ONCOLYTIC ADENOVIRUS COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Serial No. 61/782,932, filed March 14, 2013, which is incorporated by reference herein in its entirety.

STATEMENT AS TO RIGHTS UNDER FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government funding under Grant No. 5T32GM007240-35 from the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Cancer is a complex, debilitating disease that accounts for more than half a million deaths each year. There is a profound need for more effective, selective and safe treatments for cancer. Existing treatments for this pervasive, life threatening disease, such as chemotherapy and surgery, rarely eliminate all malignant cells, and often exhibit deleterious side-effects that can outweigh therapeutic benefit.

[0004] One approach that has the potential to address many of the shortcomings of current cancer treatments is oncolytic adenoviral therapy (Pesonen, S. et al., *Molecular Pharmaceutics*, 8(1): p. 12-28 (2010)). Adenovirus (Ad) is a self-replicating biological machine. It consists of a linear double-stranded 36 kb DNA genome sheathed in a protein coat. Adenoviruses invade and hijack the cellular replicative machinery to reproduce and upon assembly induce lytic cell death to escape the cell and spread and invade surrounding cells. These very same cellular controls are targeted by mutations in cancer. This knowledge can be exploited to create synthetic viruses that act like guided missiles, specifically infecting and replicating in tumor cells and bursting them apart to release thousands of virus progeny that can seek out and destroy distant metastases while overcoming possible resistance. Thus, the goal of oncolytic virus design is to generate a

virus that specifically replicates in cancer cells, but leave normal cells unharmed. However, there have been challenges in designing viruses that selectively replicate in cancer cells. Thus, there is a need for additional viruses that selectively replicate in cancer cells.

SUMMARY

[0005] Provided herein is an adenovirus comprising an E1A polypeptide comprising one or more modifications, an E4orf6/7 polypeptide comprising one or more modifications, or an E4orf1 polypeptide comprising one or more modifications or a combination thereof. Compositions and kits comprising the modified adenoviruses are also provided. Further, provided is a method of treating a proliferative disorder in a subject comprising administering to the subject an adenovirus comprising an E1A polypeptide comprising one or more modifications, an E4orf6/7 polypeptide comprising one or more modifications, or an E4orf1 polypeptide comprising one or more modifications or a combination thereof.

[0005a] In one embodiment, there is provided a recombinant adenovirus comprising:

(i) an E1A polypeptide comprising a deletion or substitution in an Rb-binding site;

(ii) a deletion of one or both of the E4orf6/7 exons; and

(iii) adenovirus E1A and E4 promoters,

wherein the recombinant adenovirus selectively replicates in Rb-deficient cells.

[0005b] In one embodiment, there is provided a recombinant adenovirus, comprising:

(i) an E1A polypeptide comprising a deletion or substitution in an Rb-binding site;

(ii) an E4orf1 polypeptide comprising a deletion in the C-terminal region, or a deletion of all of the E4orf1 gene; and

(iii) adenovirus E1A and E4 promoters,

wherein the recombinant adenovirus selectively replicates in Rb-deficient cells.

[0005c] In one embodiment, there is provided a pharmaceutical composition comprising the recombinant adenovirus as described herein and a pharmaceutically acceptable carrier.

[0005d] In one embodiment, there is provided the use of the adenovirus described herein or the pharmaceutical composition described herein, in the manufacture of a medicament for treating a proliferative disorder in a subject.

[0006] The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0007] Figure 1 is a schematic showing the general rationale of oncolytic viral cancer therapy.

[0008] Figure 2 is a schematic showing the structural features of adenovirus (Ad) and a map of the adenovirus genome with transcriptional units in boxes and labeled genes.

[0009] Figure 3 are images of immunoblots of cell lysates from infected primary small airway epithelial cells and tumor cells infected with wild type Ad5 and the "RB/p16/E2F pathway" selective viruses. Tumor and primary human cells were infected with either

wild type virus, ONYX-838 (E1A ΔLXCXE) or ONYX-411 and harvested at various time points post infection. Lysates were analyzed for the expression of viral capsid proteins (anti-ad5, three bands comprise hexon, penton and fiber) which is a measure of viral replication. ONYX-838 indiscriminately replicates in tumor and primary lung epithelial cells. ONYX-411, which combines the E1A ΔLXCXE with cellular E2F control of adenovirus E1A, E1B and E4 regions (shown in Fig. 2) demonstrates selective-replication in tumor cells vs. normal cells (Johnson et al., *Cancer Cell* 1(4):325-337 (2002)). However, the E2F promoters result in recombination and also limit replication to wild type virus levels in tumor cells.

[0010] Figures 4A and 4B are schematics showing tumor mutations and Adenovirus early proteins that converge in activating the Rb/p16 tumor suppressor pathway to activate E2F and elicit uncontrolled replication. Figure 4A shows common mutations that cause loss of Rb/p16 pathway tumor suppressor functions. Figure 4B shows adenovirus proteins that directly deregulate Rb and E2F to drive the cell into S-phase.

[0011] Figure 5 is a schematic showing multiple adenovirus encodes proteins that deregulate the Rb-E2F cell cycle checkpoint. Adenovirus E1A binds to cellular Rb through a conserved LXCXE motif, releasing E2F to activate transcription of cellular genes required for viral and cellular DNA replication. E1A is thought to be the critical mechanism through which adenovirus inactivates the Rb checkpoint and activates E2F target genes. In addition, another adenovirus protein, E4orf6/7, stabilizes E2F-DP1 abd dimerize at cellular and viral E2 promoters to enhance transcriptional activation (Schaley, J., et al., Protein J. Virol., 74(5):2084-2093 (2000); Cress, et al., Genes & Development, 7(10):1850-61 (1993)).

[0012] Figure 6 is a schematic showing construction of initial series of mutant adenoviruses in this work. The wildtype Ad5 genome was split into modules according to transcriptional units, and each of the modules was placed in different plasmids. Mutations were made on the module plasmids, and using a PCR-based approach (AdSlicR), the modules were reassembled into complete genomes enabling generation of recombinant adenoviruses.

[0013] Figures 7A and 7B are schematics showing the E4orf6/7 deletion. Two exons encode E4orf6/7. E4orf6 and E4orf6/7 utilize same start codon and share 58 amino acid N-terminal residues. The E4orf6/7 transcript splices immediately following E4orf6 stop codon. The entire second exon of E4orf6/7, including the stop codon was deleted. Figure 7A shows wildtype E4 region. Figure 7B shows resulting E4 ΔE4orf6/7 region.

[0014] Figures 8A and 8B are pictures of immunoblots of cell lysates showing expression of adenovirus early E1A from mutant Ad infected cells. Cells infected with mock (ΔE1), Ad-102 (also referred to herein as AdSyn-CO102) (wildtype), Ad-181 (also referred to herein as AdSyn-CO181) (E1A ΔLXCXE/ΔE4orf6/7), Ad-189 (also referred to herein as AdSyn-CO-189) (E1A ΔLXCXE), or ONYX-838 (E1A ΔCR2). ONYX-838 also lacks ΔLXCXE which is in the CR2 domain of E1A. For Figure 8A, quiescent human primary small airway epithelial cells (SAEC) were infected at MOI 10. Lysates were analyzed for E1A expression. Ad-102 (AdSyn-CO102) shows expected decrease of E1A levels at later times during infection. Similarly Ad-189 (AdSyn-CO189) and ONYX-838 show a decrease of E1A levels at later times during infection, but have stronger expression at the earlier timepoint. Ad-181 (AdSyn-CO181) shows stronger and continued expression of E1A throughout the infection, which is indicative of failure to progress through the adenovirus lifecycle. For Figure 8B, confluent lung adenocarcinoma cells (A549) were infected at MOI 30. Lysates were analyzed for E1A expression. All infections show expected decrease of E1A levels at later times during infection, indicative of typical adenovirus lifecycle progression.

[0015] Figures 9A and 9B are pictures of immunoblots of cell lysates showing expression of cellular cyclins and adenovirus late protein expression in infected primary and tumor cells. Cells infected with mock (ΔE1), Ad-102 (AdSyn-CO102) (wildtype), Ad-181 (AdSyn-CO181) (E1A ΔLXCXE/ΔE4orf6/7), Ad-189 (AdSyn-CO189) (E1A ΔLXCXE), or ONYX-838 (E1A ΔCR2). For Figure 9A, quiescent human primary small airway epithelial cells (SAEC) were infected at MOI 10. In contrast to wildtype and viruses with E1A mutations alone, Ad-181 (AdSyn-CO181) fails to activate E2F dependent cell cycle targets, the S phase, cyclin A and cyclin B. Furthermore, Ad-181

(AdSyn-CO181) and Ad-210 (AdSyn-CO210), which have E4orf6/7 mutations are defective for late protein expression and replication. Both of these defects are apparent to a lesser extent with Ad-210 (AdSyn-CO210). For Figure 9B, human lung adenocarcinoma cells (A549) were infected at MOI 30. In contrast to infected primary cells, there are no apparent defects in expression of late structural proteins, and cyclin A and cyclin B remain present in all infected A549 samples.

[0016] Figures 10A and 10B are FACS histograms showing DNA replication quantified with PI by FACS in infected SAEC and A549. Cells were infected with mock (ΔE1), Ad-102 (AdSyn-CO102) (wildtype), Ad-181 (AdSyn-CO181) (E1A ΔLXCXE/ΔE4orf6/7), Ad-189 (AdSyn-CO189) (E1A ΔLXCXE), or Ad-210 (AdSyn-CO210) (ΔE4orf6/7), and collected 48 hours post-infection. DNA content of uninfected cells is shown in background profile. The Y-axis is the relative abundance of cells, and the X-axis is the fluorescence from PI in the cell which is proportional to the amount of DNA. For Figure 10A, quiescent SAEC infected MOI 10. Ad-181 (AdSyn-CO181) infection reveals a strong DNA replication defect in SAEC relative to Ad-102 (AdSyn-CO102), which is linked to decreased virus replication. A modest defect is also apparent in Ad-210 (AdSyn-CO210) infected SAEC at this timepoint. For Figure 10B, human lung adenocarcinoma cells (A549) were infected at MOI 30. No DNA replication defect is apparent with any mutant virus infection.

[0017] Figures 11A and 11B are graphs showing adenovirus bursts from infected SAEC and A549. Cells were infected with mock (ΔE1), Ad-102 (AdSyn-CO102) (wildtype), Ad-181 (AdSyn-CO181) (E1A ΔLXCXE/ΔE4orf6/7), Ad-189 (AdSyn-CO189) (E1A ΔLXCXE), Ad-210 (AdSyn-CO210) (ΔE4orf6/7), or ONYX-838 (E1A ΔCR2) and the media was collected 48 and 72 hours post-infection. Infectious virus particles in the media were quantified by ELISA. For Figure 11A, quiescent SAEC were infected at MOI 10. Both Ad-181 (AdSyn-CO181) and Ad-210 (AdSyn-CO210) infection reveal strong replication defects in SAEC relative to Ad-102 (AdSyn-CO102). For Figure 11B, A549 cells were infected MOI 30. With the exception of Ad-210 (AdSyn-CO210) at these timepoints, there is no defect in virus replication.

[0018] Figures 12A and 12B are graphs showing cell viability of infected SAEC and A549 after 7 days of infection. Cells were infected with a serial dilution of mock (ΔE1), Ad-102 (AdSyn-CO102) (wildtype), Ad-181 (AdSyn-CO181) (E1A ΔLXCXE/ΔE4orf6/7), Ad-189 (AdSyn-CO189) (E1A ΔLXCXE), Ad-210 (AdSyn-CO210) (ΔE4orf6/7), or ONYX-838 (E1A ΔCR2) and the cellular metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland). As shown in Figure 12A, compared to Ad-102 (AdSyn-CO102), Ad-181 (AdSyn-CO181) presents decreased cell-killing capability in SAEC. As shown in Figure 12B, of the viruses that we have the data for, there is no defect in cell killing by mutant viruses relative to wildtype.

[0019] Figure 13A is a picture of of immunoblots of cell lysates and Figures 13B and 13C are graphs showing Ad-181 (AdSyn-CO181) has attenuated infection in normal human astrocytes (NHA). For Figure 13A, MOI 10 infected NHA were subject to immunoblot to detect adenovirus late structural protein expression and cellular cyclin induction. Ad-181 (AdSyn-CO181) does not induce cyclin A, and demonstrates delayed and decreased late viral protein expression relative to Ad-102 (AdSyn-CO102), which are indicative of replication efficiency. For Figure 13B, NHA was infected by the panel of viruses, and the number of infectious particles in the media was quantified 48 and 72 hours post-infection. Ad-181 (AdSyn-CO181) and Ad-189 (AdSyn-CO189) demonstrate a replication defect in NHA relative to Ad-102 (AdSyn-CO102). For Figure 13C, DNA replication was quantified in MOI 10 infected NHA 48 hours post-infection by PI FACS. DNA content of uninfected cells is shown in background profile of the histograms. The Y-axis is the relative abundance of cells, and the X-axis is the fluorescence from PI in the cell which is proportional to the amount of nucleic acid. Ad-181 (AdSyn-CO181) demonstrates a diminished induction of DNA replication relative to Ad-102 (AdSyn-CO102), which is linked to decreased virus replication.

