METHODS AND COMPOSITIONS FOR CANCER THERAPY USING A NOVEL ADENOVIRUS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of presently pending U.S. patent application serial number 10/888,492, filed July 9, 2004, entitled "Methods And Compositions For Cancer Therapy Using a Novel Adenovirus," which claims priority to U.S. provisional applications 60/486,219, filed July 9, 2003, both of which are hereby fully incorporated herein by reference.

FIELD OF THE INVENTION

[0002] Generally, the present invention relates to a cancer therapy. More specifically, the present invention relates to an adenovirus-based cancer therapy.

BACKGROUND

[0003] Despite advances in both diagnosis and therapy, the annual number of cancer related deaths has not decreased during the past 60 years. Although conventional cancer therapies (surgery, radiotherapy, chemotherapy) produce a high rate of cure for patients with early stage disease, many cancers recur and the majority of patients with advanced cancer eventually succumb to the disease. The limitations of conventional cancer therapies do not derive from their inability to ablate tumor, but rather from limits on their ability to do so without excessively damaging the patient. It is this consideration that constrains the extent of surgical resection, the dose of radiation and volume to be irradiated, and the dose and combination of chemotherapeutic drugs. Improving the effectiveness of a treatment is of no clinical value if there is no significant increase in the differential response between tumor and normal tissue (i.e., therapeutic index).

[0004] Nonetheless, improved methods and novel agents for treating cancer have resulted in increased survival time and survival rate for patients with various types of cancer. For example, improved surgical and radiotherapeutic procedures result in more effective removal of localized tumors. Surgical methods, however, can be limited due, for example, to the location of a tumor or to dissemination of metastatic tumor cells. Radiotherapy also can be limited by other factors that limit the dose that can be administered. Tumors that are relatively radioresistant will not be cured at such a dose.

[0005] Although a single treatment modality such as radiation therapy, chemotherapy, surgery or immunotherapy can result in improvement of a patient, superior results can be achieved when such modalities are used in combination. In particular, treatment with a combination of radiotherapy, which can be directed to a localized area containing a tumor, and chemotherapy or immunotherapy, which provide a systemic mode of treatment, can be useful where dissemination of the disease has occurred or is likely to occur. Unfortunately, the therapeutic usefulness of radiation therapy can be limited where the tumor cells are relatively radioresistant, since the dose is limited by the tolerance of normal tissue in the radiation field. Thus, there exists a need to sensitize cancer tumors to the effects of radiotherapy so that it can more effectively reduce the severity of a tumor in a patient. Further, it would be useful to develop a treatment that more specifically isolates the location of the radiation, thus preventing the effects of radiation treatment on healthy cells.

[0006] In related fashion, to mitigate unwanted effects of some chemotherapies, adenovirus vectors have been used to transduce tumor cells with so-called "chemogenes" that convert a nontoxic substance, or "prodrug", into a toxic, therapeutically effective form. Several new approaches involving gene therapy are under consideration for improving the therapeutic index of cancer therapies.

[0007] One of these approaches, so-called "suicide gene therapy," involves the transfer and expression of non-mammalian genes encoding enzymes that convert non-toxic prodrugs into toxic anti-metabolites. Two "suicide genes" that are currently being evaluated in clinical trials are the *E. coli* cytosine deaminase (CD) and herpes simplex virus type-1 thymidine kinase (HSV-1 TK) genes, which confer sensitivity to 5-fluorocytosine (5-FC) and ganciclovir (GCV), respectively. Following targeted transfer of these genes to the tumor, the 5-FC and GCV prodrugs are converted locally into potent chemotherapeutic agents resulting in significant tumor cell death (see reference 1 (and the references cited therein) in the List of References Section below). Thus, the dose-limiting systemic toxicity associated with conventional chemotherapies is mitigated.

[0008] Previously, the bacterial CD and wild-type HSV-1 TK genes have been coupled to create a novel CD/HSV-1 TK fusion gene (see reference (hereinafter "ref.") 1 in the List of References Section). The CD/HSV-1 TK fusion gene allows for combined use of CD/5-FC and HSV-1 TK/GCV suicide gene therapies. It has been previously demonstrated that CD/5-FC and HSV-1 TK/GCV suicide gene therapies render malignant cells sensitive to specific pharmacological agents and importantly, sensitize them to radiation (see refs. 1-9). Using a

novel, oncolytic, replication-competent adenovirus (Ad5-CD/TK*rep*) containing the prototype CD/HSV-1 TK fusion gene (ref. 10), the safety and efficacy of replication-competent adenovirus-mediated double suicide gene therapy without and with radiation therapy in several preclinical cancer models (refs. 10-13) and more recently, in human prostate cancer patients (refs. 14 and 15) have been demonstrated.

[0009] In these clinical trials targeting human prostate cancer, the Ad5-CD/TK*rep* virus proved to be safe up to a dose of 10^{12} Vp when combined with up to 3 weeks of 5-FC and GCV (vGCV) prodrug therapy without (ref. 14) and with (ref. 15) conventional dose (70 Gy) three dimensional conformal radiation therapy (3DCRT). Moreover, these treatment regimens have demonstrated signs of clinical activity (refs 14 and 15).

[0010] Nonetheless, despite these advances, a significant need remains for inventions that comprise effective methods and compositions for use in cancer therapies. The present invention was developed in light of these and other drawbacks.

SUMMARY OF THE INVENTION

[0011] The present invention comprises novel, improved methods and compositions for cancer therapy which comprise a novel virus that can kill mammalian cancer cells efficiently. The virus produces a novel protein that converts non-toxic prodrugs into potent chemotherapeutic agents. These chemotherapeutic agents are produced locally and help the virus kill the cancer cells as well as sensitize them to radiation. In preclinical studies, the virus has proven effective at killing a variety of human cancer cells either alone or when combined with prodrug therapy and/or radiation therapy.

[0012] The invention comprises a novel, "second-generation" adenovirus (designated "Ad5-yCD/*mut*TK_{SR39}*rep*-ADP") with at least two significant improvements relative to the previously disclosed prototype Ad5-CD/TK*rep* virus. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP contains an improved yCD/*mut*TK_{SR39} fusion gene whose product is more efficient at converting the 5-FC and GCV prodrugs into their active chemotherapeutic agents. Moreover, Ad5-yCD/*mut*TK_{SR39}*rep*-ADP expresses the Ad5 ADP protein, which significantly increases the oncolytic activity of replication-competent adenoviruses. Relative to the prototype Ad5-CDITK*rep* virus, Ad5-yCD/*mut*TK_{SR39}*rep*-ADP has demonstrated greater viral oncolytic and chemotherapeutic activity in preclinical cancer models. The data suggest that the Ad5-yCD/*mut*TK_{SR39}*rep*-ADP virus comprising the present invention will exhibit low

toxicity and significant anti-tumor activity clinically when combined with 5-FC and GCV prodrug therapy and radiation therapy.

[0013] Other aspects of the invention will be apparent to those skilled in the art after reviewing the drawings and the detailed description below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The present invention will now be described, by way of example, with reference to the accompanying drawings, in which:

[0015] FIG. 1 is a schematic representation of the Ad5-yCD/*mut*TK_{SR39}*rep*-ADP virus of the present invention.

[0016] FIG. 2 is a diagram showing an advantage of the ADP gene of the present invention.

[0017] FIGS. 3A and 3B are diagrams showing the advantage of the improved yCD/*mut*TK_{SR39} gene of the invention.

