negative control Ad vaccine. This group of animals was not boosted with gp140. * $p < 0.05$, ** $p < 0.01$, *** $p< 0.001$, **** $p < 0.0001$ by one way ANOVA in comparison to the SC-Ad6-Ebov group.

[00060] Figure 15 shows plasma HIV neutralization titers. Neutralization of the indicated viruses was performed using the TZM-bl neutralization assay. All values were calculated as compared to virus-only wells. Each dot represents the mean value for each animal.

[00061] Figure 16 shows plasma ADCC activity. Plasma samples were tested with CD16-KHYG-1 effector cells to kill CEM.NKR.CCR5.CD4+-Luc, target cells infected with $SHIV_{SF162P3}$. Each dot represents the mean value for each animal. * $p < 0.05$, *** $p< 0.001$, **** $p < 0.0001$ by one-way ANOVA vs. the SC-Ad6-Ebov group.

[00062] Figure 17 shows mucosal ADCC activity. Vaginal wash and saliva samples were tested with CD16-KHYG-1 effector cells to kill CEM.NKR.CCR5.CD4+-Luc, target cells infected with SHIVSF162P3. Each dot represents the mean value for each animal. * $p < 0.05$ by one-way ANOVA vs. the SC-Ad6-Ebov group.

[00063] Figure 18 shows IFN-γ Secreting Cells from PBMCs and Lymph Nodes. PBMCs and lymph node cells were analyzed by ELISPOT by staining for IFN-γ. Anti-Env indicates cells that were stimulated with conserved HIV Env peptides and SC-Ads. The total number of spot forming cells (SFCs) in each of the stimulated wells were counted and adjusted to control medium as background. Each dot represents the mean value for each animal. * $p < 0.05$ by one-way ANOVA. Figure 18A: Anti-Env IFN-γ SFCs and anti-Ad IFN-γ SFCs in PBMCs; Figure 18B: Anti-Env IFN-γ SFCs in PBMCs and lymph node cells.

[00064] Figure 19 shows mucosal T cell trafficking and activation. T cells were harvested from rectal biopsies collected after the second protein boost and analyzed by flow cytometry for CD4, CD8, α4β7 integrin, CD69, and FoxP3. Each dot represents the mean value for each animal. Figure 19A: α4β7 integrin CD4+ cell and α4β7 integrin CD8+ cells in rectal sites. Figure 19B: CD4+ CD69+ and CD4+ Fox P3+ cells in rectal sites

[00065] Figure 20 shows Tfh cell response in the blood and in lymph nodes. PBMCs and lymph node cells collected at week 40 were stimulated with HIV-1 Env protein and then examined for co-expression of CD3+, CD4+, CXCR5+, and IL-21. Each dot represents the mean value for each animal. * $p < 0.05$ by one-way ANOVA.

[00066] Figure 21 shows protection against repeated rectal $SHIV_{SF162P3}$ challenge. The indicated groups were challenged rectally with 4.3 TCID50 (on rhesus PBMCs) of $SHIV_{SF162P3}$ on a

weekly basis. Plasma samples were analyzed for SHIV viral RNA copies. Animals with RNA copies above 10 were considered infected and the number of challenges required to infect that animal were used as events for Kaplan-Meier survival analysis.

[00067] Figure 22 shows $SHIV_{SF162P3}$ acquisition and viral loads. A) Animals from Figure 8 were grouped by their initial SC-Ad priming route (IM or IN) yielding groups of 8 and Kaplan-Meier analysis was performed. B) Plasma SHIVSF162P3 viral RNA levels over the course of the challenge study.

[00068] Figure 23 shows SHIV viral load in tissues. RNA from PBMCs and post-mortem tissues were collected and qPCR was performed to detect analyzed for SHIV viral RNA.

[00069] Figure 24 shows single-cycle adenovirus vaccines used in Example 7. A) Cartoon of SC-Ad serotypes 6 and 657 carrying F8 and G4 clade B HIV envelope genes. B) Alignment of clade C Ad hexons including the Ad6 and 57 hexons displayed on vaccines.