[0020] Figure 14A is a picture of immunoblots of cell lysates from infected glioblastoma U87 cells and Figures 14B and 14C are graphs showing mutant Ads have no replication defects in glioblastoma U87 cells. For Figure 14A, MOI 20 infected U87 cells were subjected to immunoblot to detect adenovirus late structural protein expression and

cellular cyclin induction. B) U87 was infected by the panel of viruses, and the number of infectious particles in the media was quantified 48 and 72 hours post-infection. C) DNA replication was quantified in MOI 20 infected U87 48 hours post-infection by PI FACS. DNA content of uninfected cells is shown in background profile of the histograms. The Y-axis is the relative abundance of cells, and the X-axis is the fluorescence from PI in the cell which is proportional to the amount of nucleic acid.

[0021] Figure 15A is a picture of immunoblots of cell lysates from infected glioblastoma U118 cells and Figures 15B and 15C are graphs showing mutant Ads have no replication defects in glioblastoma U118 cells. For Figure 15A, MOI 20 infected U118 cells were subjected to immunoblot to detect adenovirus late structural proteins expression and cellular cyclin induction. B) U87 was infected by the panel of viruses, and the number of infectious particles in the media was quantified 48 and 72 hours post-infection. C) DNA replication was quantified in MOI 20 infected U118 48 hours post-infection by PI FACS. DNA content of uninfected cells is shown in background profile of the histograms. The Y-axis is the relative abundance of cells, and the X-axis is the fluorescence from PI in the cell which is proportional to the amount of nucleic acid.

[0022] Figure 16A is a picture of a gel and Figures 16B and 16C are graphs showing Ad-181 (AdSyn-CO181) has modest replication defects in human fibroblasts (IMR90). For Figure 16A, MOI 10 infected fibroblasts were subjected to immunoblot to detect late structural protein expression. Ad-181 (AdSyn-CO 181) demonstrates delayed and decreased late viral protein expression relative to Ad-102 (AdSyn-CO102), which are indicative of replication efficiency. For Figure 16B, fibroblasts were infected by the panel of viruses, and the number of infectious particles in the media was quantified 48 and 72 hours post-infection. With this assay at these timepoints, there was not a clear difference in replication capacity of the mutant adenoviruses. For Figure 16C, DNA replication was quantified in MOI 20 infected fibroblasts 48 hours post-infection by PI FACS. DNA content of uninfected cells is shown in background profile of the histograms. The Y-axis is the relative abundance of cells, and the X-axis is the fluorescence from PI in the cell which is proportional to the amount of nucleic acid. Ad-

181 (AdSyn-CO181) demonstrates a diminished induction of DNA replication relative to Ad-102 (AdSyn-CO102), which is linked to decreased virus replication.

[0023] Figure 17 is a graph showing the cell viability of infected primary normal human astrocyte cells (NHA) after 10 days of infection. Cells were infected with a serial dilution of wt and mutant viruses (see Table 1 below). The metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland). The viability of cells at three and ten infectious particles per cell show a separation of cell killing by viruses into two groups, less and more killing. The group of viruses that exhibit less killing bear both an E1A with an Rb-binding mutation and deletion of E4orf6/7. The group of viruses the exhibit more killing either have a wildtype E1 or wildtype E4.

[0024] Figure 18 is a graph showing the cell viability of infected quiescent SAEC-hTERT cells after 9 days of infection. Cells were infected with a serial dilution of wt and mutant viruses (see Table 1 below). The metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland). The viability of cells at three and ten infectious particles per cell show a separation of cell killing by viruses into two groups, less and more killing. The group of viruses that exhibit less killing bear both an E1A with an Rb-binding mutation and deletion of E4orf6/7. The group of viruses the exhibit more killing either have a wildtype E1 or wildtype E4.

[0025] Figure 19 is a graph showing the cell viability of infected proliferating SAEC-hTERT cells after 9 days of infection. Cells were infected with a serial dilution of wt and mutant viruses (see Table 1 below). The metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland). The viability of cells at three and ten infectious particles per cell show a separation of cell killing by viruses into two groups, less and more killing. The group of viruses that exhibit less killing bear both an E1A with an Rb-binding mutation and deletion of E4orf6/7. The group of viruses the exhibit more killing either have a wildtype E1 or wildtype E4.

[0026] Figure 20 is a graph showing the cell viability of infected A549 cells after 7 days of infection. Cells were infected with a serial dilution of wt and mutant viruses (see

Table 1 below). The metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland).

[0027] Figure 21 is a graph showing the cell viability of infected human breast cancer cells (MDA MB 231) after 7 days of infection. Cells were infected with a serial dilution of wt and mutant viruses (see Table 1 below). The metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland).

[0028] Figure 22 is a graph showing the cell viability of infected glioblastoma cells (U87) after 7 days of infection. Cells were infected with a serial dilution of wt and mutant viruses (see Table 1 below). The metabolic activity was quantified by WST-1 assay (Roche, Basel, Switzerland).

[0029] Figure 23 is a table showing the quantitation of cell viability assays for infected primary NHA, SAEC-hTERT (quiescent), SAEC-hTERT (proliferating), A549, MDA MB 231, and U87 cells shown in Figures 17-22.

[0030] Figure 24 are CPE microscopy images of 293 cells infected with AdSyn-CO182 and and AdSyn-CO183. Both AdSyn-CO182 and AdSyn-CO183 bear the E1A ΔLXCXE and ΔE4orf6/7 mutations, and additionally bear an insertion in the E3-12.5K orf. In AdSyn-CO182, the E3-12.5K ORF is replaced by a sequence encoding fluorescent protein mCherry. In AdSyn-CO183, the E3-12.5K ORF is replaced by a sequence encoding a non-cleavable FasL, which signals for apoptosis.

DETAILED DESCRIPTION

[0031] The Rb/p16/E2F pathway is inactivated by mutations or through other mechanisms, e.g., viral mechanisms, in almost every form of human cancer. By way of example, the pathway can be inactivated through mutations in Rb, p107 mutations, p130 mutations, p16 mutations/epigenetic silencing, cyclin mutations and amplifications, CDK mutations and amplifications, mutations that downregulate cyclin depdent kinase inhibitors, mutations that upregulate E2F transcription factors and growth factor receptor pathway mutations (EGFR, RTKs, RAS, PI-3K, PTEN, RAF, MYC). However, most

current chemotherapies are proliferative poisons that inhibit E2F transcriptional targets, but are also toxic to normal cells and have often devastating iatrogenic complications. Tumor mutations and small DNA virus' proteins converge in inactivating Rb. Studies with adenovirus E1A provided seminal insights into Rb and E2F. The original concept for an oncolytic adenovirus was an E1AΔLXCXE mutant but the agent is not selective, at least in primary cell cultures. E1A binds and inactivates Rb via a conserved (CR2) LXCXE motif (Whyte, et al., *Nature* 334(6178):124-9 (1988)), which activates E2F dependent transcription (Kovesdi et al., *PNAS* 84(8):2180-4 (1987)). This is thought to be the mechanism through which E1A activates E2F, diving expression of cellular and viral genes required for cellular and viral genome replication. Therefore, it was proposed that an adenovirus E1AΔCR2 mutant would selectively replicate in tumor cells that had mutations in the Rb/p16 tumor suppressor pathway (Heise et al., *Nat. Med.* 6(10):1134-9 (2000)). However, surprisingly, an E1AΔCR2 viral mutant still activates E2F and replicates in primary human epithelial cells (Johnson et al., *Cancer Cell* 1(4):325-337 (2002)). As described herein, it has been discovered that adenoviruses encode an additional viral protein, E4orf6/7, that activates E2F independently of E1A. Previous studies had shown that E4orf6/7 binds to E2F and DP1 to activate the transcription of viral E2 promoters (Helin and Harlow, *J. Virol.* 68(8):5027-5035 (1994)). Given that an E1A CR2 mutant still activates E2F and replicates in primary cells, it was hypothesized that E4orf6/7 activates E2F dependent cellular targets to drive S phase entry and viral replication, independently of E1A. Therefore, to design a virus that selectively replicates in tumor versus normal cells, adenoviruses were designed with mutations in both E1A and E4orf6/7. Therefore, as described in the example below, it was explored if a novel virus with E1AΔCR2 and/or ΔE4orf6/7 compound mutations would undergo selective lytic replication in tumor versus normal cells. In contrast to wild type and E1AΔCR2 viruses, E1AΔCR2/ΔE4orf6/7 and also ΔE4orf6/7 viruses replicate poorly in primary cells as evidenced by lack of capsid protein expression, failure to induce the E2F target genes-Cyclin A and B, failure to elicit S phase entry and viral replication. In contrast, these viruses replicate to wild type (WT) virus levels in A549 cells and a panel of tumor cell-lines. Therefore, the provided adenoviruses are selective cancer therapeutic agents.

[0032] Provided herein is a new Rb/p16/E1F tumor selective oncolytic viral therapy. These viruses have the potential to be self-perpetuating, kill tumor cells through regulated cell death, and produce progeny that can spread not only within the tumor but also to metastatic sites.

Definitions

[0033] The term "adenovirus" as referred to herein indicates over 52 adenoviral subtypes isolated from humans, and as many from other mammals and birds. See, e.g., Strauss, "Adenovirus infections in humans," in The Adenoviruses, Ginsberg, ed., Plenum Press, New York, N.Y., pp. 451 596 (1984). The term "adenovirus" can be referred to herein with the abbreviation "Ad" followed by a number indicating serotype, e.g., Ad5. The term optionally applies to two human serotypes, Ad2 and Ad5. Exemplary nucleic acid sequences of these adenoviruses include, but are not limited to, Human Adenovirus 5 (SEQ ID NO:7) and Human Adenovirus 2 (SEQ ID NO:8).

[0034] The term "E1A" refers to the adenovirus early region 1A (E1A) gene and polypeptides expressed from the gene. The term "E1A polypeptide" refers to the polypeptides expressed from the E1A gene and the term includes E1A polypeptides produced by any of the adenovirus serotypes. By way of example, amino acid sequences of the E1A polypeptide can be found at least at GenBank Accession Nos. CAE01147.1, AP_000161.1 (SEQ ID NO:1), and AP_000197.1 (SEQ ID NO:2). The nucleic acids encoding these polypeptides can be found at least at GenBank Accession Nos. AC_000008.1 (SEQ ID NO:7) and AC_000007.1 (SEQ ID NO:8). Also provided are E1A polypeptides comprising 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:1 or SEQ ID NO:2. E1A polypeptides have a role in viral genome replication by driving cells into the cell cycle. A comparison of E1A sequences of various human and simian adenovirus serotypes has identified three regions of conserved amino acid homology. In Ad5, conserved region 1 (CR1) maps between amino acid residues 40-80 as compared to SEQ ID NO:2, CR2 between amino acid residues 121-139 as compared to SEQ ID NO:2, and CR3 between residues 140-188 as compared to SEQ ID NO:2.

[0035] The term "E4orf1" refers to the adenovirus E4orf1 polypeptide produced from the E4 gene, which contains several open reading frames, of an adenovirus. The term "E4orf1 polypeptide" includes E4orf1 polypeptides produced by the E4 gene from any of the adenovirus serotypes. By way of example, amino acid sequences of the E4orf1 polypeptide can be found at least at GenBank Accession Nos. AP_000196.1 (SEQ ID NO:5) and AP_000232.1 (SEQ ID NO:6). The nucleic acids encoding these polypeptides can be found at least at GenBank Accession Nos. AC_000008.1 (SEQ ID NO:7) and AC_000007.1 (SEQ ID NO:8). Also provided are E4orf1 polypeptides comprising 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:5 or SEQ ID NO:6.

[0036] The term "E4orf6/7" refers to the adenovirus E4orf6/7 polypeptide produced from the E4 gene, which contains several open reading frames, of an adenovirus. The term "E4orf6/7 polypeptide" includes E4orf6/7 polypeptides produced by the E4 gene from any of the adenovirus serotypes. By way of example, amino acid sequences of the E4orf6/7 polypeptide can be found at least at GenBank Accession Nos. AP_000191.1 (SEQ ID NO:3) and AP_000227.1 (SEQ ID NO:4). The nucleic acids encoding these polypeptides can be found at least at GenBank Accession Nos. AC_000008.1 (SEQ ID NO:7) and AC_000007.1 (SEQ ID NO:8). Also provided are E4orf6/7 polypeptides comprising 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:3 or SEQ ID NO:4.

[0037] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0038] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al*., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al*., *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al*., *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0039] A particular nucleic acid sequence also implicitly encompasses "splice variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this definition. An example of potassium channel splice variants is discussed in Leicher, *et al*., *J. Biol. Chem.* 273(52):35095-35101 (1998).

