the right flank of 5- to 6-week-old female, athymic nude mice (Fredrickson Cancer Research Institute, Bethesda, Md.) by injection of 10^7 cells in 100 µl of warm phosphate-buffered saline. After one week, tumor xenografts grew to approximately 250 mm³ and were randomized to 7 animals per treatment group. Mice were injected intratumorally with 5×10⁷ PFU of R3616 using a Hamilton syringe. Tumor xenografts were measured biweekly with calipers and tumor volumes were calculated with the formula $(1\timesw\timesh)/2$, which is derived from the formula for an ellipsoid $(TMd^3)/g$ (24).

[0082] For the studies described in Example 8, tumor xenografts in athymic nude mice were established by hindlimb injection of 5×10^6 HT-caMEK, HT-dnMEK, Hep3B, or PC-3 tumor cells. At a mean volume of 115-150 mm³, the tumors were treated on days 0 and 5 by administration of R3616 via intratumoral injection of 5×10^7 PFU or intraperitoneal injection of 10^6 , 10^7 , or 10^8 PFU of R3616 recombinant virus. Tumor xenografts were measured twice weekly with calipers. Tumor volume was calculated with the formula $(1\timesw\times h)/2$, derived from the formula for the volume of an ellipsoid (d3/g). Tumor growth was measured at each time point by calculating the ratio of tumor volume (V) to initial tumor volume (V0).

[0083] Bioluminescence Imaging-HT-dnMEK and HTcaMEK tumor xenografts were established in the left and right hind limbs, respectively, of athymic nude mice by injection of 1×10^7 cells in 100 µl of warm phosphate-buffered saline. All animal studies were performed in accordance with The University of Chicago Animal Care and Use Committee standards. Once tumors grew to an average volume of 350 mm³, 9×10^8 PFU of virus R2636 in a total volume of 100 µl were injected intraperitoneally (IP) using a 30-gauge needle. At 5 days after IP injection, imaging of firefly luciferase in mice was performed on a charge-coupled device camera (Roper Scientific Photometrics, Tucson, Ariz.). Animals were injected IP with 15 mg/kg body weight with D-luciferin (Biotium, Hayward, Calif.). After 5 minutes, animals were anesthetized with IP injection of ketamine (75 mg/kg) and xylazine (5 mg/kg) for imaging, which was performed 10 minutes after the injection of D-luciferin.

[0084] Again for the studies described in Example 8, HTdnMEK and HT-caMEK xenografts were established in the right hindlimb of athymic nude mice by injection of 5×10^6 cells. At initial tumor volumes of 175 ± 60 mm³ for HT-caMEK and 131 ± 22 mm³ for HT-dnMEK, mice were injected with either intratumoral (5×10^7 PFU) or intraperitoneal (IP) (10^8 PFU) R2636. Animals were imaged on days 1, 3, 8, 12, and 22 following viral injection. Imaging was performed on a charge-coupled device camera (Roper Scientific Photometrics, Tucson, Ariz.). On days of imaging, animals were injected IP with D-luciferin (Biotium, Hayward, Calif.) at a dose of 15 mg/kg of body weight. After 5 minutes, animals were anesthetized with IP injection of ketamine (75 mg/kg) and xylazine (5 mg/kg) for imaging, which was performed 10 minutes after injection of D-luciferin.

[0085] Quantification of bioluminescence imaging data— The relative intensity of transmitted light from animals infected with virus R2636 are represented as pseudocolor images with intensity ranging from low (blue) to high (red). Gray-scale images were superimposed on the pseudocolor images using MetaMorph image analysis software (Fryer Company, Huntley, Ill.). Data for total photon flux were calculated using area under the curve analysis (MetaMorph).

Example 2

Correlation of γ_1 34.5 deficient HSV replication and MEK phenotype of host cells

[0086] The replication of R3616 ($\Delta \gamma_1 34.5$) mutant virus in human tumor cell lines is cell line dependent and correlates with constitutive activation of MEK. Replicate cultures of 13 cell lines derived from human tumors were exposed to R3616 (1 PFU/cell). The cells were harvested at 36 hours postinfection and viral yields were measured by plaque assays on Vero cell monolayers. As shown in FIG. 1, the yields of R3616 mutant virus were variable, ranging from 1×10^4 to 3×10^7 PFU/ml. To determine whether the variability in virus yields was reflected in the accumulation of viral proteins, cultures of human tumor cell lines were exposed to R3616(10 PFU/cell). Vero cells were included as an example of a nonmalignant cell type that supports replication of $\gamma_1 34.5$ deficient viruses. At 11 hours post-infection, the cells were rinsed, starved of methionine for one hour, and then incubated in methionine-free medium supplemented with 100 µCi of ³⁵S] methionine per ml for two additional hours. At 14 hours post-infection, 20 µg of equilibrated protein lysates were electrophoretically separated in denaturing polyacrylamide gels, transferred to a PVDF membrane, and exposed to autoradiography film. As shown in FIG. 2, panel A, the accumulation of viral proteins was reduced in cell lines that restricted viral replication compared to cell lines where viral yields were abundant.

[0087] To correlate the differences in the accumulation of viral proteins with the activation of PKR, replicate cultures of cell lines shown in FIG. **2**, panel A, were exposed to R3616 (10 PFU/cell) for 14 hours. Lysates were harvested and 20 µg of equilibrated whole-cell lysate were electrophoretically separated in denaturing polyacrylamide gels, transferred to PVDF membranes, and reacted with antibody specific for the phosphorylated form of PKR in which Thr446 is phosphorylated. As shown in the upper panels of FIG. **2**, B, PKR phosphorylation was elevated in the cell lines which yielded reduced viral protein accumulation (e.g., PC-3, MCF-7) and lowest in cell lines that exhibited increased levels of viral protein accumulation (e.g., HT1080, Panc-1, Hep-3B, Vero), while total PKR levels were similar.

[0088] The presence of known activating mutations within the commonly mutated oncogenic (K-, H-, N-Ras) isoforms of Ras, however, did not directly correlate with the observed differences in viral recovery from the representative cell lines identified in FIG. 1. Therefore, the constitutive activity of the downstream effectors of Ras, MEK and its substrate, ERK, which when inhibited results in the loss of the inhibitory functions of Ras on PKR (19), were examined. To determine endogenous constitutive MEK activity, uninfected cells were plated to confluence, serum-starved for 12 hours, and then immunoblotted for the phosphorylated and total forms of the MEK substrate, p42 and p44 MAPK (ERK2 and ERK1, respectively), see FIG. 2 B, lower panels. Cell lines that demonstrated increased protein synthesis and suppressed PKR activation following infection with mutant R3616 demonstrated elevated baseline levels of ERK phosphorylation. In contrast, cancer cell lines that demonstrated PKR activation, inhibited protein synthesis, and decreased viral recovery following infection with R3616 demonstrated decreased or undetectable levels of ERK phosphorylation.

Example 3

N-Ras Mutation Enabled Efficient Replication of R3616 Mutant Virus in Human Fibrosarcoma Cells

[0089] To test the hypothesis that Ras/Raf/MEK/MAPK (ERK) signaling suppresses PKR function, replication of R3616 mutant virus in two human fibrosarcoma cell lines that differ only by the expression of an oncogenic mutant allele of N-Ras were measured. HT1080 cells contain an endogenous activating mutant allele of N-Ras, whereas the MCH603 cell line, a variant of HT1080 cells in which the mutant allele has been deleted, contains only wild-type N-Ras (40). Activated MEK is a prerequisite for the Ras-dependent aggressive tumorigenic phenotype of HT1080 cells and the two cell lines differed dramatically in the constitutive levels of MEK activation, as well as in activation levels of downstream members of the Ras signaling pathway (21). The viral yields of HSV-1(F) and R3616 (1 PFU/cell) at 36 hours post-infection are shown in FIG. 3. The results led to two significant observations. First, the yield of HSV-1(F) from the MCH603 cell line was approximately 10-fold lower than that obtained from HT1080 cells $(3.1 \times 10^7 \text{ compared to } 3.5 \times 10^6)$, respectively. Second, the yield of R3616 mutant virus in HT1080 cells was similar to that of wild-type virus $(1.8 \times 10^7 \text{ versus } 3.1 \times 10^7)$, indicating that $\gamma_1 34.5$ function was not necessary during the course of infection in this cell line. In contrast, the yield of R3616 mutant virus was approximately 10-fold lower than that of wild-type virus in MCH603 cells, with yields of 4.8× 10^5 compared to 3.5×10^6 , respectively. Therefore, the presence of an activating N-Ras mutation enhanced the replication of both wild-type and mutant virus and that effect was greater on the virus lacking a functional $\gamma_1 34.5$ gene.

[0090] To determine whether virus yields correlate with overall levels of the accumulation of viral proteins, replicate cultures of HT1080 or MCH603 cells were mock-infected or exposed to viruses R3616 or HSV-1(F) (10 PFU/cell). At 11 hours post-infection, the cells were rinsed, starved of methionine for one hour, and then supplemented with 100 µCi/ml of [³⁵S] methionine for two additional hours. At 14 hours post-infection, 20 µg of equilibrated protein lysates were electrophoretically separated in denaturing polyacrylamide gels, transferred to PVDF membranes and subjected to autoradiography. The results shown in FIG. 4 are congruent with viral yields obtained from the two cell lines. Specifically, the abundance of labeled proteins in MCH603 cells infected with wild-type virus was significantly greater than that observed in the same cells infected with R3616 mutant virus, with both of the MCH603 protein yields being lower than the amounts of proteins accumulating in HT1080 cells infected with either mutant or wild-type virus.

[0091] Lastly, the correlations of each of (1) virus yields and (2) viral protein levels accumulating in infected cells with each of (3) PKR activation and (4) phosphorylation of eIF- 2α , were assessed. Electrophoretically separated proteins of lysates from cells infected with R3616 and HSV-1(F) (10 PFU/cell) were harvested at 14 hours post-infection and probed with antibodies to PKR and the phosphorylated forms of PKR (P-Thr446) and eIF2 α (P-Ser51). As shown in FIG. **5**, both PKR and eIF- 2α were phosphorylated in MCH603 cells infected with R3616 mutant virus. In contrast, only trace amounts of phosphorylated PKR and eIF-2 α were detected in infected HT1080 cells.

Example 4

Inhibition of MEK by PD98059 Resulted in Increased Levels of PKR Phosphorylation, Decreased Viral Protein Accumulation and Diminished Replication of Mutant Virus R3616

[0092] To determine if MEK mediates the observed mutant Ras-dependent suppression of PKR activation and resultant accumulation of viral proteins in HT1080 cells infected with mutant virus R3616, the relative expressions of representative a (ICP27), β (U142) and γ_2 (glycoprotein C) viral proteins in cells treated with a specific chemical inhibitor of MEK-1 (PD98059) were compared. Replicate cultures of HT1080 cells were serum-starved overnight prior to exposure to equal volumes of DMSO or PD98059 (40 µM) for 6 hours prior to infection with R3616 mutant virus (10 PFU/cell). DMSO or drug treatment was then continued until the cells were harvested at 12 hours post-infection. The cells were then lysed and the lysates were subjected to electrophoresis in denaturing polyacrylamide gels, followed by transferring to PVDF membranes and reacting with antibody to ICP27, UL42, or gC. As shown in FIG. 6, panel A, treatment with PD98059 had a slight effect on the accumulation of ICP27 and UL42 proteins but a very dramatic decrease in the amounts of gC that accumulated in HT1080 cells infected with R3616. To test whether the decrease in the accumulation of gC correlated with activation of PKR, the electrophoretically separated lysates were also probed with antibody to the auto-phosphorylated form of PKR (P-Thr446). The presence of PD98059 prior to, and during, infection with R3616 increased the amount of activated PKR in HT1080 cells (FIG. 6, panel B). [0093] These results are consistent with the earlier report that in wild-type virus-infected cells, PKR activation is concurrent with the onset of viral DNA synthesis and enhanced transcription of late genes. In R3616 mutant virus-infected cells, the phosphorylation of eIF-2 α by PKR causes a significant reduction of viral proteins whose accumulation is dependent on viral DNA synthesis (14). In contrast, viral proteins whose synthesis is not dependent on the onset of viral DNA synthesis (e.g., ICP27, UL42 protein) were minimally affected by the activation of PKR.

[0094] Finally, to determine if MEK inhibition affects viral replication, DMSO or PD98059 (40μ M) was added to replicate cultures of HT1080 cells 6 hours prior to, and during, infection with R3616 (1 PFU/cell). The cells were harvested at 36 hours post-infection and viral yields were measured by plaque assays on Vero cell monolayers. In the presence of PD98059, the yield of R3616 mutant virus was approximately 15-fold lower than in the presence of DMSO (4.14×10^6 compared to 1.67×10^5 PFU/ml).

Example 5

Viral Activation of PKR by Mutant R3616 is Suppressed in Tumor Cell Lines that Overexpressed Constitutively Active MEK, while Expression of Dominant Negative MEK Increased PKR Activation and Restricted R3616 Viral Replication

[0095] To study the potential relationship between MEK kinase activity and PKR activation in R3616-infected cancer

cells, cell lines were created that stably express either a constitutively activated mutant of MEK (caMEK) or a dominant negative mutant of MEK (dnMEK) from two tumor cell lines that differ dramatically in the magnitude of endogenous MEK activity and the ability to support R3616 viral replication. MEK is constitutively active in the HT1080 human fibrosarcoma cell line. This cell line, as shown in FIG. 1-3, is also highly permissive to R3616 viral replication and demonstrates suppressed viral activation of PKR. In contrast, the MiaPaCa2 cell line, which is derived from a patient with poorly differentiated malignant pancreatic adenocarcinoma, contains oncogenic Kras mutations in both alleles but demonstrates nearly undetectable levels of constitutively active MEK (50). The MiaPaCa2 cell line severely restricts R3616 viral replication and demonstrates robust PKR activation during R3616 viral infection.

[0096] Mutant cDNAs of human MEK-1 containing mutations in serine codons at amino acid positions 218 and 222 that resulted in codons encoding negatively charged aspartate residues have been generated. These mutations mimic the effect of phosphorylation at positions 218 and 222, resulting in constitutive activation of MEK-1 (MAPK-kinase) function (27). In contrast, alanine substitutions at the same residues functionally block phosphorylation by upstream MAPK-kinase-kinases (MAPKKKs), resulting in down-regulation of endogenous MAPK activity (34). Plasmids, designated pNC84 and pNC92, containing the respective N-terminal FLAG-tagged [Asp218, Asp222 MEK-1] or [Ala218 and Ala222 MEK-1] cDNAs under the transcriptional control of a CMV promoter and the neomycin resistance gene, were used to select for G418 resistance, FLAG-MEK expressing clonal transfectants as described in Example 1.

[0097] As shown in FIG. 7, when the mutant MEK-expressing HT1080 stable cell lines were infected with mutant R3616 (10 PFU/cell), there were appreciable differences in cytopathic effects (CPE). HT-caMEK cells exhibited CPE at 12 hours post-infection while HT-dnMEK-expressing cells did not. Both cell lines, however, exhibited CPE upon infection with HSV-1(F) (10 PFU/cell). Next, viral recoveries were compared from the stable transfectants generated from HT1080 and MiaPaCa2 cells after exposure of the cells to 1 PFU of R3616 virus per cell. There was a greater than 200fold increase in viral titer in R3616-infected caMEK cells compared with dnMEK cells, i.e., 1.18×10⁶ compared to 1.46×10⁸ PFU/m1 for the HT1080 transfectants (caMEK v. dnMEK, respectively), and 1.05×10^5 compared to 1.10×10^7 PFU/ml for the MiaPaCa2 transfectants (caMEK v. dnMEK, respectively). See FIG. 8, panels A and C.

[0098] Lastly, three series of experiments were done to determine whether the enhancement of replication of the R3616 mutant virus in caMEK cells correlated with increased accumulation of viral proteins and inhibition of PKR activation. In the first experiment, dnMEK- and caMEK-expressing cell lines and their respective parent cell lines were exposed to 10 PFU per cell of mutant virus R3616 (FIG. 8). The cells were harvested 12 hours post-infection, solubilized, subjected to electrophoresis in denaturing polyacrylamide gels and reacted with antibodies to PKR, eIF-2 α and the phosphorylated forms of PKR (P-Thr446) and eIF-2 α (P-Ser51), respectively. Baseline differential MEK activities in uninfected dnMEK- and caMEK-expressing cells and the parental cell lines were established by immunoblotting whole-cell lysates with antibody to ERK1/ERK2 and the phosphorylated form of ERK1/ERK2 (P-Thr202 and P-Tyr204, respectively),

see Panels B-1 and D-1 of FIG. **8**. As shown (Panels B-3 and D-2 of FIG. **8**), levels of phosphorylated PKR and eIF- 2α were higher in dnMEK-expressing lines infected with the R3616 mutant virus as compared with the parental cell line or the caMEK-expressing cell lines. Conversely, activated PKR was nearly undetectable in caMEK-expressing cells infected with the R3616 mutant virus.

[0099] In the second series of experiments, electrophoretically separated lysates of caMEK- or dnMEK-expressing cell lines that had been infected with the R3616 mutant virus and processed as described above were reacted with antibody to a (ICP27) and y2 (glycoprotein C) proteins. As shown in Panel B-7 and Panel D-4 of FIG. 8, the accumulation of ICP27 was similar in both the stably transfected mutant cell lines and the parental cell lines, suggesting that the expression of MEK-1 mutants did not significantly affect the accumulation of ICP27, a protein expressed prior to the onset of viral DNA synthesis. However, consistent with the result shown in FIG. 6 with chemical inhibition of MEK, the accumulation of gC was markedly decreased in dnMEK-expressing cell lines at 12 hours post-infection, compared with the parent or caMEKexpressing stable cells (Panel B-8 and Panel D-5 of FIG. 8). [0100] Lastly, caMEK- or dnMEK-over-expressing HT1080 cell lines were exposed to 10 PFU of virus HSV-1(F) or mutant R3616. At 11 hours post-infection, the cells were rinsed, starved of methionine for one hour, and then supplemented with 100 µCi/ml of [35S] methionine for two additional hours. At 14 hours post-infection, 20 µg of equilibrated protein lysates were electrophoretically separated in denaturing polyacrylamide gels, transferred to PVDF membranes, and exposed to autoradiography film. As shown in FIG. 9, the accumulation of labeled proteins was similar in HT-caMEK (lane 5) and HT-dnMEK (lane 6) cells during infection with HSV-1(F). In contrast, the accumulation of labeled proteins in HT-dnMEK cells (lane 4) was diminished compared with HT-caMEK cells (lane 3) infected with the R3616 mutant virus.

Example 6

Intratumoral Inoculation of R3616 Mutant Virus Resulted in Tumor Regression in Tumors Expressing caMEK but not in Tumors Expressing dnMEK

[0101] To determine if differential replication correlated with a reduction of tumor size, we measured tumor volumes of untreated and R3616-treated HT-caMEK and HT-dnMEK tumor xenografts. HT-dnMEK and HT-caMEK tumor xenografts were grown to an average volume of 250 mm³ and injected with a single dose of 5×10^7 PFU of R3616 or buffer on day 0. At 31 days after infection by the R3616 mutant virus, only 117 animals had a palpable HT-caMEK tumor (100 mm³), in comparison to untreated HT-caMEK tumors, which averaged (4300+/-730 mm³ (standard error of the mean (SEM))). In contrast, all (7/7) of the HT-dnMEK tumors were palpable, with an average tumor volume of (830+/-SEM 210 mm³) and untreated HT-dnMEK tumor volumes averaged (4000+/- SEM 660 mm³).

Example 7

Systemic Administration of a Recombinant Virus R2636 Expressing the gC-Luc Construct Targeted Tumor Tissue Over-Expressing Constitutively Active MEK

[0102] To determine whether differential MEK activity confers tumor-selective viral replication upon systemic deliv-

ery of a γ_1 34.5-deficient virus, bilateral hindlimb tumor xenografts were grown by injecting the left and right hindlimbs of athymic nude mice with 5×10^6 cells of the HTdnMEK and HTcaMEK cell lines, respectively. In order to image viral replication in vivo, mutant HSV R2636 was used, which is a $\gamma_1 34.5$ -deficient virus that expresses the firefly luciferase gene under the transcriptional control of the HSV-1 gC-promoter, a representative γ promoter (37). In tissue that restricts viral replication, the accumulation of the firefly luciferase gene product expressed with the kinetics of a y gene, such as gC, would be decreased over successive replicative cycles by PKR-mediated shutoff of protein synthesis. However, a $\Delta \gamma_1 34.5$ mutant virus-infected, caMEK-xenografted, tumor cells, which support viral replication and gC expression, was expected to support R2636 replication and express gC-luciferase enzyme activity. At 5 days after IP delivery of R2636, bioluminescence localized to the right hindlimb, which corresponded to the caMEK-xenografted tumor (3,692 photons/mm²/sec) while the dnMEK tumor xenograft demonstrated 95-fold less photon expression (39 photons/mm²/sec). Also, there was no detectable bioluminescence outside of the caMEK-expressing tumors by 5 days post-IP injection (FIG. 10).

Example 8

Comparative Study of Intratumoral and Systemic Delivery of Virus

[0103] A series of experiments was designed to compare the intratumoral and systemic delivery of genetically engineered virus on tumor xenografts derived by injection of isogenic tumor cells differing with respect to ectopicallyexpressed MEK activity. General experimental techniques employed have been described in Example 1, above. Tumor xenografts were established by injecting 5×10^6 HT-caMEK or HT-dnMEK tumor cells into the hindlimbs of athymic nude mice. At a mean volume of $115 \pm 13 \text{ mm}^3$, the tumors were treated on days 0 and 5 by administration of R3616 via intratumoral injection of 5×10^7 PFU or intraperitoneal injection of 10⁶, 10⁷, or 10⁸ PFU of R3616 recombinant virus. Tumor xenografts were measured twice weekly with calipers. Tumor volume was calculated with the formula $(1\times w \times h)/2$, derived from the formula for the volume of an ellipsoid. Tumor growth was measured at each time point from day 0 to day 19 by calculating the ratio of tumor volume (V) to initial tumor volume (V_0) . The results of these experiments are shown in FIG. 12. In the HT-caMEK xenografts (FIG. 12A), intraperitoneal treatment with 2×10^6 , 2×10^7 , or 2×10^8 PFU of R3616, resulted in a significant dose-dependent tumor response by 19 days (V/V₀ of 9.1 \pm 1.9, 7.3 \pm 1.6, and 1.5 \pm 0.6, respectively) compared to untreated HT-caMEK controls (V/V₀ of 14.5 ± 1 . 7) (p=0.0221, 0.0371, and 0.0007, respectively). In HT-dn-MEK xenografts (FIG. 12B), no significant effect on tumor growth was seen by day 15 with intraperitoneal administration of 2×10^6 , $2 > 10^7$, or 2×10^8 PFU of R3616 (V/V_o of 11.2±1.9, 10.4±1.6, and 9.6±0.6, respectively) compared to untreated HT-dnMEK controls (V/V₀ of 9.1±3.1) (p=0.46, 0.35, 0.14, respectively). Intratumoral administration of 108 PFU of R3616 in HT-caMEK xenografts resulted in a significant anti-tumor effect with a V/V₀ of 3.2±1.1 by day 19 (p=0.0020). Intratumoral administration of 108 PFU of R3616 in HT-dnMEK xenografts did not demonstrate a significant anti-tumor effect with V/Vo of 7.9±1.1 by day 15 (p=0.36). Thus, tumor xenografts genetically engineered to

express constitutively active MEK were susceptible to oncolysis following systemic delivery by intraperitoneal injection of R3616, while xenografts engineered to express dominantnegative MEK activity were resistant to R3616 oncolysis.

[0104] In the second set of experiments, xenografts were established in the hindlimbs of athymic nude mice consisting of Hep3B cells, a human hepatoma cell line, and PC-3 cells, a human prostate cancer cell line. As reported earlier, Hep3B expressed high MEK activity whereas the PC-3 cells expressed almost no MEK activity (Smith et al., J Virol 80:1110-1120 (2006)). Hep3B and PC-3 xenografts were established in nude mice by hindlimb injection of 5×10^6 cells per animal. Hep3B and PC-3 xenografts were grown to an average volume of 150±4 mm³, and then treated on days 0 and 5 with either intratumoral injection of 5×10^7 PFU of R3616 or intraperitoneal injection of 10⁶, 10⁷, or 10⁸ PFU of R3616. Hep3B xenografts (FIG. 12C) demonstrated a dose-dependent effect with intraperitoneal administration of 2×10^6 , 2×10^7 , and 2×10^8 PFU of R3616 which resulted in V/V_o of 4.3±1.0, 3.2±0.5, and 1.4±0.3 at 18 days compared to untreated Hep3B controls which reached a mean V/V_0 of 6.1±1 (p=0.2050, 0.0858, and 0.0135, respectively).

[0105] In PC-3 xenografts (FIG. **12**D) there was no significant difference between intraperitoneal doses of 2×10^6 , 2×10^7 , and 2×10^8 PFU of R3616 (p=0.2327, 0.0882, 0.2970, respectively) and untreated control PC-3 xenografts by day 17. Intratumoral administration of 10^8 PFU of R3616 into Hep3B xenografts (FIG. **12**C) resulted in a V/V₀ of 1.1±0.2 (p=0.0130) by day 18. In PC-3 xenografts, intratumoral administration of 10^8 PFU of R3616 did not result in a significant antitumor effect with a V/V₀ of 8.9±2.2 (p=0.102) (FIG. **12**D). These results demonstrated that tumor regrowth studies with natively high (Hep3B) and low (PC-3) MEK activity tumors were similar to the results obtained with tumors genetically engineered to express constitutively active or dominant-negative MEK activity.