[00070] Figure 25 shows saliva and vaginal HIV env binding titrations. ELISA OD450 levels are shown for the indicated samples at the indicated dilutions when tested against F8. The low level of antibodies in these mucosal samples prevent reaching saturation of the assay. For this reason, EC50 values cannot be reliably calculated for most animals. Rhesus macaque Rh13-091 in the IN-IM-IM group was the only animal in which an EC50 could be calculated (EC50 = 4580). Similar results were observed in ELISAs using SF162 gp140.

[00071] Figure 26 shows Ad657 expressing antigen genes from hepatitis C and cytomegalovirus (CMV) gB generating *in vivo* cytotoxic T lymphocyte (CTL) activity. Shown is killing of hepatitis C peptide-loaded target cells in mice vaccinated with Ad657-HCV rather than CMV gB.

[00072] Figure 27 shows expression of human GMCSF from Ad657 inducing proliferation of GMCSF-dependent TF-1 human erythroblasts.

[00073] Figure 28 is a graph showing Ad6 single IV injection vs. A549 lung tumors.

[00074] Figure 29 is a graph showing Ad6 single IV or IT injection vs. Panc1 pancreatic tumors.

[00075] Figure 30 is a graph showing Ad6 single IV injection vs. kidney cancer in immune competent hamsters.

[00076] Figure 31 is a graph showing luciferase activity in B16 melanoma and A549 lung tumor/cancer cells by Ads displaying 12.51 cell binding peptides in HVR5 of the hexon.

[00077] Figure 32 is a graph showing luciferase activity in hepatocellular carcinoma and kidney cancer by Ads displaying 12.51 cell binding peptides in HVR5 of the hexon.

[00078] Figure 33 shows a cartoon combining the insertion of individual HVRs from different Ad serotypes with the insertion of cell targeting/detargeting peptides or novel amino acids such as cysteine into the hexon for targeted chemical modification and shielding. Depicted are chimeric HVR constructs that combine different HVRs from different Ad serotypes to modulate natural interactions with cells and blood factors improve pharmacology combined with insertion of cell binding and cell detargeting peptides in different HVRs to change cell entry and cell avoidance. If one HVR is substituted from 100 Ads, this would create 100 different hexon chimeras. If all 7 HVRs each receive a different Ad HVR, this combinatorial library would equal 7^{100} variants. If one 1 peptide were introduced into 7 HVRs this would equal 7 X 7^{100} variants. If 10 different peptides were introduced into 7 HVRs, this would equal 10 X 7 X 7^{100} variants, etc.

[00079] Figure 34 shows plasmid maps for representative combinatorial hexons and peptide combinations. Shown are hexons with HVR1 from Ad6 and HVRs 2-7 from Ad57 as well as insertions of cell targeting peptides into individual HVRs.

[00080] Figure 35 shows chimeric HVR constructs that combine different HVRs from different Ad serotypes to modulate natural interactions with cells and blood factors improve pharmacology combined with insertion of cell binding and cell detargeting peptides in different HVRs to change cell entry and cell avoidance. In this example, a single cysteine amino acid is inserted into the HVR1 and HVR5 of Ad657 to modulate pharmacology and allow targeted conjugation of polymers like polyethylene glycol or other moieties like imaging agents like fluorophores.

[00081] Figure 36 shows conjugation of polyethylene glycol (PEG) to Ad657-HVR1-C. A) SDS-PAGE of Ad proteins with and without PEGylation. Arrows show size increases due to chemical attachment of PEG to hexon. B) Effects of targeted PEGylation by maleimide-PEG and non-targeting NHS-PEG on virus infection.

[00082] Figure 37 shows conjugation of polyethylene glycol (PEG) to Ad657-HVR5-C. A) SDS-PAGE of Ad proteins with and without PEGylation. B) Near infrared imaging of SDS-PAGE of Ad proteins with and without PEGylation and with and without the near infrared fluorescence imaging tag IR800. C) *In vivo* transduction after intraperitoneal injection of maleimide-PEGylated Ad657-HVR5-C by luciferase imaging.