[0040] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are near each other, and, in the case of a secretory leader, contiguous and in reading phase.

However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0041] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site or the like). Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the compliment of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0042] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0043] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a

sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al*., eds. 1995 supplement)).

[0044] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al., Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al., J. Mol. Biol*. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information, as known in the art. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the

cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0045] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0046] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0047] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0048] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0049] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles.

[0050] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g.*, Creighton, *Proteins* (1984)).

[0051] The term "recombinant" when used with reference, e.g., to a cell, virus, nucleic acid, protein, or vector, indicates that the cell, virus, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0052] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10^{o}C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x

SSC, and 1% SDS, incubating at 42^{o}C, or, 5x SSC, 1% SDS, incubating at 65^{o}C, with wash in 0.2x SSC, and 0.1% SDS at 65^{o}C.

[0053] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37^{o}C, and a wash in 1X SSC at 45^{o}C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency. Additional guidelines for determining hybridization parameters are provided in numerous reference, e.g., and *Current Protocols in Molecular Biology*, ed. Ausubel, *et al.*, John Wiley & Sons.

[0054] For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C depending on primer length. For high stringency PCR amplification, a temperature of about 62°C is typical, although high stringency annealing temperatures can range from about 50°C to about 65°C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90°C - 95°C for 30 sec - 2 min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72°C for 1 - 2 min. Protocols and guidelines for low and high stringency amplification reactions are provided, e.g., in Innis *et al.* (1990) *PCR Protocols, A Guide to Methods and Applications,* Academic Press, Inc. N.Y.).

[0055] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector has been modified by or is the result oflaboratory methods. Thus, for example, recombinant proteins include proteins produced by laboratory methods. Recombinant proteins can

include amino acid residues not found within the native (non-recombinant) form of the protein or can be include amino acid residues that have been modified, e.g., labeled.

[0056] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0057] As used herein, the term "modification" refers to a change in the sequence of a nucleic acid or polypeptide sequence. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. As used herein, the symbol Δ or delta refers to a deletion. For example, E1AΔLXCXE refers to an E1A polypeptide having a deletion of the LXCXE domain. Substitutional modifications are those in which at least one residue has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. These modifications can be prepared by modification of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the modification. Techniques for making insertion, deletion and substitution mutations at predetermined sites in DNA having a known sequence are well known. Modification techniques can involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide regions. Optionally, modification techniques include, for example, recombination, M13 primer mutagenesis and PCR mutagenesis.

[0058] The terms "transfection," "transduction," "transfecting," or "transducing," can be used interchangeably and are defined as a process of introducing a nucleic acid molecule or a protein to a cell. Nucleic acids are introduced to a cell using non-viral or viral-based methods. The nucleic acid molecule can be a sequence encoding complete proteins or functional portions thereof. Typically, a nucleic acid vector, comprising the elements necessary for protein expression (e.g., a promoter, transcription start site, etc.). Non-viral methods of transfection include any appropriate method that does not use viral DNA or viral particles as a delivery system to introduce the nucleic acid molecule into the cell. Exemplary non-viral transfection methods include calcium phosphate transfection, liposomal transfection, nucleofection, sonoporation, transfection through heat shock, magnetifection and electroporation. For viral-based methods, any useful viral vector can be used in the methods described herein. Examples of viral vectors include, but are not limited to retroviral, adenoviral, lentiviral and adeno-associated viral vectors. In some aspects, the nucleic acid molecules are introduced into a cell using an adenoviral vector following standard procedures well known in the art. The terms "transfection" or "transduction" also refer to introducing proteins into a cell from the external environment. Typically, transduction or transfection of a protein relies on attachment of a peptide or protein capable of crossing the cell membrane to the protein of interest. *See, e.g.*, Ford *et al.* (2001) *Gene Therapy* 8:1-4 and Prochiantz (2007) *Nat. Methods* 4:119-20.

[0059] A "control" or "standard control" refers to a sample, measurement, or value that serves as a reference, usually a known reference, for comparison to a test sample, measurement, or value. For example, a test sample can be taken from a patient suspected of having a given disease (e.g. an autoimmune disease, inflammatory autoimmune disease, cancer, infectious disease, immune disease, or other disease) and compared to a known normal (non-diseased) individual (e.g. a standard control subject). A standard control can also represent an average measurement or value gathered from a population of similar individuals (e.g. standard control subjects) that do not have a given disease (i.e. standard control population), e.g., healthy individuals with a similar medical background,

same age, weight, etc. A standard control value can also be obtained from the same individual, e.g. from an earlier-obtained sample from the patient prior to disease onset. One of skill will recognize that standard controls can be designed for assessment of any number of parameters (e.g. RNA levels, protein levels, specific cell types, specific bodily fluids, specific tissues, synoviocytes, synovial fluid, synovial tissue, fibroblast-like synoviocytes, macrophagelike synoviocytes, etc).

[0060] One of skill in the art will understand which standard controls are most appropriate in a given situation and be able to analyze data based on comparisons to standard control values. Standard controls are also valuable for determining the significance (e.g. statistical significance) of data. For example, if values for a given parameter are widely variant in standard controls, variation in test samples will not be considered as significant.

[0061] As used herein, the term "proliferative disorder" refers to any cellular disorder in which the cells proliferate more rapidly than normal tissue growth. A proliferative disorder includes, but is not limited to, cancer.

[0062] As used herein, the term "cancer" refers to all types of cancer, neoplasm, or malignant tumors found in mammals, including leukemia, carcinomas and sarcomas. Exemplary cancers include cancer of the brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus and Medulloblastoma. Additional examples include, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine and exocrine pancreas, and prostate cancer.

[0063] The term “leukemia” refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). The P_{388} leukemia model is widely accepted as being predictive of *in vivo* anti-leukemic activity. It is believed that a compound that tests positive in the P_{388} assay will generally exhibit some level of anti-leukemic activity *in vivo* regardless of the type of leukemia being treated. Accordingly, the present application includes a method of treating leukemia, and, preferably, a method of treating acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia.

[0064] The term “sarcoma” generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer

agent include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

[0065] The term “melanoma” is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

[0066] The term “carcinoma” refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct

carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniforni carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

[0067] As used herein, the terms “metastasis,” “metastatic,” and “metastatic cancer” can be used interchangeably and refer to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. Cancer occurs at an originating site, e.g., breast, which site is referred to as a primary tumor, e.g., primary breast cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable

tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if lung cancer metastasizes to the breast, the secondary tumor at the site of the breast consists of abnormal lung cells and not abnormal breast cells. The secondary tumor in the breast is referred to a metastatic lung cancer. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors. For example, metastatic lung cancer refers to a disease in a subject with or with a history of a primary lung tumor and with one or more secondary tumors at a second location or multiple locations, e.g., in the breast.

[0068] The terms "dose" and "dosage" are used interchangeably herein. A dose refers to the amount of active ingredient given to an individual at each administration. The dose will vary depending on a number of factors, including the range of normal doses for a given therapy, frequency of administration; size and tolerance of the individual; severity of the condition; risk of side effects; and the route of administration. One of skill will recognize that the dose can be modified depending on the above factors or based on therapeutic progress. The term "dosage form" refers to the particular format of the pharmaceutical or pharmaceutical composition, and depends on the route of administration. For example, a dosage form can be in a liquid form for nebulization, e.g., for inhalants, in a tablet or liquid, e.g., for oral delivery, or a saline solution, e.g., for injection.

[0069] As used herein, "treating" or "treatment of" a condition, disease or disorder or symptoms associated with a condition, disease or disorder refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of condition, disorder or disease, stabilization of the state of condition, disorder or disease, prevention of development of

condition, disorder or disease, prevention of spread of condition, disorder or disease, delay or slowing of condition, disorder or disease progression, delay or slowing of condition, disorder or disease onset, amelioration or palliation of the condition, disorder or disease state, and remission, whether partial or total. "Treating" can also mean prolonging survival of a subject beyond that expected in the absence of treatment. "Treating" can also mean inhibiting the progression of the condition, disorder or disease, slowing the progression of the condition, disorder or disease temporarily, although in some instances, it involves halting the progression of the condition, disorder or disease permanently. As used herein the terms treatment, treat, or treating refers to a method of reducing the effects of one or more symptoms of a disease or condition characterized by expression of the protease or symptom of the disease or condition characterized by expression of the protease. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of an established disease, condition, or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition. Further, as used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level and such terms can include but do not necessarily include complete elimination.

[0070] By "therapeutically effective dose or amount" as used herein is meant a dose that produces effects for which it is administered (e.g. treating or preventing a disease). The exact dose and formulation will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Remington: The Science and Practice of

Pharmacy, 20th Edition, Gennaro, Editor (2003), and Pickar, Dosage Calculations (1999)). For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a standard control. A therapeutically effective dose or amount may ameliorate one or more symptoms of a disease. A therapeutically effective dose or amount may prevent or delay the onset of a disease or one or more symptoms of a disease when the effect for which it is being administered is to treat a person who is at risk of developing the disease.

[0071] The term "pharmaceutically acceptable salts" or "pharmaceutically acceptable carrier" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present application contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present application contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids

like glucuronic or galactunoric acids and the like (*see, e.g.*, Berge *et al., Journal of Pharmaceutical Science* 66:1-19 (1977)). Other pharmaceutically acceptable carriers known to those of skill in the art are suitable for compositions of the present application.

[0072] A "subject," "individual," or "patient," is used interchangeably herein, which refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained *in vitro* or cultured *in vitro* are also encompassed.

[0073] As used herein, an "Rb-deficient" tumor or cell or "a tumor or cell having the phenotype of Rb-deficiency" is a tumor or cell in which the level of the tumor suppressor Rb is lower than that in a normal or control cell or in which the Rb pathway is disrupted or inactive. The terms "Rb pathway," or "Rb signaling pathway" refer to, at least in part, molecules that affect pRb activity including pRb/p107, E2F-1/-2/-3, and G1 cyclin/cdk complexes. It will be appreciated that molecules not presently known may also come within this definition.

[0074] As used herein, an "oncolytic virus" is a virus that selectively kills cells of a proliferative disorder, e.g., cancer cells. Killing of the cancer cells can be detected by any method established in the art, such as determining viable cell count, cytopathic effect, apoptosis of die neoplastic cells, synthesis of viral proteins in the cancer cells (e.g., by metabolic labeling, Western analysis of viral proteins, or reverse transcription polymerase chain reaction of viral genes necessary for replication), or reduction in size of a tumor.

[0075] As used herein, the term "replication deficient virus" refers to a virus that preferentially inhibits cell proliferation, causes cell lysis, or induces apoptosis (collectively considered killing) in a predetermined cell population with a given phenotype (e.g., tumor cells responsive to molecules in the pRb signaling pathway) which supports virus replication. Such viruses are unable to or are limited in the ability to inhibit cell proliferation, cause cell lysis, induce apoptosis, or otherwise replicate in cells that do not have the predetermined cell phenotype.

Modified Adenoviruses

[0076] Provided herein are adenoviruses (Ads) comprising an E1A polypeptide comprising one or more modifications and/or comprising an E4orf6/7 polypeptide comprising one or more modifications. The adenoviruses optionally include an E4orf1 polypeptide comprising one or more modifications. Also provided herein are adenoviruses comprising an E1A polypeptide comprising one or more modifications and comprising an E4orf1 polypeptide comprising one or more modifications. Thus, provided are modified adenoviruses with modifications in E1A, E4orf1 and E4orf6/7. The provided modified adenoviruses are oncolytic. The provided modified adenoviruses also selectively replicate in cancer cells with deregulated E2F and normal cell cycle checkpoints. The provided modified adenoviruses selectively replicate in cells with an inactive Rb/p16 tumor suppressor pathway. The provided modified adenoviruses can include one or more further modifications including those described in International Publication Nos. WO 2012/024350 and WO 2013/138505, which are incorporated by reference herein in their entireties.

[0077] The term "modified adenovirus," refers to an adenovirus having a gene sequence that is not found in nature (e.g. non-wild-type adenovirus). Optionally, the modified adenovirus is a recombinant adenovirus. As used herein, the term "modified E1A," refers to an E1A polypeptide and/or the E1A gene or nucleic acid encoding the E1A polypeptide with one or more modifications in the polypeptide or nucleic acid sequence, respectively. As used herein, the term "modified E4orf1," refers to an E4orf1 polypeptide and/or the E1orf1 gene or nucleic acid encoding the E4orf1 polypeptide with one or more modifications in the polypeptide or nucleic acid sequence, respectively. As used herein, the term "modified E4orf6/7," refers to the E4orf6/7 polypeptide and/or the E4orf6/7 gene or nucleic acid encoding the E4orf6/7 polypeptide with one or more modifications in the polypeptide or nucleic acid sequence, respectively.