[0018] FIG. 4 is a diagram showing an advantage of the ADP gene of the present invention

[0019] FIG. 5 shows Kaplan-Meier plots with Ad5-yCD/*mut*TK_{SR39}*rep*-ADP in intraprostatic LNCaP C4-2 mouse model.

DETAILED DESCRIPTION OF THE INVENTION

[0020] Generally, the present invention comprises methods and compositions for the treatment for cancer. More specifically, the present invention provides a treatment that, when administered with prodrugs, can kill cancer cells and make the remaining cancer cells more sensitive to radiation.

[0021] Embodiments of the present invention include a novel virus that produces a protein that can convert non-toxic prodrugs into chemotherapeutic agents. The prodrugs can be produced locally or administered in conjunction with the treatment. Preferably, the virus is an oncolytic, replication-competent adenovirus such as, but not limited to, Ad5-yCD/*mut*TK_{SR39}*rep*-ADP. When administered to a patient in need of such treatment, the adenovirus converts at least two prodrugs into chemotherapeutic agents. These prodrugs can include, but are not limited to, 5-fluorocytosine (5-FC) and ganciclovir (GCV and derivatives thereof).

[0022] In addition to the ability to convert the prodrugs into chemotherapeutic agents, embodiments of the present invention sensitize the cells to radiation. By sensitizing the cells, lower doses of radiation can be used without limiting the benefits of radiation. Further, the radiation therapy is more effective because the cancer cells are more sensitive to the radiation, while normal cells are not more sensitive, thus limiting the side effects of cancer treatments. The treatment of the present invention can be used in conjunction with other therapies such as surgery, chemotherapy, hormone therapy, and immunotherapy.

[0023] In preferred embodiments, the present invention comprises a novel, oncolytic, replication-competent adenovirus (Ad5-yCD/*mut*TK_{SR39}*rep*-ADP) containing a yeast cytosine deaminase (yCD)/mutant SR39 herpes simplex virus type-1 thymidine kinase (*mut*TK_{SR39}) fusion gene and the adenovirus type 5 (Ad5) adenovirus death protein (ADP) gene. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP replicates in and kills human cancer cells efficiently. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP produces a novel yCD/*mut*TK_{SR39} fusion protein that can convert two prodrugs, 5-fluorocytosine (5-FC) and ganciclovir (GCV; and GCV derivatives), into potent chemotherapeutic agents (referred to as double suicide gene therapy). Both yCD/5-FC and HSV-1 TK_{SR39} suicide gene therapies exhibit potent chemotherapeutic activity and sensitize tumor cells to ionizing radiation.

[0024] By way of example only, preclinical studies show that the Ad5-yCD/*mut*TK_{SR39}*rep*-ADP virus is effective at killing a variety of human cancer cells when used by itself or when combined with double suicide gene therapy and/or radiation therapy. In a clinical setting, the Ad5-yCD/*mut*TK_{SR39}*rep*-ADP virus could be used as a monotherapy for its virus-mediated oncolytic effect, it could be coupled with yCD/5-FC and HSV-1 Ad5-TK_{SR39}/GCV suicide gene therapies for a combined viral oncolytic/chemotherapeutic effect, or it could be coupled with yCD/5-FC and HSV-1 TK_{SR39}/GCV suicide gene therapies and radiation therapy for a combined viral oncolytic/chemotherapeutic/radiosensitization effect (referred to as trimodal therapy). Trimodal therapy could be combined with other conventional cancer treatments such as surgery, chemotherapy, hormone therapy and immunotherapy in the management of human cancer.

[0025] To develop further this gene therapy-based approach as a cancer treatment, a novel, second-generation adenovirus (Ad5-yCD/*mut*TK_{SR39}*rep*-ADP) has been developed with two significant improvements relative to the prototype Ad5-CD/TK*rep* virus. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP contains an improved yCD/*mut*TK_{SR39} fusion gene whose product is more efficient at converting the 5-FC and GCV prodrugs into their active chemotherapeutic

agents. Moreover, Ad5-yCD/*mut*TK_{SR39}*rep*-ADP expresses the Ad5 ADP protein, which significantly increases the oncolytic activity of replication-competent adenoviruses. Relative to the prototype Ad5-CDITK*rep* virus, Ad5-yCD/*mut*TK_{SR39}*rep*-ADP has demonstrated greater viral oncolytic and chemotherapeutic activity in preclinical cancer models.

[0026] Introduction of nucleic acid of the present invention by viral infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to virus' infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

[0027] Also, additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur.

[0028] Features that limit expression to particular cell types can also be included in some embodiments. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

[0029] In addition, recombinant viral vectors are useful for *in vivo* expression of the nucleic acids of the present invention because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

[0030] As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the invention will depend on desired cell type to be targeted and will be known to those skilled in the art. For example, if breast cancer is to be treated then a vector specific for such epithelial cells would be used. Likewise, if diseases or pathological conditions of the hematopoietic system are to be treated, then a viral vector that is specific for blood cells and their precursors, preferably for the specific type of hematopoietic cell, would be used.

[0031] The recombinant vector can be administered in several ways. For example, the procedure can take advantage of the target specificity of viral vectors and consequently do not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment. Administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

[0032] An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this goal. Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

[0033] The compound of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically

"effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

[0034] In the method of the present invention, the compound of the present invention can be administered in various ways. It should be noted that it can be administered as the compound and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including humans. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

[0035] It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days. The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated.

[0036] When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

[0037] Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be

used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

[0038] Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

[0039] A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

[0040] In one embodiment, the compound of the present invention can be administered initially by intravenous injection to bring blood levels to a suitable level. The patient's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity to be administered will vary for the patient being treated.

DEFINITIONS

[0041] Unless stated otherwise or suggested by context, the following terms and phrases have the meaning provided below.

[0042] The term "gene therapy" as used herein refers to the transfer of genetic material (e.g. DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (e.g. a protein, polypeptide, peptide, functional RNA, antisense) whose production in vivo is desired. For

example, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic value. The genetic material of interest can also encode a suicide gene. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

[0043] The phrase "*in vivo* gene therapy" refers to when the genetic material to be transferred is introduced into the target cells of the recipient organism *in situ*, which is within the recipient. After therapy, the genetically altered target cells express the transfected genetic material *in situ*. Such therapy also includes repairing the gene *in situ*, if the host gene is defective.

[0044] The phrase "gene expression vehicle" refers to any vehicle capable of delivery/transfer of heterologous nucleic acid into a host cell. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle. Therefore, as used herein the expression vehicle may, as needed, not include the 5'UTR and/or 3'UTR of the actual gene to be transferred and only include the specific amino acid coding region. The expression vehicle can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. Enhancers that may be required to obtain necessary transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. The expression vehicle can also include a selection gene.

EXAMPLES

[0045] 1. Description of the Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP adenovirus

[0046] The complete and partial DNA and translated protein sequences of the Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP adenovirus, yCD/*mut*TK$_{SR39}$ fusion gene and ADP gene (SEQ ID NOs. 1-5) are disclosed following the List of References Section. The following examples are presented in view of such sequences.

[0047] The Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP virus (*SEQ ID NO: 1*) of the examples is a replication-competent, type 5 adenovirus (the sequence of which is readily known and obtainable to one skilled in the art) that contains an improved yCD/*mut*TK$_{SR39}$ fusion gene in the El region and the Ad5 ADP gene in the E3 region. A schematic representation of Ad5-

yCD/*mut*TK_{SR39}*rep*-ADP is provide in Figure 1 (in Figure 1, "CMV" = human cytomegalovirus promoter; "SV40" = simian virus 40 polyadenylation sequences; and "mu" = map units.) As shown in Figure 1, the CMV-yCD/*mut*TK_{SR39}-SV40 expression cassette is located in the E1 region in place of the deleted 55 kDa E1B gene. The CMV-ADP-SV40 expression cassette is located in the E3 region in place of the deleted E3 genes.