[0106] Luciferase imaging demonstrated increased viral replication which localized to HT-caMEK tumors compared to attenuated viral replication in HT-dnMEK tumors. R2636 is a γ_1 34.5-deficient virus constructed from the R3616 backbone that expresses the firefly luciferase gene under the control of the late HSV-1 gC promoter. Using R2636, in vivo imaging of viral replication was obtained. Detectable luciferase expression in tissues connotes active viral replication because gC-driven expression marks the expression of late viral structural genes. Hindlimb xenografts were established in nude mice by the injection of 5×10^6 cells of the fibrosarcoma cell lines HT-caMEK or HT-dnMEK. At initial tumor volumes of 175±60 mm³ for HT-caMEK and 131±22 mm³ for HT-dnMEK, mice were injected with either intratumoral (5×10⁷ PFU) or intraperitoneal (10⁸ PFU) R2636. Animals were imaged on days 1, 3, 8, 12, and 22 following viral injection.

[0107] In HT-caMEK xenografts that received intratumoral injections (FIG. **13**A), an increase in luminescence remained localized to the hindlimb only. In HT-dnMEK xenografts injected intratumorally, luminescence reached a plateau early in the study and demonstrated much lower activity than their HT-caMEK counterparts injected intratumorally (FIG. **13**B). HT-caMEK tumor-bearing mice (FIG. **13**C) that received intraperitoneal R2636 demonstrated an increase in luminescence in the abdominal cavity (in the liver or spleen) on day 1 that disappeared by day 3 and remained absent up to the conclusion of the study at day 22, while a steady increase in

luminescence was observed in the hindlimb bearing xenografted tumors. HT-dnMEK tumor-bearing mice treated by intraperitoneal R2636 (FIG. **13**D) demonstrated a similar increase in luminescence in the abdominal cavity, liver and spleen, on day 1 and day 3, which abated by day 8 and remained absent up to the conclusion of the study on day 22, with no localization to the hindlimb xenografts. Luminescence was measured and relative intensity quantified as total photon flux (FIG. **14**). HT-dnMEK tumors treated with either intratumoral or intraperitoneal R2636 failed to demonstrate significantly increased luminescence above the baseline luminescence measured in untreated HT-dnMEK control tumors.

[0108] To study intratumoral distribution of R3616 in HTcaMEK tumors following IT or IP injection, xenografts were harvested 5 days after treatment with either 5×10^7 PFU of intratumoral or 108 PFU of intraperitoneal R3616. Immunohistochemistry (1HC) for HSV-1 antigen in HT-caMEK xenografts injected intratumorally demonstrated viral replication along the needle track. (FIG. 15A). In contrast, HTcaMEK xenografts treated by intraperitoneal injection demonstrated a more diffuse pattern of viral distribution with multiple foci of viral replication throughout the tumors. (FIG. 15B). No HSV-1 antigens were detected by IHC in HT-dn-MEK xenografts 5 days following intratumoral or intraperitoneal injection. To examine recovery of R3616 from HTcaMEK tumors following treatment with either intratumoral or intraperitoneal R3616, HT-caMEK xenografts were harvested 5 days post treatment with either intratumoral 5×10^7 PFU or intraperitoneal 10⁸ PFU of R3616. Viral titers from homogenized samples were determined by standard plaque formation assays on Vero cell monolayers. Intratumoral administration of 5×10^7 PFU of R3616 yielded a titer of $4 \times 10^5 \pm 1 \times 10^5$ PFU. Intraperitoneal administration of 10^8 PFU of R3616 yielded a comparable titer of $2 \times 10^5 \pm 1 \times 10^5$ PFU (FIG. 16). No detectable levels of R3616 were recovered from HT-dnMEK xenografts treated with either intraperitoneal 10^7 or 10^6 PFU at day 5.

[0109] Systemic delivery of R3616 was explored because of the observation that MEK activity suppressed PKR following tumor cell infection with R3616 and thereby increased viral recovery from tumors injected with the virus. Salient observations on the systemic administration of HSV-1 arising from the studies reported herein are: i) R3616 demonstrated greater oncolytic activity in xenografted flank tumors with high levels of active MEK as compared with tumors that expressed lower levels of active MEK. This finding held true in human tumors genetically engineered to express constitutively active MEK, as well as tumors that natively express high MEK activity. ii) The superior oncolytic effects of R3616 in high MEK-activity tumors are corroborated by in vivo imaging studies with R2636, a $\Delta \gamma_1 34.5$ mutant based on the R3616 backbone in which the late viral promoter for gC drives luciferase expression. In vivo imaging with R2636 demonstrated that systemic administration permitted $\Delta \gamma_1 34.5$ mutant virus localization to constitutively active MEK tumors with subsequent intratumoral viral replication. In contrast, in dominant-negative MEK xenografts, R2636 replication was diminished and systemic administration of R2636 did not lead to persistent intratumoral viral replication. iii) Although equal amounts of virus were recovered from caMEK-expressing tumors five days following intraperitoneal administration as compared with intratumoral administration, the kinetics of viral proliferation differed, as reflected by quantified bioluminescence imaging.

[0110] Although, intraperitoneal delivery of virus required a two-fold higher dose compared to intratumoral injection to achieve the same oncolytic efficacy, the data reported herein establish that systemic delivery of R3616 effectively treated metastases from these tumors. Also, assays of MEK activation and other kinases in tumors is expected to allow for individualized targeted therapy with R3616 or similar viruses, i.e., γ_1 34.5 deficient HSV, including $\Delta \gamma_1$ 34.5 HSV. Notably, anti-HSV-1 immune activity has not been reported to limit the use of $\Delta \gamma_1$ 34.5 mutants in human trials to date. The data disclosed herein indicate that $\Delta \gamma_1$ 34.5 mutant viruses will be useful in the treatment of disseminated metastatic disease.

[0111] The following references, numbered 1-36 and 38-50, have been cited throughout this disclosure and are hereby incorporated by reference in their entireties.

- **[0112]** 1. Anderson, M. J., G. Casey, C. L. Fasching, and E. J. Stanbridge. 1994. Evidence that wild-type TP53, and not genes on either chromosome 1 or 11, controls the tumorigenic phenotype of the human fibrosarcoma HT1080. Genes Chromosomes Cancer 9:266-81.
- [0113] 2. Andreansky, S., L. Soroceanu, E. R. Flotte, J. Chou, J. M. Markert, G. Y. Gillespie, B. Roizman, and R. J. Whitley. 1997. Evaluation of genetically engineered herpes simplex viruses as oncolytic agents for human malignant brain tumors. Cancer Res 57:1502-9.
- **[0114]** 3. Ballif, B. A., and J. Blenis. 2001. Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. Cell Growth Differ 12:397-408.
- [0115] 4. Barber, G. N., R. Jagus, E. F. Meurs, A. G. Hovanessian, and M. G. Katze. 1995. Molecular mechanisms responsible for malignant transformation by regulatory and catalytic domain variants of the interferon-induced enzyme RNA-dependent protein kinase, J. Biol. Chem. 270:17423-17428.
- [0116] 5. Barber, G. N., M. Wambach, S. Thompson, R. Jagus, and M. G. Katze. 1995. Mutants of the RNA-dependent protein kinase (PKR) lacking double-stranded RNA binding domain I can act as trans dominant inhibitors and induce malignant transformation. Mol Cell Biol 15:3138-46.
- [0117] 6. Bennett, J. J., K. A. Delman, B. M. Burt, A. Mariotti, S. Malhotra, J. Zager, H Petrowsky, S. Mastorides, H. Federoff, and Y. Fong. 2002. Comparison of safety, delivery, and efficacy of two oncolytic herpes viruses (G207 and NV 1020) for peritoneal cancer. Cancer Gene Ther 9:935-45.
- **[0118]** 7. Cassady, K. A., M. Gross, and B. Roizman. 1998. The herpes simplex virus US11 protein effectively compensates for the gamma1(34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the alpha subunit of eukaryotic translation initiation factor 2. J Virol 72:8620-6.
- [0119] 8. Chambers, R., G. Y. Gillespie, L. Soroceanu, S. Andreansky, S. Chatterjee, J. Chou, B. Roizman, and R. J. Whitley. 1995. Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a acid mouse model of human malignant glioma. Proc Natl Acad Sci (USA) 92:1411-5.

- **[0120]** 9. Chee, A. V., and B. Roizman. 2004. Herpes simplex virus 1 gene products occlude the interferon signaling pathway at multiple sites. J Virol 78:4185-96.
- [0121] 10. Cheng, G., M. E. Brett, and B. He. 2001. Val193 and Phe195 of the gamma 1 34.5 protein of herpes simplex virus 1 are required for viral resistance to interferon alpha/ beta. Virology 290:115-20.
- **[0122]** 11. Chou, J., E. R. Kern, R. J. Whitley, and B. Roizman. 1990. Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. Science 250:1262-6.
- **[0123]** 12. Chou, J., A. P. Poon, J. Johnson, and B. Roizman. 1994. Differential response of human cells to deletions and stop codons in the gamma(1)34.5 gene of herpes simplex virus. Virol 68:8304-11.
- **[0124]** 13. Chou, J., and B. Roizman. 1992. The gamma 1(34.5) gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. Proc Natl Acad Sci (USA) 89:3266-70.
- [0125] 14. Chou, J., and B. Roizman. 1994. Herpes simplex virus 1 gamma(1)34.5 gene function, which blocks the host response to infection, maps in the homologous domain of the genes expressed during growth arrest and DNA damage. Proc Natl Acad Sci (USA) 91:5247-51.
- [0126] 15. Chung, S. M., S. J. Advani, J. D. Bradley, Y. Kataoka, K. Vashistha, S. Y. Yan, J M. Markert, G. Y. Gillespie, R. I. Whitley, B. Roizman, and R. R. Weichselbaum. 2002. The use of a genetically engineered herpes simplex virus (R7020) with ionizing radiation for experimental hepatoma. Gene Ther 9:75-80.
- [0127] 16. Clemens, M. J. 2004. Targets and mechanisms for the regulation of translation in malignant transformation. Oncogene 23:3180-8.
- [0128] 17. Davies, H., G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H Woffendin, M. J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, R. Wilson, H. Jayatilake, B. A. Gusterson, C Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G. J. Riggins, D. D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A Nicholson, J. W. Ho, S. Y. Leung, S. T. Yuen, B. L. Weber, H. F. Seigler, T. L Darrow, H. Paterson, R. Marais, C. J. Marshall, R. Wooster, M. R. Stratton, and P A. Futreal. 2002. Mutations of the BRAF gene in human cancer. Nature 417:949-54.
- [0129] 18. Ejercito, P. M., E. D. Kieff, and B. Roizman. 1968. Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. J Gen Virol 2:357-64.
- [0130] 19. Farassati, F., A. D. Yang, and P. W. Lee. 2001. Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol 3:745-50.
- **[0131]** 20. Gale, M., Jr., and M. G. Katze. 1998. Molecular mechanisms of interferon resistance mediated by viraldirected inhibition of PKR, the interferon-induced protein kinase Pharmacol Ther 78:29-46.
- [0132] 21. Gupta, S., R. Plattner, C. J. Der, and E. J. Stanbridge. 2000. Dissection of Ras dependent signaling pathways controlling aggressive tumor growth of human fibrosarcoma cells: evidence for a potential novel pathway. Mol Cell Biol 20:9294-306.

- **[0133]** 22. Gupta, S., and E. J. Stanbridge. 2001. Paired human fibrosarcoma cell lines that possess or lack endogenous mutant N-ras alleles as experimental model for Ras signaling pathways. Methods Enzymol 333:290-306.
- [0134] 23. Hahn, W. C., C. M. Counter, A. S. Lundberg, R. L. Beijersbergen, M. W. Brooks, and R. A. Weinberg. 1999. Creation of human tumour cells with defined genetic elements. Nature 400:464-8.
- [0135] 24. Hallahan, D. E., H. J. Mauceri, L. P. Seung, E. J. Dunphy, J. D. Wayne, N. N. Hanna, A. Toledano, S. Hellman, D. W. Kufe, and R. R. Weichselbaum. 1995. Spatial and temporal control of gene therapy using ionizing radiation. Nat Med 1:786-91.
- **[0136]** 25. He, B., M. Gross, and B. Roizman. 1997. The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc Natl Acad Sci (USA) 94:843-8.
- [0137] 26. Hoshino, R., Y. Chatani, T. Yamori, T. Tsuruo, H. Oka, O. Yoshida, Y. Shimada, S. Ari-i, H. Wada, J. Fujimoto, and M. Kohno. 1999. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors Oncogene 18:813-22.
- [0138] 27. Huang, W., and R. L. Erikson. 1994. Constitutive activation of Mek1 by mutation of serine phosphorylation sites. Proc Natl Acad Sci (USA) 91:8960-3.
- [0139] 28. Ito, T., R. Jagus, and W. S. May. 1994. Interleukin 3 stimulates protein synthesis by regulating doublestranded RNA-dependent protein kinase. Proc Natl Acad Sci (USA) 91:7455-9.
- **[0140]** 29. Jacquemont, B., and B. Roizman. 1975. RNA synthesis in cells infected with herpes simplex virus. X. Properties of viral symmetric transcripts and of double-stranded RNA prepared from them. J Virol 15:707-13.
- [0141] 30. Katze, M. G. 1995. Regulation of the interferoninduced PKR: can viruses cope? Trends Microbiol 3:75-8.
- **[0142]** 31. Kozak, M., and B. Roizman. 1975. RNA synthesis in cells infected with herpes simplex virus. IX. Evidence for accumulation of abundant symmetric transcripts in nuclei. J Virol 15:36-40.
- **[0143]** 32. Le Gall, M., J. C. Chambard, J. P. Breittmayer, D. Grail, J. Pouyssegur, and E. Van Obberghen-Schilling. 2000. The p42/p44 MAP kinase pathway prevents apoptosis induced by anchorage and serum removal. Mol Biol Cell 11:1103-12.
- [0144] 33. Leib, D. A., M. A. Machalek, B. R. Williams, R. H. Silverman, and H. W. Virgin 2000. Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene. Proc Natl Acad Sci (USA) 97:6097-101.
- **[0145]** 34. Mansour, S. J., J. M. Candia, J. E. Matsuura, M. C. Manning, and N. G. Ahn. 1996. Interdependent domains controlling the enzymatic activity of mitogen-activated protein kinase kinase 1. Biochemistry 35:15529-36.
- [0146] 35. Marken, J. M., M. D. Medlock, S. D. Rabkin, G. Y. Gillespie, T. Todo, W. D Hunter, C. A. Palmer, F. Feigenbaum, C. Tornatore, F. Tufaro, and R. L. Martuza. 2000. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 7:867-74.
- [0147] 36. Meurs, E. F., J. Galabru, G. N. Barber, M. G. Katze, and A. G. Hovanessian. 1993. Tumor suppressor

function of the interferon-induced double-stranded RNA activated protein kinase. Proc Natl Acad Sci (USA) 90:232-6.

- [0148] 38. Nakamura, H., H. Kasuya, J. T. Mullen, S. S. Yoon, T. M. Pawlik, S Chandrasekhar, J. M. Donahue, E. A. Chiocca, R. Y. Chung, and K. K. Tanabe. 2002. Regulation of herpes simplex virus gamma(1)34.5 expression and oncolysis of diffuse liver metastases by Myb34.5. J Clin Invest 109:871-82.
- **[0149]** 39. Perkins, D. J., and G. N. Barber. 2004. Defects in translational regulation mediated by the alpha subunit of eukaryotic initiation factor 2 inhibit antiviral activity and facilitate the malignant transformation of human fibroblasts. Mol Cell Biol 24:2025-40.
- [0150] 40. Plattner, R., M. J. Anderson, K. Y. Sato, C. L. Fasching, C. J. Der, and E. J. Stanbridge. 1996. Loss of oncogenic ras expression does not correlate with loss of tumorigenicity in human cells. Proc Natl Acad Sci (USA) 93:6665-70.
- **[0151]** 41. Pouyssegur, J., V. Volmat, and P. Lenormand. 2002. Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling. Biochem Pharmacol 64:755-63.
- [0152] 42. Rampling, R., G. Cruickshank, V. Papanastassiou, J. Nicoll, D. Hadley, D. Brennan, R. Petty, A. MacLean, J. Harland, E. McKie, R. Mabbs, and M. Brown. 2000. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. Gene Ther 7:859-66.
- **[0153]** 43. Roller, R. J., and B. Roizman. 1990. The herpes simplex virus Us11 open reading frame encodes a sequence-specific RNA-binding protein. J Virol 64:3463-70.

- **[0154]** 44. Sebolt-Leopold, J. S., and R. Herrera. 2004. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat Rev Cancer 4:937-47.
- [0155] 45. Sheaffer, A. K., W. W. Hurlburt, J. T. Stevens, M. Bifano, R. K. Hamatake, R. J. Colonno, and D. J. Tenney. 1995. Characterization of monoclonal antibodies recognizing amino- and carboxy-terminal epitopes of the herpes simplex virus UL42 protein. Virus Res 38:305-14.
- [0156] 46. Shimamura, A., B. A. Ballif, S. A. Richards, and J. Blenis. 2000. Rsk1 mediates a MEK-MAP kinase cell survival signal. Curr Biol 10:127-35.
- [0157] 47. von Gise, A., P. Lorenz, C. Wellbrock, B. Hemmings, F. Berberich-Siebelt, U. R Rapp, and J. Troppmair. 2001. Apoptosis suppression by Raf-1 and MEK1 requires MEK- and phosphatidylinositol 3-kinase-dependent signals. Mol Cell Biol 21:2324-36.
- [0158] 48. Williams, B. R. 2001. Signal integration via PKR. Sci STKE 2001:RE2.
- [0159] 49. Xia, Z., M. Dickens, J. Raingeaud, R. J. Davis, and M. E. Greenberg. 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270:1326-31.
- [0160] 50. Yip-Schneider, M. T., A. Lin, D. Barnard, C. J. Sweeney, and M. S. Marshall. 1999 Lack of elevated MAP kinase (Erk) activity in pancreatic carcinomas despite oncogenic K-ras expression. Int J Oncol 15:271-9.

[0161] Numerous modifications and variations of the invention are possible in view of the above teachings and are within the scope of the invention. The entire disclosures of all publications cited herein are hereby incorporated by reference.

SEQUENCE LISTING

```
<160> NUMBER OF SEQ ID NOS: 18
<210> SEQ ID NO 1
<211> LENGTH: 2222
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEOUENCE: 1
                                                                       60
atteggeaeg agggaggaag egagaggtge tgeeeteeee eegagttgg aagegegtta
cccgggtcca aaatgcccaa gaagaagccg acgcccatcc agctgaaccc ggcccccgac
                                                                      120
ggctctgcag ttaacgggac cagctctgcg gagaccaact tggaggcctt gcagaagaag
                                                                      180
ctggaggagc tagagcttga tgagcagcag cgaaagcgcc ttgaggcctt tcttacccag
                                                                      240
aagcagaagg tgggagaact gaaggatgac gactttgaga agatcagtga gctgggggct
                                                                      300
ggcaatggcg gtgtggtgtt caaggtctcc cacaagcctt ctggcctggt catggccaga
                                                                      360
aagctaattc atctggagat caaacccgca atccggaacc agatcataag ggagctgcag
                                                                      420
gttctgcatg agtgcaactc tccgtacatc gtgggcttct atggtgcgtt ctacagcgat
                                                                      480
ggcgagatca gtatctgcat ggagcacatg gatggaggtt ctctggatca agtcctgaag
                                                                      540
aaaqctqqaa qaattcctqa acaaatttta qqaaaaqtta qcattqctqt aataaaaqqc
                                                                      600
ctgacatatc tgagggagaa gcacaagatc atgcacagag atgtcaagcc ctccaacatc
                                                                      660
                                                                      720
ctaqtcaact cccqtqqqqa qatcaaqctc tqtqactttq qqqtcaqcqq qcaqctcatc
```

18

-continued

780

gggactcatt	actctgtgca	gtcagacatc	tggagcatgg	gactgtctct	ggtagagatg	840
gcggttggga	ggtatcccat	ccctcctcca	gatgccaagg	agctggagct	gatgtttggg	900
tgccaggtgg	aaggagatgc	ggctgagacc	ccacccaggc	caaggacccc	cgggaggccc	960
cttagctcat	acggaatgga	cagccgacct	cccatggcaa	tttttgagtt	gttggattac	1020
atagtcaacg	agcctcctcc	aaaactgccc	agtggagtgt	tcagtctgga	atttcaagat	1080
tttgtgaata	aatgcttaat	aaaaaacccc	gcagagagag	cagatttgaa	gcaactcatg	1140
gttcatgctt	ttatcaagag	atctgatgct	gaggaagtgg	attttgcagg	ttggctctgc	1200
tccaccatcg	gccttaacca	gcccagcaca	ccaacccatg	ctgctggcgt	ctaagtgttt	1260
gggaagcaac	aaagagcgag	tcccctgccc	ggtggtttgc	catgtcgctt	ttgggcctcc	1320
ttcccatgcc	tgtctctgtt	cagatgtgca	tttcacctgt	gacaaaggat	gaagaacaca	1380
gcatgtgcca	agattctact	cttgtcattt	ttaatattac	tgtctttatt	cttattacta	1440
ttattgttcc	cctaagtgga	ttggctttgt	gcttggggct	atttgtgtgt	atgctgatga	1500
tcaaaacctg	tgccaggctg	aattacagtg	aaatttttgg	tgaatgtggg	tagtcattct	1560
tacaattgca	ctgctgttcc	tgctccatga	ctggctgtct	gcctgtattt	tcggactttg	1620
acatttgaca	tttggtggac	tttatcttgc	tgggcatact	ttctctctag	gagggagcct	1680
tgtgagatcc	ttcacaggca	gtgcatgtga	agcatgcttt	gctgctatga	aaatgagcat	1740
cagagagtgt	acatcatgtt	attttattat	tattatttgc	ttttcatgta	gaactcagca	1800
gttgacatcc	aaatctagcc	agagecette	actgccatga	tagctggggc	ttcaccagtc	1860
tgtctactgt	ggtgatctgt	agacttctgg	ttgtatttct	atatttattt	tcagtatact	1920
gtgtgggata	cttagtggta	tgtctcttta	agttttgatt	aatgtttctt	aaatggaatt	1980
atttgaatgt	cacaaattga	tcaagatatt	aaaatgtcgg	atttatcttt	ccccatatcc	2040
aagtaccaat	gctgttgtaa	acaacgtgta	tagtgcctaa	aattgtatga	aaatcctttt	2100
aaccatttta	acctagatgt	ttaacaaatc	taatctctta	ttctaataaa	tatactatga	2160
aataaaaaaa	aaaggagaaa	gctaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	2220
aa						2222
<210> SEQ : <211> LENG <212> TYPE <213> ORGAN <400> SEQUI	ID NO 2 IH: 393 : PRT NISM: Homo ENCE: 2	sapiens				
Met Pro Ly: 1	s Lys Lys P: 5	ro Thr Pro	Ile Gln Leu 10	Asn Pro Ala	a Pro Asp 15	
Gly Ser Ala	a Val Asn G 20	ly Thr Ser	Ser Ala Glu 25	Thr Asn Leu 30	ı Glu Ala	
Leu Gln Ly: 35	s Lys Leu G	lu Glu Leu 40	Glu Leu Asp	Glu Gln Glr 45	n Arg Lys	
Arg Leu Glu 50	ı Ala Phe L	eu Thr Gln 55	Lys Gln Lys	Val Gly Glu 60	ı Leu Lys	
Asp Asp Asp 65	p Phe Glu L 7	ys Ile Ser D	Glu Leu Gly 75	Ala Gly Asr	n Gly Gly 80	