[0078] The term "Rb/p16/E2F replication impaired or deficient," as used herein, means that, upon infection of a cell, adenovirus replication is partially or fully attenuated in the presence of normal levels of functional cellular "pocket protein family" members

including Rb/p107/p130/p16/E2F/CDK-cyclin checkpoints. For example, if the infected cell is Rb/p16 pathway impaired or deficient (i.e. the infected cell does not express normal levels of fully functional Rb or other proteins in the Rb/p16 pathway), replication of the Rb/p16/E2F pathway replication impaired adenovirus will proceed normally. Conversely, if a cell expresses normal levels of functional Rb (e.g. Rb with normal activity, also referred to herein as an "Rb expressing cell"), replication of the Rb replication impaired or deficient adenovirus is attenuated or prevented. A cell may be Rb impaired or deficient by failing to express normal levels of Rb (e.g. a mutation to the regulatory (e.g. promoter) region of the Rb gene) or expressing mutated Rb having below normal Rb activity. Normal levels of Rb and normal Rb activity levels are found in healthy, non-diseased cells of the same type. Thus, the Rb impaired cell includes a mutated Rb gene. Optionally, the Rb impaired cell includes a genome wherein the Rb gene is wholly or partially deleted. The Rb impaired cell may be a cancer (e.g. neoplastic) cell. Other genomic lesions that can result in the loss of normal Rb function include, but are not limited to, CDK mutations, cyclin mutations and amplifications, p16 mutations and/or epigenetic silencing, p107 mutations, p130 mutations, and growth factor receptor pathway mutations.

[0079] The term "Rb/p16 tumor suppressor pathway" or "Rb/p16 pathway" refers to the entire signaling pathway of molecular signaling that includes retinoblastoma protein (RB), and other protein/protein families in the pathway, including but not limited to Cdk, E2f, atypical protein kinase C, and Skp2. The term "Rb/p16 tumor suppressor pathway impaired or deficient" means that one or more molecules in the signaling pathway are impaired or deficient, e.g., by failing to express normal levels or a protein or expressing mutated proteins having below normal activity, such that the pathway functions abnormally. Such defects result in high expression levels of free E2F and high activity of the E2F promoter. Thus, a cell may be Rb/p16 pathway impaired or deficient by failing to express normal levels of a protein or expressing mutated proteins having below normal activity in the Rb/p16 tumor suppressor pathway.

[0080] The terms "E1A impaired," "E1A deficient," "E4orf1 deficient," "E4orf1 impaired," "E4orf6/7 impaired," and "E4orf6/7 deficient" as used herein, means the adenovirus is not capable of producing normal levels and/or fully functional E1A, E4orf1 or E4orf6/7 gene product. For example, a virus may be E1A, E4orf1, or E4 orf6/7 deficient or impaired by failing to express normal levels of E1A, E4orf1 or E4orf6/7 gene product (e.g. a mutation to the regulatory (e.g. promoter) region of the E1A, E4orf1 or E4orf6/7 gene) or expressing a mutated E1A, E4orf1 or E4orf6/7 gene product having below normal E1A, E4orf1 or E4orf6/7 gene product activity. Thus, the E1A, E4orf1 and/or E4orf6/7 deficient adenovirus includes a mutated E1A, E4orf1 and/or E4orf6/7 gene. Optionally, the E1A, E4orf1 and/or E4orf6/7 deficient adenovirus includes a genome wherein the E1A, E4orf1 and/or E4orf6/7 gene is wholly or partially deleted. The E1A and E4 regions of adenoviruses are known and can be modified using the methods described throughout and in the example and others known in the art. See, for example, International Publication No. WO 1998/046779, U.S. Patent No. 8465732, and International Publication No. 2012/024350, which are incorporated by reference herein in their entireties. By way of example, amino acid sequences of the E1A polypeptide can be found at least at GenBank Accession Nos. CAE01147.1, AP_000161.1 (SEQ ID NO:1), and AP_000197.1 (SEQ ID NO:2) and amino acid sequences of the E4orf6/7 polypeptide can be found at least at GenBank Accession Nos. AP_000191.1 (SEQ ID NO:3) and AP_000227.1 (SEQ ID NO:4). Amino acid sequence of the E4orf1 polypeptide can be found at least at GenBank Accession Nos. AP_000196.1 (SEQ ID NO:5) and AP_000232.1 (SEQ ID NO:6). The nucleic acids encoding these polypeptides can be found at least at GenBank Accession Nos. AC_000008.1 (SEQ ID NO:7) and AC_000007.1 (SEQ ID NO:8). Also provided are E1A polypeptides comprising 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:1 or SEQ ID NO:2, E4orf1 polypeptides comprising 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:5 or SEQ ID NO:6 and E4orf6/7 polypeptide comprising 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:3 or SEQ ID NO:4.

[0081] Various assays for determining levels and activities of protein (such as Rb, E1A gene product, E4orf1 gene product, E4orf6/7 gene product) are available, such as amplification/expression methods, immunohistochemistry methods, FISH and shed antigen assays, southern blotting, or PCR techniques. Moreover, the protein expression or amplification may be evaluated using *in vivo* diagnostic assays, e.g. by administering a molecule (such as an antibody) which binds the protein to be detected and is tagged with a detectable label (e.g. a radioactive isotope) and externally scanning the patient for localization of the label. Thus, methods of measuring levels of protein levels in cells are generally known in the art and may be used to assess protein levels and/or activities in connection with the methods and compositions provided herein as applicable. These assays can be used to determine the effect of modifications in the E1A, E4orf1, and E4orf6/7 polypeptides and combinations thereof, e.g., to determine if the modifications result in adenoviruses not capable of producing normal levels or fully functional gene products of the polypeptide(s) and to confirm adenoviruses comprising a deletion of all or part of one or more of the E1A, E4orf1 and E4orf6/7 polypeptides or combinations thereof.

[0082] Provided are adenoviruses (Ads) that selectively replicate in Rb-deficient cells. Specifically, provided are adenoviruses comprising an E1A polypeptide comprising one or more modifications, comprising an E4orf6/7 polypeptide comprising one or more modifications, comprising an E4orf1 polypeptide comprising one or more modifications and various combinations thereof. Also provided are adenoviruses comprising a genome comprising a deletion of all or part of the E1A gene, the E4orf1 gene, and/or the E4orf6/7 gene. Thus, provided are adenoviruses comprising a genome lacking a nucleic acid sequence encoding the E4orf1 polypeptide and/or lacking a nucleic acid sequence encoding a E4orf6/7 polypeptide and comprising a nucleic acid encoding E1A with one or more modifications. As discussed above, the term "modification" refers to a modification in a nucleic acid sequence of a gene or an amino acid sequence. Modifications include, but are not limited to, insertions, substitutions and deletions. Amino acid sequence modifications typically fall into one or more of three classes:

substitutional, insertional, or deletional modifications. Insertions include amino and/or terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once. Substitutional modifications are those in which at least one residue has been removed and a different residue inserted in its place. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct.

[0083] Optionally, the modification of E1A comprises a modification in the Rb binding site of E1A. Optionally, the modification of E1A comprises a modification in one or more of amino acid residues 122-126 of the E1A polypeptide, e.g., amino acid residues 122-126 as compared to SEQ ID NO:1 or SEQ ID NO:2. Optionally, the modification is a deletion. Thus, optionally, the modification of E1A and/or E4orf6/7 comprises a deletion. Optionally, the modification of E1A is a deletion of amino acid residues 122-126 of E1A, e.g., as compared to SEQ ID NO:1 or SEQ ID NO:2. Optionally, the modification is a deletion in the conserved LXCXE motif of E1A, referred to throughout as ΔLXCXE. By way of example, the conserved motif can be found at amino acid residues 122-126 of SEQ ID NO:1 or SEQ ID NO:2. The E4orf6/7 is encoded by two exons, shown in Figures 2 and 6. Optionally, the modification of E4orf6/7 comprises a modification in one or both of the E4orf6/7 exons. Optionally, the modification of E4orf6/7 is a deletion of one or both of the E4orf6/7 exons. Optionally, the modification of E4orf6/7 is ΔE4orf6/7. Optionally, the adenovirus comprises E1A ΔLXCXE and ΔE4orf6/7, referred to throughout as Ad E1A ΔLXCXE/ ΔE4orf6/7 or AdSyn-CO181.

[0084] Thus, provided is an adenovirus comprising an E1A polypeptide comprising one or more modifications and/or comprising an E4orf6/7 polypeptide comprising one or more modifications. The E1A polypeptide can comprise a modification in an Rb binding site of E1A. The E1A polypeptide can comprise two Rb binding sites and wherein the E1A polypeptide comprises a modification in both Rb binding sites. Optionally, the E1A polypeptide comprises a modification in one or more of amino acid residues 120-130 of

the E1A polypeptide, a modification in one or more of amino acid residues 122-126 of the E1A polypeptide, a modification in one or more of amino acid residues 35-55 of the E1A polypeptide, a modification in one or more of amino acid residues 37-49 of the E1A polypeptide, or combinations thereof. For example, the modifications can be occur in one or more of amino acid residues 120-130, 122-126, 35-55, 37-49, or combinations thereof as compared to SEQ ID NO:1 or SEQ ID NO:2. By way of example, the E1A polypeptide can comprise a modification in one or more of amino acid residues 122-126 and in one or more of amino acid residues 37-49 of the E1A polypeptide, wherein the E1A optionally comprises SEQ ID NO:1 or SEQ ID NO:2. Thus, the provided E1A polypeptides can comprise one or more substitutions. Optionally, the E1A polypeptide comprises a substitution at residue Y47, residue C124 or at both residues Y47 and C124, wherein the E1A optionally comprises SEQ ID NO:1 or SEQ ID NO:2. Alternatively or additionally, the E1A polypeptide comprises a deletion. Optionally, the deletion is a deletion of amino acid residues 122-126 of the E1A polypeptide and/or a deletion of amino acid residues 2-11 of the E1A polypeptide. Optionally, the E1A polypeptide comprises the deletion ΔLXCXE. As discussed above, the E1A polypeptide to be modified can comprise SEQ ID NO:1 or SEQ ID NO:2.

[0085] In the provided adenoviruses, the E4orf6/7 polypeptide can comprise a modification in one or both of the E4orf6/7 exons. Thus, the E4orf6/7 polypeptide can comprise one or more modifications including insertions, substitutions and deletions and combinations thereof. Optionally, the E4orf6/7 polypeptide comprises a deletion of one or both of the E4orf6/7 exons. Optionally, the E4orf6/7 comprises an N-terminal deletion selected from the group consisting of 4 to 38, 4 to 58 or 38 to 58 N-terminal amino acids, e.g., as compared to SEQ ID NOs:3 or 4. See, e.g., Schaley et al., *J. Virol.* 79(4):2301-8 (2005), which is incorporated by reference herein in its entirety. Optionally, the E4orf6/7 polypeptide comprises a modification selected from the group consisting of dl355, dl356, and dl366 (Huang and Hearing, *Genes & Development* 3:1699-1710 (1989), which is incorporated by reference herein in its entirety). As discussed above, the E4orf6/7 polypeptide for modification can comprise SEQ ID NO:3 or SEQ ID NO:4.

[0086] In the provided adenoviruses, the adenoviruses may comprise an E4orf1 polypeptide comprising one or more modifications. Optionally, the E4orf1 polypeptide comprises one or more deletions. Optionally, the E4orf1 polypeptide comprises a deletion in the C-terminal region of E4orf1. Optionally, the E4orf1 polypeptide comprises a deletion of the last four amino acids in the C-terminal region of the E4orf1 polypeptide. Optionally, the E4orf1 polypeptide comprises a deletion of residues 125-128 of the E4orf1 polypeptide, optionally, wherein the E4orf1 polypeptide comprises SEQ ID NO:5 or SEQ ID NO:6. Optionally, the E4orf1 polypeptide comprises a modification selected from the group consisting of D68A, P17A, Y26A, L109A, P117A, E3A, L5A, G13T, P31A, G58T, E85A, and L86A (Chung et al., *J. Virol.* 81(9):4787-97 (2007), which is incorporated by reference herein in its entirety). As discussed above, the E4orf1 polypeptide for modification can comprise SEQ ID NO:5 or SEQ ID NO:6. Thus, also provided is an adenovirus comprising an E1A polypeptide comprising one or more modifications and comprising an E4orf1 polypeptide comprising one or more modifications. As noted above, the adenovirus can further include an E4orf6/7 polypeptide comprising one or more modifications.

[0087] Also provided herein are nucleic acids encoding the modified adenoviruses described above. Optionally, one nucleic acid is provided encoding the modified adenovirus (e.g. a plasmid). Optionally, a plurality of nucleic acids is provided encoding the modified adenovirus (e.g. a plurality of plasmids).