[0048] Ad5-yCD/*mut*TK_{SR39}*rep*-ADP contains a 1,255 base pair (bp) deletion (bases 2,271 to 3,524) in the 55 kDa E1B gene (*see SEQ ID NO: 2*). Using methods known to those of ordinary skill in the art, two premature translation stop codons were engineered into the 55 kDa E1B gene resulting in the production of a truncated, non-functional, 78 amino acid E1B protein. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP expresses the wild-type Ad5 E1A and 19 kDa E1B proteins. The yCD/*mut*TK_{SR39} fusion gene (*SEQ ID NO: 4*) was inserted in place of the deleted 55 kDa E1B gene. Expression of the yCD/*mut*TK_{SR39} fusion gene is driven by the human cytomegalovirus (CMV) promoter and utilizes simian virus 40 (SV40) polyadenylation elements. The yCD/*mut*TK_{SR39} fusion gene codes for a 59 kDa yCD/*mut*TK_{SR39} fusion protein, which is capable of enzymatically converting 5-flurocytosine (5-FC) into fluorouracil (5-FU) and ganciclovir (GCV) and its derivatives into their corresponding monophosphates (e.g. GCV-MP). The downstream metabolic products of 5-FU and GCV-MP are potent inhibitors of DNA replication and result in the death of dividing cells. These downstream metabolic products are also potent radiosensitizers and can markedly increase the therapeutic effect of radiation therapy (see refs. 1-14). Cells that express the yCD/*mut*TKs_{R39} fusion protein, as well as neighboring cells via the bystander effect, are killed by yCD/5-FC and HSV-1 TK_{SR39}/GCV suicide gene therapies and are sensitized to the killing effects of ionizing radiation.

[0049] Ad5-yCD/*mut*TK_{SR39}*rep*-ADP also contains a 2.68 kb deletion in the E3 region (bases 28,133 to 30,181), which affects genes that suppress the host immune response but are unnecessary for virus replication (*see SEQ ID NO: 3*). Ad5-yCD/*mut*TK_{SR39}*rep*-ADP contains an Ad5 ADP expression cassette in place of the natural Ad5 E3 genes. Expression of the ADP gene (*SEQ ID NO: 5*) is driven by the human cytomegalovirus (CMV) promoter and utilizes simian virus 40 (SV40) polyadenylation elements. The authentic 111.6 kDa Ad5 ADP protein is produced, which significantly increases the oncolytic activity of replication-competent adenoviruses. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP lacks all other known Ad5 E3 genes (gp19, 10.4 kDa, 14.5 kDa and 14.7 kDa genes).

[0050] 2. Construction of the Ad5 -yCD/*mut*TK$_{SR39}$*rep*-ADP adenovirus

[0051] Plasmids containing adenoviral sequences used in the construction of Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP were obtained from Microbix (Toronto, Canada). To generate the pCMV-yCD/*mut*TK$_{SR39}$ expression plasmid (left-end vector), the mutant SR39 HSV-1 TK gene (ref. 16) was generated by the polymerase chain reaction (PCR) using linearized pET23d:HSVTK$_{SR39}$ as template. The following primer pair was used to generate the *mut*TK$_{SR39}$ PCR product:

5'-GATCGGATCCCTCGAGATC2CTAGCATGGCTTCGTACCCCGGC-3 (SEQ ID NO: 8)

5'-GATCGAATTCTTCCGTGTTTCAGTTAGCCTC-3 (SEQ ID NO: 9)

[0052] The resulting 1,128 bp fragment was digested with *BamHI* (GGATCC) + *EcoRI* (GAATTC) and cloned between the *BamHI* + *EcoRI* sites of pCA14-CDglyTK-E1aE1b (ref. 10) after removal of the prototype CD/HSV-1 TK fusion gene generating pCA14-CMV-*mut*TK$_{SR39}$-E1aE1b. The yCD gene (ref. 17) was generated by PCR using linearized pBAD-ByCD as template. The following primer pair was used to generate the yCD PCR product:

5'-GATCCTCGAGCCACCATGGTGACAGGGGGAATG-3' (SEQ ID NO: 10)

5'-GATCGCTAGCACCTCCCCCACCGCCTCtCCCTCCACCCTCACCAATATCT TC-3' (SEQ ID NO: 11)

[0053] The resulting 526 bp fragment was digested with *XhoI* (CTCGAG) + *NheI* (GCTAGC) and cloned between the *XhoI* + *NheI* sites of pCA14-CMV-*mut*TK$_{SR39}$-E1aE1b generating pCA14-CMV-yCD/*mut*TK^{SR39}-E1aE1b.

[0054] To generate pBHG10-PacImod-CMV-ADP (right-end vector), the ADP gene was generated by PCR and cloned between the *PacI* and *SwaI* sites of pBHG10-*PacImod*. pBHG10-*PacImod* is a derivative of pBHG10 (Microbix; Toronto, Canada) and contains *PacI* and *SwaI* sites in the E3 region to facilitate directional cloning.

[0055] pBHG10 is a plasmid that contains the entire adenovirus type 5 genome minus bases 188 to 1,339 in the El region and bases 28,133 to 30,818 in the E3 region. Using wild-type Ad5 DNA as template, a 333 bp PCR product containing the ADP gene was generated. The following primer pair was used to generate the ADP PCR product:

5'-GATCGGATCCCCTGCTCCAGAGATGACCGGC.3' (SEQ ID NO: 12)

5'-GATCAAGCTTGGAATCATGTCTCAMAATC-3' (SEQ ID NO: 13)

[0056] The resulting 333 bp PCR product was digested with *BamHI* (GGATCC) + *HindIII* (AAGCTT) and cloned into *BamHI-HindIII* digested pCA14 (Microbix; Toronto, Canada) generating pCA14-ADP. The entire CMV-ADP-SV40 polyA expression cassette was generated by PCR using the following primer pair:

5'-GATCATTTAAATAATTCCCTGGCATTATGCCCAGTA-3' (SEQ ID NO: 14)
5'-GATCTTAATTAATCGATGCTAGACGATCCAGACATG-3' (SEQ ID NO: 15)

[0057] A *SwaI* restriction site (ATTTAAAT) was introduced upstream of the CMV promoter in the 5' primer and a *PacI* restriction site (TTAATTAA) was introduced downstream of the SV40 poly A region with the 3' primer. The PCR product was digested with *SwaI* and *PacI* and cloned into *SwaI-PacI* digested pBGH10-*PacImod* generating pBGH10-*PacImod*-CMV-ADP.

[0058] To generate Ad5-yCD/*mut*TK_{SR39}*rep*-ADP virus, pCA14-CMV-yCD/*mut*TK_{SR39}-E1aE1b (10 μg) was linearized by *PvuI* digestion and co-transfected with *ClaI*-linearized pBHG10-*PacImod*-CMV-ADP (30 μg) into HEK 293 cells (Microbix) using the $CaP0_4$-DNA precipitation method. Isolated plaques were harvested 7 - 14 days later and plaque-purified a second time on HEK 293 cells. Virus form twice purified plaques was used to infect HEK 293 cells to generate crude viral supernatants and CsCl gradient-purified adenovirus.