[00083] Figure 38 shows luciferase imaging of nude mice. A) 1, 4, 7, 14, 28, and 42 days after single I.V. injection of Ad6 treatment vs. distant DU145 prostate tumors. B) 3, 7, and 19 days after I.V. injection of replicating Ad5-GFPLUC into mice bearing LNCaP prostate tumors.

[00084] Figure 39 shows a schematic of cancer-specific conditionally-replicating Ads (CRAds) dl1101+dl1107 having a modification in the E1A gene.

[00085] Figure 40 is a graph showing that Ad6 and Ad657 can both be used as CRAds for targeted cancer therapy.

[00086] Figure 41 is a schematic showing Ad therapeutic cycles. A) A schematic of serotype-switching with Ads. B) A schematic of an exemplary therapeutic cycle where Ad6 and Ad657 can be used for multiple rounds of treatment by serotype-switching in combination with covalent polymer conjugation.

[00087] Figure 42 demonstrates serotype-switching and on-target luciferase activity in the DU145 prostate tumors after a single IV injection of Ad6 and Ad6-F35 with deletions in E3A genes (12.5K, 6.7K, 19K, 11.6K), but retention of E3B genes (10.4K, 14.5K, and 14.7K) and retention of E4 34K. Mice whose tumors resisted prior single IV injection with Ad657 and CRAd657 both with intact E3 genes were injected with the indicated vectors by single IV injection.

[00088] Figure 43 is a schematic of a replication competent Ad (RC-Ad), wherein E1 expression is controlled by the native E1 promoter; a variant CRAd –Probasin-E1A (Ad-PB), wherein E1 expression is controlled by prostate-specific probasin promoter; CRAd-dl1101, wherein p300 pathway binding ablated, susceptible to IFN pathway in normal cells; CRAd-dl1107, wherein pRB binding ablated allows virus to kill cancer cells with RB pathway disruptions, but is repressed in RB+ normal cells; CRAd-dl1101/07, wherein p300 pathway binding ablated, susceptible to IFN pathway pRB binding ablated allows virus to kill cancer cells with RB pathway disruptions, but is repressed in RB+ normal cells.

[00089] Figure 44 (A and B) shows the effect of infection with replication-competent Ad5, Ad6, Ad657 on non-cancerous cells and modification of Ad6 and Ad657 to be conditionally-replicating Ads (CRAds).

[00090] Figure 45 demonstrates killing of cancerous cells by replication-competent Ad5, Ad6, Ad657, and the indicated CRAds.

[00091] Figure 46 demonstrates modification of Ad6 and Ad657 to be conditionally-replicating Ads (CRAds).

[00092] Figure 47 demonstrates modification of Ad6 and Ad657 to be conditionally-replicating Ads (CRAds).

[00093] Figure 48 demonstrates *in vivo* effects of replication-competent Ad6 or the indicated CRAds on growth of DU145 tumors in mice.

[00094] Figure 49 demonstrates *in vivo* effects of replication-competent Ad657 and conditionally-replicating Ad657-dl1101/07 both with intact E3 regions *in vivo* after a single intravenous injection in mice bearing human prostate tumors.

[00095] Figure 50 demonstrates that PEGylation de-targets adenovirus to liver *in vivo.*

[00096] Figure 51 demonstrates modification of Ad657 with the shorter fiber from chimpanzee AdC68 and the addition of a codon-wobbled E4 34.K gene changes *in vitro* efficacy.

[00097] Figure 52 demonstrates 6/56/6 virus killing human prostate cancer cells with and without CRAd modifications.

[00098] Figure 53 demonstrates the production of antibody responses against the human cancer antigen folate receptor alpha after a single intramuscular immunization of BALB/c mice by CRAd657-dl1101/07-FOLR with an intact E3 region.

[00099] Figure 54 depicts sites on Ad HVRs which may be modified, for example, by PEGylation or "BAPylation" with biotin acceptor peptides (BAPs).

[000100] Figure 55 is a schematic of variants of Ads having mutations in the E1 protein to convert the virus to a conditionally-replicating Ad (CRAd).

[000101] Figure 56 shows amino acid sequences of the N-terminal portion of the wild-type E1A polypeptide and the E1A N-terminus of the CRAd variants, dl1101, dl1107 and dl1101/1107.