[0088] Modifications are generated in the nucleic acid of a virus using any number of methods known in the art. For example, site directed mutagenesis can be used to modify a nucleic acid sequence. One of the most common methods of site-directed mutagenesis is oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, an oligonucleotide encoding the desired change(s) in sequence is annealed to one strand of the DNA of interest and serves as a primer for initiation of DNA synthesis. In this manner, the oligonucleotide containing the sequence change is incorporated into the newly synthesized strand. See, for example, Kunkel, 1985, Proc. Natl. Acad. Sci. USA, 82:488; Kunkel et al., 1987, Meth. Enzymol., 154:367; Lewis & Thompson, 1990, Nucl.

Acids Res., 18:3439; Bohnsack, 1996, Meth. Mol. Biol., 57:1; Deng & Nickoloff, 1992, Anal. Biochem., 200:81; and Shimada, 1996, Meth. Mol. Biol., 57:157. Other methods are routinely used in the art to introduce a modification into a sequence. For example, modified nucleic acids are generated using PCR or chemical synthesis, or polypeptides having the desired change in amino acid sequence can be chemically synthesized. See, for example, Bang & Kent, 2005, Proc. Natl. Acad. Sci. USA, 102:5014-9 and references therein. Selection on a cell type on which virus is not usually grown (e.g., human cells) and/or chemical mutagenesis (see, for example, Rudd & Lemay, 2005, J. Gen. Virology, 86:1489-97) also can be used to generate modifications in the nucleic acid of a virus.

[0089] Also provided is a cell that has been infected with the modified adenovirus described throughout. The cell can be transformed by the modified adenovirus described above. Optionally, the cell has been genetically altered as a result of the uptake, incorporation and expression of the genetic material of the modified adenovirus described above. Optionally, the cell is a mammalian cell, such as a human cell. The adenovirus can be a mammalian adenovirus such as a human adenovirus. Optionally, the cell is an amphibian cell (e.g. a frog cell) or a reptilian cell (e.g. a snake cell).

Compositions

[0090] Provided herein are compositions comprising the modified viruses (or one or more nucleic acids encoding the modified adenovirus). The compositions are, optionally, suitable for formulation and administration *in vitro* or *in vivo*. Optionally, the compositions comprise one or more of the provided agents and a pharmaceutically acceptable carrier. Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy, 22nd Edition*, Loyd V. Allen *et al*., editors, Pharmaceutical Press (2012). By pharmaceutically acceptable carrier is meant a material that is not biologically or otherwise undesirable, i.e., the material is administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained. If administered to a subject, the carrier is optionally selected to minimize degradation of the active ingredient and to minimize adverse side effects in the subject.

[0091] The modified viruses (or one or more nucleic acids encoding the modified adenovirus) are administered in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, intratumoral or inhalation routes. The administration may be local or systemic. The compositions can be administered via any of several routes of administration, including topically, orally, parenterally, intravenously, intra-articularly, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, intrahepatically, intracranially, nebulization/inhalation, or by installation via bronchoscopy. Thus, the compositions are administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

[0092] The compositions for administration will commonly comprise an agent as described herein (e.g. a modified adenovirus or one or more nucleic acids encoding the modified adenovirus) dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

[0093] Pharmaceutical formulations, particularly, of the modified viruses can be prepared by mixing the modified adenovirus (or one or more nucleic acids encoding the modified adenovirus) having the desired degree of purity with optional pharmaceutically

acceptable carriers, excipients or stabilizers. Such formulations can be lyophilized formulations or aqueous solutions.

[0094] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used. Acceptable carriers, excipients or stabilizers can be acetate, phosphate, citrate, and other organic acids; antioxidants (e.g., ascorbic acid) preservatives low molecular weight polypeptides; proteins, such as serum albumin or gelatin, or hydrophilic polymers such as polyvinylpyllolidone; and amino acids, monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents; and ionic and non-ionic surfactants (e.g., polysorbate); salt-forming counter-ions such as sodium; metal complexes (e. g. Zn-protein complexes); and/or non-ionic surfactants. The modified adenovirus (or one or more nucleic acids encoding the modified adenovirus) can be formulated at any appropriate concentration of infectious units.

[0095] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the modified adenovirus suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

[0096] The modified adenovirus (or one or more nucleic acids encoding the modified adenovirus), alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation.

Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0097] Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intratumoral, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the provided methods, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically intratumorally, or intrathecally. Parenteral administration, intratumoral administration, and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[0098] Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced or infected by adenovirus or transfected with nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

[0099] The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. Thus, the pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges.

Methods of Treatment

[0100] The provided modified adenoviruses and/or compositions comprising the modified adenoviruses can be administered for therapeutic or prophylactic treatments.

[0101] Thus, provided is a method of treating a proliferative disorder in a subject. The method includes administering the provided adenoviruses or compositions to the subject. As described throughout, the adenovirus or pharmaceutical composition is administered in any number of ways including, but not limited to, intravenously, intravascularly, intrathecally, intramuscularly, subcutaneously, intraperitoneally, or orally. Optionally, the method further comprising administering to the subject one or more additional therapeutic agents. Optionally, the therapeutic agent is a chemotherapeutic agent.

[0102] As described throughout, the proliferative disorder can be cancer. Optionally, the proliferative disorder is selected from the group consisting of lung cancer, prostate cancer, colorectal cancer, breast cancer, thyroid cancer, renal cancer, liver cancer and leukemia. Optionally, the proliferative disorder is metastatic. As discussed above, cancers include an abnormal state or condition in a warm-blooded animal characterized by rapidly proliferating cell growth or neoplasm. Neoplastic diseases include malignant or benign neoplasms, including diffuse neoplasms such as leukemia, as well as malignant or benign cancers and tumors (including any carcinoma, sarcoma, or adenoma). A neoplasm is generally recognized as an abnormal tissue that grows by cellular proliferation more rapidly than normal, and can continue to grow after the stimuli that initiated the new growth has ceased. Neoplastic diseases include, for example, tumors such as tumors of the mammary, pituitary, thyroid, or prostate gland; tumors of the brain, liver, meninges, bone, ovary, uterus, cervix, and the like; as well as both monocytic and myelogenous leukemia, adenocarcinoma, adenoma, astrocytoma, bladder tumor, brain tumor, Burkitt lymphoma, breast carcinoma, cervical carcinoma, colon carcinoma, kidney carcinoma, liver carcinoma, lung carcinoma, ovarian carcinoma, pancreatic carcinoma, prostate carcinoma, rectal carcinoma, skin carcinoma, stomach carcinoma, testis carcinoma, thyroid carcinoma, chondrosarcoma, choriocarcinoma, fibroma, fibrosarcoma, glioblastoma, glioma, hepatoma, histiocytoma, leiomyoblastoma, leiomyosarcoma, lymphoma, liposarcoma cell, mammary tumor, medulloblastoma, myeloma, plasmacytoma, neuroblastoma, neuroglioma, osteogenic sarcoma, pancreatic tumor, pituitary tumor, retinoblastoma, rhabdomyosarcoma, sarcoma, testicular tumor, thymoma,

Wilms tumor. Tumors include both primary and metastatic solid tumors, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract, (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma) and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). In some aspects, solid tumors may be treated that arise from hematopoietic malignancies such as leukemias (i.e. chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia) as well as in the treatment of lymphomas (both Hodgkin's and non-Hodgkin's lymphomas). In addition, treatments may be useful in the prevention of metastases from the tumors described herein.

[0103] In therapeutic applications, compositions are administered to a subject suffering from a proliferative disease or disorder (e.g., cancer) in a “therapeutically effective dose.” Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. A "patient" or “subject” includes both humans and other animals, particularly mammals. Thus the methods are applicable to both human therapy and veterinary applications.

[0104] An effective amount of a virus having a modified sequence is determined on an individual basis and is based, at least in part, on the particular virus used; the individual's size, age, gender; and the size and other characteristics of the proliferating cells. For example, for treatment of a human, approximately 10^3 to 10^{12} plaque forming units (PFU) of a virus is used, depending on the type, size and number of proliferating cells or

neoplasms present. The effective amount can be from about 1.0 pfu/kg body weight to about 10^{15} pfu/kg body weight (e.g., from about 10^{2} pfu/kg body weight to about 10^{13} pfu/kg body weight). A virus is administered in a single dose or in multiple doses (e.g., two, three, four, six, or more doses). Multiple doses are administered concurrently or consecutively (e.g., over a period of days or weeks). Treatment with a virus having a modified sequence lasts from several days to several months or until diminution of the disease is achieved.

[0105] Optionally, the provided methods include administering to the subject one or more additional therapeutic agents. Thus, the provided methods can be combined with other cancer therapies, radiation therapy, hormone therapy, or chemotherapy. Suitable additional therapeutic agents include, but are not limited to, therapeutic agent is selected from the group consisting of chemotherapeutic agents, CDK inhibitors, anti-inflammatory agents, antibiotics, antiviral agents immunological agents, vitamins, growth factors, and hormones. Thus, the provided methods include, optionally, administering to the subject known anticancer compounds or chemotherapeutic agents. Chemotherapeutic agents agents, include, but are not limited to 5-fluorouracil; mitomycin C; methotrexate; hydroxyurea; cyclophosphamide; dacarbazine; mitoxantrone; anthracyclins (epirubicin and doxurubicin); antibodies to receptors, such as herceptin; etoposide; pregnasome; hormone therapies such as tamoxifen and anti-estrogens; interferons; aromatase inhibitors; progestational agents; and LHRH analogs. CDK (Cyclin-dependent kinase) inhibitors are agents that inhibit the function of CDKs. Suitable CDK inhibitors for use in the provided methods include, but are not limited to, AG-024322, AT7519, AZD5438, flavopiridol, indisulam, P1446A-05, PD-0332991, and P276-00 (See., e.g., Lapenna et al., *Nature Reviews*, 8:547-566 (2009), which is incorporated by reference herein in its entirety). The choice of agent and dosage can be determined readily by one of skill in the art based on the given disease being treated. The combined administrations contemplates coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Combinations

of agents or compositions can be administered either concomitantly (e.g., as a mixture), separately but simultaneously (e.g., via separate intravenous lines) or sequentially (e.g., one agent is administered first followed by administration of the second agent). Thus, the term combination is used to refer to concomitant, simultaneous or sequential administration of two or more agents or compositions.

[0106] According to the methods taught herein, the subject is administered an effective amount of one or more of the agents provided herein. The terms effective amount and effective dosage are used interchangeably. The term effective amount is defined as any amount necessary to produce a desired physiologic response (e.g., killing of a cancer cell). Therapeutic agents are typically administered at the initial dosage of about 0.001 mg/kg to about 1000 mg/kg daily. A dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied depending upon the requirements of the subject, the severity of the condition being treated, and the compound being employed. For example, dosages can be empirically determined considering the type and stage of cancer diagnosed in a particular subject. The dose administered to a subject, in the context of the provided methods should be sufficient to affect a beneficial therapeutic response in the patient over time. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Thus, effective amounts and schedules for administering the agent may be determined empirically by one skilled in the art. The dosage ranges for administration are those large enough to produce the desired effect in which one or more symptoms of the disease or disorder are affected (e.g., reduced or delayed). The dosage should not be so large as to cause substantial adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex, type of disease, the extent of the disease or disorder, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosages can vary and can be administered in one or more dose administrations daily, for one or several days.

Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

[0107] As used herein the terms treatment, treat, or treating refers to a method of reducing the effects of one or more symptoms of a disease or condition characterized by expression of the protease or symptom of the disease or condition characterized by expression of the protease. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of an established disease, condition, or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition. Further, as used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level and such terms can include but do not necessarily include complete elimination.

Kits

[0108] Provided herein are kits comprising one or more of the provided modified adenoviruses and/or compositions comprising the modified adenoviruses. Thus, provided are kits comprising adenoviruses (Ads) comprising an E1A polypeptide comprising one or more modifications and/or comprising an E4orf6/7 polypeptide comprising one or more modifications and/or compositions comprising the adenoviruses. Optionally, the adenoviruses further include an E4orf1 polypeptide comprising one or more modifications. Provided are also kits comprising adenoviruses (Ads) comprising an E1A polypeptide comprising one or more modifications and comprising an E4orf1 polypeptide comprising one or more modifications and/or compositions comprising the adenoviruses. Optionally, the composition is a pharmaceutical composition. Optionally, the kit further includes one or more additional therapeutic agents. Optionally, the therapeutic agent is a

chemotherapeutic agent. Provided herein are kits comprising one or more of the provided pharmaceutical compositions and instructions for use. Optionally, the kit comprises one or more doses of an effective amount of a composition comprising an adenovirus that selectively replicates in Rb/p16 tumor suppressor pathway deficient cells. Optionally, the adenovirus selectively replicates in cells with upregulated E2F activity. Optionally, the composition is present in a container (e.g., vial or packet). Optionally, the kit comprises a means of administering the composition, such as, for example, a syringe, needle, tubing, catheter, patch, and the like. The kit may also comprise formulations and/or materials requiring sterilization and/or dilution prior to use.

[0109] Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a method is disclosed and discussed and a number of modifications that can be made to a number of molecules including the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

[0110] Publications cited herein and the material for which they are cited are hereby specifically incorporated by reference in their entireties.