[0059] 3. Advantage of the ADP gene contained in Ad5-yCD/*mut*TK_{SR39}*rep*-ADP *in vitro*

[0060] Human DU145 prostate adenocarcinoma cells were plated in a 24-well plate at a concentration of 5 x 10^4 cells/well and were infected with graded amounts of thee Ad5-CD/TK*rep* (lane 1) and Ad5-yCD/*mut*TK_{SR39}*rep*-ADP viruses (lane 2). Five days later, cells were fixed and stained with crystal violet. The results (as shown in Figure 2, "Vp" = viral particles) clearly demonstrate that replication-competent adenoviruses containing the Ad5 ADP gene and expressing the ADP protein (i.e. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP) possess significantly greater oncolytic activity than adenoviruses that lack ADP. In other words, the presence of the Ad5 ADP gene significantly increased the oncolytic activity of replication competent adenoviruses. These results demonstrate, *in vitro*, the advantage of the ADP gene contained in Ad5-yCD/*mut*TK_{SR39}*rep*-ADP.

[0061] 4. Advanta ge of the yCD/*mut*TK$_{SR39}$ gene contained in Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP *in vitro*

[0062] A. CD Assays

[0063] LNCaP C4-2 cells were mock-infected (lanes 1 & 5), or infected with Ad5-CD/TK*rep* (lanes 2 & 6), Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP (lanes 3 & 7), Ad5-yCD/*mut*TK$_{SR39}$ *rep*-hNIS (lanes 4 & 8) at a MOI of 10. Seventy two hours later, cells were examined for CD activity using [^{14}C]-cytosine (lanes 1-4) and [^{3}H]-5-FC (lanes 4-8) as substrates. The results are shown in Figure 3A [(Cytosine (lower left arrow), uracil (upper left arrow), 5-FC (upper right arrow), 5-FU (lower right arrow)]. As shown by Figure 3A, recombinant adenoviruses that express the improved yCD/*mut*TK$_{SR39}$*rep* gene, such as Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP, demonstrate greater conversion of 5-FC into 5-FU, but not cytosine into uracil, than viruses expressing the CD/HSV-1 TK fusion gene contained in the prototype Ad5-CD/TK*rep* virus.

[0064] B. Cytopathic Effect Assay

[0065] Cells (10^6 cells, 60 mm dish) were mock-infected or infected with Ad5-CD/TK*rep* or Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP at an MOI of 3. The next day, cells were replated (24 well plate) in medium containing varying concentrations of 5-FC (wells 3 - 7 and 15 - 19, going left to right, top to bottom) or GCV (wells 8 – 12 and 20 - 24, going left to right, top to bottom) in μg/ml. Cells were stained with crystal violet 9 days later. The results (as shown in Figure 3B) demonstrate that recombinant adenoviruses expressing the improved yCD/*mut*TK*rep* gene, such as Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP, achieve greater cell kill when combined with 5-FC prodrug therapy than viruses expressing the CD/HSV-1 TK fusion gene contained in the prototype Ad5-yCD/TK*rep* virus. Together, the results of Figures 3A and 3B show, *in vitro,* the advantage of the yCD/*mut*TK$_{SR39}$ gene, which is contained in Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP.

[0066] The results of this example also demonstrate that yCD/5-FC and HSV-1 TK$_{SR39}$/GCV suicide gene therapies can be used to increase the therapeutic effect of the Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP virus itself. Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP contains a novel yCD/*mut*TK$_{SR39}$ fusion gene whose product has improved catalytic activity relative to the CD/HSV-1 TK fusion protein produced by the prototype Ad5-CD/TK*rep* virus. Recombinant adenoviruses that express the improved yCD/*mut*TK$_{SR39}$ fusion protein demonstrate significantly greater conversion of 5-FC into 5-FU, and possibly GCV into GCV-MP, than viruses that express the prototype CD/HSV-1- TK fusion protein. Thus,

yCD/5-FC and HSV-1 TK_{SR39}/GCV suicide gene therapies can be used independently and together to augment the tumor destructive effects of the Ad5-yCD/*mut*TK_{SR39}*rep*-ADP virus.

[0067] 5. Advantage of the ADP gene contained in Ad5-yCD/*mut*TK_{SR39}*rep*-ADP *in vivo*

[0068] Intramuscular (leg) C33A tumors (150 – 200 mm^3) were injected with 10^{10} vp of Ad5-CD/TK*rep* or Ad5-CD/TK*rep*-ADP on Days 0, 2 and 4 (arrowheads in Figure 4). 5-FC (500 mg/kg/day) and GCV (30 mg/kg/day) were administered on Days 5 – 11 (hatched bar in Figure 4). Tumor volume was monitored every other day. The predetermined endpoint was 500 mm^3. Survival is defined as an animal having no tumor (cure) or a tumor < 500 mm^3 on Day 90. The results (as shown in Figure 4 and Table 1 below) show greater destruction of tumor cells *in vivo* and thus demonstrate the advantage of the ADP gene, which is contained in Ad5-yCD/*mut*TK_{SR39}*rep*-ADP. In other words, the presence of the Ad5 ADP gene significantly increased the oncolytic activity of replication competent adenoviruses *in vivo* as well as *in vitro*.

Table 1. Summary of Results with Ad5-CD/TK*rep*-ADP in C33A Tumor Model.

Group	Median Survival	Tumor Care (%)	Pvalue	
			Log Rank (Survival)	Fisher Exact (Tumor Cure)
PBS	17	0(0/13)		
Ad5-CD/TK*rep*	26	0(0/12)		
Ad5-CD/TK*rep* + 5-FC + GCV	33	9(1/11)		
Ad5-CD/TK*rep*-ADP	>90	8(1/12)	0.022[b]	1.000[b]
Ad5-CD/TK*rep*-ADP + 5-FC + GCV	>90	70(7/10)	0.026[c]	.008[c]

[a]Median survival is in days.
[b]Ad5-CD/TK*rep*-ADP vs. Ad5-CD/TK*rep*
[c]Ad5-CD/TK*rep*-ADP + 5-FC + GCV vs. Ad5-CD/TK*rep* + 5-FC + GCV

[0069] 6. Effectiveness of Ad5-yCD/*mut*TK_{SR39}*rep*-ADP *in vivo* in Mouse Model

[0070] Male SCID mice bearing intraprostatic LNCaP C4-2 tumors (~25 - 50 mm^3 in size) were injected with about 10^9 vp of Ad5-yCD/*mut*TK_{SR39}*rep*-ADP on Day 0 (arrowhead in Figure. 5). 5-FC (500 mg/kg/day) and GCV (30 mg/kg/day) were administered on Days 3 - 9 (hatched bar in Figure 5). Serum PSA was measured weekly. The predetermined

endpoint was PSA = 500 ng/ml. The results (as shown in Figure 5 and Table 2) show an increase in median survival time and/or tumor cure in mouse model using Ad5-yCD/*mut*TK_{SR39}*rep*-ADP of the present invention.

Table 2 - Results with Ad5-yCD/*mut*TK_{SR39}*rep*-ADP in LNCaP C4-2 Tumor Model.

Group	Median Survival	Tumor cure	P value	
	(days)	(%)	Log Rank (Survival)	Fisher Exact (Tumor Cure)
PBS	5	0 (0/8)		
Ad5-yCD/*mut*TK_{SR39}*rep*-ADP	17	0 (0/11)	.038[a]	NA[a]
Ad5-yCD/*mut*TK_{SR39}*rep*-ADP + 5-FC + GCV	> 90	80 (8/10)	<0.001[b]	<0.001[b]

[a]Ad5-yCD/*mut*TK_{SR39}*rep*-ADP vs. PBS;
[b]Ad5-yCD/*mut*TK_{SR39}*rep*-ADP + 5-FC + GCV vs. PBS.