gactccatgg ccaactcctt cgtgggcaca aggtcctaca tgtcgccaga aagactccag

aor	· +-	4	\mathbf{r}		\sim	
COL	ι.			L.	-	C 3

											-	con	tin	ued							
Val	Val	Phe	Lys	Val 85	Ser	His	Lys	Pro	Ser 90	Gly	Leu	Val	Met	Ala 95	Arg						
ГÀа	Leu	Ile	His 100	Leu	Glu	Ile	Lys	Pro 105	Ala	Ile	Arg	Asn	Gln 110	Ile	Ile						
Arg	Glu	Leu 115	Gln	Val	Leu	His	Glu 120	Сув	Asn	Ser	Pro	Tyr 125	Ile	Val	Gly						
Phe	Tyr 130	Gly	Ala	Phe	Tyr	Ser 135	Asp	Gly	Glu	Ile	Ser 140	Ile	Суз	Met	Glu						
His 145	Met	Asp	Gly	Gly	Ser 150	Leu	Asp	Gln	Val	Leu 155	Lys	Lys	Ala	Gly	Arg 160						
Ile	Pro	Glu	Gln	Ile 165	Leu	Gly	Lys	Val	Ser 170	Ile	Ala	Val	Ile	Lys 175	Gly						
Leu	Thr	Tyr	Leu 180	Arg	Glu	Lys	His	Lys 185	Ile	Met	His	Arg	Asp 190	Val	Lys						
Pro	Ser	Asn 195	Ile	Leu	Val	Asn	Ser 200	Arg	Gly	Glu	Ile	Lys 205	Leu	Суз	Asp						
Phe	Gly 210	Val	Ser	Gly	Gln	Leu 215	Ile	Asp	Ser	Met	Ala 220	Asn	Ser	Phe	Val						
Gly 225	Thr	Arg	Ser	Tyr	Met 230	Ser	Pro	Glu	Arg	Leu 235	Gln	Gly	Thr	His	Tyr 240						
Ser	Val	Gln	Ser	Asp 245	Ile	Trp	Ser	Met	Gly 250	Leu	Ser	Leu	Val	Glu 255	Met						
Ala	Val	Gly	Arg 260	Tyr	Pro	Ile	Pro	Pro 265	Pro	Asp	Ala	Lys	Glu 270	Leu	Glu						
Leu	Met	Phe 275	Gly	Сүз	Gln	Val	Glu 280	Gly	Asp	Ala	Ala	Glu 285	Thr	Pro	Pro						
Arg	Pro 290	Arg	Thr	Pro	Gly	Arg 295	Pro	Leu	Ser	Ser	Tyr 300	Gly	Met	Asp	Ser						
Arg 305	Pro	Pro	Met	Ala	Ile 310	Phe	Glu	Leu	Leu	Asp 315	Tyr	Ile	Val	Asn	Glu 320						
Pro	Pro	Pro	Lys	Leu 325	Pro	Ser	Gly	Val	Phe 330	Ser	Leu	Glu	Phe	Gln 335	Asp						
Phe	Val	Asn	Lys 340	Сүз	Leu	Ile	Lys	Asn 345	Pro	Ala	Glu	Arg	Ala 350	Asp	Leu						
Lys	Gln	Leu 355	Met	Val	His	Ala	Phe 360	Ile	Lys	Arg	Ser	Asp 365	Ala	Glu	Glu						
Val	Asp 370	Phe	Ala	Gly	Trp	Leu 375	Суз	Ser	Thr	Ile	Gly 380	Leu	Asn	Gln	Pro						
Ser 385	Thr	Pro	Thr	His	Ala 390	Ala	Gly	Val													
<210 <213 <213 <213	0> SI L> LI 2> T 3> OF	EQ I ENGT YPE : RGAN	D NO H: 1 DNA ISM:	3 611 Hom	o saj	pien	S														
<40)> SI	EQUE	NCE:	3																	
acat	caatt	ttc	tgga	geee	tg t	acca	acgt	g tg	gcca	cata	ttc	tgtc	agg a	aacco	ctgtgt	6	0				
gato	catgo	gtc	tgga	tctg	ca a	cacg	ggcc.	a ggo	ccaa	agtc	aca	gatc	ttg a	agato	cacagg	12	0				
tggi	gttę	gag	cage	aggc.	ag g	cagg	caat	c ggi	cccga	agtg	gct	gtcg	gct (cttca	agetet	18	0				
ccg	ctcg	gcg	tctt	cctt	cc t	ctcc	cggt	c ago	cgtc	ggcg	gct	gcac	cgg (cggc	gggcag	24	0				

continued	
teetgeggga ggggggacaa gagetgagge geggeegeeg agegtegage teagegegge	300
ggaggeggeg geggeeegge agecaacatg geggeggegg eggegggggg egegggeeeg	360
gagatggtcc gcgggcaggt gttcgacgtg gggccgcgct acaccaacct ctcgtacatc	420
ggcgagggcg cctacggcat ggtgtgctct gcttatgata atgtcaacaa agttcgagta	480
gctatcaaga aaatcagccc ctttgagcac cagacctact gccagagaac cctgagggag	540
ataaaaatct tactgcgctt cagacatgag aacatcattg gaatcaatga cattattcga	600
gcaccaacca tcgagcaaat gaaagatgta tatatagtac aggacctcat ggaaacagat	660
ctttacaagc tettgaagae acaacaeete ageaatgaee atatetgeta ttttetetae	720
cagateetca gagggttaaa atatateeat teagetaaeg ttetgeaeeg tgaeeteaag	780
ccttccaacc tgctgctcaa caccacctgt gatctcaaga tctgtgactt tggcctggcc	840
cgtgttgcag atccagacca tgatcacaca gggttcctga cagaatatgt ggccacacgt	900
tggtacaggg ctccagaaat tatgttgaat tccaagggct acaccaagtc cattgatatt	960
tggtctgtag gctgcattct ggcagaaatg ctttccaaca ggcccatctt tccagggaag	1020
cattatettg accagetgaa teacattttg ggtattettg gateeecate acaagaagae	1080
ctgaattgta taataaattt aaaagctagg aactatttgc tttctcttcc acacaaaaat	1140
aaggtgccat ggaacaggct gttcccaaat gctgactcca aagctctgga cttattggac	1200
aaaatgttga cattcaaccc acacaagagg attgaagtag aacaggctct ggcccaccca	1260
tatctggagc agtattacga cccgagtgac gagcccatcg ccgaagcacc attcaagttc	1320
gacatggaat tggatgactt gcctaaggaa aagctaaaag aactaatttt tgaagagact	1380
gctagattcc agccaggata cagatcttaa atttgtcagg acaagggctc agaggactgg	1440
acgtgctcag acatcggtgt tcttcttccc agttcttgac ccctggtcct gtctccagcc	1500
cgtcttggct tatccacttt gactcctttg agccgtttgg agggggggtt tctggtagtt	1560
gtggctttta tgctttcaaa gaatttcttc agtccagaga attcactggc c	1611
<210> SEQ ID NO 4 <211> LENGTH: 360 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 4	
~ Met Ala Ala Ala Ala Ala Gly Ala Glv Pro Glu Met Val Arc Glv	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
Gln Val Phe Asp Val Gly Pro Arg Tyr Thr Asn Leu Ser Tyr Ile Gly 20 25 30	
Glu Gly Ala Tyr Gly Met Val Cys Ser Ala Tyr Asp Asn Val Asn Lys 35 40 45	
Val Arg Val Ala Ile Lys Lys Ile Ser Pro Phe Glu His Gln Thr Tyr 50 55 60	
Cys Gln Arg Thr Leu Arg Glu Ile Lys Ile Leu Leu Arg Phe Arg His 65 70 75 80	
Glu Asn Ile Ile Gly Ile Asn Asp Ile Ile Arg Ala Pro Thr Ile Glu 85 90 95	
Gln Met Lys Asp Val Tyr Ile Val Gln Asp Leu Met Glu Thr Asp Leu 100 105 110	
Tyr Lys Leu Leu Lys Thr Gln His Leu Ser Asn Asp His Ile Cys Tyr	

-continued

	115					120					125								
Phe Leu	u Tyr	Gln	Ile	Leu	Arg	Gly	Leu	Lys	Tyr	Ile	His	Ser	Ala	Asn					
130		_	_	_	135	_	_	_	_	140	_	_							
Val Leu 145	u His	Arg	Asb	Leu 150	гда	Pro	Ser	Asn	Leu 155	Leu	Leu	Asn	Thr	160					
CAa YaF	p Leu	Lys	Ile	Сүз	Asp	Phe	Gly	Leu	Ala	Arg	Val	Ala	Asp	Pro					
			165					170					175						
Asp His	s Asp	His 180	Thr	Gly	Phe	Leu	Thr 185	Glu	Tyr	Val	Ala	Thr 190	Arg	Trp					
Tyr Arg	g Ala	Pro	Glu	Ile	Met	Leu	Asn	Ser	Lys	Gly	Tyr	Thr	Lys	Ser					
-	195					200			-	-	205		-						
Ile Asp 21(p Ile 0	Trp	Ser	Val	Gly 215	Сүз	Ile	Leu	Ala	Glu 220	Met	Leu	Ser	Asn					
Arg Pro	~ ~ T] ~	Dha	Pro	Glv	Lare	ціа	Tur	T.011	Agn	Gln	I.011	Agn	Uio	TIA					
225 225	o iie	FIIe	FIO	230	цур	птр	тут	шец	235 235	GIII	цец	ASII	птр	240					
Leu Gly	y Ile	Leu	Gly	Ser	Pro	Ser	Gln	Glu	Asp	Leu	Asn	Cys	Ile	Ile					
_	_		245	_	_	_	_	250	_	_		_	255	_					
Asn Leu	u Lys	Ala 260	Arg	Asn	Tyr	Leu	Leu 265	Ser	Leu	Pro	His	Lys 270	Asn	Lys					
Val Pro	o Trp	Asn	Arg	Leu	Phe	Pro	Asn	Ala	Asp	Ser	Lys	Ala	Leu	Asp					
	275					280					285								
Leu Leu 29(u Asp 0	Lys	Met	Leu	Thr 295	Phe	Asn	Pro	His	Lys 300	Arg	Ile	Glu	Val					
Glu Glr	n∆la	1.011	۵la	Ніс	Pro	Tvr	I.011	Glu	Gln	Tvr	Tvr	Agn	Pro	Ser					
305		Lou		310	110	- / -	Doa	oru	315	- / -	- / -	11010	110	320					
Asp Glu	u Pro	Ile	Ala	Glu	Ala	Pro	Phe	Lys	Phe	Asp	Met	Glu	Leu	Asp					
3 T			325		T	T	a 1	330	T] -	Dl	a 1	61	335						
Авр Гел	u pro	цув 340	GIU	гда	Leu	гуа	345	Leu	шe	Pne	GIU	350	Inr	AIa					
Arg Phe	e Gln	Pro	Gly	Tyr	Arg	Ser													
	355					360													
<210> \$	SEQ II	D NO	5																
<211> I <212> 7	LENGTI TYPE :	H: 1 DNA	759																
<213> 0	ORGAN	ISM:	Hom	o saj	pien	s													
<400> \$	SEQUE	NCE :	5																
cccctgo	cctc	tcgg	acto	aa a	ctgc	ggcgi	t caq	geeti	tctt	cgg	geet	cgg (cage	ggtagc	60	I			
ggctcgd	ctcg	cctc	agcc	cc a	gcgc	ccct	c gg	ctac	cctc	ggc	ccag	gcc (cgca	gegeeg	120	I.			
cccgcco	ctcg g	geeg	acce	ga c	geeg	gcct	a aa	ccgcé	ggcc	gca	gece	cgg g	gctc	gcgtag	180	I.			
gegeega	accg (ctcc	cggc	cc g	cccc	ctate	a aa	caca	ggct	aga	ggcg	ccg (ccgc	cgccgg	240	I			
cccgcgg	gagc (cccg	atge	tg g	cccg	gagga	a ago	ccggi	tgct	gcc	ggcg	ctc a	accat	caacc	300	I			
ctaccat	tcgc (cgage	ggcc	ca t	cccc.	tacca	a gco	gagg	gege	ctc	cgag	gca a	aacci	ggtgg	360				
acctoca	- agaa (gaaq	ctqa	ag a	agct	ggaa	tto	gaco	agca	gca	gaaq	aaq (egget	ggaaq	420	I			
cetter	tcac	ccad	aaad	cc a	agat	caac	а аа	otca	aada	caal	taac	tto /	Jaaa	gatet	480	1			
cacacct	taaa		uaas	a	22c.	-99~;	y ta	acca		cca	- 940	ada (acat	raaaca	540	1			
cayayou	-999 '	-y-y	gyca.	ue y	90999	99199	9	acca	ayı	cca	gcac.	uya (-99900	540				
tcatcat	tggc (agg	aage	cg a	ccca	cctt	g aga	atcaa	agcc	ggc	catc	cgg i	aacca	agatca	600				
tccgcga	agct g	gcag	gtcc	tg c	acga	atge	a act	tcgc	cgta	cat	cgtg	ggc 1	ttcta	acgggg	660				

	tgacg	Iggga	g at	cago	cattt	gca	atgga	laca	cate	ggaco	ldc d	gete	cctgg	720
accaggtgct	gaaag	laggc	c aa	agago	gatto	ccç	jagga	ıgat	cctç	gggga	iaa ç	gtcag	gcatcg	780
cggttctccg	gggct	tggc	g ta	accto	ccgaç	aga	agca	icca	gato	catgo	ac c	gaga	itgtga	840
agccctccaa	catco	tcgt	g aa	actct	agag	ada	gagat	caa	gcto	gtgtg	jac t	tcgg	ıggtga	900
gcggccagct	catag	actc	c at	ggco	caact	cct	tcgt	ggg	cacç	geget	.cc t	acat	ggete	960
cggagcggtt	gcagg	Igcac	a ca	attad	ctage	l tgo	agto	gga	cato	ctgga	igc a	tggg	jeetgt	1020
ccctggtgga	gctgg	lccgt	c go	gaago	gtacc	cca	atcco	ccc	gcco	cgaco	jec a	aaga	igctgg	1080
aggccatctt	tggcc	ggcc	c gt	ggt	cgaco	aaa	jaaga	agg	agaç	geete	ac a	igcat	ctcgc	1140
ctcggccgag	gcccc	ccgg	g cg	jacad	cgtca	geg	gtca	ıcgg	gato	ggata	ıgc o	ggco	tgcca	1200
tggccatctt	tgaac	tcct	g ga	actat	attg	ı tga	acga	ıgcc	acct	ccta	ag d	tgcc	caacg	1260
gtgtgttcac	ccccg	Jactt	c ca	aggag	gtttg	tca	ataa	iatg	ccto	catca	ag a	acco	agcgg	1320
agcgggcgga	cctga	agat	g ct	caca	aaacc	aca	acctt	cat	caaç	geggt	cc ç	Jaggt	ggaag	1380
aagtggattt	tgccg	gctg	g tt	gtgt	aaaa	ccc	tgcg	gct	gaad	ccago	aa g	gcac	accca	1440
cgcgcaccgc	cgtgt	gaca	g to	gccó	gggct	ccc	tgcg	ftcc	cgct	ggtg	jac d	tgcc	caccg	1500
tccctgtcca	tgccc	egee	c tt	ccag	yctga	gga	cago	lctg	gcgo	cctcc	ac c	caco	ctcct	1560
gcctcacccc	tgcgg	lagag	c ac	cgto	gaada	l ddo	gaca	ıgcg	cate	gcago	jaa c	adda	gtctc	1620
ctctcctgcc	cgtcc	tggc	c go	gggtg	geete	tgg	gggad	ada	cgao	gete	get g	gtgtg	jtggtc	1680
tcagaggctc	tgctt	cctt	a go	yttad	caaaa	caa	aaca	ıggg	agag	gaaaa	ag d	aaaa	iaaaaa	1740
aaaaaaaaaa	aaaaa	aaaa												1759
<210> SEQ <211> LENG <212> TYPE <213> ORGA	ID NO TH: 40 : PRT NISM:	6 00 Homo	sap	biens	3									
<400> SEQU	ENCE :	6												
<400> SEQU Met Leu Al 1	ENCE: a Arg	6 Arg 5	Lys	Pro	Val	Leu	Pro 10	Ala	Leu	Thr	Ile	Asn 15	Pro	
<400> SEQU Met Leu Al 1 Thr Ile Al	ENCE: a Arg a Glu 20	6 Arg 5 Gly	Lys Pro	Pro Ser	Val Pro	Leu Thr 25	Pro 10 Ser	Ala Glu	Leu Gly	Thr Ala	Ile Ser 30	Asn 15 Glu	Pro Ala	
<400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35	ENCE: a Arg a Glu 20 l Asp	6 Arg 5 Gly Leu	Lys Pro Gln	Pro Ser Lys	Val Pro Lys 40	Leu Thr 25 Leu	Pro 10 Ser Glu	Ala Glu Glu	Leu Gly Leu	Thr Ala Glu 45	Ile Ser 30 Leu	Asn 15 Glu Asp	Pro Ala Glu	
<400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35 Gln Gln Ly 50	ENCE: a Arg a Glu 20 l Asp s Lys	6 Arg 5 Gly Leu Arg	Lys Pro Gln Leu	Pro Ser Lys Glu 55	Val Pro Lys 40 Ala	Leu Thr 25 Leu Phe	Pro 10 Ser Glu Leu	Ala Glu Glu Thr	Leu Gly Leu Gln 60	Thr Ala Glu 45 Lys	Ile Ser 30 Leu Ala	Asn 15 Glu Asp Lys	Pro Ala Glu Val	
<400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35 Gln Gln Ly 50 Gly Glu Le 65	ENCE: a Arg a Glu 20 l Asp s Lys u Lys	6 Arg 5 Gly Leu Arg Asp	Lys Pro Gln Leu Asp 70	Pro Ser Lys Glu 55 Asp	Val Pro Lys 40 Ala Phe	Leu Thr 25 Leu Phe Glu	Pro 10 Ser Glu Leu Arg	Ala Glu Glu Thr Ile 75	Leu Gly Leu Gln 60 Ser	Thr Ala Glu 45 Lys Glu	Ile Ser 30 Leu Ala Leu	Asn 15 Glu Asp Lys Gly	Pro Ala Glu Val Ala 80	
<400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35 Gln Gln Ly 50 Gly Glu Le 65 Gly Asn Gl	ENCE: a Arg a Glu 20 l Asp s Lys u Lys y Gly	6 Arg 5 Gly Leu Arg Asp 85	Lys Pro Gln Leu Asp 70 Val	Pro Ser Lys Glu 55 Asp Thr	Val Pro Lys 40 Ala Phe Lys	Leu Thr 25 Leu Phe Glu Val	Pro 10 Ser Glu Leu Arg Gln 90	Ala Glu Glu Thr Ile 75 His	Leu Gly Leu Gln 60 Ser Arg	Thr Ala Glu 45 Lys Glu Pro	Ile Ser 30 Leu Ala Leu Ser	Asn 15 Glu Asp Lys Gly 95	Pro Ala Glu Val Ala 80 Leu	
<400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35 Gln Gln Ly 50 Gly Glu Le 65 Gly Asn Gl Ile Met Al	ENCE: a Arg a Glu 20 l Asp s Lys u Lys y Gly a Arg 100	6 Arg 5 Gly Leu Arg Asp Val 85 Lys	Lys Pro Gln Leu Asp 70 Val Leu	Pro Ser Lys Glu 55 Asp Thr Ile	Val Pro Lys 40 Ala Phe Lys His	Leu Thr 25 Leu Phe Glu Val Leu 105	Pro 10 Ser Glu Leu Arg Gln 90 Glu	Ala Glu Glu Thr Ile Ile	Leu Gly Leu Gln 60 Ser Arg Lys	Thr Ala Glu 45 Glu Pro Pro	Ile Ser 30 Leu Ala Leu Ser Ala 110	Asn 15 Glu Asp Lys Gly Sly 95 Ile	Pro Ala Glu Val Ala 80 Leu Arg	
<400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35 Gln Gln Ly Gly Glu Le 65 Gly Asn Gl Ile Met Al Asn Gln Il	ENCE: a Arg a Glu 20 l Asp s Lys u Lys y Gly a Arg 100 e Ile 5	6 Arg 5 Gly Leu Arg Asp 5 Val 85 Lys Arg	Lys Pro Gln Leu Asp 70 Val Leu Glu	Pro Ser Lys Glu 55 Asp Thr Ile Leu	Val Pro Lys 40 Ala Phe Lys His Gln 120	Leu Thr 25 Leu Phe Glu Val Leu 105 Val	Pro 10 Ser Glu Leu Arg Gln 90 Glu Leu	Ala Glu Glu Thr Ile 75 Ile His	Leu Gly Leu Gln 60 Ser Arg Lys Glu	Thr Ala Glu Lys Glu Pro Pro Cys 125	Ile Ser 30 Leu Ala Leu Ser Ala 110 Asn	Asn 15 Glu Asp Lys Gly 95 Ile Ser	Pro Ala Glu Val Ala 80 Leu Arg Pro	
<pre><400> SEQU Met Leu Al 1 Thr Ile Al Asn Leu Va 35 Gln Gln Ly Gly Glu Le 65 Gly Asn Gl Ile Met Al Asn Gln Il 11 Tyr Ile Va 130</pre>	ENCE: a Arg a Glu 20 l Asp s Lys u Lys y Gly a Arg 100 e Ile 5 l Gly	6 Arg 5 Gly Leu Arg Asp Val 85 Lys Arg Phe	Lys Pro Gln Leu Asp 70 Val Leu Glu	Pro Ser Lys Glu 55 Asp Thr Ile Leu Gly 135	Val Pro Lys 40 Ala Phe Lys His Gln 120 Ala	Leu Thr 25 Leu Phe Glu Val Leu 105 Val Phe	Pro 10 Ser Glu Leu Arg Gln Glu Leu Tyr	Ala Glu Glu Thr Thr Ile His Ile His Ser	Leu Gly Leu Gln 60 Ser Arg Lys Glu Asp 140	Thr Ala Glu 45 Lys Glu Pro Cys 125 Gly	Ile Ser 30 Leu Ala Leu Ser Ala 110 Asn Glu	Asn 15 Glu Asp Lys Gly 95 Ile Ser Ile	Pro Ala Glu Val Ala 80 Leu Arg Pro Ser	

-	$1 \cap n$	+	•	n	11	\sim	~
- (1			ι.	_	
-			_		~	~	-

Glu	Ala	Lys	Arg	Ile 165	Pro	Glu	Glu	Ile	Leu 170	Gly	Lys	Val	Ser	Ile 175	Ala	
Val	Leu	Arg	Gly 180	Leu	Ala	Tyr	Leu	Arg 185	Glu	Lys	His	Gln	Ile 190	Met	His	
Arg	Asp	Val 195	Lys	Pro	Ser	Asn	Ile 200	Leu	Val	Asn	Ser	Arg 205	Gly	Glu	Ile	
Lys	Leu 210	Cys	Asp	Phe	Gly	Val 215	Ser	Gly	Gln	Leu	Ile 220	Asp	Ser	Met	Ala	
Asn 225	Ser	Phe	Val	Gly	Thr 230	Arg	Ser	Tyr	Met	Ala 235	Pro	Glu	Arg	Leu	Gln 240	
Gly	Thr	His	Tyr	Ser 245	Val	Gln	Ser	Aap	Ile 250	Trp	Ser	Met	Gly	Leu 255	Ser	
Leu	Val	Glu	Leu 260	Ala	Val	Gly	Arg	Tyr 265	Pro	Ile	Pro	Pro	Pro 270	Asp	Ala	
Lys	Glu	Leu 275	Glu	Ala	Ile	Phe	Gly 280	Arg	Pro	Val	Val	Asp 285	Gly	Glu	Glu	
Gly	Glu 290	Pro	His	Ser	Ile	Ser 295	Pro	Arg	Pro	Arg	Pro 300	Pro	Gly	Arg	Pro	
Val 305	Ser	Gly	His	Gly	Met 310	Asp	Ser	Arg	Pro	Ala 315	Met	Ala	Ile	Phe	Glu 320	
Leu	Leu	Asp	Tyr	Ile 325	Val	Asn	Glu	Pro	Pro 330	Pro	Lys	Leu	Pro	Asn 335	Gly	
Val	Phe	Thr	Pro 340	Asp	Phe	Gln	Glu	Phe 345	Val	Asn	Lys	Суз	Leu 350	Ile	Lya	
Asn	Pro	Ala 355	Glu	Arg	Ala	Asp	Leu 360	Lys	Met	Leu	Thr	Asn 365	His	Thr	Phe	
Ile	Lys 370	Arg	Ser	Glu	Val	Glu 375	Glu	Val	Asp	Phe	Ala 380	Gly	Trp	Leu	Сүа	
Lys 385	Thr	Leu	Arg	Leu	Asn 390	Gln	Pro	Gly	Thr	Pro 395	Thr	Arg	Thr	Ala	Val 400	
<210 <211 <212 <213 <400)> SE L> LE 2> TY 3> OF 0> SE	EQ II ENGTH (PE : RGAN] EQUEN	D NO H: 19 DNA SM: NCE:	7 963 Homo 7	sar	iens	3									
gaaa	acgto	ecc g	gtgtg	ggag	ia aa	gcggg	gtetg	g ggt	gegg	gctg	ccgo	catga	act (cgtgg	yttcgg	60
aggo	cccad	gt g	ggeeg	99990	g gg	ygact	cago	g cgo	ctgg	jcag	ccga	actga	att a	acgta	agcggg	120
cggg	ggeeg	gga a	agtgo	ccgct	c ct	tggt	gggg	g gct	gtto	atg	gcgg	gttco	gg a	ggtct	ccaac	180
attt	ttco	cg g	gtete	gtggt	c ct	aaat	ctgt	c cca	aago	aga	ggca	agtgo	gag (cttga	aggttc	240
ttgo	ctggt	gt g	gaaat	gact	g ag	gtaca	aact	: ggt	ggtg	gtt	ggag	gcago	gtg o	gtgtt	gggaa	300
aago	cgcad	tg a	acaat	ccag	gc ta	atco	agaa	a cca	lcttt	gta	gato	gaata	atg a	atcco	caccat	360
agag	ggatt	ct t	acag	jaaaa	ac aa	gtgg	yttat	aga	atggt	gaa	acct	gttt	gt 1	ggad	atact	420
ggat	acag	get g	ggaca	agaa	ng ag	gtaca	agtgo	c cat	gaga	igac	caat	cacat	ga q	ggaca	aggcga	480
aggo	ettec	etc t	gtgt	attt	g co	catca	ataa	a tag	gcaag	ytca	tttç	gegga	ata 1	taad	cctcta	540
cago	ggago	ag a	attaa	agcga	ng ta	aaaq	gacto	gga	atgat	gta	ccta	atggt	gc t	cagto	Jggaaa	600
caag	gtgtg	gat t	tgco	caaca	a go	Jacas	yttga	a tac	caaaa	acaa	gcco	cacga	ac 1	zggco	aagag	660