[0111] A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made. Accordingly, other embodiments are within the scope of the claims.

Example

Example 1. Oncolytic adenoviruses that selectively replicate in tumor cells that have deregulated E2F activity

[0112] Modified adenoviruses were made with the below referenced components. Gateway DONR vectors were employed. From human Ad5 DNA, the E1 module was obtained by PCR and inserted into the vector pDONR P1P4 using SLIC. The pDONR P1P4 vector backbone including attL1 and attL4 recombination sites was amplified using PCR and combined with the Ad5 E1 module by SLIC. The E3 module was obtained by PCR to generate a product flanked by attB5 and attB3r recombination sites. The product was inserted into the pDONR P5P3r vector by gateway BP reaction. The E4 module was obtained by PCR to generate a product flanked by attB3 and attB2 recombination sites. The product was inserted into the pDONR P3P2 vector by gateway BP reaction. The attR5-ccdB-Cm(r)-attR2 fragment from the pDONR P5P2 vector was amplified by PCR and inserted into the Adsembly DEST vector. See "MultiSite Gateway® Pro Plus", Cat# 12537-100; and Sone, T. et al. J Biotechnol. 2008 Sep 10;136(3-4):113-21. The Adsembly method is described in International Publication No. WO 2012/024351, which is incorporated by reference herein in its entirety.

[0113] The vector backbone for the Adsembly DEST vector is composed of parts from three different sources. The Amp(r) cassette and lacZ gene was amplified from plasmid pUC19. This was combined with the p15A origin of replication, obtained from plasmid pSB3K5-I52002, part of the BioBricksiGEM 2007 parts distribution. The p15A ori, which maintains plasmids at a lower (10-12) copy number is necessary to reduce E1 toxicity. Lastly, in order to create a self-excising virus, the mammalian expression cassette for the enzyme ISceI was PCR'd from plasmid pAdZ5-CV5-E3+. This cassette was cloned into the vector backbone to create the vector called p15A-SceI. This is the vector used to start genome assembly. The gene modules were all obtained from either

DNA purified from wild type Ad5 virus or the plasmid pAd/CMV/V5/DEST (Invitrogen).

[0114] Regarding the DEST vector for human Ad5, the E2 and L3 modules were inserted into plasmid p15A-SceI by 3-fragment SLIC. The counterselection marker expressing ccdB and Chlor(r) flanked by attR5 and attR2 sites was obtained by PCR from plasmid pDONR P5P2. The second counterselection marker was obtained by PCR from the vector pDONR P1P4. The two counterselection markers were inserted on the right and left sides of p15A-SceI E2-L4 by SLIC after cutting with unique restriction enzymes engineered to the ends of the E2 and L4 modules to create the DEST vector.

[0115] Regarding Amp(r) cassette: plasmid pUC19, the p15A ori: plasmid pSB3K5-I52002 was part of the BioBricksiGEM 2007 parts distribution. Regarding the adenoviral gene modules, either the DNA purified from Ad5 particles, or plasmid pAd/CMV/V5/DEST (Invitrogen). The DONR vectors pDONR P1P4, P5P2, P5P3R, P3P2 were received from Jon Chesnut (Invitrogen).

[0116] Regarding PCRs, all PCRs were performed using the Phusion enzyme (NEB). PCRs to obtain the ADENOVIRAL GENE modules from Ad5 were performed with 1x HF buffer, 200μM each dNTP, 0.5μM each primer, and 10 ng of template. For the E2-L2 module, 3% DMSO was also added. Template was either plasmid pAd/PL-DEST (Invitrogen; for E2-L2, L3-L4, and E4 modules) or Ad5 genomic DNA (for E1 and E3 modules). PCR conditions were as follows. E2-L2 and L3-L4: 98°C 30sec - 10 cycles of 98°C 10sec, 65°C 30sec (decrease temp 1°C every 2 cycles), 72°C 7min - 29 cycles of 98°C 10sec, 60°C 30sec, 72°C 8min - 72°C 10min - 4°C hold. E3: 98°C 30sec - 10 cycles of 98°C 10sec, 70°C 30sec (decrease temp 0.5°C every cycle), 72°C 2min30sec - 25 cycles of 98°C 10sec, 68°C 30sec, 72°C 2min30sec - 72°C 10min - 4°C hold. E4: 98°C 30sec - 6 cycles of 98°C 10sec, 63°C 30sec (decrease temp 0.5°C every cycle), 72°C 2min - 29 cycles of 98°C 10sec, 60°C 30sec, 72°C 2min - 72°C 5min - 4°C hold.

[0117] Regarding obtaining viral genomic DNA from purified virus, up to 100μl of purified virus is added to 300μl of lysis buffer containing 10mM Tris pH8, 5mM EDTA,

200mM NaCl, and 0.2% SDS. Mix is incubated at 60°C for 5 min, followed by addition of 5µl of proteinase K stock (~20mg/mL) and further incubated at 60°C for 1 hour. Samples are then placed on ice for 5 min, followed by spinning at 15K x g for 15min. Supernatant is removed and added to an equal volume of isopropanol, mixed well, and spun at 15K x g for 15min at 4°C. Pellet is washed with 70% ethanol and respun for 15min at 4°C. The pellet is dried and resuspended for use.

[0118] Regarding SLIC, linear fragments are exonuclease treated for 20min at room temp in the following 20µl reaction: 50mM Tris pH8, 10mM MgCl2, 50µg/mL BSA, 200mM Urea, 5mM DTT, and 0.5µl T4 DNA polymerase. The reaction is stopped by addition of 1µl 0.5M EDTA, followed by incubation at 75°C for 20min. An equal amount of T4-treated DNAs are then mixed to around 20µl in volume in a new tube. For SLIC combining 2 fragments, 10µl of each reaction is used. For SLIC combining 3 fragments, 7µl of each reaction is used. Fragments are annealed by heating to 65°C for 10min, followed by a slow cool down decreasing the temperature 0.5°C every 5 seconds down to 25°C. After annealing, 5µl of the reaction is transformed and clones are screened.

[0119] Regarding AdSlicR, a 3-fragment SLIC reaction is performed using 100ng of T4-treated p15A-SceI (linearized by PCR), and 300ng of each of the E2 and L3 modules (obtained by PCR from their respective entry vectors). This creates vector p15A-SceI E2-L4. Five µg of p15A-SceI E2-L4 is cut with SwaI and gel purified using Qiagen QiaexII. The E3 and E4 modules are obtained by PCR from their respective entry vectors. Each of the linearized vector (450ng) and PCR products (200ng) are treated with T4 DNA polymerase and SLIC performed as normal, using 150-200ng of vector and ~100ng of each module PCR. After isolation of positive clones, 5µg of the new vector is cut with PacI and gel purified, then combined with an E1 PCR product (100ng of T4-treated) in a new SLIC reaction. This completes the genome assembly, and the plasmid is ready for transfection to reconstitute virus.

[0120] Regarding the construction of E1 and E4 mutant regions, manipulation was carried out on the individual module entry vectors. The E1 module with vector backbone was PCR'd with primers to generate a product lacking the LTCHE sequence (residues

122-126), then circularized using SLIC to generate pENTR-E1-E1A-ΔLXCXE. Alternatively, the E1 module with vector backbone was PCR'd with primers to generate products with E1A codon changes to mutate Y47 to H, residue C124 to G, or to delete residues 2-11 to generate pENTR-E1-E1A-Y47H, pENTR-E1-E1A-C124G, or pENTR-E1-E1A-Δ2-11 respectively. These products were used as the template for further PCR mutation to generate combinations of these mutations: pENTR-E1-E1A-Y47H-C124G and pENTR-E1- E1A-Y47H-C124G-Δ2-11. The E4 module with vector backbone was PCR'd with primers to generate a product lacking the E4orf6/7-specific exon sequence (297 bp) downstream of the E4orf6 stop codon to generate pENTR-E4-ΔE4orf6/7. This product was also used as the template for PCR with primers to generate products either lacking the PDZ-binding motif of E4orf1, or the entire E4orf1 sequence (pENTR-E4-ΔE4orf6/7-E4orf1ΔPDZb and pENTR-E4-ΔE4orf6/7-ΔE4orf1 respectively).

[0121] To generate complete virus genomes bearing the mutations, AdSlicR was performed using p15A-SceI E2-L4 in combination with the wt E3 module and the wt E4 or a mutant E4 module, then with either the wt E1 or mutant E1. The wildtype AdSlicR adenoviruses are designated in Table 1 shown below.

Table 1. Adenovirus Modifications in E1 and E4.Ad	E1	E4
Ad-102 [AdSyn-CO102]	wt	wt
Ad-210 [AdSyn-CO210]	wt	dE4orf6/7
AdSyn-CO283	wt	E4orf1 dPDZb, dE4orf6/7
AdSyn-CO284	wt	dE4orf1, dE4orf6/7
Ad-189 [AdSyn-CO189]	E1A dLXCXE	wt
Ad-181 [AdSyn-CO181]	E1A dLXCXE	dE4orf6/7

AdSyn-CO285	E1A dLXCXE	E4orf1 dPDZb, dE4orf6/7
AdSyn-CO286	E1A dLXCXE	dE4orf1, dE4orf6/7
AdSyn-CO235	E1A C124G	wt
AdSyn-CO287	E1A C124G	dE4orf6/7
AdSyn-CO288	E1A C124G	E4orf1 dPDZb, dE4orf6/7
AdSyn-CO289	E1A C124G	dE4orf1, dE4orf6/7
AdSyn-CO236	E1A d2-11	wt
AdSyn-CO290	E1A d2-11	dE4orf6/7
AdSyn-CO291	E1A d2-11	E4orf1 dPDZb, dE4orf6/7
AdSyn-CO292	E1A d2-11	dE4orf1, dE4orf6/7
AdSyn-CO238	E1A Y47H, C124G	wt
AdSyn-CO293	E1A Y47H, C124G	dE4orf6/7
AdSyn-CO294	E1A Y47H, C124G	E4orf1 dPDZb, dE4orf6/7
AdSyn-CO295	E1A Y47H, C124G	dE4orf1, dE4orf6/7
AdSyn-CO244	E1A Y47H, C124G, d2-11	wt
AdSyn-CO296	E1A Y47H, C124G, d2-11	dE4orf6/7
AdSyn-CO297	E1A Y47H, C124G, d2-11	E4orf1 dPDZb, dE4orf6/7
AdSyn-CO298	E1A Y47H, C124G, d2-11	dE4orf1, dE4orf6/7

[0122] Regarding virus production, concentration and purification, 293 E4 cells are infected with infectious particles, and approximately 48 hours post-infection when CPE is apparent, the cells are collected and isolated by centrifugation at 500 x g for 5 minutes. The cells are lysed in TMN buffer **(**10 mM TrisCl pH 7.5, 1 mM $MgCl_2$, 150 mM NaCl) via 3x freeze/thaws, and the cell debris is removed by two rounds of centrifugation at 3K x g and 3.5K x g for 15 minutes. A cesium chloride gradient (0.5 g/mL) is used to band virus particles via ultracentrifugation at 37K x g for 18-24 hours. The band is collected and dialyzed in a 10k MWCO Slide-A-Lyzer® dialysis cassette (Thermo Scientific) in TMN with 10% glycerol overnight (12-18 h) at 4 °C, then stored at -80 °C. The titer of the purified virus is determined versus a titered wildtype standard by a cell-based serial dilution infection ELISA with anti-adenovirus type 5 primary antibody (ab6982, Abcam), and ImmunoPure anti-rabbit alkaline phosphatase secondary antibody (Thermo Scientific).

[0123] Regarding evaluation of adenovirus protein expression during infection of primary human small airway epithelial cells (SAEC), quiescent SAEC in 12-well plates were infected with MOI 30 adenovirus, and the media is replaced on the cells 4 hours after infection. At 24, 36, and 48 hours after infection, cells were washed with cold PBS, harvested in 500 uL cold PBS, pelleted at 5K rpm for 5 min at 4 °C, snap frozen and stored at -80 °C. Cell pellets were lysed in RIPA buffer (100 mM Tris pH7.4, 300 mM NaCl, 2mM EDTA, 2% Triton X, 2% deoxycholate, 2 mM NaF, 0.2 mM $NaVO_4$, 2 mM DTT) for 1 hour at 4 °C, including sonication in a cup sonicator (2x 60 s pulses at 60 amplitude at 4 °C). Cell debris was pelleted by centrifuging at 13K rpm for 20 min at 4 °C. Protein concentration was determined using Bio-rad's *DC*™ Protein Assay, and the protein concentration of the samples were normalized. Gel electrophoresis was performed using Life Technologies' Novex® NuPAGE® SDS-PAGE gels, as per the manufacturer's protocol. Proteins were detected by Western blot. The primary antibodies used to detect proteins follows: E1A (ab28305, Abcam), β-actin (A5441, Sigma), Ad5 late proteins (ab6982, Abcam), cyclin A (Ab-6 6E6, NeoMarkers), cyclin B (Ab-3 GNS1,

NeoMarkers). Life Technologies' Alexa Fluor® antibodies were used as secondary antibodies, and the signal was detected using a LI-COR ODYSSEY® instrument. Regarding evaluation of adenovirus protein expression during infection of lung adenocarcinoma A549 cells and normal human astrocyte cells (NHA), confluent cells in 12-well plates were infected with MOI 10 adenovirus, and similarly processed as described for SAEC. Regarding evaluation of adenovirus protein expression during infection of glioblastoma U87 cells, glioblastoma U118 cells, human vascular endothelial cells (HuVEC), and human fibroblasts, confluent cells in 12-well plates were infected with MOI 20 adenovirus, and similarly processed as described for SAEC.