[000102] Figure 57 shows as schematic of different E3 immune evasion genes in Ad species C exemplar Ad6 and Ad species D exemplar Ad26. Both Ads express size and sequence variants of E3 12.5K, 6.7K, 19K, 10.4K (RIDα), 14.5K (RIDβ), and 14.7K genes, as well as a depiction of the functions of these E3 encoded proteins.

[000103] Figure 58 demonstrates the effects of PEGylation and E3 deletion on oncolytic viral anti-tumor activity by Ad6-Luc viruses in immunocompetent hamsters. Ad6-Luc and Ad6-Luc-20K PEG both have all E3 genes and E4 34K intact. Ad6-deltaE3-Luc has partial deletion of E3 12.5K and E4 34K and full deletion of E3 6.7K, 19K, 11.6K (ADP), 10.4K (RIDα), 14.5K (RIDβ), and 14.7K genes. Oncolytic efficacy is lost in this immunocompetent animal model when these immune evasion genes are not present in oncolytic adenovirus.

[000104] Figure 59 is a plasmid map of Ad657 with partial deletion of E3 12.5K and E4 34K and full deletion of E3 6.7K, 19K, 11.6K (ADP), 10.4K (RIDα), 14.5K (RIDβ), and 14.7K genes.

[000105] Figure 60 depicts CRAd 657 constructs with and without dl1101/1107 CRAd modifications and with and without deletions of selected E3 immune evasion genes.

[000106] Figure 61 depicts CRAd657 with E3 insertion site. These are with and without dl1101/1107 CRAd modifications described herein and with and without E3 immune evasion modifications.

[000107] Figure 62 depicts CRAd657 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide. These are with and without dl1101/1107 CRAd modifications described in previous slides and with and without E3 immune evasion modifications. In some cases, a codon-wobbled E4 34K gene is included after E4 and before fiber to compensate for E4 34K partial deletion when deleted E3B genes.

[000108] Figure 63 depicts CRAd657 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide. These are with and without dl1101/1107 CRAd modifications described in previous slides and with and without E3 immune evasion modifications.

[000109] Figure 64 depicts CRAd657 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide Expressing Folate Receptor alpha.

[000110] Figure 65 depicts CRAd657 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide Expressing Granulocyte Macrophage Colony Stimulating Factor (GMCSF).

[000111] Figure 66 depicts CRAd657 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide Expressing 4-1BBL or GMCSF or IL21 or CD40L and combinations in one virus.

[000112] Figure 67 depicts Ad6/57 with Ad6 HVR1 and Ad57 HVRs2-7 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide.

[000113] Figure 68 depicts Ad6/57/6 with Ad6 HVR1, Ad57 HVRs2-6, Ad6 HVR7 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide.

[000114] Figure 69 depicts Ad6/57/6 with Ad6 HVR1, Ad57 HVRs2-6, Ad6 HVR7 +/- Ad35 Fiber or Chimpanzee C68 Fiber +/- K7 peptide expressing GFPLuciferase.

[000115] Figure 70 shows luciferase imaging after serotype switching. Mice bearing LNCaP prostate tumors on their flanks were treated by a single IV injection of Ad657 or CRAd657. Mice with residual tumors 5 months after single IV injection were treated by serotype-switching of the indicated Ad6/57/6 variants expressing GFPLuciferase with and without variant fibers and a codon-optimized E4 34K gene. The indicated Ad6/57/6 variants include Ad6/57/6 virus having different

fiber modifications including an added 7 lysine on fiber (K7), chimpanzee C68 fiber grafted onto Ad6 fiber after its KKTK flexibility domain and with an Ad35 fiber. Mice were imaged for luciferase activity 7 days later.

[000116] Figure 71 shows A549 human lung cancer cells which were treated with the indicated viral particles (vp) per cell of Ad6/57/6 with and without variant fibers and a codon-optimized E4 34K gene. 7 days later, the cells were stained with crystal violet and the wells were analyzed in a plate reader. High OD indicates the presence of viable cells. Low OD indicates death and loss of adherent cells.