-concinued	
ttacgggatt ccattcattg aaacctcagc caagaccaga cagggtgttg aagatgcttt	720
ttacacactg gtaagagaaa tacgccagta ccgaatgaaa aaactcaaca gcagtgatga	780
tgggactcag ggttgtatgg gattgccatg tgtggtgatg taacaagata cttttaaagt	840
tttgtcagaa aagagccact ttcaagctgc actgacaccc tggtcctgac ttcctggagg	900
agaagtattc ctgttgctgt cttcagtctc acagagaagc teetgetact teeceagete	960
tcagtagttt agtacaataa tctctatttg agaagttctc agaataacta cctcctcact	1020
tggctgtctg accagagaat gcacctcttg ttactccctg ttatttttct gccctgggtt	1080
cttccacagc acaaacacac ctcaacacac ctctgccacc ccaggttttt catctgaaaa	1140
gcagttcatg tctgaaacag agaaccaaac cgcaaacgtg aaattctatt gaaaacagtg	1200
tettgagete taaagtagea actgetggtg attittitt tettittaet gitgaaetta	1260
gaactatgcc taatttttgg agaaatgtca taaattactg ttttgccaag aatatagtta	1320
ttattgetgt ttggtttgtt tataatgtta teggetetat tetetaaaet ggeatetget	1380
ctagattcat aaatacaaaa atgaatactg aattttgagt ctatcctagt cttcacaact	1440
ttgacgtaat taaatccaac ttttcacagt gaagtgcctt tttcctagaa gtggtttgta	1500
gacteettta taatatttea gtggaataga tgteteaaaa ateettatge atgaaatgaa	1560
tgtctgagat acgtctgtga cttatctacc attgaaggaa agctatatct atttgagagc	1620
agatgccatt ttgtacatgt atgaaattgg ttttccagag gcctgttttg gggctttccc	1680
aggagaaaga tgaaactgaa agcatatgaa taatttcact taataatttt tacctaatct	1740
ccactttttt cataggttac tacctataca atgtatgtaa tttgtttccc ctagcttact	1800
gataaaccta atattcaatg aacttccatt tgtattcaaa tttgtgtcat accagaaagc	1860
tctacatttg cagatgttca aatattgtaa aactttggtg cattgttatt taatagctgt	1920
gatcagtgat tttcaaacct caaatatagt atattaacaa att	1963
<210> SEQ ID NO 8 <211> LENGTH: 189 <212> TYPE: PRT <213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 8	
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Gly Gly Val Gly Lys 1 5 10 15	
Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr 20 25 30	
Asp Pro Thr Ile Glu Asp Ser Tyr Arg Lys Gln Val Val Ile Asp Gly 35 40 45	
Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Ala Gly Gln Glu Glu Tyr 50 55 60	
Ser Ala Met Arg Asp Gln Tyr Met Arg Thr Gly Glu Gly Phe Leu Cys	
65 70 75 80	
Val Phe Ala Ile Asn Asn Ser Lys Ser Phe Ala Asp Ile Asn Leu Tyr 85 90 95	
Arg Glu Gln Ile Lys Arg Val Lys Asp Ser Asp Asp Val Pro Met Val 100 105 110	
Leu Val Gly Asn Lys Cys Asp Leu Pro Thr Arg Thr Val Asp Thr Lys 115 120 125	

Gln Ala His Glu Leu Ala Lys Ser Tyr Gly Ile Pro Phe Ile Glu Thr 130 135 140 Ser Ala Lys Thr Arg Gln Gly Val Glu Asp Ala Phe Tyr Thr Leu Val 145 150 155 160 Arg Glu Ile Arg Gln Tyr Arg Met Lys Lys Leu Asn Ser Ser Asp Asp 165 170 175 Gly Thr Gln Gly Cys Met Gly Leu Pro Cys Val Val Met 180 185 <210> SEQ ID NO 9 <211> LENGTH: 5775 <212> TYPE: DNA <213> ORGANISM: Homo sapiens <400> SEQUENCE: 9 60 teetaggegg eggeegegge ggeggaggea geageggegg eggeagtgge ggeggegaag gtggcggcgg ctcggccagt actcccggcc cccgccattt cggactggga gcgagcgcgg 120 cgcaggcact gaaggcggcg gcgggggccag aggctcagcg gctcccaggt gcgggagaga 180 ggcctgctga aaatgactga atataaactt gtggtagttg gagcttgtgg cgtaggcaag 240 agtgccttga cgatacagct aattcagaat cattttgtgg acgaatatga tccaacaata 300 gaggatteet acaggaagea agtagtaatt gatggagaaa eetgtetett ggatattete 360 gacacagcag gtcaagagga gtacagtgca atgagggacc agtacatgag gactggggag 420 ggetttettt gtgtatttge cataaataat actaaateat ttgaagatat teaceattat 480 aqaqaacaaa ttaaaaqaqt taaqqactct qaaqatqtac ctatqqtcct aqtaqqaaat 540 aaatgtgatt tgccttctag aacagtagac acaaaacagg ctcaggactt agcaagaagt 600 660 720 aaqaaaaaqa aqtcaaaqac aaaqtqtqta attatqtaaa tacaatttqt acttttttct 780 taaggcatac tagtacaagt ggtaattttt gtacattaca ctaaattatt agcatttgtt 840 ttagcattac ctaattttt tcctgctcca tgcagactgt tagcttttac cttaaatgct 900 tattttaaaa tgacagtgga agttttttt tcctcgaagt gccagtattc ccagagtttt 960 ggtttttgaa ctagcaatgc ctgtgaaaaa gaaactgaat acctaagatt tctgtcttgg 1020 ggtttttggt gcatgcagtt gattacttct tatttttctt accaagtgtg aatgttggtg 1080 tgaaacaaat taatgaaget tttgaateat eeetattetg tgttttatet agteacataa 1140 atggattaat tactaatttc agttgagacc ttctaattgg tttttactga aacattgagg 1200 gacacaaatt tatgggcttc ctgatgatga ttcttctagg catcatgtcc tatagtttgt 1260 catccctgat gaatgtaaag ttacactgtt cacaaaggtt ttgtctcctt tccactgcta 1320 1380 ttagtcatgg tcactctccc caaaatatta tattttttct ataaaaagaa aaaaatggaa aaaaattaca aggcaatgga aactattata aggccatttc cttttcacat tagataaatt 1440 actataaaga ctcctaatag ctttttcctg ttaaggcaga cccagtatga atgggattat 1500 tatagcaacc attttggggc tatatttaca tgctactaaa tttttataat aattgaaaag 1560 attttaacaa gtataaaaaa attctcatag gaattaaatg tagtctccct gtgtcagact 1620 getettteat agtataaett taaatetttt etteaaettg agtetttgaa gatagtttta 1680

-continued	
attctgcttg tgacattaaa agattatttg ggccagttat agcttattag gtgttgaaga	1740
gaccaaggtt gcaagccagg ccctgtgtga accttgagct ttcatagaga gtttcacagc	1800
atggactgtg tgccccacgg tcatccgagt ggttgtacga tgcattggtt agtcaaaaat	1860
ggggagggac tagggcagtt tggatagete aacaagatae aateteaete tgtggtggte	1920
ctgctgacaa atcaagagca ttgcttttgt ttcttaagaa aacaaactct tttttaaaaa	1980
ttacttttaa atattaactc aaaagttgag attttggggt ggtggtgtgc caagacatta	2040
atttttttt taaacaatga agtgaaaaag ttttacaatc tctaggtttg gctagttctc	2100
ttaacactgg ttaaattaac attgcataaa cacttttcaa gtctgatcca tatttaataa	2160
tgetttaaaa taaaaataaa aacaateett ttgataaatt taaaatgtta ettattttaa	2220
aataaatgaa gtgagatggc atggtgaggt gaaagtatca ctggactagg ttgttggtga	2280
cttaggttct agataggtgt cttttaggac tctgattttg aggacatcac ttactatcca	2340
tttcttcatg ttaaaagaag tcatctcaaa ctcttagttt tttttttta cactatgtga	2400
tttatattcc atttacataa ggatacactt atttgtcaag ctcagcacaa tctgtaaatt	2460
tttaacctat gttacaccat cttcagtgcc agtcttgggc aaaattgtgc aagaggtgaa	2520
gtttatattt gaatateeat tetegtttta ggaetettet teeatattag tgteatettg	2580
cctccctacc ttccacatgc cccatgactt gatgcagttt taatacttgt aattccccta	2640
accataagat ttactgctgc tgtggatatc tccatgaagt tttcccactg agtcacatca	2700
gaaatgeeet acatettatt tteeteaggg eteaagagaa tetgacagat accataaagg	2760
gatttgacct aatcactaat tttcaggtgg tggctgatgc tttgaacatc tctttgctgc	2820
ccaatccatt agcgacagta ggatttttca accctggtat gaatagacag aaccctatcc	2880
agtggaagga gaatttaata aagatagtgc agaaagaatt ccttaggtaa tctataacta	2940
ggactactcc tggtaacagt aatacattcc attgttttag taaccagaaa tcttcatgca	3000
atgaaaaata ctttaattca tgaagcttac ttttttttt ttggtgtcag agtctcgctc	3060
ttgtcaccca ggctggaatg cagtggcgcc atctcagctc actgcaacct tccatcttcc	3120
caggttcaag cgattctcgt gcctcggcct cctgagtagc tgggattaca ggcgtgtgca	3180
ctacactcaa ctaatttttg tatttttagg agagacgggg tttcacctgt tggccaggct	3240
ggtctcgaac tcctgacctc aagtgattca cccaccttgg cctcataaac ctgttttgca	3300
gaactcattt attcagcaaa tatttattga gtgcctacca gatgccagtc accgcacaag	3360
gcactgggta tatggtatcc ccaaacaaga gacataatcc cggtccttag gtactgctag	3420
tgtggtctgt aatatcttac taaggccttt ggtatacgac ccagagataa cacgatgcgt	3480
attttagttt tgcaaagaag gggtttggtc tctgtgccag ctctataatt gttttgctac	3540
gattccactg aaactcttcg atcaagctac tttatgtaaa tcacttcatt gttttaaagg	3600
aataaacttg attatattgt ttttttattt ggcataactg tgattctttt aggacaatta	3660
ctgtacacat taaggtgtat gtcagatatt catattgacc caaatgtgta atattccagt	3720
tttctctgca taagtaatta aaatatactt aaaaattaat agttttatct gggtacaaat	3780
aaacagtgcc tgaactagtt cacagacaag ggaaacttct atgtaaaaat cactatgatt	3840
tetgaattge tatgtgaaac tacagatett tggaacaetg tttaggtagg gtgttaagae	3900
ttgacacagt acctcgtttc tacacagaga aagaaatggc catacttcag gaactgcagt	3960