[0071] 7. Radiosensitize d Human Cancer Cells using yCD/5-FC and HSV-1 TK_{SR39}/GCV

[0072] As shown in previous experiments by the inventors (see refs. 1-14), yCD/5-FC and HSV-1 TK_{SR39}/GCV suicide gene therapies can also be used to radiosensitize human cancer cells. Ad5-yCD/*mut*TK_{SR39}*rep*-ADP contains a novel yCD/*mut*TK_{SR39} fusion gene whose product has improved catalytic activity relative to the CD/HSV-1 TK fusion protein produced by the prototype Ad5-CD/TK*rep* virus. The previous studies demonstrated that CD/5-FC and HSV-1 TK/GCV suicide gene therapies can sensitize human tumor cells to ionizing radiation. Thus, since Ad5-yCD/*mut*TK_{SR39} *rep*-ADP expresses an improved yCD/*mut*TK_{SR39} fusion protein, it may result in greater tumor cell radiosensitization *in vivo*.

[0073] Throughout this application, various references are noted by reference numbers. A numbered list of these references with their full citations is provided below. The disclosures of these references in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

[0074] While the present invention has been particularly shown and described with reference to the foregoing preferred and alternative embodiments, and examples, it should be understood by those skilled in the art that various alternatives to the embodiments of the

invention described herein may be employed in practicing the invention without departing from the spirit and scope of the invention as defined in the following claims. It is intended that the following claims define the scope of the invention and that the method and composition within the scope of these claims and their equivalents be covered thereby. This description of the invention should be understood to include all novel and non-obvious combinations of elements described herein, and claims may be presented in this or a later application to any novel and non-obvious combination of these elements. The foregoing embodiments are illustrative, and no single feature or element is essential to all possible combinations that may be claimed in this or a later application. Where the claims recite "a" or "a first" element of the equivalent thereof, such claims should be understood to include incorporation of one or more such elements, neither requiring nor excluding two or more such elements.

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Complete DNA Sequence of Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP Virus
(SEQ ID NO: 1)

CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGGGGGTGGAGTTTGTGACGTGGCGCGGGGCGTGGGAACGGGGCGG
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Partial DNA sequence of Ad5-yCD/*mut*TK_{SR39}*rep*-ADP (base 1 → 5100) (E1 Region)
(SEQ ID NO: 2)

yCD sequence is italicized; glycine polylinker is bolded; *mut*TK_{SR39} sequence is regular text; mutations in *mut*TK_{SR39} are indicated

10 20 30 40 50 60 70 80 90 100
CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGGGGGTGGAGTTTGTGACGTGGCGCGGGGCGTGGGAACGGGGCGG
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110 120 130 140 150 160 170 180 190 200
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210 220 230 240 250 260 270 280 290 300
GAAGTGACAATTTTCGCGCGGTTTTAGGCGGATGTTGTAGTAAATTTGGGCGTAACCGAGTAAGATTTGGCCATTTTCGCGGGAAAACTGAATA
AGAGGA

*Bgl*II/*Bam*HI
310 320 330 340 350 360 370 380 390 400
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TGGAG

E1A TATA
410 420 430 440 450 460 470 480 490 500
ACTCGCCCAGGTGTTTTTCTCAGGTGTTTTCCGCGTTCCGGGTCAAAGTTGGCGTTTTATTATTATAGTCAGCTGACGTGTAGTGTATTTATACCC
GGTG

+1 E1A mRNA E1A start
510 520 530 540 550 560 570 580 590 600
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CACGG

610 620 630 640 650 660 670 680 690 700
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CACCT

710 720 730 740 750 760 770 780 790 800
ACCCTTCACGAACTGTATGATTTAGACGTGACGGCCCCCGAAGATCCCAACGAGGAGGCGGCTTCGCAGATTTTTCCCGACTCTGTAATGTTGG
GGTGC

810 820 830 840 850 860 870 880 890 900
AGGAAGGGATTGACTTACTCACTTTTCCGCCGGCGCCCGGTTCTCCGGAGCCGCCTCACCTTTCCCGGCAGCCCGAGCAGCCGGAGCAGAGAGC
CTTGGG

E1A SD

910 920 930 940 950 960 970 980 990 1000
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GAGGAG

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
TTTGTGTTAGATTATGTGGAGCACCCCGGGCACGGTTGCAGGTCTTGTCATTATCACCGGAGGAATACGGGGGACCCAGATATTATGTGTTCGCT
TTGCT

E1A SD

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
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TTTTT

E1A SA

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
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AAGAC

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
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GGTCC

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
TTCTAACACACCTCCTGAGATACACCCGGTGGTCCCGCTGTGCCCCATTAAACCAGTTGCCGTGAGAGTTGGTGGGCGTCGCCAGGCTGTGGAAT
GTATC

E1A stop

1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
GAGGACTTGCTTAACGAGCCTGGGCAACCTTTGGACTTGAGCTGTAAACGCCCCAGGCCATAAGGTGTAAACCTGTGATTGCGTGTGTGGTTAA
CGCCTT

E1A polyadenylation E1A mRNA 3' terminus

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
TGTTTGCTGAATGAGTTGATGTAAGTTTAATAAAGGGTGAGATAATGTTTAACTTGCATGGCGTGTTAAATGGGGCGGGGCTTAAAGGGTATAIA
ATGCG

+1 E1B mRNA 19 kDa E1B start

1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
CCGTGGGCTAATCTTGGTTACATCTGACCTCATGGAGGCTTGGGAGTGTTTGGAAGATTTTTCTGCTGTGCGTAACTTGCTGGAACAGAGCTCTA
ACAGT

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
ACCTCTTGGTTTTGGAGGTTTCTGTGGGGCTCATCCCAGGCAAAGTTAGTCTGCAGAATTAAGGAGGATTACAAGTGGGAATTTGAAGAGCTTTT
GAAAT

1910 1920 1930 1940 1950 1960 1970 1980 1990 2000

CCTGTGGTGAGCTGTTTGATTCTTTGAATCTGGGTCACCAGGCGCTTTTCCAAGAGAAGGTCATCAAGACTTTGGATTTTTCCACACCGGGGCGC
GCTGC

55 kDa E1B start

2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
GGCTGCTGTTGCTTTTTTGAGTTTTATAAAGGATAAATGGAGCGAAGAAACCCATCTGAGCGGGGGGTACCTGCTGGATTTTCTGGCCATGCATC
TGTGG

2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
AGAGCGGTTGTGAGACACAAGAATCGCCTGCTACTGTTGTCTTCCGTCCGCCCGGCGATAATACCGACGGAGGAGCAGCAGCAGCAGCAGGAG
GAAGCCA

55 kDa premature stops
19 kDa E1B stop *Bgl*II

2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
GGCGGCGGCGGCAGGAGCAGAGCCCATGGAACCCGAGAGCCGGCCTGGACCCTCGGGAATGAATGTTGTATAGGTGGCTTAACTGTATAGATC
TAATTCC

2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTG
GCAG

2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
TACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCA
ACGGG

CMV TATA

2510 2520 2530 2540 2550 2560 2570 2580 2590 2600

ACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGT
GAACCG

+1 CMV *Bam*HI *Xho*I

2610 2620 2630 2640 2650 2660 2670 2680 2690 2700

TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTGGGGATCTTCGAGTCGAGGGATCCC
TCGAGC