DETAILED DESCRIPTION OF THE INVENTION

[000117] This invention provides methods and materials for nucleic acid delivery, vaccination, and/or treating cancer. For example, this invention provides methods and materials for nucleic acid delivery of proteins/polypeptides, vaccination, and/or treating cancer using one or more recombinant Ads (*e.g.*, Ad657 and variants thereof) as an oncolytic agent.

[000118] An adenovirus icosahedron is made up of 720 copies of its hexon protein. The virus does not use this protein to bind receptors, but this nano-lattice of repeating proteins provides a matrix for interactions (e.g., natural interactions and unnatural interactions) with proteins, cells, and drugs. Antibodies that can neutralize Ads can target hypervariable regions (HVRs) of the hexon polypeptide on an Ads.

[000119] In some cases, this invention provides recombinant Ads having oncolytic anti-cancer activity. For example, a recombinant Ad can be derived from a first Ad and can include hexon HVRs from one or more different Ads. The HVRs may be derived from any species C Ads, for example Ad1, Ad2, Ad5, Ad6 and Ad57. In an embodiment, a recombinant Ad can be derived from a first Ad and can include one or more hexon HVRs from at least one other Ad, wherein at least one hexon HVR is different from the HVR(s) of the first Ad. The first Ad strain can be a human Ad6 strain, and the second Ad strain can be a human Ad57 strain. Hexon shuttle plasmid maps (Figure 34) show the combination of the insertion of individual HVRs from different Ad serotypes with the insertion of cell targeting/detargeting peptides or novel amino acids such as cysteine into the hexon for targeted chemical modification and shielding. In an embodiment, the recombinant Ads comprise amino acid substitutions, for example, substitution of cysteines into polypeptides, and modifications such as PEGylation and BAPylation. The ability to target polymer and other chemical modifications to cysteines inserted in Ad657 hexon is demonstrated herein.

[000120] Ad657 as an oncolytic against human prostate cancer is demonstrated. The Ad6 HVRs were replaced with those from Ad57 to generate a chimeric species C oncolytic virus called Ad657. Ad657 and Ad6 were tested as systemic oncolytic therapies by single i.v. injection in nude mice bearing human cancerous tumors. Ad657 may be used as a local or systemic oncolytic virotherapy for cancers. These data also demonstrate surprising effects of serotype-switching with oncolytic species C Ads.

[000121] In some cases, this invention provides methods for using one or more recombinant Ads provided herein to treat a mammal having, or at risk of having, cancer, an infectious disease, and/or a genetic disease. For example, one or more recombinant Ads can be administered to a mammal having, or at risk of having, cancer to reduce the number of cancer cells (*e.g.*, by infecting and killing cancer cells) in the mammal (*e.g.*, a human). For example, one or more recombinant Ads can be administered to a mammal having, or at risk of having, cancer to stimulate anti-cancer immune responses in the mammal (*e.g.*, a human).

[000122] In some cases, recombinant Ads described herein (e.g., recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657 and variants thereof) are not destroyed by a mammal's immune system. For example, a recombinant Ad is not destroyed by antigen presenting cells (APCs), macrophages, and/or other immune cells in a mammal that the recombinant Ad is administered to.

[000123] In some cases, recombinant Ads described herein (e.g., recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657 and variants thereof) can be administered for multiple (e.g., two or more) rounds of treatment. For example, a first recombinant Ad described herein can avoid antibodies that can neutralize a second recombinant Ad described herein, and vice versa. In cases where a mammal having cancer is treated with one or more recombinant Ads described herein, the mammal can be administered a first round of treatment with a first recombinant Ad and can subsequently be administered a second round of treatment with a second recombinant Ad.

[000124] In some cases, recombinant Ads described herein (*e.g.*, recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657 and variants thereof) can be replication competent Ads (RC-Ads). For example, a RC-Ad can be a RC-Ad that includes a nucleic acid encoding an E1 polypeptide (e.g., an E1+ RC-Ad). For example, a RC-Ad can be a single-cycle Ad (SC-Ad) that includes a deletion of one or more nucleic acids encoding one or more polypeptides associated with the production of infectious viral progeny (e.g., pIIIa and E3). For example, a RC-

Ad can be a conditionally-replicating Ad (CRAd). Examples of single-cycle Ads and how to make and use them are provided elsewhere (International Patent Application Publication No. WO2009/111738).