pottatgagg ggatattag gootottga tuttgatg agatggoat titttag Agrogita tacottia gigaactig aatggita caasagatt dittiga agattiaa ggggagaat tolgaaat aatgtacot aatattaca gootaag Agattiaa ggggagaat tolgaaat asgtacot aatgatact tugaotaa Agagtatca gootaatt googacta tatgatact tugaotaa dicocaag Alao aagatta googacta tatgatcat tugaotaa dicocaag Alao aagatta googacta tatgatcat tugaotaa dicocaag Alao aagatta googacta tatgatcat tugaotaa dicocaag Alao aagatta googacta tatgatcat tugaotaa dicocaag Alao Aaaggtat aagattag cacgtaga cattaga gootaaga dicocaag Alao Alao aagatta gootaa cattatga cacgtaa tatgatcat tugaotaa Alao	-continued	
<pre>sagiggtaa tiacetta gigaactig atggttaa caaagatta gittiga gigagagaa tilgagaal atggatta gottaaaga 1400</pre>	gcttatgagg ggatatttag gcctcttgaa tttttgatgt agatgggcat ttttttaagg	4020
agattttaa gyggggaat tctagaat atgttact attgtact gocttaaga 140 caaattctt tgtggagtt tttttaaas agactaat tacatgact taggcatta 200 aggcattcta goctcatt aactgagca catgcatag gaattagaa theocaad 2200 aggcattcta goctcatt aactgagca cacgtcatag gaattagaa cotaacttag 380 ttttgtggg aggaata tggcacattge cacgtteg cacatteg to catattat actagaad 440 tttttttttt ttttgggga aggaatag actgraag gattagaa gagattagaa gattagaa gattagaa gattagaa gattaga	tagtggttaa ttacctttat gtgaactttg aatggtttaa caaaagattt gtttttgtag	4080
caesantoot tyttyaagit tittitaaaa aagaotaat taotagoot tagoottaa 4200 caeguttyg gaagaatata goagoota attgratoot tugotyaa gitcoccaegi 420 atagotato goetotati actgrato caegaota gaattaga otaaatt 420 atagotato aaastgitg tooccatig caeattig cotaatta caetagaac 430 tittigugogo atgitaagit acagityoo caagitata tatactit tootatgat 440 tittigugogo atgitaagit acagityoo caegitat tatactit tootatgat 440 tittigugo agoaaaaa otatogaag attoota tatattig toocaaagi 450 tittiguga goetaaaa otatogaag attoota tatactit tutagogaa 450 tittiguga agoaaaaa otatogaag attoota tatactit tutagogaa 470 tittigua cutagtat tutagita acagiga googaatta 470 catatagat tagatagit gittitaga cocagagit acotgaag ofgaatta 470 catatagaa tugadoot gittigut tatagitg aagigootgi tugogaaagi 470 catatagaa tugadoot gittitagit aatagitg aagigootgi tugogaatagi 470 catatogaa tugadoot gittitagit aatagitg aagigootgi tugogaatagi 470 catatogaa tugadoot gittitagit aatagitg aagigootgi tugogaatagi 470 catatogaa tugadoot gittitagit aatagitgi aagigotogi tugogaatagi 470 catatogaa tugadoot gittitagi aasaagita cocgacigot ottitaata cacatotoco cocaacoo cacaagaota acoggita agigitut ogaaagit 490 caattoota tagitatat tottatgi aacaigita ocigootgi ottitagigi 100 caattogaa gitaacagi tgittacagi aacaagita cocgootgo ottitagigi 100 caattogaa gitaacagi tgittacagi tagitagaa tutgigi 00 caattogaa gitaacagi tgittacad tutgigaa tugogaa agaattoo 230 caatagagi agitaatat tootagaa aacagita cocgootga cigaactagi 520 caattagaa gaacatot tutaigaaa taagotat goegacta gaacatagi 520 caatagagi atoittai goagatagi agigataci titattit tutootta 530 caatagagi atoittai goagatagi agigataci tutaitagaa gaacacaa agigaacia a soo aacacaat tigootagi gittigoti aacaatit cocatagi agigacaca i 520 caatagagi atoittai goagatagi acogacagi tagacaca i 520 caatagagi atoittai goagatagi agigaacia tutaitati tutootta 550 caatagagi atoittai goagatagi agigaaca tutai goocaaagi gicacaagi 570 gootatica aggacaga taigacaci atacatai titattoi taacaacai 570 soo sipoues i 520 Dib 10 10 521 Dib 100 10 521 Dib 100 10 521 Dib 100 10	agattttaaa gggggagaat tctagaaata aatgttacct aattattaca gccttaaaga	4140
catglitigtig gaagsatata googsogtat attglateat titgagigaat giteccaagi 4260 aggetateta goetatti aactgagita eestigeata gaattagaa cotaactit 1300 attaggitate aaaactgitig teeseatige acattitigt ootaatata actagaaae 1300 tittigtaggiga atgetaagit acattitigt eestigata tetaactitt tittaggiggat 4500 tittittigaa eageataa etateggaa atteestiga eestigaag otgaatta 4500 tittigtaa ooteetigata atetagata etateggaa etateggaa etagaag etagaag etagaag otgaatta 4500 eataactag taagateti tittittiga eestigaga etagaag etagaag etagaag etagaag etagaag etagaag etagaagata 4500 eataactag taagateti tiggiggaa aaaagtat eegggeta agtiggiteti eegagaata 4500 eataetatg tittigtig tittiggig aagtigetit eegagaatag 4500 eataetaga ttagateti tiggiggaa aaaagtat eigeggeta ditgiggigge 4600 eataetata tittigtig aagtigetit eegagaag etagagetit 4500 eataetata tittigtig aaateetit eegagaate 4500 eataetata tittigtig aagtigetit eegagaata 4500 eataetata tittigtig aaateetit eegageetit eitittiggiggi 4500 eataetata tittigtig aaateetit eegageetit eitittiggiggi 4500 eatateaat gaecaetee attelgaaat taeetittaa atgittatag 5200 eatateaat gaecaetee attelgaat taeetittaa atgittatag 5200 eataetatatit geagegaata tiggataeti taetittittit teaaag	caaaaatcct tgttgaagtt tttttaaaaa aagactaaat tacatagact taggcattaa	4200
agocaticta gotictatti aartgagica cartgostag gaattisgaa octaactitti 420 atagotati aaaatgitig toocoatig acadtitig octaattat actaigaaa 440 tittitta aaaatgitig toocoatig acadtitig octaattat actaigaaa 440 tittittit titti titti tittika aaagitig aartgosta takaattit tittiggggat 450 tittittig ocgaaaaaa cittiggaa oroogoogit acotigaaag ofgaatta 420 tittittig orgaaaaaa cittigaag atticaatti gicaaaaagt aagigatig 460 cittittigaa tittigatag tittigaa oroogoogit acotigaag attigaagaatg 460 cittittigaa tittigatgaa tittigaaga acaagita takagitti gaagaagatg 460 cittittigaa tittigatgaa tittigaaga agatactaa agagitti gaagaagatg 470 cacaactaga titagatagi tittigaaga agatactaa agagitti gaagaagatg 470 cacaactaga titagatagaa titagaggaaa aaaagitta oroogogitti gitgaggaa a gaaggitat tiagatgaat titagagggaa aaaaagitta oroogogitti gitgaggaa cacattocoo cocaagaoco acaagagta actoggitta agotgitti goggatatg 490 cacattocoo cocaagaoco acaagagta actoggitta agotgitti tootgaagitti 490 cacattocoo cocaagaoco actoggi agaatto oroogocogi titgaagittig 500 cacattocoo cocaagaoco actoggi agaatta citggitg gotgaacta ggaatgitg 5100 cacattocoo cocaagoco actoggi agaatta citggitg gotgaacta ggaatgitg 5100 cacatactaa citaaaata gaccacot titaatgaaa taagotta tagotatag 520 gagtatgig tiggaagig toocaaasta gaccacot titaatgaat taagotat gagaatgitg 5100 cacatactaa aataaaa gaccacot titaatgaat taagotat gacatcaca 530 catattatg caagitaa tigaaggaa tiggaatat gagaatat taagotat gacagag figogatat agaagag gigtitoo acaaggig agaatact citaattata titaagota gacaaggi aattgaaco caacaggig tiggaagatta taagatata tactoo fiso caaaaagaata tiggaataa tigaataat agataact catattat titaagagaag figogaatat agagaaga taagataca tattatta titattoo titaagagaag figogaatat agagaaga taagatac tattatta titattoo fiso caaaaaga agot goodiaga agatagat agataga gacagaaga 570 gotittis aggaagaa tatgaatoa tacaattat titattota taactaata figo figogaaga tatgaataa tigaatgat agatagat agataga gacagaaga 570 gotittis aggaagataa tigaatgat agatagat agatagaaga figo figo figo figo figo figo figo figo figo figo figo	catgtttgtg gaagaatata gcagacgtat attgtatcat ttgagtgaat gttcccaagt	4260
ataggitate aaactgitg teacattge acatttge ectattat acatagaace 4380 tttggggge atgttaggt acagttgee eagtetee teattgat teeatgat 4440 ttttttte tetaaact tttttetea aaccgitat tataacttt tttaggggat 4500 ttttttage eageaaaas etateggat atteeat geeaaag atgattet 4560 tgataateg graggaats tttggata geegaat eegagatet geeaaag atgattat 4500 attagtae tegggatg tttttaga eegagate geegagate gaggtete gaagatag 4600 ecgeeaaatega taagseeg teeteeaaga agateeea argagtete gaagatag 4600 ecgeeaaatega taagseeg teeteeaaga agateeea argagetet gaagatag 4700 gataggtaat tagatgat teegagaa agaaagtat eigeagtet gaagatag 4800 gataggtaat tagatgat teegagaa aasaagtat eigeagtet gagagee 4800 ecaatteea taggtgat teegagaa acaaggeta acgggttat eigeaggee 4800 ecaatteee eegeeaa argegeta acgggtaa acaagget 4920 ecaattee eigeegge geegaa aasaagtat eigeagtet geeggee 4800 ecaattee eigeegge geegaa aasaagtat eigeagtete eigeaggee 4800 ecaattee eigeegge geegaa aasaagtat eigeagtete eigeaggee 4800 ecaattee eigeegge geggaaga aasaagtet eigeegtete eigeaggee 4800 ecaattee eigeegge geggaaga aasaagtet eigeegtete eigeaggee 4800 ecaattee eigeegge geggaaga aasaagtet eigeegtete eigeeggee 4800 ecaattee eigeegge geggaaga attegee eigeegeegge 220 gagatgege gegaagga eigeaaa attegeaa tettgeegtee tettage 5200 ecaattee eigeegge eigeaggee eigeagee eigeegee eigeegee geegee 520 aaaattegaa tigteegee gaaggeta eigeagete gaagetee tittatee gaegee 520 aaaaetage gegetete gegaegte eigeaetet gagataet tettette taaagage 5520 aaaaetage gegetete geaaegae attegete eigeegee 5520 aaaaetaat eigeeettag eegaaaaa aggataee tittatte titteettta 5580 teaaetaagg aattegatee eaeaagte eigeeetae graegee 5520 aaaeetaate igeeettag geaaega eigetete geeeeaag tageeeaag 5700 geetattee aggeeaga tageaeet eiteegte geeeaagg tageeage 5520 aaaeetaate aiggaaata tigaaagt agtegete gitaageee gieaeeaage 5700 geetattee aggeeaga tageaeea tagetee eiteeettee 5700 eitea eitee eigees eigees eigees eigees eigees eigees eigees 5700 eitea eigees	aggcatteta ggetetattt aactgagtea caetgeatag gaatttagaa eetaaetttt	4320
ttggggge atgttagtt acagtttgen eagttegen eagttegen tenttgent teenttgent teenttgent 4440 tttttttte teenaacat ttttettee aaacagtate tataactttt ttagggget 4500 ttggtaattge gaggaatg ttttggen genggaatg eergenaacg	ataggttatc aaaactgttg tcaccattgc acaattttgt cctaatatat acatagaaac	4380
<pre>tttttttt to totaaxaat tottotca aaxaagtat tataactttt totaggggat 4500 tttttttag cagcaaxaa otatotgaag attocatt gotaaxaagt axtgattte 4560 tttttttaga cagcaaxaa otatotgaag attocatt gotaaxaagt axtgattet 4500 ttttttagtaa totoggtaat titttagaa cocagcagt acottgaag otgaattat 4620 atttagtaat totoggtat atcotgata gotagatte tgottgag aactgaatagt 4680 cotaatcaag attaagatot gottttagt tataagttg aagtaccac atgagtett gagaatagt 4740 cotaatcaga ttaagatog tgtttagt tatagttg aagtaccac atgagtett gagaatagt 4800 gataggtaat ttagatgaat ttaggggaa aaaagtta cigcagtta gitgaggge 4660 cotatcocc cocacaceee cacagageta actgggtac agtgtttat cogaaagtt 4920 cocattotec cocacaceee cacagageta actgggtac agtgtttat cogaaagtt 4920 cocattotec cocacaceee cacagageta actgggtac agtgtttat cogaaagtt 4920 cocattotec cocacaceee cacagageta actgggtae agtgtttat cogaagtt 4920 cocattotec cocacacee cacagageta actgggtae agtgtttat cogaagtt 4920 cocattotec cocacacee cacagageta actgggtae agtgttte tott titgtggg 5100 cotataccaa cattaaaa gocactet totaagaa tactggte gotgacet ggaagtgg 5100 cotataccaa cattaaaaat gaccactet taaggaat tagetatte 5280 cotattage tgtgaagtg totaaatt gotaattt tgtoatgac tgtactacte 5280 cotattage tgtgaagtg totaaatt gaagtace tittatte taagecatag 5400 ctaatcaag tatgatae gagtgtat tggataett tatagecatag 5400 ctaatcaag at atgtacae aaaagta toggtacet tagagatag 5520 aaaacaaate tgeetttag cacaaaaaa aggataact tattatta titeettta 5580 ctaattagg attgaaagg attgaatge tiggatage ftggtttag garaggage 5640 aaactata taggaaata tiggaatgit agtaagta gitaage gitageaggag 5640 aaactata taggaaata tiggaatgit agtatgit gitaagee gitageaggag 5775 c100 SEQUENCE 10 Met The Glu Pyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 1 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2</pre>	tttgtggggc atgttaagtt acagtttgca caagttcatc tcatttgtat tccattgatt	4440
ttittitaga cagcaaaaa citateigaag atticeatti gicaaaagi aatgatitei 4560 kigataattigi gicagigaatgi ttittitagaa eecaageagi aceetigaaa eetigaaa deegaattati 4620 attiagiaae teetigigita ateetiggata geatgaatte tigeateigaga aaetgaatagi 4680 eetigeataa atgettitti teetaagaa agataetee tigeateigaga aaetgaatagi 4740 eetaaetaga tiaagateig tijttitagit taatagittig aagigeetigi tigggataat 4800 gataggtaat tiaagatiga titaggegaa aaeaagitta eigeagitta eigeagitta 4920 eeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeee	ttttttttt ttctaaacat tttttcttca aaacagtata tataactttt tttaggggat	4500
tgataatig gtagtgaag tittitgaa cocagoogit accttgaag cdga atttagtaac thotgtgtta tatactggaa agtacttoc atgggttot gagaatagt 4680 cotgtcataaa atgottott toctgtgata agtactoca atgggttott gagaatagt 4680 catactaga ttaggtott ttaggtott tgaggtott 4800 gatagtadt ttaggtott ttaggtott 4800 catactaga ttaggtott ttaggtott 4800 catactaga ttaggtott ttaggtott 4800 catactaga ttaggtott ttaggtott 4900 catactaga ttaggtot dtaggtott 4900 caatttott ctagtact ttoctgagtg 4900 caatttott ctagtact ttoctgagtg 4900 caatttott ctagtact ttoctgagtg 5000 cattagagt ttoctgagtot ttoctgagtg 5000 cattagaat gaccactat ttagtott 5200 cattactag catactat gagtagtot ttagtaat 520 caaattott ttgotact	tttttttaga cagcaaaaaa ctatctgaag atttccattt gtcaaaaagt aatgatttct	4560
attragtaac ttotgtgtta atactggata goatgaatto tgoattgaga aactgaatag 4680 ctgtcataaa atgottott tootaaagaa agatactoa atgagtott gaagaatagt 4740 cataactaga ttaagatotg tgttttagtt taatagttig aagtgootgt tiggggataat 4800 gataggtaat ttagatgaat ttaggggaaa aaaaagtta otgoagttat gitgagggoo 4860 catototoco ocoacacco cacagagota actgggtta agtgtttat cogaagttt 4920 coaattoota tgtottgtgt ttocatgtig aaatactit tgoattito ottgaggg 4980 caattota otagacat ttottaatgt acatgtita ootgootgt ottitaacta 5040 ttittgtata gigtaaactg aaacatgoac attitgtaca tigtgootto ttitagigg 5100 caatatoaa dagtacag tgtttocat cattiggtig ootgacca ggaatgtig 5100 caatataaa ggtaactg aaacatgoac attitgtaca tigtgootto ttitagig 5220 gagtatgto tgigaagtga totaaaatt gaatattt tgicatgaac tgaactatag 5220 gagtatgto tgigaagtga totaaaatt gaatattt tgicatgaac tgiactacco 5280 ctaattaga agtatata aaatagta cagtgata taggtgtgt titatoatgo 5340 aaattigaa tgittgooco gaaaggata tggatactt taatggaat gaacatatag 5400 tatacoagi gattotag tgigttoot aacatgit agagatact titattoatgo 5400 tatacoagi gattotag cagaatggat tggaagtat tggatactt ataagocata gaacatatag 5400 tatacoagi gattotag cacaaaaat aggatacat tattatta titoottta 5580 toaataagg agtgttigot aacaaaat aggatacat tattatta titoottta 5580 toaataaggi aattgataca cacaoggig citiggit agacaa tattita gaagagaag 5520 aaaacaaat tgoottai gaaaaaa taggatacat tattatta titoottta 5580 toaataaggi aattgataca cacaoggig citiggit gita agocaagg tagocagaga 5700 ggotattica aggoagaag taatgacto atoatata titattita titoottita 5760 taaacataat acgag 5775 *210> SPQ ID NO 10 *2112 LDNOTE: 18 *212> PTPE: PTP *212> Orgonism: Homo sapiens *400> SPQUENCE: 10 Met Thi Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lig 17 1 0 10 10 *212	tgataattgt gtagtgaatg ttttttagaa cccagcagtt accttgaaag ctgaatttat	4620
ctgtcataa atgetttett teetaaagaa agataeteae atgagttett gaagatagt 4740 cataactaga ttaagatedt gtgtttagtt taatgittg aagtgeetgt ttgggataat 4800 gataggtaat ttagatgaat ttaggggaaa aaaagttat etgeggttat gtgggggee 4860 cateteee ee	atttagtaac ttctgtgtta atactggata gcatgaattc tgcattgaga aactgaatag	4680
catactaga ttaagatctg tgttttagt taatgttt aatgtttg aagtgeetgt ttgggataat 4800 gataggtaat ttagatgaat ttaggggaaa aaaaagtta etgeggtta gtgggggee 4860 cateteetee eeeeeeeeeeeeeeeeeeeeeeeeeeeee	ctgtcataaa atgctttctt tcctaaagaa agatactcac atgagttctt gaagaatagt	4740
gataggtaat ttagatgaat ttaggggaaa aaaagttat ctgcagttat gttgagggoc 4860 caatctetee eeeeeeeeeeeeeeeeeeeeeeeeeeeee	cataactaga ttaagatctg tgttttagtt taatagtttg aagtgcctgt ttgggataat	4800
catctctcc cocacaccc cacagagcta actgggtta agtgtttat cogaaagtt 4920 coaattccac tgtcttgtg tttcatgttg aaatactt tgcattttc ctttgggte 4980 caattctta ctggtactat ttcttaatgt accaggtta ctggcttt tgtgggt 5100 catatgcagt gtgtaccagt tgtttccat cattggttg ogctgaccta ggaatgttgg 5160 tcatatcaa cattaaaaat gaccactct ttaatgaaat taactttaa atgttatag 5220 gagtatgge tgtgaagtga tctaaaatt gtaatattt tgtcatgaac tgtactact 5280 ctaattcaa cattaaaaat gaccactct ttaatgaaat taactttaa atgttatag 5240 saatttga tagtaata aaaatagta cagtgactat gagtgtgtat ttattcatg 5340 aaatttga tagtaata aaaatagta cagtgactat gagtgtgtat tattacag 5400 tataccagtg aatctttat goagctgt tgtgaagtat tggatact tataagcaat gaccactatg 5400 tataccagtg aatctttat goagctgt agaagtat tggatact tattagaagtagge 5520 aaaataaat atgaaagge gtgttgct aaacaattt ccatattag aagtagagcg 5520 aaaataaat atgaaagge gtgttgct aaacaatt tattatta tttccttta 5580 tcaataagg aattgaaca caacagggg cttggttta ggacaacat tatttatta tttccttta 5580 tcaataagg aattgaaca caacaggg cttggttta ggacacat tattatta tttcctttta 5580 tcaataagg aattgaaca caacaggg cttggttt ggacaagag tagcagcage 5640 aacattaata atggaaata ttgaatagt agttagtat gttaatgcca gtcaccagca 5700 ggctattca aggtcagag taatgactco atacatata tttattct tacatacatt 5760 taaacattaa cagg cacasaata tcagg cacasaata tcagg cacasaata tcagg tagceca atactata tttatttot taactacatt 5760 taaacatta ccagg 10 NO 10 call> LBNNFN 188 call> LBNNFN 188 call> LBNNFN 188 call> LBNNFN 188 call> LBNNFN 189 call> LBNNFN	gataggtaat ttagatgaat ttaggggaaa aaaaagttat ctgcagttat gttgagggcc	4860
ccaattocad tydettydy titeatydy aaataetti tydattite etityggy 4980 caattocta etigetaeta titetaatyd aacaegyta eetiggeetyd etittaacta 5040 titttydata gydaacey aacaegoad attiggaca tydgoetde tittgyggy 5160 caataecaa eataaaaa gaceaetet taatggaad taaetttaa atgytaag 5220 gagtatgge tydgaagyg tetaaaatti gaaatatti tydeada tydeetae 5280 ceaattogad eyigaagyg tetaaaatti gaatatti tydeada eigeetae 5340 caattegaa tydeetae eigeetae 5340 caattegaa tydeetae eigeetae gaagydyg 5460 taateeegy aactittat geagettyd agaagtaeet tittatte taaaagydge 5460 tittaecagy aactittat geagettyd agaagtaee tittattet taaaggage 5460 teaataacag gydeeteetae gaagaga aggaaaaaat aggataaeat tattette taaaagydge 5520 aaaacaaate tydeetaeg acaaaaaaa aggataaeat tattattat titeettita 5580 teaataagg aattgaaca caacaggga etiggttud gydaaeaat tattatta titeettita 5580 teaataagg aattgaaca caacaggga etiggttud gydaaeaat tattatta titeettita 5580 teaataagg aattgaaca caacaggga etiggttig gitaageacaa tattattet taaeexaatt 5760 sacaattaata atggaaataa tigaatagti agtatgat gitaatgee giceacaagg 5775 <210> SKO ID NO 10 <211> LSMOFH: 188 <212> TYPE: PET <213> OKGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thi GU Tyi Lyv Leu Val Val Gly Ala Cyv Gly Val Gly Lyv 15 See Ala Leu Thi Ile Gln Leu Ile Gln Aen His Phe Val Aep Glu Tyr	catctctccc cccacacccc cacagagcta actgggttac agtgttttat ccgaaagttt	4920
caatttotta ctagtactat ttottaatgt aacatgtta cctggootgt ottttaacta 5040 tttttgtata gtgtaaactg aacatgcac atttgtgtaca ttgtgottto ttttgtgggt 5160 catatgcagt gtgatocagt tgttttocat cattggttg ogotgacota ggaatgttgg 5220 gagtatgtgo tgtgaagtga totaaaattt gtaatattt tgtcatgaac tgtactacto 5280 ctaattatg taatgtaata aaaatagtta cagtgactat gagtgtgtat ttattcatgo 5340 aaatttgaac tgttgocoog gaaatggata tggatacttt ataagccata gacactatag 5400 tataccagtg atotttat goagettgt agaagtatoc ttttattte taaaaggtgo 5460 tgtggatatt atgtaaagg dtgttgott aaacaattt cocatattag aagtagatgo 5520 aaaacaaato tgocottatg acaaaaaat aggataacat tatttatta tttocttta 5580 tcaataatg aatgataca caacaggga cttggttta ggtaatgga tagtagcaga 5640 aacattaata atggaaataa ttgaatagt aggatate ggtaatgca gtcaccagca 5700 ggotatttoa aggtoagaag taatgacto atacaatta tttatttat ttocttta 5760 taaaccatat ccagg 5775 c210> SEQ ID NO 10 c210> SEQ UD NO 10 c210> SEQ UD NO 10 c210> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 5 10 Lasan Ala Leu Thr Lie Gln Leu Lie Gin Aan Hie Phe Val Abp Glu Tyr	ccaattccac tgtcttgtgt tttcatgttg aaaatacttt tgcatttttc ctttgagtgc	4980
ttttgtata gtgtaaactg aaacatgcac atttgttaca ttgtgctttc ttttgtgggt 5100 catatgcagt gtgtaccagt tgttttccat catttggttg cgctgaccta ggaatgttgg 5160 tcatatcaaa cattaaaaat gaccactctt ttaatgaaat taacttttaa atgttatag 5220 gagtatgtge tgtgaagtga tctaaaattt gtaatattt tgtcatgaac tgtactactc 5280 cctaattattg taatgtaata aaaatagtta cagtggactat gagtgtgtat ttattcatgc 5340 aaatttggaac tgtttgccce gaaatggata tggatactt ataagccata gacactatag 5400 tataccagtg aatctttat gcagettgt agaagtatee ttttattte taaaaggtge 5520 aaaacaagt agtgaaaaaaa aggataacat tattatta tttecttta 5580 tcaataagg aattggatac caacaggtga cttggttta ggccaaagg tagcagcag 5640 aaacataata atggaaataa ttgaatagt agtatgtat gttaatgcca gtcaccaga 5700 ggetattca aggtcagaag taatgactec atacatatta tttatteta taaccacatt 5760 taaacattaata atggaaataa ttgaataget agtatgtat gttaatgcca gtcaccaga 5700 ggetattte aggtcagaag taatgactec atacatatta tttatteta taactacatt 5760 taaacatta ccagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 116 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	caatttetta etagtaetat ttettaatgt aacatgttta eetggeetgt ettttaaeta	5040
catatgcagt gtgatccagt tgttttccat catttggttg cgctgaccta ggaatgttgg tcatatcaaa cattaaaaat gaccacttt ttaatgaaat taacttttaa atgttatag gagtatgtgc tgtgaagtga tctaaaatt gtaatattt tgtcatgaac tgtactactc 5280 ctaattattg taatgtaata aaaatagtta cagtgactat gagtgtgtat ttattcatgc 540 aaatttggaac tgtttgcccc gaaatggata tggatactt ataagccata gaccatatag full scatcagtg aatctttat gcagcttgtt agaagtatcc ttttatttc taaaaggtgc 5460 tgtgggatatt atgtaaaggc gtgttgctt aaacaattt ccatattag aagtagatgc 5520 aaaacaaaact tgcctttatg acaaaaaat aggataacat tattattat tttccttta 5580 tcaataaggt aattgataca caacaggtga cttggttta ggcccaaagg tagcagcagc 5640 aacattaata atggaaataa ttgaatagtt agttatgat gttaatgcca gtcaccagca 5700 ggctattca aggtcagaag taatgactce atacatata tttatttat tatcattat 5760 callo SEQ ID No 10 callo LENCTH: 188 callo SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 10 1 5 10 10 587 Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	tttttgtata gtgtaaactg aaacatgcac attttgtaca ttgtgctttc ttttgtgggt	5100
tcatatcaaa cattaaaaat gaccactctt ttaatgaaat taacttttaa atgtttatag 5220 gagtatgtgc tgtgaagtga tctaaaattt gtaatattt tgtcatgaac tgtactactc 5280 ctaattattg taatgtaata aaaatagtta cagtgactat gagtgtgtat ttattcatgc 5340 aaatttggaac tgtttgcccc gaaatggata tggatacttt ataagccata gaccatatag 5400 tataccagtg aatctttat gcagcttgtt agaagtatcc ttttatttc taaaaggtgc 5460 tgtgggatatt atgtaaaggc gtgttgctt aaacaattt ccatattag aagtagatgc 5520 aaaaacaaatc tgcctttatg acaaaaaat aggataacat tatttatta tttcctttta 5580 tccaataaggt aattgataca caacaggtga cttggttta ggcccaaagg tagcagcagc 5640 aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca 5700 ggctattca aggtcagaag taatgactcc atacatatta tttattta taactacatt 5760 taaatcatta ccagg 5775 <210> SEQ ID NO 10 <211> LENOTH: 188 <210> SEQ UD NO 10 <211> LENOTH: 188 <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	catatgcagt gtgatccagt tgttttccat catttggttg cgctgaccta ggaatgttgg	5160
<pre>gagtatgtgc tgtgaagtga tctaaaattt gtaatattt tgtcatgaac tgtactactc 5280 ctaattattg taatgtaata aaaatagtta cagtgactat gagtgtgtat ttattcatgc 5340 aaatttgaac tgtttgococ gaaatggata tggatactt ataagccata gacactatag 5400 tataccagtg aatotttat gcagcttgtt agaagtacc tttatttc taaaaggtgc 5460 tgtgggatatt atgtaaaggc gtgtttgott aaacaattt cotatttag aagtagagtag 5520 aaaacaaaatc tgocttatg acaaaaaaat aggataacat tattattat tttocttta 5580 tcaataaggt aattgataca caacaggtga cttggttta ggccaaagg tagcagcagc 5640 aaacttaata atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca 5700 ggctattca aggtcagaag taatgactoc atacatatta tttatttca taactacatt 5760 taaatcatta ccagg 5775 </pre>	tcatatcaaa cattaaaaat gaccactctt ttaatgaaat taacttttaa atgtttatag	5220
ctaattattg taatgtaata aaaatagtta cagtgactat gagtgtgtat ttattcatge 5340 aaatttgaac tgtttgococ gaaatggata tggatacttt ataagccata gacactatag 5400 tataccagtg aatctttat gcagcttgtt agaagtatoc ttttatttc taaaaggtge 5460 tgtgggatatt atgtaaagge gtgtttgott aaacaattt coatattag aagtagatge 5520 aaaaacaaato tgoctttatg acaaaaaat aggataacat tatttatta tttoctttta 5580 tcaataaggt aattgataca caacaggtga cttggttta ggoccaaagg tagcagcage 5640 aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca 5700 ggotatttca aggtcagaag taatgactoc atacatatta tttatttota taactacatt 5760 taaatcatta ccagg 5775 <210> SEQ ID N0 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	gagtatgtgc tgtgaagtga tctaaaattt gtaatatttt tgtcatgaac tgtactactc	5280
aaatttgaac tgttgoecc gaaatggata tggatacttt ataagecata gacactaag 5400 tataeccagtg aatetttat geagettgtt agaagtatee ttttattte taaaaggtge 5460 tgtggatatt atgtaaagge gtgtttgett aaacaatttt eeatattag aagtagatge 5520 aaaacaaaate tgeetttatg acaaaaaaat aggataacat tatttatta ttteettta 5580 teaataaggt aattgataea caacaggtga ettggttta ggeecaaagg tageageage 5640 aaacattaata atggaaataa ttgaatagtt agttatgtat gttaatgeea gteaecagea 5700 ggeetatteea aggteagaag taatgaetee ataeatatta tttattet taaectaeatt 5760 taaateatta eeagg sagtageedeeaga 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15	ctaattattg taatgtaata aaaatagtta cagtgactat gagtgtgtat ttattcatgc	5340
tataccagtg aatcttttat geagettgtt agaagtatee ttttattte taaaaggtge 5460 tgtgggatatt atgtaaagge gtgtttgett aaacaattt eeatattag aagtagatge 5520 aaaacaaate tgeetttatg acaaaaaaat aggataacat tatttattta ttteetttta 5580 teaataaggt aattgataca caacaggtga ettggttta ggeecaaagg tageageage 5640 aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgeea gteaecagea 5700 ggetatttea aggteagaag taatgaetee atacatatta tttatttea taaetaeatt 5760 taaateatta eeagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	aaatttgaac tgtttgcccc gaaatggata tggatacttt ataagccata gacactatag	5400
tgtggatatt atgtaaagge gtgtttgett aaacaattt eeatatttag aagtagatge 5520 aaaacaaate tgeetttatg acaaaaaaat aggataacat tatttattta ttteetttta 5580 teaataaggt aattgataca caacaggtga ettggtttta ggeecaaagg tageageage 5640 aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgeea gteaecagea 5700 ggetatteea aggteagaag taatgaetee atacatatta tttatteta taaetaeatt 5760 taaateatta eeagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	tataccagtg aatcttttat gcagcttgtt agaagtatcc ttttattttc taaaaggtgc	5460
aaaacaaatc tgootttatg acaaaaaat aggataacat tatttattta tttootttta 5580 tcaataaggt aattgataca caacaggtga ottggttta ggoocaaagg tagoagcago 5640 aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgooa gtoaccagoa 5700 ggootattooa aggtoagaag taatgactoo atacatatta tttattoota taactacatt 5760 taaaatoatta ocagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	tgtggatatt atgtaaaggc gtgtttgctt aaacaatttt ccatatttag aagtagatgc	5520
tcaataaggt aattgataca caacaggtga cttggtttta ggcccaaagg tagcagcagc 5640 aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca 5700 ggctatttca aggtcagaag taatgactcc atacatatta tttatttcta taactacatt 5760 taaatcatta ccagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	aaaacaaatc tgcctttatg acaaaaaaat aggataacat tatttattta tttcctttta	5580
aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca 5700 ggctatttca aggtcagaag taatgactcc atacatatta tttatttcta taactacatt 5760 taaatcatta ccagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	tcaataaggt aattgataca caacaggtga cttggtttta ggcccaaagg tagcagcagc	5640
ggctatttca aggtcagaag taatgactcc atacatatta tttatttcta taactacatt 5760 taaatcatta ccagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	aacattaata atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca	5700
taaatcatta ccagg 5775 <210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	ggctatttca aggtcagaag taatgactcc atacatatta tttatttcta taactacatt	5760
<pre><210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr</pre>	taaatcatta ccagg	5775
<400> SEQUENCE: 10 Met Thr Glu Tyr Lys Leu Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	<210> SEQ ID NO 10 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Homo sapiens	
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15 Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	<400> SEQUENCE: 10	
Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr	Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Cys Gly Val Gly Lys 1 5 10 15	
20 25 30	Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr 20 25 30	

- con	t	Т	n	11	e	C

-continued	
Asp Pro Thr Ile Glu Asp Ser Tyr Arg Lys Gln Val Val Ile Asp Gly 35 40 45	
Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Ala Gly Gln Glu Glu Tyr 50 55 60	
Ser Ala Met Arg Asp Gln Tyr Met Arg Thr Gly Glu Gly Phe Leu Cys 65 70 75 80	
Val Phe Ala Ile Asn Asn Thr Lys Ser Phe Glu Asp Ile His His Tyr 85 90 95	
Arg Glu Gln Ile Lys Arg Val Lys Asp Ser Glu Asp Val Pro Met Val 100 105 110	
Leu Val Gly Asn Lys Cys Asp Leu Pro Ser Arg Thr Val Asp Thr Lys 115 120 125	
Gln Ala Gln Asp Leu Ala Arg Ser Tyr Gly Ile Pro Phe Ile Glu Thr 130 135 140	
Ser Ala Lys Thr Arg Gln Gly Val Asp Asp Ala Phe Tyr Thr Leu Val 145 150 155 160	
Arg Glu Ile Arg Lys His Lys Glu Lys Met Ser Lys Asp Gly Lys Lys	
Lys Lys Lys Ser Lys Thr Lys Cys Val Ile Met 180 185	
<210> SEQ ID NO 11 <211> LENGTH: 571 <212> TYPE: DNA <213> ORGANISM: Homo sapiens	
CHOON BEQUENCE. II	
catgacggaa tataagctgg tggtggtggg cgccggcggt gtgggcaaga gtgcgctgac catccagctg atccagaacc attttgtgga cgaatacgac cccactatag aggattccta	60 120
ccggaagcag gtggtcattg atggggagac gtgcctgttg gacateetgg atacegeegg	180
ccaggaggag tacagegeca tgegggaeca gtacatgege aceggggagg getteetgtg	240
tgtgtttgcc atcaacaaca ccaagtcttt tgaggacatc caccagtaca gggagcagat	300
caaacgggtg aaggactcgg atgacgtgcc catggtgctg gtggggaaca agtgtgacct	360
ggetgeaege aetgtggaat eteggeagge teaggaeete geeegaaget aeggeateee	420
ctacatcgag acctcggcca agacccggca gggagtggag gatgccttct acacgttggt	480
gcgtgagatc cggcagcaca agctgcggaa gctgaaccct cctgatgaga gtggccccgg	540
ctgcatgagc tgcaagtgtg tgctctcctg a	571
<210> SEQ ID NO 12 <211> LENGTH: 189 <212> TYPE: PRT <213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 12	
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Gly Gly Val Gly Lys 1 5 10 15	
Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp Glu Tyr 20 25 30	
Asp Pro Thr Ile Glu Asp Ser Tyr Arg Lys Gln Val Val Ile Asp Gly 35 40 45	
Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Ala Gly Gln Glu Glu Tyr	

-continued	
50 55 60	
Ser Ala Met Arg Asp Gln Tyr Met Arg Thr Gly Glu Gly Phe Leu Cys65707580	
Val Phe Ala Ile Asn Asn Thr Lys Ser Phe Glu Asp Ile His Gln Tyr 85 90 95	
Arg Glu Gln Ile Lys Arg Val Lys Asp Ser Asp Asp Val Pro Met Val 100 105 110	
Leu Val Gly Asn Lys Cys Asp Leu Ala Ala Arg Thr Val Glu Ser Arg 115 120 125	
Gln Ala Gln Asp Leu Ala Arg Ser Tyr Gly Ile Pro Tyr Ile Glu Thr 130 135 140	
Ser Ala Lys Thr Arg Gln Gly Val Glu Asp Ala Phe Tyr Thr Leu Val 145 150 155 160	
Arg Glu Ile Arg Gln His Lys Leu Arg Lys Leu Asn Pro Pro Asp Glu 165 170 175	
Ser Gly Pro Gly Cys Met Ser Cys Lys Cys Val Leu Ser 180 185	
<210> SEQ ID NO 13 <211> LENGTH: 2477 <212> TYPE: DNA <213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 13	
cgcctccctt ccccctcccc gcccgacage ggccgctcgg gccccggctc tcggttataa	60
gatggeggeg etgageggtg geggtggtgg eggegeggag eegggeeagg etetgtteaa	120
cggggacatg gageeegagg eeggegeegg egeeggegee geggeetett eggetgegga	180
ccctgccatt ccggaggagg tgtggaatat caaacaaatg attaagttga cacaggaaca	240
tatagaggcc ctattggaca aatttggtgg ggagcataat ccaccatcaa tatatctgga	300
ggcctatgaa gaatacacca gcaagctaga tgcactccaa caaagagaac aacagttatt	360
ggaatetetg gggaaeggaa etgatttte tgtttetage tetgeateaa tggataeegt	420
tacatettet teetetteta geettteagt getaeettea tetettteag ttttteaaaa	480
tcccacagat gtggcacgga gcaaccccaa gtcaccacaa aaacctatcg ttagagtctt	540
cctgcccaac aaacagagga cagtggtacc tgcaaggtgt ggagttacag tccgagacag	600
tctaaagaaa gcactgatga tgagaggtct aatcccagag tgctgtgctg	660
tcaggatgga gagaagaaac caattggttg ggacactgat atttcctggc ttactggaga	720
agaattgcat gtggaagtgt tggagaatgt tccacttaca acacaaact ttgtacgaaa	780
aacgtttttc accttagcat tttgtgactt ttgtcgaaag ctgcttttcc agggtttccg	840
ctgtcaaaca tgtggttata aatttcacca gcgttgtagt acagaagttc cactgatgtg	900
tgttaattat gaccaacttg atttgctgtt tgtctccaag ttctttgaac accacccaat	960
accacaggaa gaggcgteet tageagagae tgeeetaaca tetggateat eeeetteege	1020
accegeeteg gaetetattg ggeeecaaat teteaceagt eegteteett caaaateeat	1080
tccaattcca cagcccttcc gaccagcaga tgaagatcat cgaaatcaat ttgggcaacg	1140
agacegatee teateagete ceaatgtgea tataaacaca atagaacetg teaatattga	1200
tgacttgatt agagaccaag gatttcgtgg tgatggagga tcaaccacag gtttgtctgc	1260