[0124] Regarding cell cycle analysis, cells were infected with the same MOI as for protein expression. Fourty-eight hours post-infection, cells were trypsinized off the plate and washed with cold PBS. Cells were resuspended in 500 uL cold PBS, and fixed with 3 mL cold 70% EtOH/15 mM glycine, pH 2.8. Samples were kept at 4 °C, and prior to FACS, the cells were pelleted, washed in cold PBS, and resuspended in propidium iodide (PI)/RNase A solution, then incubated at 37 °C for 1 h. At least 10K events were collected by FACS for each sample.

[0125] Regarding adenovirus bursts from infection, quiescent cells in 12-well plates were infected with MOI 1 or 10 adenovirus, and the media is replaced on the cells 4 hours after infection. Media from the wells is collected 48 and 72 hours post-infection, flash frozen and thawed once, and centrifuged at 7K rpm at 4 °C for 5 min to pellet cellular debris. The volume of the media is measured, and is flash frozen and stored at -80 °C. The titer of the virus in the media is determined versus a titered wildtype standard by a cell-based serial dilution infection ELISA with anti-adenovirus type 5 primary antibody (ab6982, Abcam), and ImmunoPure anti-rabbit alkaline phosphatase secondary antibody (Thermo Scientific).

[0126] Regarding cell viability assays, cells were seeded in 96-well plates, and in infected in triplicate at serial dilutions at MOI 30. Following infection at 7-10 days when there is complete CPE in the MOI 10 infected wells, metabolic activity is measured using cell proliferation reagent WST-1 (Roche) as per manufacturer's specifications.

[0127] The data are shown in Figures 1-16. As discussed above, cancer continues to be a problematic disease in need of additional therapeutic treatments. One such treatment includes oncolytic viruses. See Figure 1 for a schematic showing the general rationale of oncolytic viral cancer therapy. Adenoviruses are one of the viruses being explored for use as an oncolytic virus. Figure 2 is a schematic showing the structural features of adenovirus (Ad) and a map of the adenovirus genome with transcriptional units in boxes and labeled genes. The retinoblastoma (Rb) tumor suppressor pathway function is lost in almost every human cancer either by direct mutation of Rb, by loss of CDK-inhibitor p16 function due to mutation/methylation, or by amplification of CDK/cyclins. In normal, non-dividing cells, Rb remains hypophosphorylated and binds to transcription factor E2F at its target promoters, suppressing transcription by masking the E2F transactivation domain as well as recruiting chromatin-remodeling complexes and histone-modifying activities. During the G1-S transition of the cell cycle, CDKs phosphorylate Rb which relieves E2F suppression. Adenoviruses express early viral oncoproteins that inactivate the Rb tumor suppressor pathway to force cells to replicate and concomitantly reproduce the viral genome. Adenovirus E1A binds Rb, in part, via an LXCXE motif, deregulating its tumor suppressor activities. It was thus proposed that deleting the LXCXE motif in E1A would eliminate Rb inactivation, and make a selectively replicating virus (ONYX-838). However, Johnson et al., *Cancer Cell*, 1(4):325-337 (2002) provided evidence that Ad E1A LXCXE mutation is not sufficient to prevent S-phase entry, viral DNA replication, and late protein expression, consistent with results from experiments described herein. Even though the Rb-selectivity of the E1A mutant is controversial, this mutation has been carried forward as the basis for an oncolytic virus (DNX-2401) that is moving into phase II clinical trials for malignant brain tumors. In an attempt to achieve higher Rb-selectivity, an adenovirus was generated that replaced the promoters for the Adenovirus E1 and E4 regions with E2F promoters and combined it with the E1A ΔLXCXE motif to generate ONYX-411 (Johnson et al., *Cancer Cell*, 1(4):325-337 (2002); and Dubensky et al., *Cancer Cell*, 1(4):307-309 (2002)). To test these viruses, tumor and primary human cells were infected with either wild type virus, ONYX-838 (E1A ΔLXCXE) or ONYX-411 and harvested at various time points post infection (Fig.

3). As shown in Figure 3, ONYX-838 indiscriminately replicates in tumor and primary lung epithelial cells. ONYX-411, which combines the E1A ΔLXCXE with cellular E2F control of adenovirus E1A, E1B and E4 regions (shown in Fig. 2) demonstrates selective-replication in tumor cells vs. normal cells (Johnson et al., Cancer Cell 1(4):325-337 (2002)). However, the E2F promoters result in recombination and also limit replication to wild type virus levels in tumor cells. Thus, these viruses remain problematic

[0128] As described herein, independently of E2F release from Rb suppression by E1A, there is another Ad protein, E4orf6/7, that further stabilizes E2F proteins at cellular and Ad promoters. Together, E1A and E4orf6/7 drive E2F-mediated transcription, causing S-phase initiation, concomitantly propagating the viral DNA genome. Therefore, provided herein is an Adenovirus bearing both E1A modifications and E4orf6/7 modifications that is a selective oncolytic viral therapy for tumor cells lacking functional Rb. Specifically, compound mutations in E1A/E4orf6/7 were engineered to determine if they selectively replicate in tumor cells, but not primary cells. It is proposed that the combination of these mutations result in an effective, self-amplifying therapy for cancer. Figures 4A and 4B are schematics showing tumor mutations and Adenovirus early proteins converge in activating the Rb pathway to elicit uncontrolled replication. A) Common mutations that cause loss of Rb-tumor suppressor functions. B) Adenovirus proteins directly deregulate Rb and E2F to drive the cell into S-phase. Figure 5 is a schematic showing Adenovirus encodes multiple proteins to deregulate the Rb-E2F cell cycle checkpoint. Adenovirus E1A binds to cellular Rb, releasing E2F to activate transcription. Adenovirus E4orf6/7 stabilizes E2F at promoters to enhance expression of downstream genes. Figure 6 is a schematic showing construction of mutant adenoviruses in this work. The wildtype Ad5 genome was split into modules according to transcriptional units, and each of the modules was placed in different plasmids. Mutations were made on the module plasmids, and using AdSlicR, the modules were reassembled into complete genomes enabling generation of recombinant adenoviruses. Figure 7 is a schematic showing the E4orf6/7 deletion. Two exons encode E4orf6/7. E4orf6 and E4orf6/7 utilize same start codon and share 58 amino acid N-terminal residues. The

E4orf6/7 transcript splices immediately following E4orf6 stop codon. The entire second exon of E4orf6/7, including the stop codon was deleted. Figure 7A shows the wildtype E4 region. Figure 7B shows the resulting E4 ΔE4orf6/7 region.

[0129] To test these viruses, cells infected with mock (ΔE1), Ad-102 (AdSyn-CO102) (wildtype), Ad-181 (AdSyn-CO181) (E1A ΔLXCXE/ΔE4orf6/7), Ad-189 (AdSyn-CO189) (E1A ΔLXCXE), or ONYX-838 (E1A ΔCR2). ONYX-838 also lacks ΔLXCXE which is in the CR2 domain of E1A. Quiescent human primary small airway epithelial cells (SAEC) were infected at MOI 10. Ad-102 (AdSyn-CO102) shows expected decrease of E1A levels at later times during infection (Fig. 8A). Similarly Ad-189 (AdSyn-CO189) and ONYX-838 show a decrease of E1A levels at later times during infection, but have stronger expression at the earlier timepoint. Ad-181 (AdSyn-CO181) shows stronger and continued expression of E1A throughout the infection, which is indicative of failure to progress through the adenovirus lifecycle. Confluent lung adenocarcinoma cells (A549) were infected at MOI 30. All infections show expected decrease of E1A levels at later times during infection, indicative of typical adenovirus lifecycle progression (Fig. 8B). Testing of adenovirus late protein and cyclin expression of the mutant adenoviruses in infected cells is shown in Figure 9. There are no apparent defects in expression of late structural proteins, and cyclin remains present in all infected A549 samples. DNA replication of infected SAEC and A549 cells is shown in Figure 10. No DNA replication defect is apparent with any mutant virus infection. Figure 11 shows adenovirus bursts from infected SAEC and A549. With the exception of Ad-210 (AdSyn-CO210) at these timepoints with this burst assay, there is no defect in virus replication. Figure 12 shows the cell viability of infected SAEC and A549 after 7 days of infection. Of the viruses there is no defect in cell killing by mutant viruses relative to wildtype. Figure 13 shows that the mutant viruses do have attenuated infection in normal human astrocytes (NHA) which Figures 14 and 15 show that the mutant adenoviruses have no replication defects in glioblastoma U87 and U118 cells, respectively.

[0130] Thus, the data show that, in contrast to wild type and E1AΔCR2 viruses, E1AΔCR2/ΔE4orf6/7 and also ΔE4orf6/7 viruses replicate poorly in primary cells as

evidenced by lack of capsid protein expression, failure to induce the E2F target genes-Cyclin A and B, failure to elicit S phase entry and viral replication. See, e.g., Figures 9, 10, and 11. In contrast, these viruses replicate to wild type (WT) virus levels in A549 cells and a panel of tumor cell-lines. Therefore, the provided adenoviruses are selective cancer therapeutic agents.

[0131] Results of the replication specificity of the larger set of mutant adenoviruses, including mutations in E4orf1 (see Table 1) are shown in Figures 17-22 and summarized in Figure 23. Figure 17 is a graph showing the cell viability of infected primary normal human astrocytes (NHA) after 10 days of infection. Figure 18 is a graph showing the cell viability of infected quiescent normal small airway epithelial cells (SAEC-hTERT) after 9 days of infection. Figure 19 is a graph showing the cell viability of infected proliferating SAEC-hTERT cells after 10 days of infection. Figure 20 is a graph showing the cell viability of infected human lung adenocarcinoma cells (A549) after 7 days of infection. Figure 21 is a graph showing the cell viability of infected human breast cancer cells (MDA MB 231) after 7 days of infection. Figure 22 is a graph showing the cell viability of infected glioblastoma cells (U87) after 7 days of infection. Figure 23 is a heatmap table showing the quantitation of cell viability assays for infected primary NHA, SAEC-hTERT (quiescent), SAEC-hTERT (proliferating), A549, MDA MB 231, and U87 cells after 7 days of infection. These data show that the combination of various modifications of E1A and E4orf6/7 results in selective oncolytic adenovirus that specifically replicate in cancer cells with a defective Rb tumor suppressor pathway.

EMBODIMENTS

Embodiment 1. An adenovirus comprising an E1A polypeptide comprising one or more modifications and comprising an E4orf6/7 polypeptide comprising one or more modifications.

Embodiment 2. The adenovirus of embodiment 1, wherein the E1A polypeptide comprises a modification in an Rb binding site of E1A.

Embodiment 3. The adenovirus of embodiment 1, wherein the E1A polypeptide comprises two Rb binding sites and wherein the E1A polypeptide comprises a modification in both Rb binding sites.

Embodiment 4. The adenovirus of embodiment 1, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 120-130 of the E1A polypeptide.

Embodiment 5. The adenovirus of embodiment 1, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 122-126 of the E1A polypeptide.

Embodiment 6. The adenovirus of any one of embodiments 1-5, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 35-55 of the E1A polypeptide.

Embodiment 7. The adenovirus of any one of embodiments 1-6, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 37-49 of the E1A polypeptide.

Embodiment 8. The adenovirus of any one of embodiments 1-7, wherein the E1A polypeptide comprises a deletion.

Embodiment 9. The adenovirus of embodiment 8, wherein the deletion is a deletion of amino acid residues 122-126 of the E1A polypeptide.

Embodiment 10. The adenovirus of embodiment 8, wherein the deletion is a deletion of amino acid residues 2-11 of the E1A polypeptide.

Embodiment 11. The adenovirus of embodiment 1, wherein the E1A polypeptide comprises the deletion ΔLXCXE.

Embodiment 12. The adenovirus of any one of embodiments 1-11, wherein the E1A polypeptide comprises one or more substitutions.

Embodiment 13. The adenovirus of embodiment 12, wherein the E1A polypeptide comprises a substitution at residue Y47, residue C124 or at both residues Y47 and C124.

Embodiment 14. The adenovirus of embodiment 12, wherein the E1A polypeptide comprises the substitution Y47H.

Embodiment 15. The adenovirus of embodiment 12, wherein the E1A polypeptide comprises the substitution C124G.