[000125] In some cases, recombinant Ads described herein (e.g., recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657 and variants thereof) can be replication defective Ads (RD-Ads). For example, a RD-Ad can be a RD-Ad that includes a deletion of a nucleic acid encoding an E1 polypeptide (e.g., an E1-deleted RD-Ad).

[000126] It is demonstrated in the examples herein that CRAd 657 and variants thereof are conditionally-replicating Ads (CRAds) in cancerous cells and that infection of cells with CRAd 657 and variants thereof reduces cell viability and tumor volume. Thus, CRAd 657 and variants thereof may be used as a local or systemic oncolytic virotherapy in subjects with cancer.

[000127] What is more, it is demonstrated that CRAds can be used for expression of antigens and used as a vaccine for vaccinating against viruses, for example, against Human Immunodeficiency Virus (HIV), Human Papilloma Virus (HPV) and Hepatitis C Virus (HCV).

[000128] In some cases, recombinant Ads described herein (*e.g.*, recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657 and variants thereof) can bind to a cell surface receptor (*e.g.*, to facilitate viral entry to a cell). For example, a recombinant Ad described herein can bind to coxsackie-adenovirus receptors (CARs) and/or Fc receptors (e.g., FcμR and FcγR), complement receptors (e.g., CR3 and/or C2qR).

[000129] In an aspect of the invention, CRAds may comprise nucleic acids encoding polypeptides heterologous to the Ad, for example, antigens, cell surface receptors, cell targeting polypeptides and the like. For example, CRAd-657-dl1101/1107-FolR is a recombinant Ad comprising intact E3 and expressing the human folate receptor alpha. It is demonstrated herein that CRAds may be used to generate antibodies against known cancer antigens, for example, folate receptor alpha.

[000130] In some cases, recombinant Ads described herein (*e.g.*, recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657s) can avoid binding (e.g., do not bind) to a scavenger receptor (*e.g.*, to facilitate viral entry to a cell). For example, a recombinant Ad described herein avoid binding to a SREC receptors and/or SR-A receptors.

[000131] In some cases, recombinant Ads described herein (e.g., recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657s) can avoid phagocytosis.

[000132] In some cases, recombinant Ads described herein (*e.g.*, recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657s) are non-pathogenic (*e.g.*, to a mammal being treated as described herein).

[000133] In some cases, recombinant Ads described herein (*e.g.*, recombinant Ads having oncolytic anti-cancer activity such as recombinant Ad657s) can infect dividing cells (*e.g.*, can infect only dividing cells).

[000134] A recombinant Ad described herein can be any appropriate recombinant Ad (e.g., a recombinant Ad having oncolytic anti-cancer activity) generated by recombinant DNA technology and methods known to those skilled in the art. A recombinant Ad can be any Ad generated by recombining material (e.g., nucleic acid and/or polypeptide) from any organism other than the Ad from which the recombinant Ad is derived. For example, a recombinant Ad can include one or more materials that do not naturally occur in that Ad (*e.g.*, do no naturally occur in that Ad prior to recombination). In some cases, a recombinant Ad provided herein can be a chimeric Ad (e.g., can include viral elements from two or more (e.g., two, three, four, five, or more) different Ad genomes).

[000135] These embodiments have been applied also in the context of Ads which combine different HVRs from different Ads (i.e., shuffling HVRs). For example, HVR1 of Ad6 with HVRs 2-7 of Ad57 or HVR1 and 7 of Ad6 with HVRs 2-6 of Ad57, or HVRs 1 and 7 from Ad6 and HVRs 2-6 from Ad657.