-concinued	
	1320
aggaceteag egagaaagga agteatette ateeteagaa gacaggaate gaatgaaaae	1380
acttggtaga cgggactcga gtgatgattg ggagattcct gatgggcaga ttacagtggg	1440
acaaagaatt ggatctggat catttggaac agtctacaag ggaaagtggc atggtgatgt	1500
ggcagtgaaa atgttgaatg tgacagcacc tacacctcag cagttacaag ccttcaaaaa	1560
tgaagtagga gtactcagga aaacacgaca tgtgaatatc ctactcttca tgggctattc	1620
cacaaagcca caactggcta ttgttaccca gtggtgtgag ggctccagct tgtatcacca	1680
tctccatatc attgagacca aatttgagat gatcaaactt atagatattg cacgacagac	1740
tgcacagggc atggattact tacacgccaa gtcaatcatc cacagagacc tcaagagtaa	1800
taatatattt cttcatgaag acctcacagt aaaaataggt gattttggtc tagctacagt	1860
gaaatctcga tggagtgggt cccatcagtt tgaacagttg tctggatcca ttttgtggat	1920
ggcaccagaa gtcatcagaa tgcaagataa aaatccatac agctttcagt cagatgtata	1980
tgcatttgga attgttctgt atgaattgat gactggacag ttaccttatt caaacatcaa	2040
caacagggac cagataattt ttatggtggg acgaggatac ctgtctccag atctcagtaa	2100
ggtacggagt aactgtccaa aagccatgaa gagattaatg gcagagtgcc tcaaaaagaa	2160
aagagatgag agaccactct ttccccaaat tctcgcctct attgagctgc tggcccgctc	2220
attgccaaaa attcaccgca gtgcatcaga accctecttg aategggetg gtttccaaac	2280
agaggatttt agtctatatg cttgtgcttc tccaaaaaca cccatccagg cagggggata	2340
tggtgcgttt cctgtccact gaaacaaatg agtgagagag ttcaggagag tagcaacaaa	2400
aggaaaataa atgaacatat gtttgcttat atgttaaatt gaataaaata ctctctttt	2460
ttttaaggtg aaccaaa	2477
<210> SEQ ID NO 14 <211> LENGTH: 766 <212> TYPE: PRT <213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 14	
Met Ala Ala Leu Ser Gly Gly Gly Gly Gly Gly Ala Glu Pro Gly Gln 1 5 10 15	
Ala Leu Phe Asn Gly Asp Met Glu Pro Glu Ala Gly Ala Gly Ala Gly 20 25 30	
Ala Ala Ser Ser Ala Ala Asp Pro Ala Ile Pro Glu Glu Val Trp 35 40 45	
Asn Ile Lys Gln Met Ile Lys Leu Thr Gln Glu His Ile Glu Ala Leu 50 55 60	
Leu Asp Lys Phe Gly Gly Glu His Asn Pro Pro Ser Ile Tyr Leu Glu	
65 70 75 80	
Ala Tyr Glu Glu Tyr Thr Ser Lys Leu Asp Ala Leu Gln Gln Arg Glu 85 90 95	
Gln Gln Leu Leu Glu Ser Leu Gly Asn Gly Thr Asp Phe Ser Val Ser 100 105 110	
Ser Ser Ala Ser Met Asp Thr Val Thr Ser Ser Ser Ser Ser Leu 115 120 125	
Ser Val Leu Pro Ser Ser Leu Ser Val Phe Gln Asn Pro Thr Asp Val 130 135 140	

Ala 145	Arg	Ser	Asn	Pro	Lys 150	Ser	Pro	Gln	Lys	Pro 155	Ile	Val	Arg	Val	Phe 160	
Leu	Pro	Asn	Lys	Gln 165	Arg	Thr	Val	Val	Pro 170	Ala	Arg	Сүз	Gly	Val 175	Thr	
Val	Arg	Asp	Ser 180	Leu	Lys	Lys	Ala	Leu 185	Met	Met	Arg	Gly	Leu 190	Ile	Pro	
Glu	Cys	Cys 195	Ala	Val	Tyr	Arg	Ile 200	Gln	Asp	Gly	Glu	Lys 205	Lys	Pro	Ile	
Gly	Trp 210	Aab	Thr	Asp	Ile	Ser 215	Trp	Leu	Thr	Gly	Glu 220	Glu	Leu	His	Val	
Glu 225	Val	Leu	Glu	Asn	Val 230	Pro	Leu	Thr	Thr	His 235	Asn	Phe	Val	Arg	Lys 240	
Thr	Phe	Phe	Thr	Leu 245	Ala	Phe	Cys	Aab	Phe 250	Cys	Arg	Lys	Leu	Leu 255	Phe	
Gln	Gly	Phe	Arg 260	Сүз	Gln	Thr	Суз	Gly 265	Tyr	Lys	Phe	His	Gln 270	Arg	Суз	
Ser	Thr	Glu 275	Val	Pro	Leu	Met	Cys 280	Val	Asn	Tyr	Asp	Gln 285	Leu	Asp	Leu	
Leu	Phe 290	Val	Ser	Lys	Phe	Phe 295	Glu	His	His	Pro	Ile 300	Pro	Gln	Glu	Glu	
Ala 305	Ser	Leu	Ala	Glu	Thr 310	Ala	Leu	Thr	Ser	Gly 315	Ser	Ser	Pro	Ser	Ala 320	
Pro	Ala	Ser	Asp	Ser 325	Ile	Gly	Pro	Gln	Ile 330	Leu	Thr	Ser	Pro	Ser 335	Pro	
Ser	ГЛа	Ser	Ile 340	Pro	Ile	Pro	Gln	Pro 345	Phe	Arg	Pro	Ala	Asp 350	Glu	Aap	
His	Arg	Asn 355	Gln	Phe	Gly	Gln	Arg 360	Asp	Arg	Ser	Ser	Ser 365	Ala	Pro	Asn	
Val	His 370	Ile	Asn	Thr	Ile	Glu 375	Pro	Val	Asn	Ile	Asp 380	Asp	Leu	Ile	Arg	
Asp 385	Gln	Gly	Phe	Arg	Gly 390	Asp	Gly	Gly	Ser	Thr 395	Thr	Gly	Leu	Ser	Ala 400	
Thr	Pro	Pro	Ala	Ser 405	Leu	Pro	Gly	Ser	Leu 410	Thr	Asn	Val	Lys	Ala 415	Leu	
Gln	Lys	Ser	Pro 420	Gly	Pro	Gln	Arg	Glu 425	Arg	Lys	Ser	Ser	Ser 430	Ser	Ser	
Glu	Asp	Arg 435	Asn	Arg	Met	Lys	Thr 440	Leu	Gly	Arg	Arg	Asp 445	Ser	Ser	Asp	
Asp	Trp 450	Glu	Ile	Pro	Asp	Gly 455	Gln	Ile	Thr	Val	Gly 460	Gln	Arg	Ile	Gly	
Ser 465	Gly	Ser	Phe	Gly	Thr 470	Val	Tyr	Lys	Gly	Lys 475	Trp	His	Gly	Asp	Val 480	
Ala	Val	Lys	Met	Leu 485	Asn	Val	Thr	Ala	Pro 490	Thr	Pro	Gln	Gln	Leu 495	Gln	
Ala	Phe	Lys	Asn 500	Glu	Val	Gly	Val	Leu 505	Arg	Lys	Thr	Arg	His 510	Val	Asn	
Ile	Leu	Leu 515	Phe	Met	Gly	Tyr	Ser 520	Thr	Lys	Pro	Gln	Leu 525	Ala	Ile	Val	
Thr	Gln 530	Trp	Суз	Glu	Gly	Ser 535	Ser	Leu	Tyr	His	His 540	Leu	His	Ile	Ile	

Glu Thr Lys Phe Glu Met Ile Lys Leu Ile Asp Ile Ala Arg Gln Thr545550555560
Ala Gln Gly Met Asp Tyr Leu His Ala Lys Ser Ile Ile His Arg Asp 565 570 575
Leu Lys Ser Asn Asn Ile Phe Leu His Glu Asp Leu Thr Val Lys Ile 580 585 590
Gly Asp Phe Gly Leu Ala Thr Val Lys Ser Arg Trp Ser Gly Ser His
Gln Phe Glu Gln Leu Ser Gly Ser Ile Leu Trp Met Ala Pro Glu Val
610 615 620 Ile Arg Met Gln Asp Lys Asn Pro Tyr Ser Phe Gln Ser Asp Val Tyr
625 630 635 640 Ala Phe Gly Ile Val Leu Tyr Glu Leu Met Thr Gly Gln Leu Pro Tyr
$\begin{array}{c} 645 \\ 650 \\ 655 \end{array}$
Ser Asn Ile Asn Asn Ang Asp Gln Ile Ile Phe Met Val Gly Arg Gly 660 665 670
Tyr Leu Ser Pro Asp Leu Ser Lys Val Arg Ser Asn Cys Pro Lys Ala 675 680 685
Met Lys Arg Leu Met Ala Glu Cys Leu Lys Lys Arg Asp Glu Arg 690 695 700
Pro Leu Phe Pro Gln Ile Leu Ala Ser Ile Glu Leu Leu Ala Arg Ser705710715720
Leu Pro Lys Ile His Arg Ser Ala Ser Glu Pro Ser Leu Asn Arg Ala
Gly Phe Gln Thr Glu Asp Phe Ser Leu Tyr Ala Cys Ala Ser Pro Lys
Thr Pro Ile Gln Ala Gly Gly Tyr Gly Ala Phe Pro Val His
755 760 765
<210> SEQ ID NO 15 <211> LENGTH: 5916 <212> TYPE: DNA <213> ORGANISM: Homo sapiens
<400> SEQUENCE: 15
gcccctccct ccgcccgccc gccggcccgc ccgtcagtct ggcaggcagg caggcaatcg 60
gteegagtgg etgteggete tteagetete eegeteggeg tetteettee teeteeeggt 120
cagegtegge ggetgeaeeg geggeggege agteeetgeg ggagggggega caagagetga 180
geggeggeeg eegagegteg ageteagege ggeggaggeg geggeggeee ggeageeaae 240
atggeggegg eggeggegge gggegeggge eeggagatgg teegegggea ggtgttegae 300
gtggggccgc gctacaccaa cctctcgtac atcggcgagg gcgcctacgg catggtgtgc 360
tctgcttatg ataatgtcaa caaagttcga gtagctatca agaaaatcag cccctttgag 420
caccagacct actgccagag aaccctgagg gagataaaaa tcttactgcg cttcagacat 480
gagaacatca ttggaatcaa tgacattatt cgagcaccaa ccatcgagca aatgaaagat 540
gtatatatag tacaggacct catggaaaca gatetttaca agetettgaa gacacaacac 600
ctcagcaatg accatatctg ctattttctc taccagatcc tcagagggtt aaaatatatc 660
cattcagcta acgttctgca ccgtgacctc aagccttcca acctgctgct caacaccacc 720

				-contir	lued		
acagggttcc	tgacagaata	tgtggccaca	cgttggtaca	gggctccaga	aattatgttg	840	
aattccaagg	gctacaccaa	gtccattgat	atttggtctg	taggctgcat	tctggcagaa	900	
atgettteta	acaggcccat	ctttccaggg	aagcattatc	ttgaccagct	gaaccacatt	960	
ttgggtattc	ttggatcccc	atcacaagaa	gacctgaatt	gtataataaa	tttaaaagct	1020	
aggaactatt	tgctttctct	tccacacaaa	aataaggtgc	catggaacag	gctgttccca	1080	
aatgctgact	ccaaagctct	ggacttattg	gacaaaatgt	tgacattcaa	cccacacaag	1140	
aggattgaag	tagaacaggc	tctggcccac	ccatatctgg	agcagtatta	cgacccgagt	1200	
gacgagccca	tcgccgaagc	accattcaag	ttcgacatgg	aattggatga	cttgcctaag	1260	
gaaaagctca	aagaactaat	ttttgaagag	actgctagat	tccagccagg	atacagatct	1320	
taaatttgtc	aggacaaggg	ctcagaggac	tggacgtgct	cagacatcgg	tgttcttctt	1380	
cccagttctt	gacccctggt	cctgtctcca	gcccgtcttg	gcttatccac	tttgactcct	1440	
ttgagccgtt	tggaggggcg	gtttctggta	gttgtggctt	ttatgctttc	aaagaatttc	1500	
ttcagtccag	agaattcctc	ctggcagccc	tgtgtgtgtc	acccattggt	gacctgcggc	1560	
agtatgtact	tcagtgcacc	tactgcttac	tgttgcttta	gtcactaatt	gctttctggt	1620	
ttgaaagatg	cagtggttcc	tccctctcct	gaatcctttt	ctacatgatg	ccctgctgac	1680	
catgcagccg	caccagagag	agattettee	ccaattggct	ctagtcactg	gcatctcact	1740	
ttatgatagg	gaaggctact	acctagggca	ctttaagtca	gtgacagccc	cttatttgca	1800	
cttcaccttt	tgaccataac	tgtttcccca	gagcaggagc	ttgtggaaat	accttggctg	1860	
atgttgcagc	ctgcagcaag	tgetteegte	tccggaatcc	ttggggagca	cttgtccacg	1920	
tcttttctca	tatcatggta	gtcactaaca	tatataaggt	atgtgctatt	ggcccagctt	1980	
ttagaaaatg	cagtcatttt	tctaaataaa	aaggaagtac	tgcacccagc	agtgtcactc	2040	
tgtagttact	gtggtcactt	gtaccatata	gaggtgtaac	acttgtcaag	aagcgttatg	2100	
tgcagtactt	aatgtttgta	agacttacaa	aaaaagattt	aaagtggcag	cttcactcga	2160	
catttggtga	gagaagtaca	aaggttgcag	tgctgagctg	tgggcggttt	ctggggatgt	2220	
cccagggtgg	aactccacat	gctggtgcat	atacgccctt	gagctacttc	aaatgtgggt	2280	
gtttcagtaa	ccacgttcca	tgcctgagga	tttagcagag	aggaacactg	cgtctttaaa	2340	
tgagaaagta	tacaattctt	tttccttcta	cagcatgtca	gcatctcaag	ttcatttttc	2400	
aacctacagt	ataacaattt	gtaataaagc	ctccaggagc	tcatgacgtg	aagcactgtt	2460	
ctgtcctcaa	gtactcaaat	atttctgata	ctgctgagtc	agactgtcag	aaaaagctag	2520	
cactaactcg	tgtttggagc	tctatccata	ttttactgat	ctctttaagt	atttgttcct	2580	
gccactgtgt	actgtggagt	tgactcggtg	ttctgtccca	gtgcggtgcc	tcctcttgac	2640	
ttccccactg	ctctctgtgg	tgagaaattt	gccttgttca	ataattactg	taccctcgca	2700	
tgactgttac	agctttctgt	gcagagatga	ctgtccaagt	gccacatgcc	tacgattgaa	2760	
atgaaaactc	tattgttacc	tctgagttgt	gttccacgga	aaatgctatc	cagcagatca	2820	
tttaggaaaa	ataattctat	ttttagcttt	tcatttctca	gctgtccttt	tttcttgttt	2880	
gatttttgac	agcaatggag	aatgggttat	ataaagactg	cctgctaata	tgaacagaaa	2940	
tgcatttgta	attcatgaaa	ataaatgtac	atcttctatc	ttcacattca	tgttaagatt	3000	
cagtgttgct	tteetetgga	tcagcgtgtc	tgaatggaca	gtcaggttca	ggttgtgctg	3060	

-continued	
aacacagaaa tgctcacagg cctcactttg ccgcccaggc actggcccag cacttggatt	3120
tacataagat gagttagaaa ggtacttctg tagggtcctt tttacctctg ctcggcagag	3180
aatcgatgct gtcatgttcc tttattcaca atcttaggtc tcaaatattc tgtcaaaccc	3240
taacaaagaa geeeegacat etcaggttgg atteeetggt tetetetaaa gagggeetge	3300
ccttgtgccc cagaggtgct gctgggcaca gccaagagtt gggaagggcc gccccacagt	3360
acgcagteet caccacceag eccagggtge teacgeteae cacteetgtg getgaggaag	3420
gatagetgge teateetegg aaaacagaee cacateteta ttettgeeet gaaataegeg	3480
cttttcactt gcgtgctcag agctgccgtc tgaaggtcca cacagcattg acgggacaca	3540
gaaatgtgac tgttaccgga taacactgat tagtcagttt tcatttataa aaaagcattg	3600
acagttttat tactcttgtt tctttttaaa tggaaagtta ctattataag gttaatttgg	3660
agteetette taaatagaaa accatateet tggetaetaa catetggaga etgtgagete	3720
cttcccattc cccttcctgg tactgtggag tcagattggc atgaaaccac taacttcatt	3780
ctagaatcat tgtagccata agttgtgtgc tttttattaa tcatgccaaa cataatgtaa	3840
ctgggcagag aatggtccta accaaggtac ctatgaaaag cgctagctat catgtgtagt	3900
agatgcatca ttttggctct tcttacattt gtaaaaatgt acagattagg tcatcttaat	3960
tcatattagt gacacggaac agcacctcca ctatttgtat gttcaaataa gctttcagac	4020
taatagettt tttggtgtet aaaatgtaag caaaaaatte etgetgaaae atteeagtee	4080
tttcatttag tataaaagaa atactgaaca agccagtggg atggaattga aagaactaat	4140
catgaggact ctgtcctgac acaggtcctc aaagctagca gagatacgca gacattgtgg	4200
catctgggta gaagaatact gtattgtgtg tgcagtgcac agtgtgtggt gtgtgcacac	4260
tcattccttc tgctcttggg cacaggcagt gggtgtagag gtaaccagta gctttgagaa	4320
gctacatgta gctcaccagt ggttttctct aaggaatcac aaaagtaaac tacccaacca	4380
catgccacgt aatatttcag ccattcagag gaaactgttt tctctttatt tgcttatatg	4440
ttaatatggt ttttaaattg gtaactttta tatagtatgg taacagtatg ttaatacaca	4500
catacatacg cacacatget ttgggteett ceataataet tttatatttg taaateaatg	4560
ttttggagca atcccaagtt taagggaaat atttttgtaa atgtaatggt tttgaaaatc	4620
tgagcaatcc ttttgcttat acatttttaa agcatttgtg ctttaaaatt gttatgctgg	4680
tgtttgaaac atgatactcc tgtggtgcag atgagaagct ataacagtga atatgtggtt	4740
totottaogt catocacott gacatgatgg gtoagaaaca aatggaaato cagagoaagt	4800
cctccagggt tgcaccaggt ttacctaaag cttgttgcct tttcttgtgc tgtttatgcg	4860
tgtagagcac tcaagaaagt tctgaaactg ctttgtatct gctttgtact gttggtgcct	4920
tottggtatt gtaccocaaa attotgoata gattatttag tataatggta agttaaaaaa	4980
tgttaaagga agattttatt aagaatctga atgtttattc attatattgt tacaatttaa	5040
cattaacatt tatttgtggt atttgtgatt tggttaatct gtataaaaat tgtaagtaga	5100
aaggtttata tttcatctta attcttttga tgttgtaaac gtacttttta aaagatggat	5160
tatttgaatg tttatggcac ctgacttgta aaaaaaaaaa	5220
atcattaaat tgtgtccctg tattaccaaa ataacacagc accgtgcatg tatagtttaa	5280
ttgcagtttc atctgtgaaa acgtgaaatt gtctagtcct tcgttatgtt ccccagatgt	5340

cttccagatt tgctctgcat gtggtaactt gtgttagggc tgtgagctgt tcctcgagtt gaatggggat gtcagtgctc ctagggttct ccaggtggtt cttcagacct tcacctgtgg gggggggggt aggcggtgcc cacgcccatc tcctcatcct cctgaacttc tgcaacccca ctgctgggca gacatcctgg gcaacccctt ttttcagagc aagaagtcat aaagatagga tttcttggac atttggttct tatcaatatt gggcattatg taatgactta tttacaaaac aaagatactg gaaaatgttt tggatgtggt gttatggaaa gagcacaggc cttggaccca tccagctggg ttcagaacta ccccctgctt ataactgcgg ctggctgtgg gccagtcatt ctgcgtctct gctttcttcc tctgcttcag actgtcagct gtaaagtgga agcaatatta cttgccttgt atatggtaaa gattataaaa atacatttca actgttcagc atagtacttc aaagcaagta ctcagtaaat agcaagtctt tttaaa <210> SEQ ID NO 16 <211> LENGTH: 360 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 16 Met Ala Ala Ala Ala Ala Gly Ala Gly Pro Glu Met Val Arg Gly Gln Val Phe Asp Val Gly Pro Arg Tyr Thr Asn Leu Ser Tyr Ile Gly 20 25 30 Glu Gly Ala Tyr Gly Met Val Cys Ser Ala Tyr Asp Asn Val Asn Lys Val Arg Val Ala Ile Lys Lys Ile Ser Pro Phe Glu His Gln Thr Tyr Cys Gln Arg Thr Leu Arg Glu Ile Lys Ile Leu Leu Arg Phe Arg His Glu Asn Ile Ile Gly Ile Asn Asp Ile Ile Arg Ala Pro Thr Ile Glu Gln Met Lys Asp Val Tyr Ile Val Gln Asp Leu Met Glu Thr Asp Leu Tyr Lys Leu Leu Lys Thr Gln His Leu Ser Asn Asp His Ile Cys Tyr Phe Leu Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asn Val Leu His Arg Asp Leu Lys Pro Ser Asn Leu Leu Leu Asn Thr Thr Cys Asp Leu Lys Ile Cys Asp Phe Gly Leu Ala Arg Val Ala Asp Pro Asp His Asp His Thr Gly Phe Leu Thr Glu Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Ile Met Leu Asn Ser Lys Gly Tyr Thr Lys Ser Ile Asp Ile Trp Ser Val Gly Cys Ile Leu Ala Glu Met Leu Ser Asn Arg Pro Ile Phe Pro Gly Lys His Tyr Leu Asp Gln Leu Asn His Ile Leu Gly Ile Leu Gly Ser Pro Ser Gln Glu Asp Leu Asn Cys Ile Ile