Embodiment 16. The adenovirus of embodiment 12, wherein the E1A polypeptide comprises the substitution Y47H and C124G.

Embodiment 17. The adenovirus of any one of embodiments 12-16, wherein the E1A polypeptide further comprises a deletion of amino acid residues 2-11.

Embodiment 18. The adenovirus of embodiment 1, wherein the E1A polypeptide comprises a deletion of amino acid residues 122-126 of E1A and a substitution at residue Y47.

Embodiment 19. The adenovirus of any one of embodiments 1-18, wherein the E1A polypeptide comprises SEQ ID NO:1.

Embodiment 20. The adenovirus of any one of embodiments 1-18, wherein the E1A polypeptide comprises SEQ ID NO:2.

Embodiment 21. The adenovirus of any one of embodiments 1-20, wherein the E4orf6/7 polypeptide comprises a modification in one or both of the E4orf6/7 exons.

Embodiment 22. The adenovirus of any one of embodiments 1-20, wherein the E4orf6/7 polypeptide comprises a deletion of one or both of the E4orf6/7 exons.

Embodiment 23. The adenovirus of any one of embodiments 1-22, wherein the E4orf6/7 polypeptide comprises SEQ ID NO:3.

Embodiment 24. The adenovirus of any one of embodiments 1-22, wherein the E4orf6/7 polypeptide comprises SEQ ID NO:4.

Embodiment 25. The adenovirus of any one of embodiments 1-24, further comprising an E4orf1 polypeptide comprising one or more modifications.

Embodiment 26. The adenovirus of embodiment 25, wherein the E4orf1 polypeptide comprises one or more deletions.

Embodiment 27. The adenovirus of embodiment 25, wherein the E4orf1 polypeptide comprises a deletion in the C-terminal region of E4orf1.

Embodiment 28. The adenovirus of embodiment 25, wherein the E4orf1 polypeptide comprises a deletion of residues 125-128 of the E4orf1 polypeptide.

Embodiment 29. The adenovirus of any one of embodiments 25-28, wherein the E4orf1 polypeptide comprises SEQ ID NO:5.

Embodiment 30. An adenovirus comprising an E1A polypeptide comprising one or more modifications and comprising an E4orf1 polypeptide comprising one or more modifications.

Embodiment 31. The adenovirus of embodiment 30, wherein the E4orf1 polypeptide comprises one or more deletions.

Embodiment 32. The adenovirus of embodiment 31, wherein the E4orf1 polypeptide comprises a deletion in the C-terminal region of E4orf1.

Embodiment 33. The adenovirus of embodiment 31, wherein the E4orf1 polypeptide comprises a deletion of residues 125-128 of the E4orf1 polypeptide.

Embodiment 34. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a modification in an Rb binding site of E1A.

Embodiment 35. The adenovirus of embodiment 34, wherein the E1A polypeptide comprises two Rb binding sites and wherein the E1A polypeptide comprises a modification in both Rb binding sites.

Embodiment 36. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 120-130 of the E1A polypeptide.

Embodiment 37. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 122-126 of the E1A polypeptide.

Embodiment 38. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 35-55 of the E1A polypeptide.

Embodiment 39. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a modification in one or more of amino acid residues 37-49 of the E1A polypeptide.

Embodiment 40. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a deletion.

Embodiment 41. The adenovirus of embodiment 40, wherein the deletion is a deletion of amino acid residues 122-126 of the E1A polypeptide.

Embodiment 42. The adenovirus of embodiment 40, wherein the deletion is a deletion of amino acid residues 2-11 of the E1A polypeptide.

Embodiment 43. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises the deletion ΔLXCXE.

Embodiment 44. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises one or more substitutions.

Embodiment 45. The adenovirus of embodiment 44, wherein the E1A polypeptide comprises a substitution at residue Y47, residue C124 or both Y47 and C124.

Embodiment 46. The adenovirus of embodiment 44, wherein the E1A polypeptide comprises the substitution Y47H.

Embodiment 47. The adenovirus of embodiment 44, wherein the E1A polypeptide comprises the substitution C124G.

Embodiment 48. The adenovirus of embodiment 44, wherein the E1A polypeptide comprises the substitution Y47H and C124G.

Embodiment 49. The adenovirus of any one of embodiments 44-48, wherein the E1A polypeptide further comprises a deletion of amino acid residues 2-11.

Embodiment 50. The adenovirus of any one of embodiments 30-33, wherein the E1A polypeptide comprises a deletion of amino acid residues 122-126 of E1A and a substitution at residue Y47.

Embodiment 51. The adenovirus of any one of embodiments 30-50, wherein the E1A polypeptide comprises SEQ ID NO:1.

Embodiment 52. The adenovirus of any one of embodiments 30-50, wherein the E1A polypeptide comprises SEQ ID NO:2.

Embodiment 53. The adenovirus of any one of embodiments 1-52, wherein the adenovirus selectively replicates in Rb-deficient cells.

Embodiment 54. A pharmaceutical composition comprising the adenovirus of any one of embodiments 1-53 and a pharmaceutically acceptable carrier.

Embodiment 55. A kit comprising the pharmaceutical composition of embodiment 54 and instructions for use.

Embodiment 56. The kit of embodiment 55, further comprising one or more additional therapeutic agents.

Embodiment 57. The kit of embodiment 56, wherein the therapeutic agent is a chemotherapeutic agent.

Embodiment 58. A method of treating a proliferative disorder in a subject comprising administering the adenovirus of any one of embodiments 1-53 or the pharmaceutical composition of embodiment 54 to the subject.

Embodiment 59. The method of embodiment 58, wherein the adenovirus or pharmaceutical composition is administered intravenously, intravascularly, intrathecally, intramuscularly, subcutaneously, intraperitoneally, or orally.

Embodiment 60. The method of embodiment 58 or 59, further comprising administering to the subject one or more additional therapeutic agents.

Embodiment 61. The method of embodiment 60, wherein the therapeutic agent is a chemotherapeutic agent.

Embodiment 62. The method of any one of embodiments 58-61, wherein the proliferative disorder is selected from the group consisting of lung cancer, prostate cancer, colorectal cancer, breast cancer, thyroid cancer, renal cancer, liver cancer and leukemia.

Embodiment 63. The method of any one of embodiments 58-62, wherein approximately 103 to 1012 plaque forming units of the adenovirus is administered to the subject.

Embodiment 64. The method of any one of embodiments 58-63, wherein the proliferative disorder is metastatic.

Embodiment 65. An adenovirus comprising E1A comprising one or more modifications and comprising E4orf6/7 comprising one or more modifications.

Embodiment 66. The adenovirus of embodiment 65, wherein the modification of E1A comprises a modification in the Rb binding site of E1A.

Embodiment 67. The adenovirus of embodiment 65, wherein the modification of E1A comprises a modification in one or more of amino acid residues 122-126 of the E1A polypeptide.

Embodiment 68. The adenovirus of embodiment 65, wherein the modification of E1A comprises a deletion.

Embodiment 69. The adenovirus of embodiment 65, wherein the deletion is a deletion of amino acid residues 122-126 of E1A.

Embodiment 70. The adenovirus of embodiment 65, wherein the modification of E1A is ΔLXCXE.

Embodiment 71. The adenovirus of any one of embodiments 65-70, wherein the modification of E4orf6/7 comprises a modification in one or both of the E4orf6/7 exons.

Embodiment 72. The adenovirus of any one of embodiments 65-70, wherein the modification of E4orf6/7 is a deletion of one or both of the E4orf6/7 exons.

Embodiment 73. The adenovirus of any one of embodiments 65-70, wherein the modification of E4orf6/7 is ΔE4orf6/7.

Embodiment 74. The adenovirus of embodiment 65, wherein the adenovirus comprises E1A ΔLXCXE and ΔE4orf6/7.

Embodiment 75. The adenovirus of any one of embodiments 65-74, wherein the adenovirus selectively replicates in Rb-deficient cells.

Embodiment 76. A pharmaceutical composition comprising the adenovirus of any one of embodiments 65-75 and a pharmaceutically acceptable carrier.

Embodiment 77. A kit comprising the pharmaceutical composition of embodiment 76 and instructions for use.

Embodiment 78. The kit of embodiment 77, further comprising one or more additional therapeutic agents.

Embodiment 79. The kit of embodiment 78, wherein the therapeutic agent is a chemotherapeutic agent.

Embodiment 80. A method of treating a proliferative disorder in a subject comprising administering the adenovirus of any one of embodiments 65-75 or the pharmaceutical composition of embodiment 76 to the subject.

Embodiment 81. The method of embodiment 80, wherein the adenovirus or pharmaceutical composition is administered intravenously, intravascularly, intrathecally, intramuscularly, subcutaneously, intraperitoneally, or orally.

Embodiment 82. The method of embodiment 80 or 81, further comprising administering to the subject one or more additional therapeutic agents.

Embodiment 83. The method of embodiment 82, wherein the therapeutic agent is a chemotherapeutic agent.

Embodiment 84. The method of any one of embodiments 65-83, wherein the proliferative disorder is selected from the group consisting of lung cancer, prostate cancer, colorectal cancer, breast cancer, thyroid cancer, renal cancer, liver cancer and leukemia.

Embodiment 85. The method of any one of embodiments 65-84, wherein approximately 103 to 1012 plaque forming units of the adenovirus is administered to the subject.

Embodiment 86. The method of any one of embodiments 65-85, wherein the proliferative disorder is metastatic.

WHAT IS CLAIMED IS:

1. A recombinant adenovirus comprising:

(i) an E1A polypeptide comprising a deletion or substitution in an Rb-binding site;

(ii) a deletion of one or both of the E4orf6/7 exons; and

(iii) adenovirus E1A and E4 promoters,

wherein the recombinant adenovirus selectively replicates in Rb-deficient cells.

2. The recombinant adenovirus of claim 1, wherein the E1A polypeptide comprises two Rb-binding sites and the E1A polypeptide comprises a deletion or substitution in both Rb-binding sites.

3. The recombinant adenovirus of claim 1 or claim 2, wherein the E1A polypeptide comprises a deletion of an LXCXE motif.

4. The recombinant adenovirus of claim 3, wherein the E1A polypeptide comprises a deletion of amino acid residues 122-126, a deletion of amino acid residues 2-11, or both.

5. The recombinant adenovirus of any one of claims 1-4, wherein the E1A polypeptide comprises a substitution at residue Y47, a substitution at residue C124, or both.

6. The recombinant adenovirus of claim 5, wherein the substitution at residue Y47 is a Y47H substitution, or the substitution at residue C124 is a C124G substitution, or both.

7. The recombinant adenovirus of any one of claims 1-6, wcomprising a deletion of one of the E4orf6/7 exons.

8. The recombinant adenovirus of any one of claims 1-7, further comprising:

an E4orf1 polypeptide comprising a deletion in the C-terminal region; or

a deletion of all of the E4orf1 gene.

9. The recombinant adenovirus of claim 8, wherein the E4orf1 polypeptide comprises a deletion in the C-terminal region.

10. The recombinant adenovirus of claim 9, wherein the E4orf1 polypeptide comprises a deletion of the last four amino acids in the C-terminal region.

11. The recombinant adenovirus of claim 9, wherein the E4orf1 polypeptide comprises a deletion of residues 125-128.

12. A recombinant adenovirus, comprising:

(i) an E1A polypeptide comprising a deletion or substitution in an Rb-binding site;

(ii) an E4orf1 polypeptide comprising a deletion in the C-terminal region, or a deletion of all of the E4orf1 gene; and

(iii) adenovirus E1A and E4 promoters,

wherein the recombinant adenovirus selectively replicates in Rb-deficient cells.

13. The recombinant adenovirus of claim 12, wherein the E1A polypeptide comprises two Rb-binding sites and the E1A polypeptide comprises a deletion or substitution in both Rb-binding sites.

14. The recombinant adenovirus of claim 12 or claim 13, wherein the E1A polypeptide comprises a deletion of an LXCXE motif.

15. The recombinant adenovirus of claim 14, wherein the E1A polypeptide comprises a deletion of amino acid residues 122-126, a deletion of amino acid residues 2-11, or both.

16. The recombinant adenovirus of any one of claims 12-15, wherein the E1A polypeptide comprises a substitution at residue Y47, a substitution at residue C124, or both.

17. The recombinant adenovirus of claim 16, wherein the substitution at residue Y47 is a Y47H substitution, or the substitution at residue C124 is a C124G substitution, or both.

18. The recombinant adenovirus of any one of claims 12-17, wherein the E4orf1 polypeptide comprises a deletion in the C-terminal region.

19. The recombinant adenovirus of claim 18, wherein the E4orf1 polypeptide comprises a deletion of the last four amino acids in the C-terminal region.

20. The recombinant adenovirus of claim 18, wherein the E4orf1 polypeptide comprises a deletion of residues 125-128.

21. A pharmaceutical composition comprising the recombinant adenovirus of any one of claims 1-20 and a pharmaceutically acceptable carrier.