[000136] Nucleic acid and/or polypeptides that do not naturally occur in the Ad can be from any appropriate source. In some cases, a nucleic acid and/or a polypeptide that does not naturally occur in that Ad can be from a non-viral organism. In some cases, a nucleic acid and/or a polypeptide that does not naturally occur in that Ad can be from a virus other than an Ad. In some cases, a nucleic acid and/or a polypeptide that does not naturally occur in that Ad can be from an Ad obtained from a different species. In some cases, a nucleic acid and/or a polypeptide that does not naturally occur in that Ad can be from a different strain of Ad (*e.g.*, serotypically distinct strains). In some cases, a nucleic acid and/or a polypeptide that does not naturally occur in that Ad can be a synthetic nucleic acid and/or a synthetic polypeptide.

[000137] A recombinant Ad described herein (*e.g.*, a recombinant Ad having oncolytic anti-cancer activity such as a recombinant Ad657) can be derived from (*e.g.*, can include a genomic backbone from) any appropriate Ad. In some cases, a recombinant Ad described herein can be derived from an Ad having low seroprevalence. For example, 50% or fewer (e.g., 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or fewer) of mammals (e.g., human) can have been exposed

to an Ad from which a recombinant Ad described herein is derived. With regard to seroprevalence, species C adenoviruses, Ad6 and Ad657 have lower prevalence than archetype Ad5 virus. In some cases, a recombinant Ad described herein can be derived from an Ad having reduced or eliminated side effects (e.g., phagocytosis and liver damage). A recombinant Ad can be derived from an Ad isolated from any appropriate species of animal. For example, Ads can be isolated from humans, non-human primates (*e.g.*, monkeys such as Old World monkey species like rhesus macaques), fish, frogs, and snakes. In some cases, a recombinant Ad described herein can be derived from a human Ad (HAd or HAdV). A recombinant Ad can be derived from any species of Ad (e.g., A, B, C, D, E, F, or G). In some cases, a recombinant Ad described herein can be derived from an Ad C species (e.g., a human Ad C species (HAd-C)). A recombinant Ad can be derived from any appropriate Ad serotype (e.g., 2, 5, 6, or 57). In some cases, a recombinant Ad described herein can be derived from an Ad serotype 6 (Ad6; e.g., a human Ad6).

[000138] In some cases, a recombinant Ad described herein (e.g., a recombinant Ad having oncolytic anti-cancer activity such as a recombinant Ad657 and variants thereof) can include an Ad genome containing one or more modifications to one or more nucleic acids encoding a polypeptide (or fragments thereof) and/or one or more viral elements of the Ad genome. The one or more modifications can be any appropriate modification. In some cases, a modification can be effective to inhibit the ability of the modified polypeptide to bind another polypeptide such as p300 and/or pRB. In some cases, a modification can be effective to neutralize one or more interferon pathways. Examples of modifications that can be made to a nucleic acid encoding a polypeptide or to a viral element include, without limitation, substitutions, deletions, insertions, and mutations.

[000139] Ads, for example Ad657 and variants thereof, may be modified and retain all E1A genes, or modified to delete selected regions and functions of their encoded proteins.

[000140] Figure 57 shows as schematic of different E3 immune evasion genes in Ad species C exemplar Ad6 and Ad species D exemplar Ad26, as well as a depiction of the functions of these E3 encoded proteins. Both Ads express size and sequence variants of E3 12.5K, 6.7K, 19K, 10.4K (RIDα), 14.5K (RIDβ), and 14.7K genes. 19K reduces display of MHC I and MIC proteins on the cell surface to protect infected cells from T cells and NK cells. RID proteins protect infected cells from death-inducing ligands (FAS, TRAIL, TNFR, and EGFR). 14.7K inhibits intrinsic activation of apoptosis in infected cells. Species C Ads also express the 11.6K known as the adenovirus death protein (ADP). Over-expression of ADP accelerates cell death, but overall cell death is equal. Species D viruses also express two novel variants called 49K and 31K. The secreted form of 49K

binds to CD46 on T cells and NK cells leading to down-regulation of these cells and less-efficient cell killing of cells deficient in class I MHC by NK cells. Ad657 plasmids have been modified to retain all native E3 immune evasion genes (12.5K, 6.7K, 19K, 11.6K (ADP), 10.4K (RIDα), 14.5K (RIDβ), and 14.7K) and E4 34K or to delete selected regions. Ad657 and its variants are also modified with the addition of 49K and 31K to provide these extra functions to these species C viral platforms.