-continued

Val Pro Trp Ann Arg Leu Pho Pro Ann Ala Arg far Lyn Ala Leu Arg 275 Leu Leu App Lyn Met Leu Tra Pho Ann Pro His Lyn Arg He Giu Val 280 Giu Gin Ala Leu Ala His Pro Tyr Leu Giu Gin Tyr Tyr An Pro Sen 200 Ang Giu Pro He Ala Giu Ala Pro Phe Lyn Phe Ang Met Giu Leu Ang 235 Ang Leu Pro Lyn Giu Lyn Leu Lyn Giu Lu He Pho Giu Giu Tir Ala 350 Arg Pho Gin Pro Gil Tyr Arg Sen 200 Callo SRO ID NO 17 Callo SRO ID NO 17 Callo DIN 10 17 Callo SRO ID NO 17 <th>Asn Leu Lys Ala Arg Asn Tyr Leu Leu Ser Leu Pro His Lys Asn Lys 260 265 270</th> <th></th>	Asn Leu Lys Ala Arg Asn Tyr Leu Leu Ser Leu Pro His Lys Asn Lys 260 265 270	
Leu Leu Ap Lya Pic Lie The Pic An Pic Ni by Arg Tie Glu Val 200 Glu Ala Lieu Ala His Pico Tyr Lieu Glu Glu Jin Tyr Tyr Asp Pic 320 Aeg Glu Pico Ile Ala Glu Ala Pico Pic Lyg The Ang Net Glu Leu Ap 325 Arg Leu Pico Lyg Glu Lyg Leu Lyg Glu Leu Ile Pic Glu Glu Jir Tr Ala 140 415 Pico Gly Tyr Arg Ser 325 47 Pico Gly Tyr Arg Ser 325 47 Pico Gly Tyr Arg Ser 326 410 SEO ID No 17 411 Liberrin: 147 411 Li	Val Pro Trp Asn Arg Leu Phe Pro Asn Ala Asp Ser Lys Ala Leu Asp	
299 M. S. M. E. 200 M. S. M. K. M. 200 M. M. Y. Tyr Amp Pro Ser 305 M. 200 M. S. M.	275 280 285	
Gin Gin Ala Len Ala Kin Pro Tyr Leu Gin Gin Tyr Tyr App Pro Ser 315App Gin Pro11e Ala Gin Ala ProPheLyp Phe App Met Gin Leu App 335App Leu ProLyg Gin Lyp Leu Lyg Gin Leu IIe Phe Gin Gin Ulu Tala 346Arg Phe Gin Pro Gily Tyr Arg Ser 3552100 SEG ID NO 17 2111 LENDIFF 14992115 GINADIFF 14992115 GINADIFF 14992116 Sequence conservation2100 SEGUENCE 17geoceccccc cogeocece cogeogeoce cogeogeog extectore toctcocegi2100 SEQUENCE 17geoceccccc cogeocece geoggocege cogeoggo cogeoggoce gagogagea cogageta gigogoggoc cogagecteg agetcageog gagogageg cogagegage agageta dagegoceg geoggocege cogeoggo ggeoggoggo cogagegage agageta gigogoggoc geoggoceg geoggoggog cogagegage gagogage daggeta gigoggoceg cogagecteg agetcageog ggeoggoggo cogagegage gagegagea cogagetica gigoggoceg cogagecteg agetcageag geoggoggo cogagegage gagegage cogagegage daggeta gigoggoceg cogagecteg agetcageag gagegagea cogagageta gigoggoceg cogagecteg agetcageag accetagag gagetaaaa tottactog cottcagaa dagaacaca tigogaaca gatettate dagecaca categagea categagea agageta gigoacacaa cottageaag accetaga gacetaga gacetaaa agetcaga gagetacaa dagaacaca tiggaacaa gacetaga gacetacaa categagea categagea agageta gigoacacaa categaga cottagag gacetacaa gacetaa agateta tiggacaaca gatettate dagecaca accetagea gaacacaa dagaacaata tiggacaca gacetaga gacetagaa gacetaa agatetag toa gacegate categaga gacetaat tiggacaaca categagaa atatatat dagaacaata tiggacaaca gacetaga gacetaat tiggacaaca gacetaat tiggacaaca gacetaat tiggacaaca gacetaat tiggacaaca gacetaat tiggacaaca gacetaatatig tigaacaaca dagaacaat tiggacacaa gacetaatat gacaatat tiggacaac	290 295 300	
Ang Giu Pro lie Ala Giu Ala Pro Phe bye Phe Ang Net Giu Leu Ang 325 Ang Leu Pro bye Giu bye Leu bye Giu Leu Ile Phe Giu Giu Thr Ala 350 Arg Phe Gin Pro Giy Tyr Ang Ser 355 7360 *2010 SEQ ID NO 17 *2015 SEQUENCE: 17 geoceteeet egocogoece geoggeecge cegteagtet ggeageage cagocaate 60 greegagegg eggeeggeeg ageteagee ggeageage ggagggeeg e gocaeceae 240 atgeoggeegg eggeeggeeg ageteagee ggeagageg ggegeggee g gocaeceae 240 atgeoggeegg eggeeggeeg ageteagee ggeagageg ggeegeggee gitte cegteage 300 greggeeggeeg eggeeggeeg ageteagee ggeagageg eggeeggee 300 greggeeggeeg eggeeggeeg ggeeggeegge ageteageag cagocaate 400 atgeoggeegg eggeeggeeg ggeeggeegge ageteageag agaatate tettaetge ettaaget 400 gagaacatea teggaacaa eettetagte teageteta agaaataag ecettag 420 caceagaeet aetgeocaag aaceetgag gagataaaa tettaetge ettaagaata gagaacatea teggaacaa gacettae agetetaga agetaagaat 540 gtatatatag tacaagaace gatetate cagagaeet aagetetaga agetaaata fato eatgebaat ageattate cagageete aceetgage ageteageag 700 ettageaatg aceatateg ettageetta teggaaceaa ceategagea aatagaata fato aggateta ettageacet aagetetta agetettaa agetetaga aataagat 540 gtatatatag tacaagaace gatettae eggeaceaa ceategagea aatagaata 540 gtatatatag tacaagaace gatettae eggeaceaa ceategagea aatagaat 540 gtatatatag tacaagaace categaaca gatettae agetettaa agetetaga aceatagea 780 acaaggatee takaagaaca gteettga ettageetta aagetetaga acaatagea 780 atteeaag geteaaceaa gteettga ettgeeetg gaegtegaa aatagat 540 atteeaag geteaaceaa gteettga ettgeeetg agetegaa attagteg 840 atteeaag geteaaceaa gteettga ettgeeetg agetegaa attagteg 180 atteeaag geteaaceaa gteettga ettgeeetg agetegaa attagteg 180 atteeaag geteaaceaa gteettaga agettaat tgacadeag ecatagaaca 180 atgetteta aagaetet ettgeeetga agettaate tgacadeag ecatagaata 180 aatgetgaet etaaagaata tetgeeetag agetgaaca getgetgae 180 aatgetgaet etaaagaet etageaetag attagatag etagetaga etageaetag 180 aatgetgaet etaaagaet etageaetag agetgaatag tegeeggaa attagatag 180 aatgetgaet etaaagaet etageetaga attagatag etageetgaet 180 aatgetgaet etaaaagaet eta	Glu Gln Ala Leu Ala His Pro Tyr Leu Glu Gln Tyr Tyr Asp Pro Ser305310315320	
Arp Leu Pro Ly Gui Lys Leu Lys Giu Leu The Phe Glu Glu Thr Ala Arp Leu Pro Ly Gui Lys Leu Lys Glu Leu The Phe Glu Glu Thr Ala 340 Arg Phe Gln Pro Gly Tyr Arg Ser 355 -210- SEO ID NO 17 -211- LENKTH: 1499 -222- TYPE: DNA -222- SQUECE: 17 geocetcect cegeogeece geoggeogge cegtcagtet ggeaggagg caggeateg 400- SQUECE: 17 geocetcect cegeoggeog ggeoggeog agtocagge dattectect cecceoggt 120 caggetgge ggstgacaeg ggeoggeogge agtocagge gggeoggeog caggeateg 400- SQUECE: 17 geocetcect cegeoggeogge ggeoggeogge cegtoagge tettectte cecceoggt 120 caggetgge ggstgacaeg ggeoggeogge agtocagge ggeoggeog aggeolage 240 atggeoggeog ggeoggeogg gggeoggeog caggeolagg cegoggage aggeolagg 300 gtggggeogge ggeoggeogg gggeoggeog caggeolagg tettectte agaata tetggeoggeog ggeoggeogg gggeoggeog aggeolagg teggeoggeolagg 300 gtggggeogge ggaeggeogg gggeoggeog aggeolagg teggeoggeolagg 300 gtggggeog geoggeogge gggeoggeogg aggeolagg teggeoggeolagg 300 gtgggacaet attggeagaa accetgagg gagaaaa tettactgeo adaaatt 400 ggaacaeta ttggaatea tgacattatt cegageaaa gatettae agaatatag cocettgag 400 attgatatag taaggacet catggaaaa gatettae agetttgaa gacaacaec 600 cteageaatg accatteg catttette taceagate teagaggt aagaataat 660 catteageat acgatetga cuttggeolag gocoggeolagg caggeolaga 700 atggatetaa agatetgg attggeeca gtggedea gggeolaga catgaagat 340 atteaagg getaaceaa gatettge cattggaca ggeolaga catatagt 340 atgetata acaggeoca tetteagag ageattate tigaceaga attatgt 340 atteaagg getaaceaa gacettag teggeolaga ggetegag accetga attatgt 340 atgettett acaeggeoca tetteagag ageattate tigaceaga fall atteaagg getaaceaa gacettat teggeolag ggeolaga ggetega 300 atgettett acaeggeoca tettegaaga attggeolag ggeolaga ggetegaa 300 atgettett acaeggeoca tettegaaga attggeolaga ggetegaa 1200 aggaacatt tyettetet tecacaaaa ataggtg catggaacag getytteea 1080 atgotgate ceaagad tiggeocae catatedg gaagattat gacecaacaa 1100 aggaacgate teggeolaga accettaag tegacaaga gatagata gacageolaga 1120 gaaagteaa tagataga tegacaaga accetaag agaagtag tegacagag getytteea 1080 ata	Asp Glu Pro Ile Ala Glu Ala Pro Phe Lys Phe Asp Met Glu Leu Asp 325 330 335	
300345350Arg Phe Cln Pro Cly Tyr Arg Ser 3553602110 bERGTH: 14992111 bERGTH: 14992115 bERGTH: 1499 <td< td=""><td>Asp Leu Pro Lys Glu Lys Leu Lys Glu Leu Ile Phe Glu Glu Thr Ala</td><td></td></td<>	Asp Leu Pro Lys Glu Lys Leu Lys Glu Leu Ile Phe Glu Glu Thr Ala	
Ang Phe Gin Peo Giy Tyr Arg Ser 3350<210> SEQ ID NO 17 <211> LENGTH: 1499 >211> TIPE: DNA <211> CONCERCE: 17geocetcect cogreegeee geogeogee cogteagte geogeogee agtectee tecteeege agtogage gigtegaeeg geogeogge ggoogageg gagedaed atog googage gadecaea cetetegta atoggooga gadecaeg acategte teagatet agaatata togacatata togacatata togacatata gacatatat cagacataa actetatega gaatataa attatatg acacacae 700gtatatatg tacagaac catggoaca gatette agacataa actetgagag taaaatata600gtatatatg tacagaaca cattete tacagacaa cactegaga taaaatata600catacagaa tagaatata togacatata agacatata totacagaata attata taaaatata600catacagaata tagaatat gacattat tagacacaa cactegaga taaatata600catacagag acactateg catggaaca gactettea acctegaca catagate 700700catacagag acactateg catggaaca gtocaga gacataca 700700catacagag attatte tagacataa gacattat tagacatata 100700attatat tagacagaat taggocaa gtocaga gacacaca 700700attatata tagacacaa gtocattat tattagtoc tagacaga attattata 1000700attatata tagacagaat gtocaaaa actattag tagacagaa 700700attatata tagacagaa tagacatat tagacataa 100700attatata tagacacaaa gtocataa gacattat tagacagaa gacacaaa 700700attatata tagacacaa gtocaaaa gacattat tagacagaa 700700attatata tagacacaa gtocaaaa agacagaa gacacaaaa 700700attatata t	340 345 350	
 Allo SEQ ID NO 17 Allo LENGTH: 1499 Allo SEQUENCE: 17 gococtocol ocgocogoo googgocogo cogtagtat ggoaggaag caggoaatog 60 gtocgagtag otgtocgoto ttoagotot cogtoggo ggoggag caggoagat 240 atgocggocg cogagogtog agtocaceg ggoggago ggoggag cagagoaatog 120 caggotoggo gotgcaceg ggoggogo agtocotgo ggoggago gagocacaa 240 atgocggocg cogagogtog agtocago ggoggago goggoggag cagagotaga 180 gtoggoggog gotgcacea cottogta atoggogag goggogga dagtotto tootoo tootoo gu gtoggagog gotgcacea cottogta atoggogag gogocaga cottogtag 120 cacagacta atgocaga accotaga gtagtata agaaaatag cocttaga 420 atgacacta ttggaatca tgacatat cgagacaa cottogaa atgaagat 540 gtatatat atagtaca tagacata gacttata caggottag agaccaaca 600 ctagcatag accatatog catttoot taccagato tagagogt cagaacaa 720 tggattata agatotgo agtocaga ggocogg goocgttg cagatocag actagaga 720 atgagattat agtocaga atcotgg gactta agactaa acttagtog aaccataca 720 tggattata agtocgo attagocag agactata ttggocaca catogtoga aattatto 660 cattagoag ct tagoagaa tggocaga ggocogg cagactag cataga gat 120 cacaggatto tagaacaa tgtoga cagagottg cagatocag actagtaga 900 attoagga catatot o cattago agactaa ggottoga actaga agactaaa 720 tggattata aggocot attggocag agactaat ttgacoga agaccaaa 720 tggattata aggocot datggocag agactaat ttgacag agactaaa 720 tggattata aggocot datggocag agactaa ttgacaga ggotocag attattg 900 attoagga gatacaca gtocaga agactaga gactaa ggotocaga actagtaga 140 attoagga catacto ggocotag agactaa ttgacaga gactaa 120 aggattat tgettot tocacaaa ataaggt catggacag ttgacaaa 720 tggatta aggaccat ttgocaa agactaga gactgaa ggotocag aattatt 900 attoaggat ctgaacaa gtocataga gactgaa ggactaa ttgacagat 1200 aggatgaat tagatagac tacaagaa gactgaat gtagaatag tgacaaaag 1140 aggatgaat tagacagag tagacaaga ttgacaga gacgaag gtotgaag 1200 gaaaattat tgo	Arg Phe Gln Pro Gly Tyr Arg Ser 355 360	
-210- SEQ ID NO 17 -211- LEKRYN : 1499 -212- TYPE: DNA -213- ORCANISM: Homo sapiens <400-> SEQUENCE: 17 gcccctcoct cogocogoco googooogo cogteagtot ggoaggaag cagageaateg 60 gtoogagtgg cgtgcaccog geoggoogg cgtocotgo ggaggagg cagageateg 120 caagegtogg cgtgcaccog geoggoogg egtocotgo ggaggagg cagageateg 120 caagegtogg cgtgcaccog geoggoogg egtocotgo ggaggagg cggaggoog cagagetga 180 gcccctact cogocogoco geoggoogg egtocogg egtocogg cggagagg ggogoggoc cggagactag 240 atggoggoog cgtcaccacaa cototogtaa toggoogg ggogooggo coggagateg tocogogg agtgotoga 300 gtggggoog gotacaccaa cototogtaa toggoogg ggogooggo cataggotgt 360 totgettatg ataatgtcaa caaagttoga gtagtatea agaaaateag cocottgag 420 caccagaect actgocagag aaccotagg gagtaaaaa tottactgog ottaagaat 540 gtatatatag tacaggacot catggaaaca gatottaca agocottgaa gacacaaca 600 otcageatg accatatot otatttoto taccagatot tacagaggt aaaaatate 660 attacageta acgtotga cgttagetg googgtgt googgtca catgagetga 9900 attacagta agtotgga ctttggoctg geogotgt gagtacaa actagtog aaccataca 780 acaggttot gacagaata tytggocca cgttggtaa ggotccag aattatytg 840 aattocaagg gctacacca gtocategg agactatot ttgaccagt totggoaga 900 atgottota acaggcoot ottocagga agoctgaa ggoctgaacag googt gacacacat 960 ttgggtatto tagagacat ottocagag agoctgaa ggoctgaa ggogotg gacacacat 960 ttgggtatto tagagacat dttggocag agtgtaa ggotccaga aattatytg 840 aattocaagg gctacaccaa gtocataga gactgaat gtaataaa tttaaagot 1020 aggaacatat tgottot tocacaaaa aataaggtg catggaacag gotgtocca 1080 aattota acaggcoct ctgoccac cattatag gacagaa gotgtaccaa gitagacaag 1140 aggaactat tgottot tocacaagaa aataaggt tagactaa cacacacag 1140 aggaactaat tttggaagg tagaagga tagagaatat tagacagag gtgtocaag 1260 gaagatgaag tagaacag totggaaga accatagg gacagaag 1260 gaagattaa tottot toccaagaa accatagg gacagaagg 1260 aggaacaaggttaga tagaagga cacacaag agactaaga 1260<		
212:2 TTPE: DNA 213:3 ORGANISS: Homo appients 2400: SEQUENCE: 17 gococtocot cogocogoco gocogocogo cogtoagta gocagocago adagocatog fo gococtocot cogocogoco gocogocogo adtoctogo gogogogo acaagagotga 120 cagogtogo gotgocacog gogogogo adtoctogo gogogogoc agagocaco 240 atgogocogoco cogagogtog agotagocogo cogtagata tococogogo attoctoto totococogi 300 gtogogogo gotgocacoa cottocgta attogogogo gogocogoc agotagocaco 300 gtogogocog cotacaccaa cottocgta atogogogo gogococaco 240 atgogocogo gotacaccaa cottocgta atogogogo gogococaco 300 gtogocogo gotacaccaa cottocgta atogogogo gogococaco 240 atagocagoca acadotta atogocago gogocogo cogagatagi tococogo agotagocaco 480 gagaacata tuggaacaa cattata cogagocaca catocaga atoga atogaagat 540 gtatatatag tacagagoca cogtagaca gatottaca agotataga atotatago atogaagat 540 gtatatatag tacagacat catogo cogtogo gococogo acacogado atagaagat 660 ctacacagact attigocacagi acctago gocogogo gococogo acacocaco 720 tgtgatota agatotiga cuttigoco gococogo gocogo atacagocaca 780 acaaggitoc tacagoca gitcatago a cytiggita agactata tiggococa cittiggicagi anatatato 1020 aggaacatt tiggatoca tittigocogi acctagagi agotataa attagoci 1200 aggaacatt tiggatoco atcacagaa gaccitagi gacagatagi tocacacacagi 1100 aggaacatt tiggatoco digacaa ataaggi catgacagi gocigocigi 1200 adagattaga tagaacago totgococa catattig gacagacagi gocigocigi 1200 agaacatti tigattoct gaccitagi gacagatagi tocacacacagi 1120 aggaacatti tigattoco digacaaa ataaggi catgacagi gocigocigi 1200 aggaacatti tigattoci gaccitatig gacaaagi tigacagi gocigocigi 1200 agaacatat tigattaca gaccitagi tigacaga gocigacagi 1200 agaacatat tittagaga accitagi tigacagi attogacagi 1200 agaacatati tittagaagi accitaagi tigacagagi attogacagi 1200 agaacatat tittagaagi accitaagi tigacagi attogacagi 1200 agaacatati tittagaagi accitaagi tigacagi attogacagi 1200 agaacatata	<210> SEQ ID NO 17 <211> LENGTH: 1499	
 <400> SEQUENCE: 17 gococtcot o cocococto georgeoco o costeadet georgeogo capacata (20) gococtcot o costeadet o costeadet georgeoco georgeoco (20) gococtcot o costeadet o costeadet georgeoco georgeoco (20) gococtcot o costeadet o costeadet georgeoco (20) gococtcot o costeadet o costeadet georgeoco (20) gococtcot o costeadet o costeadet o costeadet (20) gococtcot o costeadet o costeadet (20) gococtcot o costeadet o	<212> TYPE: DNA <213> ORGANISM: Homo sapiens	
gcccctccctccgcccgcccgccggcgcgccgtcggcgccgtcggcgfalgtccgagtggctgtcggccccgcgcggggagcctcgggggggagcctcggggggggagcctcgggggggggggggggggfalgcgggggggccgggfalgtgggggggccggggggggggggggggggggggggggggggggggfalgtgggggggggggggggggggggggggggggggggfalgtgggggggggggggggggggggggggggggggggfalgtgggggggggggggggggggggggggggggggggfalfttggtattgatagtgcagccctgggaggggaccacafalggaacacattggaacagacttattggaccacafalgtgggtccgcacacaacatggaaagatttatafalgtgggtctcfalfalfalfalgtgggtctcacatgtcggggggggggacacacacfalgtggaacacattggaacagatttatagacacacacafalgtgggtctcgacacatactctggcccacatggaggggcccacaggtgggttcgacagatagtggcccaggggggggacacatacfalgtggattcgacagatagtggcccaggggggggacacacafalattggggggacacacagtggggggacacacafalfalgagaacatattggactggccggggggacacacafalfalgtgggtgacatgtgggccgtgggaccatacfalfalattggggggacacacagttggggggacacacafalfal <trr< td=""><td><400> SEQUENCE: 17</td><td></td></trr<>	<400> SEQUENCE: 17	
gtccgagtggctgtcggctettoagtcteccgcgtcggggtcctgagtgggtctgtagtgggtctgtagtgggtctgtagtgggtctgtagtgggtcgggggggtgggggggggtggggggggggggggggggggggggggggggg	gececteect eegecegeee geeggeeege eegteagtet ggeaggeagg eaggeaate	g 60
caqcqtcqgc ggctqcaccq ggcqgcqg agtcctqg ggqqgqg caqqqcqa180gcqgcqgcqc ccqaqcqtcq agctcaqcq ggcqqqcq ggcqgqcq cgqcqqcaca240atgqqqqcqc ggcqgcqc gggqqqg cggqqgc ccgqaqtqg tccqcqgqca ggtqttcqa300gtqgqqqcq gttcaccaa cctctqtaa atgqqqq ggcqtaca ggatqttcqa360tctqcttat atatqtcaa caaqttcqa gtaqctaca agaaatcag ccctttqa420gaqaactaa ttgqaataa tgacatta cgaccaa catcqqaa atgaaqat540gtatatatq tacaqgact catggaaca gatcttaca agatctqa gaccaaaca600ctcaqcaat acttctga catttt cgaccaca catcqqcq aatgaaqt780gtqgqtcqc tgacqaat tggccaca cgttgttg cagtccaa actqqqca780atatccaqqg cttacacaa gtccttqg agctqgc tctggcaga900atttccaqg gttaccca gtccttqg agctqg agctgaca ggtttcqa900atttccaqg cttaccaa tctccqq agctgtaca ggctccaqa attagt840atttccaqg gttacccaa gtccttqg acgtggt caggqcgaa900atttccaqg gttacccaa gtccttqg agctgaat ggcctcaqa attagt900atttccaqg gttacccaa tcttccaqg agctgaat gtacaaat1020aqgqattt tggtccc atcacaaa aataggtc catggaaca ggctgtcca1080attggtqgc g dgacqga tctggccaa cgtgtqg agcattat cgaccagag1200aggattgaa tagaacagc tctggccaa ccattgg agcattat gacatta cgaccaaca1200aggattgaa tagaacag tctggccaa ccatattg agcatgat cacaacaca1200aggatgaa tagaacaga tcggaaca gtcgtagt tccagcagg tgttcca1200aggattgaa tagaacag tctggccaa ccatattg agcagtat ccacacaag1200aggatgaa tagaacag tccgcaa acttag ttggaatg attgacagg taccaaca1200aggatgaacaa tttttgaag accatat tgcaatg attggaag taccaacat1200gaaaagta agaacaat ttttgaag accgtag tccacacag ttccacaagt	gtccgagtgg ctgtcggctc ttcagctctc ccgctcggcg tcttccttcc tcctcccgg	t 120
gegggggege ecggaegteg ageteagee gegeggggeg geggeggee ecggaggee geggeggee gegaegtea240atggeggege eggeggege gggegggge ecggagatg teegegggea ggtgttegae300gtggggeege getacaccaa ectetegtae ateggeggag gegeetaegg eagtgttegae360tetgettatg ataatgteaa eaagttega gtagetatea agaaaateag eccetttgag420caccagacet actgeeagag aaceetgagg gagataaaaa tettaetgeg etteagaeat480gagaacatea ttggaateaa tgaeattatt egageaceaa ecategagea aatgaagat540gtatatatg taeaggaeet eatggaaca gatettaea agetettgaa gaeacaacae600ecteageatg aceetatetg ettettee taeeagtee teagaggt eaaattatte660catteageta aegtetega ecgtgaeetg geeegtgttg eagateeaga ecatgatee780acagggttee tgaeagaat tgtggecae egttggtea gggeteeaga agtettee940atteeteagg getacacea gteettea agaeettee tegaeagaa geettee960attgettete tegaeagaat tgtggecae egttggte eagateeaga ecatgaea1020aggaattatt tgettetet tecaeagaa gaeetgaat gtataataa ttaaaagte1020aggaattet tggateee ateeagaa gaeetgaat gtatataaa ttaaaagte1020aggaattat tgettetet tecaeaaa aataagte eatggaaeg gegetgeetg1200aggaatgae tagaacage tetggeeea ecatateg ageagtatte egaeceaga1200aggaattat tgettetet teeacaaa aataagte eatggaaeg getgeteeag1200aggaageeea tegeegaag aceetteag teegeegg aatteggaag tegeegeegge1200gaaaagetea aagaactaat ttttgaaga actgetaga teegeegg atacagate1320taaattege dagaecage teggeeege eegeegg atacagag geegeegeetgeette1380taaattege agtateteg agtatate agtaggete ageaggag geegeegeetgeette1340	cagegtegge ggetgeaceg geggeggege agteeetgeg ggagggggga caagagetga	a 180
atggggggg cggggggg cggggggg cgggggg cgggggg	geggeggeeg eegagegteg ageteagege ggeggaggeg geggeggeee ggeageeaa	c 240
gtggggccg gtaacacaa cctctgtac atcggcgag gcgctacgg catggtgtg360tctgcttatg ataatgtcaa caaagttcga gtagctata agaaaatcag cocctttgag420caccagacct actgccagag aacctgagg gagataaaa tcttactgcg cttcagacat480gagaacatca ttggaatcaa tgacattat cgagcaccaa ccatcgagca aatgaaagat540gtatatatag tacaggacct catggaaaca gatcttaca agotctgaa gacacaacac600ctcagcaatg accatactg ctatttct taccagatce tcagagggt aaaatatat660cattcagcta acgttctgca ccgtgacct aagcetcca acctgctgct caacaccace720tgtgatcta agatctgtga cttggcctg gcccgtgttg cagatccaga actagtatg840aattccaagg gctaaccaa gtccattga attggtgta gggctccaga aattagttg840attgttttta acaggcccat cttccagga agcattat ttggccg gacctgact gagctgag aattagtg900atgctttcta acaggccat ctttccaaga gacctgaat gtacacaa gtccatga ggctgcag1020aggaactat tggttctct tccacacaaa aataaggtg catggacag gctgttccca1080aatgctgac ccaaaggt ctggccca ccatactgg agcattat cgacccgag 1140aggattgaag tagaacagg tctggccca ccatactgg agattata cgaccgagt1200gacgagccca tgccgaaga accattaag ttcgacagg attggatg attggatga cttgcctag1320gagaacatat tttgtaaga actgttaga attggatga cttgcctag1380ttaaattgtc aggtacctg agtttaata agtaggtc tagcaaggg gcgctgcct1340	atggcggcgg cggcggcggc gggcgcgggc ccggagatgg tccgcgggca ggtgttcgad	c 300
tctgcttatg ataatgtcaa caaagttcga gtagctatca agaaatcag cccctttgag420caccagacct actgccagag aaccctgagg gagtaaaaa tcttactgcg cttcagacat480gagaacatca ttggaatcaa tgacattatt cgagcaccaa ccatcgagca aatgaaagat540gtatatatag tacaggacct catggaaca gatcttaca agctcttgaa gaccacaaca600ctcagcaatg accatatctg ctatttctc taccagatce tcagagggt aaaatatate660cattcagcta acgttctgca ccgtgaccta agccttcca acctgctgct caacaccacc720tgtgatcta agatctgtga ctttggectg gcccgtgttg cagatccaga acttagttg840aattccaagg gctacaccaa gtccattgat attggtctg taggetgcat tctggcaga900atgctttcta acaggccca cttccaaga gaccttact ttgaccagt gaccacatt960ttgggattc ttggatccc acacacaag agcctgatt gtatataaa tttaaaagt1020aggaactatt tgcttctct tccacaaaa aataagtgc catggacag gctgtccaa1140aggattgaag tagaacage tctggccca ccattcgg agcagtatta cgaccagag1200gacagagcca aggactaat ttttgaagg actgcatgg attcgaga tctagccaga1320taaatttgc aggatcaca gagtattac agcaggag gccgccctt1380	gtggggccgc gctacaccaa cctctcgtac atcggcgagg gcgcctacgg catggtgtg	c 360
caccagacct actgocaga aacctgag gagataaaa tottactgog ottoagacat 480 gagaacatca ttggaatca tgacattatt ogagcaccaa coatogagca aatgaaagat 540 gtatatatag tacaggacot catggaaaca gatotttaca agotottgaa gacacaacac 600 cotcagcaatg accatactog otatttoto taccagatoc tcagagggt aaaatatato 660 catcagota acgtotgaa cogtgacota agoottoca acotgotgot caacacacac 720 tgtgatotca agatotgtga ottiggocag gocogtgtg cagatocaga coatgatcac 780 acagggtto tgacagaata tgtggocaca ogttggtaca gggotocaga aattatgtg 840 aattocaagg gotacaccaa gtocattgat attoggotg taggotgocat totggoagaa 900 atgotttota acaggoccat ottocaggg aagoattato ttgacagot gaaccacat 960 ttgggtatto ttggatocca atcacagaa gacctgaatt gtataataaa tttaaaagot 1020 aggaactat tgotttott tocacacaaa aataaggtgo catggaacag gotgttocca 1080 aatgotgact caaagote ggacttattg gacaaatgt tgacattcaa cocacacaag 1140 aggattgaag tagaacaggo totggoccac coatatotgg agcagtatta ogaccogat 1200 gacgagocca togocgaag accattcaag ttogacatgg aattggatga ottgoccag 1260 gaaaagotca aagaactaat ttttgaagg actgotagat tocagocagg tacagact 1320 taaatttigo aggtacotgg agttatata agtgagotta tocagocagg tacagatot 1320 taaatttigo agatactga tuttgaagg cogtagat tocagacagg gocgtocot 1380	totgottatg ataatgtcaa caaagttoga gtagotatca agaaaatcag ocootttga	g 420
gagaacatca ttggaatcaa tgacattatt cgagcaccaa ccatcgagca aatgaagat540gtatatatag tacaggacct catggaaca gatctttaca agctcttgaa gacacaacac600ctcagcaatg accatactg ctatttete taccagatee tcagagggtt aaaatatate660cattcagcta acgttetgaa cegtgacete aageetteca acctgetget caacaccace720tgtgatetea agatetgtga etttggeetg geeegtgttg cagateeaga ceatgatea780acagggttee tgacagaata tgtggeeaca egttggtaca gggeteeaga aattatgttg840aatteeaagg getacaccaa gteeattgat atteggetg taggetgeet tetggeagaa900atgettteta acaggeeea etteegagaa gacettate ttgaceage gacecatt960ttgggatete ttggateece atecacagaa gacetgatt gtataataa tttaaaaget1020aggaactatt tgetteet tecacacaaa aataaggtge catggaacag getgtteeca1080aatgettgaag tagaacagge tetggeecae ceatatetgg ageagtatta egaceegag1200gacgageeea tegeegaage accatteaag ttegacatgg aattggatga ettgeetaag1200gaaaagetea aagaactaat ttttgaagag actgetaga teeageegag attegagette1320taaatttgte aggtacetgg agtttaatae agtagagetet ageaggag geetgeet1380ttgttteter agataetgg agtttaatae egategagg geetgeet1340	caccagacct actgccagag aaccctgagg gagataaaaa tcttactgcg cttcagaca	t 480
gtatatatag tacaggacct catggaaaca gatetttaca agetettgaa gacacaacac600ctcagcaatg accatatetg etatttete taccagatee teagagggtt aaaatatate660catteageta aegteetgea eegtgaeete aageetteea aeetgetget eaacaceace720tgtgatetea agatetgtga etttggeetg geeegtgttg eagateeaga eeatgateae780acagggttee tgacagaata tgtggeeaca egttggtaca gggeteeaga aattatgttg840aatteeaagg getacaceaa gteeattgat atttggtetg taggetgeat tetggeeaga900atgetttet aeaggeeeat ettegeeaga ageettate ttgaceaget gaaceacatt960ttgggtatte ttggateee ateacaagaa gacetgaatt gtataataa tttaaaaget1020aaggaattatt tgetteet teecacaaaa aataaggtge catggaacag getgteeea1080aatgetgae teeggeeea eeaatteg gacaaaatg tgacattae egaceegagt1200gacgageeea tegeegaage accatteaag teegacatgg aattggatga ettgeetaag1200gacagageeea tegeegaage accatteaag teegacatgg aattggatga ettgeetaag1200gaaaagetea aagaactaat ttttgaagag actgetagt teeacaegag ateagatet1320taaatttgte aggtacetgg agtttaata agtgagetet ageaggagg geegetgeett1380ttgtteteg aatattat teefeeagg agttatate teefeaggg geegetgeett1440	gagaacatca ttggaatcaa tgacattatt cgagcaccaa ccatcgagca aatgaaaga	t 540
ctcagcatg accatatetg etatttete taccagatee teagagggtt aaaatatate660catteageta acgttetgea cegtgacete aageetteea acetgetget eaacaceaee720tgtgatetea agatetgtga etttggeetg geeegtgttg eagateeaga ecatgateae780acagggttee tgacagaata tgtggeeaea egttggtaca gggeteeaga aattatgttg840aatteeaagg getacaceaa gteeattgat atteggtetg taggetgeat tetggeagaa900atgetteta acaggeeea ettecagga aageattate ttgaceaget gaaceaeatt960ttgggtatte ttggateee ateeaeaa gaeetgaatt gtataataaa tttaaaaget1020aggaactatt tgetteete teeaeaaaa aataaggtge eatggaacag getgteeea1080aatgetgaat egaacage tetggeeeae ecatatetg ageagtata egaeeegga1140aggaatgaag tagaacage tetggeeeae ecatatetgg aattggatga ettgeetag1320gaaaagetea aagaactaat ttttgaaga actgetagat teeageegg atacagatet1320taaattege aggtacetgg agttaataa agtgagete ageaaggag geetgeett1380ttgtttetag aatatateg teectagag ecettattt gtatettette eeageteet1440	gtatatatag tacaggacct catggaaaca gatctttaca agctcttgaa gacacaaca	c 600
cattcageta acgttetgea cegtgacete aageetteea acetgetget eaacaceaee720tgtgatetea agatetgtga etttggeetg geeegtgttg eagateeaga ecatgateae780acagggttee tgacagaata tgtggeeaea egttggtaca gggeteeaga aattatgttg840aatteeaagg getacaceaa gteeattgat atttggtetg taggetgeat tetggeagaa900atgettteta acaggeeeat etteeaggg aageattate ttgaceaget gaaceaeat960ttgggtatte ttggateee ateaeaagaa gaeetgaatt gtataataaa tttaaaaget1020aggaactatt tgetteet teeacacaaa aataaggtge catggaacag getgtteeea1080aatgettgaag tagaacage tetggeeeae ceatatetgg ageagtatta egaceegagt1200gaeaggeeea tegeegaage aceatteeag ttegaeatgg aattggatga ettgeetaag1200gaaaagetea aagaactaat ttttgaagag actgetagat teeageegag taeeagate1320taaattgte aggtacetgg agtttaatae agtgagetet ageaaggag geegetgeett1380ttgtttetag aatattatgt teeteaaggt ceattatttt gtattetttt ceaageetge1440	ctcagcaatg accatatctg ctattttctc taccagatcc tcagagggtt aaaatatat	c 660
tgtgatetea agatetgtga etttggeetg geeegtgttg eagateeaga eeatgateae780acagggttee tgacagaata tgtggeeaca egttggtaca gggeteeaga aattatgttg840aatteeaagg getacaeeaa gteeattgat atttggtetg taggetgeat tetggeagaa900atgettteta acaggeeeat ettteeagg aageattate ttgaceaget gaaceaeatt960ttgggtatte ttggateeee ateaeagaa gaeetgaatt gtataataaa tttaaaaget1020aggaaetatt tgettteet teeaeaaaa aataaggtge eatggaaeag getgtteeea1080aatgetgaet eeaagee tetggeeeae eeatategg ageagtatta egaeegagt1200gaegageeea teggeegaa eeaatteag ttegaeatgg ageagtatta egaeegagt1200gaeaagetea aagaaetaat ttttgaagag aetgetagg aattggatga ettgeetaag1200gaaaagetea aagaaetaat ttttgaagag aetgetaga teeageegag geegetgeett1320taaatttgte aggtaeetgg agtttaatae agtgagetet ageaaggag geegetgeett1380ttgttteteag aatattatgt teeteaaggt eeattattt gtattetttt ceaageteet1440	cattcageta acgttetgea cegtgacete aageetteea acetgetget caacaceace	c 720
acagggtttee tgacagaata tgtggeeaca egttggtaca gggetceaga aattatgttg840aatteeaagg getacaecaa gteeattgat atttggtetg taggetgeat tetggeagaa900atgettteta acaggeecat ettteeaggg aageattate ttgaceaget gaaceacatt960ttgggtatte ttggateece ateaeaagaa gaeetgaatt gtataataaa tttaaaaget1020aggaactatt tgetttetet teeaeaaaa aataaggtge catggaacag getgtteeea1080aatgetgaet ecaaagete ggaettattg gaeaaaatgt tgaeattea eceaeaag1140aggaattgaag tagaacagge tetggeecae ecatatetgg ageagtatta egaeeegagt1200gaeaaagetea aagaactaat ttttgaagag actgetagat teeageeagg atacagatet1320taaatttgte aggtaectgg agttaatae agtgagetet ageaaggag geetgeett1380ttgtttetag aatattatgt teeteaaggt ceattatttt gtattetttt ecaageteet1440	tgtgatctca agatctgtga ctttggcctg gcccgtgttg cagatccaga ccatgatca	c 780
aatteeaagg getadaccaa gteeattgat atttggtetg taggetgeat tetggeagaa900atgettteta acaggeecat ettteeaggg aageattate ttgaccaget gaaccacatt960ttgggtatte ttggateece ateacaagaa gaeetgaatt gtataataaa tttaaaaget1020aggaactatt tgettteet teeacaaaa aataaggtge catggaacag getgtteeca1080aatgetgaet ecaagetet ggaettattg gaeaaaatgt tgaeatteaa eceacaeag1140aggaattgaag tagaacagge tetggeecae ecatatetgg ageagtatta egaeeegagt1200gaegageeca tegeegaage accatteaag ttegaeatgg aattggatga ettgeetaag1320taaatttgte aggtaectgg agttaatae agtgageet ageagggg geetgeett1380ttgtttefeag aatattatgt teefeaaggt ceattatttt gtattetttt ecaagefeet1440	acagggttcc tgacagaata tgtggccaca cgttggtaca gggctccaga aattatgttg	g 840
algettleta acaggeccat etteccaggg aageattate tigaccaget gaaccacatt960tigggtatte tiggatecee ateacaagaa gaeetgaatt gtataataaa titaaaaget1020aggaactatt tgettletet tecacacaaa aataaggtge catggaacag getgttecea1080aatgetgaet ecaaagetet ggaettattg gaeaaaatgt tgaeatteaa eceacacaag1140aggaatgaag tagaacagge tetggeceae ecatatetgg ageagtatta egaeeegagt1200gaegageeea tegeegaage accatteaag tiegacatgg aattggatga ettgeetaag1260gaaaagetea aagaactaat tittgaagag actgetagat teeageeagg atacagatet1320taaattigte aggtaeetgg agttaatae agtgagetet ageaagggag geetgeett1380tigttletag aatattatgt teeteaaggt ceattattit gtattettitt ecaageteet1440	aatteeaagg getacaceaa gteeattgat atttggtetg taggetgeat tetggeaga	a 900
aggaactatt tgetttett teesaagaa gaeetgaatt gtataataa tttaaaaget 1020 aatgetgaet eesaagetet ggaettattg gaeaaaatgt tgaeatteaa eesaageteea 1080 aatgetgaet eesaagetet ggaettattg gaeaaaatgt tgaeatteaa eesaacaag 1140 aggattgaag tagaacagge tetggeeeae eesaatetgg ageagtatta egaeeegagt 1200 gaegageeea tegeegaage aceatteaag ttegaeatgg aattggatga ettgeetaag 1260 gaaaagetea aagaactaat ttttgaagag aetgetagat teeageeagg atacagatet 1320 taaatttgte aggtaeetgg agttaatae agtgagetet ageaagggag gegetgeett 1380 ttgtttetag aatattatgt teeteaaggt ceattatttt gtattetttt eesaageteet 1440	augurutera acaggeeear ettecaggg aageattate ttgaceaget gaaceacat	L 960
astgetgaet egetetete teeaaaaa aataayyyy eatyyaaday getgeteeaa 1000 aatgetgaet egetetete egettattg gacaaaatgt tgacatteaa eecaacaaag 1140 aggattgaag tagaacagge tetggeecae eeataetgg ageagtatta egaecegagt 1200 gacgageeca tegeegaage accatteaag ttegaeatgg aattggatga ettgeetaag 1260 gaaaagetea aagaactaat tittgaagag actgetagat teeageeagg ataeagatet 1320 taaattigte aggtaeetgg agttaatae agtgagetet ageaagggag gegetgeett 1380 tigttletag aatattatgt teeteaaggt ceattattit gtattettit eeaageteet 1440	angesetett trattetet trassesses solografia astronogar activities	a 1020
aggattgaag tagaacagge tetggeecae ceatatetgg ageagtatta egaecegagt 1200 gaegageeca tegeegaage aceatteaag ttegaeatgg aattggatga ettgeetaag 1260 gaaaagetea aagaactaat ttttgaagag aetgetagat teeageeagg atacagatet 1320 taaatttgte aggtaeetgg agtttaatae agtgagetet ageaagggag gegetgeett 1380	ayyaactatt tyotteett teaaataa aataayytye catyyaacay getgtteee	n 1140
gacgageeega tegeegaage accatteaag ttegacatgg aattggatga ettgeetaag 1260 gaaaagetea aagaactaat ttttgaagag actgetagat teegacagg atacagatet 1320 taaatttgte aggtaeetgg agtttaatae agtgagetet ageaagggag gegetgeett 1380	adaptigadi telaacado totooccan costatotoo adcadatta doccada	5 1200
gaaaagetea aagaaetaat ttttgaagag actgetagat teegeecagg atacagatet 1320 taaatttgte aggtaeetgg agtttaatae agtgagetet ageaagggag gegetgeett 1380	acquarcea terecoaade accatteaad treacated aattgaatga etteretaa	a 1260
taaatttgtc aggtacetgg agtttaatac agtgagetet ageaagggag gegetgeett 1380	gaaaaqctca aagaactaat ttttgaagag actgctagat tccagccagg atacagatc	t 1320
ttgtttctag aatattatgt tootcaaggt coattatttt gtattotttt graaggtoot 1440	taaatttqtc aqqtacctqq aqtttaatac aqtqaqctct aqcaaqqqaq qcqctqcct	t 1380
	ttgtttctag aatattatgt teeteaaggt ceattatttt gtattetttt ceaagetee	t 1440