CD start

2710 2720 2730 2740 2750 2760 2770 2780 2790 2800

CACC*ATGGTGACAGGGGGAATGGCAAGCAAGTGGGATCAGAAGGGTATGGACGTTGCCTATGAGGAGGCGGCCTTAGGTTACAAAGAGGGTGGTGTTC*
CT

2810 2820 2830 2840 2850 2860 2870 2880 2890 2900

ATTGGCGGATGTCTTATCAATAACAAAGACGGAAGTGTTCTCGGTCGTGGTCACAACATGAGATTTCAAAAGGGATCCGCCACACTACATGGTGAGATCT

2910 2920 2930 2940 2950 2960 2970 2980 2990 3000

CCACTTTGGAAAACTGTGGGAGATTAGAGGGCAAAGTGTACAAAGATACCACTTTGTATACGACGCTGTCTCCATGCGACATGTGTACAGGTGCCATCAT

3010 3020 3030 3040 3050 3060 3070 3080 3090 3100

CATGTATGGTATTCCACGCTGTGTTGTCGGTGAGAACGTTAATTTCAAAAGTAAGGGCGAGAAATATTTACAAACTAGAGGTCACGAAGTTGTTGTTGTT

end of yCD polylinker

3110 3120 3130 3140 3150 3160 3170 3180 3190 3200

*GACGATGAGAGGTGTAAAAAGATCATGAAACAATTTATCGATGAAAGACCTCAGGATTGGTTTGAAGATATTGGTGAG***GGTGGAGGGGGAGGCGGTGG**
GG

*Nhe*I TK start

3210 3220 3230 3240 3250 3260 3270 3280 3290 3300

GAGGTGCTAGCATGGCTTCGTACCCCGGCCATCAACACGCGTCTGCGTTCGACCAGGCTGCGCGTTCTCGCGGCCATAGCAACCGACGTACGGC
GTTGCG

3310 3320 3330 3340 3350 3360 3370 3380 3390 3400

CCCTCGCCGGCAGCAAGAAGCCACGGAAGTCCGCCCGGAGCAGAAAATGCCCACGCTACTGCGGGTTTATATAGACGGTCCCCACGGGATGGG
GAAAACC

3410 3420 3430 3440 3450 3460 3470 3480 3490 3500

ACCACCACGCAACTGCTGGTGGCCCTGGGTTCGCGCGACGATATCGTCTACGTACCCGAGCCGATGACTTACTGGCGGGTGCTGGGGGCTTCCG
AGACAA

3510 3520 3530 3540 3550 3560 3570 3580 3590 3600

TCGCGAACATCTACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACAA
TGGGCAT

mutTK_{SR39} mutations

3610 3620 3630 3640 3650 3660 3670 3680 3690 3700

GCCTTATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGGAGGCTGGGAGCTCACATGCCCCGCCCCCGGCCCTCACCATCTTCCTCG
ACCGC

mutTK_{SR39} mutations

3710 3720 3730 3740 3750 3760 3770 3780 3790 3800

CATCCCATCGCCTTCATGCTGTGCTACCCGGCCGCGCGGTACCTTATGGGCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTGGCCCTCATCCC
GCCGA

3810 3820 3830 3840 3850 3860 3870 3880 3890 3900

CCTTGCCCGGCACCAACATCGTGCTTGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAAACGCCAGCGCCCCGGCGAGCGGCTGGA
CCTGGC

3910 3920 3930 3940 3950 3960 3970 3980 3990 4000
TATGCTGGCTGCGATTCGCCGCGTTTACGGGCTACTTGCCAATACGGTGCGGTATCTGCAGTGCGGCGGGTCGTGGCGGGAGGACTGGGGACAG
CTTTCG

4010 4020 4030 4040 4050 4060 4070 4080 4090 4100
GGGACGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACGCGGGCCCACGACCCCATATCGGGGACACGTTATTTACCCTGTTTCGGGCCC
CCGAGT

4110 4120 4130 4140 4150 4160 4170 4180 4190 4200
TGCTGGCCCCCAACGGCGACCTGTATAACGTGTTTGCCTGGGCCTTGGACGTCTTGGCCAAACGCCTCCGTTCCATGCACGTCTTTATCCTGGATT
ACGA

4210 4220 4230 4240 4250 4260 4270 4280 4290 4300
CCAATCGCCCGCCGGCTGCCGGGACGCCCTGCTGCAACTTACCTCCGGGATGGTCCAGACCCACGTCACCACCCCCGGCTCCATACCGACGATAT
GCGAC

TK stop *Eco*RI *Hind*III
4310 4320 4330 4340 4350 4360 4370 4380 4390 4400
CTGGCGCGCACGTTTGCCCGGGAGATGGGGGAGGCTAACTGAAACACGGAAGAATTCAAGCTTGTCGACTTCGAGCAACTTGTTTATTGCAGCT
TATAAT

SV40 polyadenylation SV40 3' terminus
4410 4420 4430 4440 4450 4460 4470 4480 4490 4500

GGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
TATC

*Bgl*II *Bam*HI/*Bgl*II

4510 4520 4530 4540 4550 4560 4570 4580 4590 4600
ATGTCTGGATCGTCTAGCATCGAAGATCTGGATCTGGGCGTGGTTAAGGGTGGGAAAGAATATATAAGGTGGGGGTCTTATGTAGTTTTGTATC
GTTTT

E1B SA

4610 4620 4630 4640 4650 4660 4670 4680 4690 4700
GCAGCAGCCGCCGCCGCCATGAGCACCAACTCGTTTGATGGAAGCATTGTGAGCTCATATTTGACAACGCGCATGCCCCCATGGGCCGGGGTGC
GTCAGA

4710 4720 4730 4740 4750 4760 4770 4780 4790 4800
ATGTGATGGGCTCCAGCATTGATGGTCGCCCCGTCCTGCCCGCAAACTCTACTACCTTGACCTACGAGACCGTGTCTGGAACGCCGTTGGAGAC
GCAGC

4810 4820 4830 4840 4850 4860 4870 4880 4890 4900
CTCCGCCGCCGCTTCAGCCGCTGCAGCCACCGCCCGCGGGATTGTGACTGACTTTGCTTTCCTGAGCCCGCTTGCAAGCAGTGCAGCTTCCCGTT
CATCC

4910 4920 4930 4940 4950 4960 4970 4980 4990 5000
GCCCGCGATGACAAGTTGACGGCTCTTTTGGCACAATTGGATTCTTTGACCCGGGAACTTAATGTCGTTTCTCAGCAGCTGTTGGATCTGCGCCA
GCAGG

E1B polyadenylation E1B mRNA 3' terminus

5010 5020 5030 5040 5050 5060 5070 5080 5090 5100
TTTCTGCCCTGAAGGCTTCCTCCCCTCCCAATGCGGTTTAAAACATAAATAAAAACCAGACTCTGTTTGGATTTGGATCAAGCAAGTGTCTTGC
TGTCT

Partial DNA sequence of Ad5-yCD/*mut*TK_{SR39}*rep*-ADP (base 29,163 → 30,079) (E3 Region)
(SEQ ID NO: 3)