[000141] Ads, for example Ad657 and variants thereof, may be modified to retain all E3 immune evasion genes, or to delete selected regions and functions of their encoded proteins. With respect to E3 mutations: 19k downregulates MHCI and MIC proteins on infected cells; ADP over expression accelerates cell death, but does not increase the number of cells that are killed; 10k and 14k proteins (RIDα and RIDβ) combine to block cell killing by extrinsic apoptosis proteins like FAS, TRAIL, TNF, TNFR, and EGFR; 14.7k protein inhibits intrinsic apoptosis signaling.

[000142] Retaining these E3 proteins may allow oncolytic to persist longer, and deleting them may increase immune stimulation.

[000143] Data testing oncolytic efficacy suggests intact E3 mediates better efficacy.

[000144] DE3 constructs have deleted part of 12.5k through and including 14.7k, DE3A constructs have deleted part of E3 12.5k through and including 19k, and DE3ADP constructs have deleted part of E3 12.5k through and including ADP.

[000145] Surprisingly, deleting all E3 genes makes the oncolytic virus less effective in repressing tumor growth.

[000146] In an embodiment, the invention encompasses single-cycle adenovirus, for example SC-Ad657 and variants thereof. Recombinant SC-Ad viruses with heterologous nucleic acids encoding polypeptides were evaluated for use as a vaccine. SC-Ad657 vaccines by themselves generated significant circulating antibody titers against an HIV envelope protein after only a single immunization.

[000147] Similarly, Ad657 carrying genes for hepatitis B and C antigens is able to generate cytotoxic T lymphocyte (CTL) responses against hepatitis and cytomegalovirus.

highly variable (Figure 17). Despite this, higher ADCC activity was observed in the IN-IN-IN group when compared to control animals ($p < 0.05$ by ANOVA).

Systemic Cellular Immune Responses after One Protein Boost

[000278] Week 38 PBMCs were assayed for T cells against Env and against adenovirus by ELISPOT on samples collected just prior to a second F8 Env protein boost. All Env-immunized animals had Env-specific IFN-γ secreting cells in their PBMCs (Figure 18A). The level of Env-specific IFN-γ SFCs were generally increased in animals that received at least one mucosal immunization. However, IFN-γ SFCs were only significantly higher only in the IN-IN-IN SC-Ad group when they were compared to SC-Ad Ebola immunized control animals ($p<0.05$ by ANOVA). Anti-Ad SFCs were relatively low in all groups when compared to anti-Env SFCs at this time point.

Systemic Cellular Immune Responses after a Second Protein Boost

[000279] At week 40, PBMCs and inguinal lymph node cells were assayed for Env-specific IFN-γ SFCs by ELISPOT (Figure 18B). This protein boost increased Env-specific SFCs in PBMCs and in lymph nodes to similar levels in all of the Env-immunized animals.

Mucosal Cellular Trafficking

[000280] Flow cytometry on rectal biopsy samples at week 40 showed similar numbers of α4β7+ CD4 cells and α4β7+ CD8 cells in rectal sites (Figure 19A). There was a trend towards increasing numbers in the IN primed groups, but these did not reach significance. The numbers of activated CD69+ CD4+ cells in rectal tissues were similar between the groups (Figure 19B). Similarly, FoxP3+ CD4+ cells in this mucosal site were not appreciably different (Figure 19B).

Antigen-specific Tfh Cell Distributions

[000281] CXCR5+ IL-21+ CD4+ T follicular helper (Tfh) cells were measured in PBMCs and lymph node samples at week 40 (Figure 20). The animals that were immunized by Ad and protein by only the IM route had significantly higher peripheral Tfh (pTfh) cells in PBMCs than other groups (Figure 20). In lymph nodes, Tfh cells were lowest in the control and IM only group. In contrast, approximately one half of the animals that received at least one IN mucosal immunization have detectable Tfh in their lymph nodes after the last protein boost (Figure 20).