1499

<210> SEQ ID NO 18 <211> LENGTH: 360 <212> TYPE: PRT <213> ORGANISM: Homo sapiens															
<400	<400> SEQUENCE: 18														
Met 1	Ala	Ala	Ala	Ala 5	Ala	Ala	Gly	Ala	Gly 10	Pro	Glu	Met	Val	Arg 15	Gly
Gln	Val	Phe	Asp 20	Val	Gly	Pro	Arg	Tyr 25	Thr	Asn	Leu	Ser	Tyr 30	Ile	Gly
Glu	Gly	Ala 35	Tyr	Gly	Met	Val	Cys 40	Ser	Ala	Tyr	Asp	Asn 45	Val	Asn	Lys
Val	Arg 50	Val	Ala	Ile	Lys	Lys 55	Ile	Ser	Pro	Phe	Glu 60	His	Gln	Thr	Tyr
Cys 65	Gln	Arg	Thr	Leu	Arg 70	Glu	Ile	Lys	Ile	Leu 75	Leu	Arg	Phe	Arg	His 80
Glu	Asn	Ile	Ile	Gly 85	Ile	Asn	Asp	Ile	Ile 90	Arg	Ala	Pro	Thr	Ile 95	Glu
Gln	Met	Lys	Asp 100	Val	Tyr	Ile	Val	Gln 105	Asp	Leu	Met	Glu	Thr 110	Asp	Leu
Tyr	ГЛа	Leu 115	Leu	Lys	Thr	Gln	His 120	Leu	Ser	Asn	Asp	His 125	Ile	Суз	Tyr
Phe	Leu 130	Tyr	Gln	Ile	Leu	Arg 135	Gly	Leu	ГЛа	Tyr	Ile 140	His	Ser	Ala	Asn
Val 145	Leu	His	Arg	Asp	Leu 150	ГЛа	Pro	Ser	Asn	Leu 155	Leu	Leu	Asn	Thr	Thr 160
Суз	Asp	Leu	Lys	Ile 165	Суз	Asp	Phe	Gly	Leu 170	Ala	Arg	Val	Ala	Asp 175	Pro
Asp	His	Asp	His 180	Thr	Gly	Phe	Leu	Thr 185	Glu	Tyr	Val	Ala	Thr 190	Arg	Trp
Tyr	Arg	Ala 195	Pro	Glu	Ile	Met	Leu 200	Asn	Ser	Lys	Gly	Tyr 205	Thr	Lys	Ser
Ile	Asp 210	Ile	Trp	Ser	Val	Gly 215	Суз	Ile	Leu	Ala	Glu 220	Met	Leu	Ser	Asn
Arg 225	Pro	Ile	Phe	Pro	Gly 230	Lys	His	Tyr	Leu	Asp 235	Gln	Leu	Asn	His	Ile 240
Leu	Gly	Ile	Leu	Gly 245	Ser	Pro	Ser	Gln	Glu 250	Asp	Leu	Asn	Суз	Ile 255	Ile
Asn	Leu	Lys	Ala 260	Arg	Asn	Tyr	Leu	Leu 265	Ser	Leu	Pro	His	Lys 270	Asn	Lys
Val	Pro	Trp 275	Asn	Arg	Leu	Phe	Pro 280	Asn	Ala	Asp	Ser	Lys 285	Ala	Leu	Asp
Leu	Leu 290	Asp	Lys	Met	Leu	Thr 295	Phe	Asn	Pro	His	Lys 300	Arg	Ile	Glu	Val
Glu 305	Gln	Ala	Leu	Ala	His 310	Pro	Tyr	Leu	Glu	Gln 315	Tyr	Tyr	Asp	Pro	Ser 320
Asp	Glu	Pro	Ile	Ala 325	Glu	Ala	Pro	Phe	Lys 330	Phe	Asp	Met	Glu	Leu 335	Asp

tattggaagg tatttttta aatttagaat taaaaattat ttagaaagtt acatataaa

-continued

Asp	Leu	Pro	Lys 340	Glu	ГЛЗ	Leu	Lys	Glu 345	Leu	Ile	Phe	Glu	Glu 350	Thr	Ala
Arg	Phe	Gln 355	Pro	Gly	Tyr	Arg	Ser 360								

What is claimed is:

1. A method of treating a cell proliferation disorder comprising administration of an effective amount of a γ_1 34.5 deficient herpes simplex virus-1 comprising at least one expressible coding region of the MAPK pathway to a subject in need.

2. The method according to claim 1 wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for MEK.

3. The method according to claim **2** wherein the MEK is selected from the group consisting of MEK1 and MEK2.

4. The method according to claim 1 wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for ERK.

5. The method according to claim **4** wherein the ERK is selected from the group consisting of ERK1 and ERK2.

6. The method according to claim 1 wherein the $\gamma_1 34.5$ deficient herpes simplex virus-1 comprises a coding region for Raf.

7. The method according to claim **6** wherein the Raf is selected from the group consisting of Raf-1, A-Raf and B-Raf.

8. The method according to claim **1** wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for Ras.

9. The method according to claim 1 wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for a protein selected from the group consisting of MEK Kinase-1, mos and Tpl-2.

10. The method according to claim **1** wherein the coding region for the MAPK pathway encodes a variant of a member of the pathway.

11. The method according to claim 10 wherein the variant is selected from the group consisting of K-Ras V12, K-Ras D12, K-Ras G12, H-Ras V12, K-Ras D13, N-Ras V12, Raf S338A, Raf S339A, B-Raf V600E, Raf-CAAX, Raf BXB, Δ N3MKK1 S218E/S222D, Δ N3MKK2 S218E/S222D, ERK2 E58Q, ERK2 D122A, ERK2 S151A, ERK2 S221A, ERK2 S151D ERK L73P and a full-length MEK-ERK fusion.

12. A method of treating a cell proliferation disorder comprising administration of an effective amount of a $\gamma_1 34.5$ deficient herpes simplex virus-1 comprising at least one expressible coding region encoding a protein selected from the group consisting of a catalytically inactive mutant of PKR, a catalytically inactive mutant of eIF-2 α , a growth factor and an active mutant of a tyrosine kinase receptor.

13. The method according to claim 1 wherein the γ_1 34.5 deficient herpes simplex virus-1 lacks any γ_1 34.5 gene.

14. The method according to claim 1 wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a γ_1 34.5 gene with a point mutation.

15. The method according to claim **1** wherein the treating ameliorates at least one symptom associated with the cell proliferation disorder.

16. The method according to claim **1** wherein the cell proliferation disorder is a cancer.

17. Use of a γ_1 34.5 deficient HSV comprising at least one expressible coding region of the MAPK pathway in the preparation of a medicament for the treatment of a patient with a cell proliferation disorder.

18. A γ_1 34.5 deficient HSV comprising at least one expressible coding region of the MAPK pathway. **19**. The γ_1 34.5 deficient HSV according to claim **18**

19. The $\gamma_1 34.5$ deficient HSV according to claim 18 wherein the $\gamma_1 34.5$ deficient herpes simplex virus-1 comprises a coding region for MEK.

20. The γ_1 34.5 deficient HSV according to claim **19** wherein the MEK is selected from the group consisting of MEK1 and MEK2.

21. The γ_1 34.5 deficient HSV according to claim **18** wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for ERK.

22. The $\gamma_1 34.5$ deficient HSV according to claim 21 wherein the ERK is selected from the group consisting of ERK1 and ERK2.

23. The γ_1 34.5 deficient HSV according to claim **18** wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for Raf.

24. The γ_1 34.5 deficient HSV according to claim 23 wherein the Raf is selected from the group consisting of Raf-1, A-Raf and B-Raf.

25. The γ_1 34.5 deficient HSV according to claim **18** wherein the coding region encodes a protein selected from the group consisting of MEK Kinase-1, mos and Tpl-2.

26. The γ_1 34.5 deficient HSV according to claim **18** wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a coding region for Ras.

27. The γ_1 34.5 deficient HSV according to claim 18 wherein the coding region of the MAPK pathway encodes a variant of a member of the pathway.

28. The γ_1 34.5 deficient HSV according to claim **27** wherein the variant is selected from the group consisting of IC-Ras V12, K-Ras D12, H-Ras V12, K-Ras D13, N-Ras V12, Raf S338A, Raf S339A, B-Raf V600E, Raf-CAAX, Raf BXB, Δ N3MKK1 S218E/S222D, Δ N3MKK2 S218E/S222D, ERK2 E58Q, ERK2 D122A, ERK2 S151A, ERK2 S221A, ERK2 S151D ERK L73P and a full-length MEK-ERK fusion.

29. The γ_1 34.5 deficient HSV according to claim **18** wherein the coding region encodes a protein selected from the group consisting of a catalytically inactive mutant of PKR, a catalytically inactive mutant of eIF-2 α , a growth factor and an active mutant of a tyrosine kinase receptor.

30. The γ_1 34.5 deficient HSV according to claim **18** wherein the γ_1 34.5 deficient herpes simplex virus-1 lacks any γ_1 34.5 gene.

31. The γ_1 34.5 deficient HSV according to claim **18** wherein the γ_1 34.5 deficient herpes simplex virus-1 comprises a γ_1 34.5 gene with a point mutation.

32. A composition comprising the γ_1 34.5 deficient HSV according to claim **18** in combination with a pharmaceutically acceptable adjuvant, carrier, or diluent.

* * * * *