ADP sequence is bolded

*Swa*I

10 20 30 40 50 60 70 80 90 100

ATTTAAATAATTCCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG
GTGA

110 120 130 140 150 160 170 180 190 200

TGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTT
TGGCA

CMV TATA

210 220 230 240 250 260 270 280 290 300

CCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCT

+1 CMV

310 320 330 340 350 360 370 380 390 400

CGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTGGGGATCTTCG
AGTCGA

*Bam*HI ADP start

410 420 430 440 450 460 470 480 490 500

GGGATCCCCTGCTCCAGAG**ATGACCGGCACAACCAACGCGGCCGCCGCTACCGGACTTACATCTACCACAAATACACCCCAAGTTTCTGC**
CTTTGTCAAT

510 520 530 540 550 560 570 580 590 600

AACTGGGATAACTTGGGCATGTGGTGGTTCTCCATAGCGCTTATGTTTGTATGCCTTATTATTATGTGGCTCATCTGCTGCCTAAAGCGC
AAACGCGCCC

610 620 630 640 650 660 670 680 690 700
GACCACCCATCTATAGTCCCATCATTGTGCTACACCCAAACAATGATGGAATCCATAGATTGGACGGACTGAAACACATGTTCTTTTCTC
TTACAGTATG

ADP stop *Hind*III SV40 poly A SV40 3' t
710 720 730 740 750 760 770 780 790 800
ATTAAATGAGACATGATTCCAAGCTTGTCGACTTCGAGCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTT
CACAA

*Pac*I
810 820 830 840 850 860 870 880 890
TAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCGTCTAGCATTTAATTAA

DNA Sequence and Translation of yCD/*mut*TK$_{SR39}$ Fusion Gene (SEQ ID NO: 4)

yCD sequence is italicized; glycine polylinker is bolded; *mut*TK$_{SR39}$ sequence is regular text; mutations in *mut*TK$_{SR39}$ are indicated

```
            10                  20                  30
M V T G G M A S K W D Q K G M D V A Y E E A A L G Y K E G G
ATGGTGACAGGGGGAATGGCAAGCAAGTGGGATCAGAAGGGTATGGACGTTGCCTATGAGGAGGCGGCCTTAGGTTACAAAGAGGGTGGT
   10    20    30    40    50    60    70    80    90

            40                  50                  60
V P I G G C L I N N K D G S V L G R G H N M R F Q K G S A T
GTTCCTATTGGCGGATGTCTTATCAATAACAAAGACGGAAGTGTTCTCGGTCGTGGTCACAACATGAGATTTCAAAAGGGATCCGCCACA
   100   110   120   130   140   150   160   170   180

            70                  80                  90
L H G E I S T L E N C G R L E G K V Y K D T T L Y T T L S P
CTACATGGTGAGATCTCCACTTTGGAAAACTGTGGGAGATTAGAGGGCAAAGTGTACAAAGATACCACTTTGTATACGACGCTGTCTCCA
   190   200   210   220   230   240   250   260   270

            100                 110                 120
C D M C T G A I I M Y G I P R C V V G E N V N F K S K G E K
TGCGACATGTGTACAGGTGCCATCATCATGTATGGTATTCCACGCTGTGTTGTCGGTGAGAACGTTAATTTCAAAAGTAAGGGCGAGAAA
   280   290   300   310   320   330   340   350   360

            130                 140                 150
Y L Q T R G H E V V V V D D E R C K K I M K Q F I D E R P Q
TATTTACAAACTAGAGGTCACGAAGTTGTTGTTGTTGACGATGAGAGGTGTAAAAAGATCATGAAACAATTTATCGATGAAAGACCTCAG
  -370   380   390   400   410   420   430   440   450

            160                 170                 180
D W F E D I G E G G G G G G G G A S M A S Y P G H Q H A S
GATTGGTTTGAAGATATTGGTGAGGGTGGAGGGGGAGGCGGTGGGGGAGGTGCTAGCATGGCTTCGTACCCCGGCCATCAACACGCGTCT
```

460 470 480 490 500 510 520 530 540

190 200 210
A F D Q A A R S R G H S N R R T A L R P R R Q Q E A T E V R
GCGTTCGACCAGGCTGCGCGTTCTCGCGGCCATAGCAACCGACGTACGGCGTTGCGCCCTCGCCGGCAGCAAGAAGCCACGGAAGTCCGC
550 560 570 580 590 600 610 620 630

220 230 240
P E Q K M P T L L R V Y I D G P H G M G K T T T T Q L L V A
CCGGAGCAGAAAATGCCCACGCTACTGCGGGTTTATATAGACGGTCCCCACGGGATGGGGAAAACCACCACCACGCAACTGCTGGTGGCC
640 650 660 670 680 690 700 710 720

250 260 270
L G S R D D I V Y V P E P M T Y W R V L G A S E T I A N I Y
CTGGGTTCGCGCGACGATATCGTCTACGTACCCGAGCCGATGACTTACTGGCGGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCTAC
730 740 750 760 770 780 790 800 810

280 290 300
T T Q H R L D Q G E I S A G D A A V V M T S A Q I T M G M P
ACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACAATGGGCATGCCT
820 830 840 850 860 870 880 890 900

310 320 330
WT HSV-1 TK L I F
WT HSV-1 TK C A T
Y A V T D A V L A P H I G G E A G S S H A P P P A L T I F L
TATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGAGGCTGGGAGCTCACATGCCCCGCCCCCGGCCCTCACCATCTTCCTC
910 920 930 940 950 960 970 980 990

340 350 360
WT HSV-1 TK A L
WT HSV-1 TK GC C C

D R H P I A F M L C Y P A A R Y L M G S M T P Q A V L A F V
GACCGCCATCCCATCGCCTTCATGCTGTGCTACCCGGCCGCGCGGTACCTTATGGGCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTG
1000 1010 1020 1030 1040 1050 1060 1070 1080

370 380 390
A L I P P T L P G T N I V L G A L P E D R H I D R L A K R Q
GCCCTCATCCCGCCGACCTTGCCCGGCACCAACATCGTGCTTGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAAACGCCAG
1090 1100 1110 1120 1130 1140 1150 1160 1170

400 410 420
R P G E R L D L A M L A A I R R V Y G L L A N T V R Y L Q C
CGCCCCGGCGAGCGGCTGGACCTGGCTATGCTGGCTGCGATTCGCCGCGTTTACGGGCTACTTGCCAATACGGTGCGGTATCTGCAGTGC
1180 1190 1200 1210 1220 1230 1240 1250 1260

430 440 450
G G S W R E D W G Q L S G T A V P P Q G A E P Q S N A G P R
GGCGGGTCGTGGCGGGAGGACTGGGGACAGCTTTCGGGGACGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACGCGGGCCCACGA
1270 1280 1290 1300 1310 1320 1330 1340 1350

460 470 480
P H I G D T L F T L F R A P E L L A P N G D L Y N V F A W A
CCCCATATCGGGGACACGTTATTTACCCTGTTTCGGGCCCCCGAGTTGCTGGCCCCCAACGGCGACCTGTATAACGTGTTTGCCTGGGCC
1360 1370 1380 1390 1400 1410 1420 1430 1440

490 500 510
L D V L A K R L R S M H V F I L D Y D Q S P A G C R D A L L
TTGGACGTCTTGGCCAAACGCCTCCGTTCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCGCCGGCTGCCGGGACGCCCTGCTG
1450 1460 1470 1480 1490 1500 1510 1520 1530

520 530 540
Q L T S G M V Q T H V T T P G S I P T I C D L A R T F A R E
CAACTTACCTCCGGGATGGTCCAGACCCACGTCACCACCCCCGGCTCCATACCGACGATATGCGACCTGGCGCGCACGTTTGCCCGGGAG

1540 1550 1560 1570 1580 1590 1600 1610 1620

545
M G E A N
ATGGGGGAGGCTAAC
1630

DNA Sequence and Translation of ADP gene (SEQ ID NO: 5)

```
            10                  20                  30
M T G T T N A A A A T G L T S T T N T P Q V S A F V N N W D
ATGACCGGCACAACCAACGCGGCCGCCGCTACCGGACTTACATCTACCACAAATACACCCCAAGTTTCTGCCTTTGTCAATAACTGGGAT
    10    20    30    40    50    60    70    80    90

            40                  50                  60
N L G M W W F S I A L M F V C L I I M W L I C C L K R K R A
AACTTGGGCATGTGGTGGTTCTCCATAGCGCTTATGTTTGTATGCCTTATTATTATGTGGCTCATCTGCTGCCTAAAGCGCAAACGCGCC
    100   110   120   130   140   150   160   170   180

            70                  80                  90
R P P I Y S P I I V L H P N N D G I H R L D G L K H M F F S
CGACCACCCATCTATAGTCCCATCATTGTGCTACACCCAAACAATGATGGAATCCATAGATTGGACGGACTGAAACACATGTTCTTTTCT
    190   200   210   220   230   240   250   260   270

  93
L T V
CTTACAGTATGA
    280
```

SEQ ID NO: 6

Met Gly Thr Gly Gly Met Ala Ser Lys Trp Asp Gln Lys Gly Met Asp
1 5 10 15

Ile Ala Tyr Glu Glu Ala Ala Leu Gly Tyr Lys Glu Gly Gly Val Pro
20 25 30

Ile Gly Gly Cys Leu Ile Asn Asn Lys Asp Gly Ser Val Leu Gly Arg
35 40 45

Gly His Asn Met Arg Phe Gln Lys Gly Ser Ala Thr Leu His Gly Glu
50 55 60

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

Asp Thr Thr Leu Tyr Thr Thr Leu Ser Pro Cys Asp Met Cys Thr Gly
85 90 95

Ala Ile Ile Met Tyr Gly Ile Pro Arg Cys Val Val Gly Glu Asn Val
100 105 110

Asn Phe Lys Ser Lys Gly Glu Lys Tyr Leu Gln Thr Arg Gly His Glu
115 120 125

Val Val Val Val Asp Asp Glu Arg Cys Lys Lys Ile Met Lys Gln Phe
130 135 140

Ile Asp Glu Arg Pro Gln Asp Trp Phe Glu Asp Ile Gly Glu Gly Gly
145 150 155 160

Gly Gly Gly Gly Gly Gly Gly Ala Ser Met Ala Ser Tyr Pro Cys His
165 170 175

Gln His Ala Ser Ala Phe Asp Gln Ala Ala Arg Ser Arg Gly His Ser
180 185 190

Asn Arg Arg Thr Ala Leu Arg Pro Arg Arg Gln Gln Glu Ala Thr Glu
195 200 205

Val Arg Leu Glu Gln Lys Met Pro Thr Leu Leu Arg Val Tyr Ile Asp
210 215 220

Gly Pro His Gly Met Gly Lys Thr Thr Thr Thr Gln Leu Leu Val Ala
225 230 235 240

Leu Gly Ser Arg Asp Asp Ile Val Tyr Val Pro Glu Pro Met Thr Tyr
245 250 255

Trp Gln Val Leu Gly Ala Ser Glu Thr Ile Ala Asn Ile Tyr Thr Thr
260 265 270

Gln His Arg Leu Asp Gln Gly Glu Ile Ser Ala Gly Asp Ala Ala Val
275 280 285

Val Met Thr Ser Ala Gln Ile Thr Met Gly Met Pro Tyr Ala Val Thr
290 295 300

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

Ala Pro Pro Pro Ala Leu Thr Ile Phe Leu Asp Arg His Pro Ile Ala
325 330 335

Phe Met Leu Cys Tyr Pro Ala Ala Arg Tyr Leu Met Gly Ser Met Thr
340 345 350

Pro Gln Ala Val Leu Ala Phe Val Ala Leu Ile Pro Pro Thr Leu Pro
355 360 365

Gly Thr Asn Ile Val Leu Gly Ala Leu Pro Glu Asp Arg His Ile Asp
370 375 380

Arg Leu Ala Lys Arg Gln Arg Pro Gly Glu Arg Leu Asp Leu Ala Met
385 390 395 400

Leu Ala Ala Ile Arg Arg Val Tyr Gly Leu Leu Ala Asn Thr Val Arg
405 410 415

Tyr Leu Gln Gly Gly Gly Ser Trp Trp Glu Asp Trp Gly Gln Leu Ser
420 425 430

Gly Thr Ala Val Pro Pro Gln Gly Ala Glu Pro Gln Ser Asn Ala Gly
435 440 445

Pro Arg Pro His Ile Gly Asp Thr Leu Phe Thr Leu Phe Arg Ala Pro
450 455 460

Glu Leu Leu Ala Pro Asn Gly Asp Leu Tyr Asn Val Phe Ala Trp Ala
465 470 475 480

Leu Asp Val Leu Ala Lys Arg Leu Arg Pro Met His Val Phe Ile Leu
485 490 495

Asp Tyr Asp Gln Ser Pro Ala Gly Cys Arg Asp Ala Leu Leu Gln Leu
500 505 510

Thr Ser Gly Met Val Gln Thr His Val Thr Thr Pro Gly Ser Ile Pro
515 520 525

Thr Ile Cys Asp Leu Ala Arg Thr Phe Ala Arg Glu Met Gly Glu Ala
530 535 540

Asn
545

SEQ ID NO: 7

Met Thr Gly Ser Thr Ile Ala Pro Thr Thr Asp Tyr Arg Asn Thr Thr
1 5 10 15

Ala Thr Gly Leu Thr Ser Ala Leu Asn Leu Pro Gln Val His Ala Phe
20 25 30

Val Asn Asp Trp Ala Ser Leu Asp Met Trp Trp Phe Ser Ile Ala Leu
35 40 45

Met Phe Val Cys Leu Ile Ile Met Trp Leu Ile Cys Cys Leu Lys Arg
50 55 60

Arg Arg Ala Arg Pro Pro Ile Tyr Arg Pro Ile Ile Val Leu Asn Pro
65 70 75 80

His Asn Glu Lys Ile His Arg Leu Asp Gly Leu Lys Pro Cys Ser Leu
85 90 95

Leu Leu Gln Tyr Asp
100

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence of a yeast cytosine deaminase/mutant SR 39 herpes simplex virus type 1 thymidine kinase fusion gene, comprising the nucleotide sequence of SEQ ID NO: 4.

2 A recombinant adenovirus comprising an isolated polynucleotide comprising a nucleotide sequence of a yeast cytosine deaminase/mutant SR 39 herpes simplex virus type 1 thymidine kinase fusion gene and comprising an adenovirus type 5 adenovirus death protein gene, wherein the adenovirus type 5 adenovirus death protein gene comprises the nucleotide sequence of SEQ ID NO: 5.

3. An isolated polypeptide comprising an amino acid sequence encoded by an isolated polynucleotide comprising a nucleotide sequence of a yeast cytosine deaminase/mutant SR 39 herpes simplex virus type 1 thymidine kinase fusion gene, which converts prodrugs, 5 fluorocytosine and ganciclovir, into active chemotherapeutic agents, comprising an amino acid sequence of SEQ ID NO: 6.

4. A recombinant adenovirus comprising an isolated polynucleotide comprising a nucleotide sequence of a yeast cytosine deaminase/mutant SR 39 herpes simplex virus type 1 thymidine kinase fusion gene and an adenovirus type 5 adenovirus death protein gene, comprising the nucleotide sequence of SEQ ID NO: 1.