



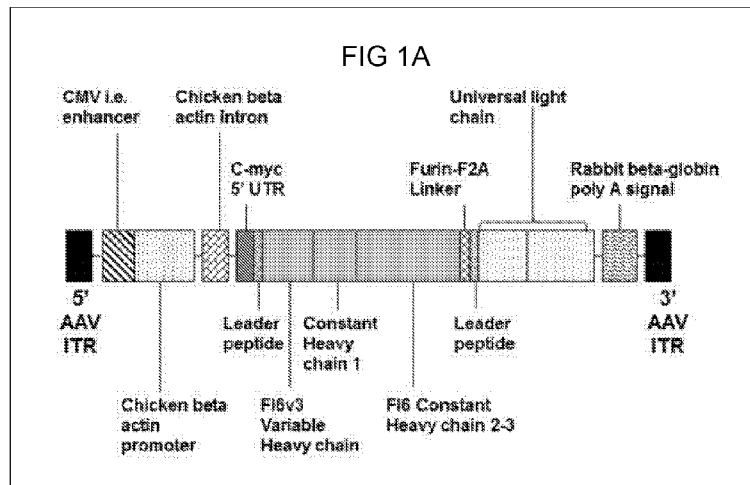
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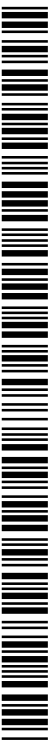
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(54) Title: AAV-MEDIATED EXPRESSION OF ANTI-INLUENZA ANTIBODIES AND METHODS OF USE THEREOF



(57) Abstract: AAV vectors expressing anti-influenza antibodies are provided. Also described are pharmaceutical compositions useful in delivery same for prophylactic or anti-viral purposes. Methods of delivering such vectors are provided.



AAV-MEDIATED EXPRESSION OF ANTI-INFLUENZA ANTIBODIES AND METHODS OF USE THEREOF

5 INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED IN ELECTRONIC FORM

Applicant hereby incorporates by reference the Sequence Listing material filed in electronic form herewith. This file is labeled "UPN_15-7484PCT_ST25.txt".

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STATEMENT OF FEDERALLY SPONSORED RESEARCH

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BACKGROUND OF THE INVENTION

Influenza infections are the seventh leading cause of death in the US, equating to 49,000 deaths per year, a significant proportion of almost 500,000 deaths worldwide [Prevention, C.f.D.C.a. Estimates of deaths associated with seasonal influenza: United States, 1976–2007. 2010; Available from: MMWR Morb. Mortal. Wkly. Rep. 59:1057–1062]. The economic burden of annual influenza epidemics is estimated to be in the order of \$87 billion. More than half of this cost covers the hospital care required for almost 1 million patients, of which 70% are elderly patients (>65 years of age) [Molinari, N.A., et al., Vaccine, 2007. 25(27): p. 5086-96]. In addition, immunocompromised individuals, such as patients with HIV/AIDS, organ transplant recipients, or those suffering from an autoimmune disease are considered high-risks groups for influenza and have an increased susceptibility to infection, as well as its complications, which include fatal pneumonia and acute respiratory distress syndrome [Oliveira, E.C., et al., J Intensive Care Med, 2003. 18(2): p. 80-91.]. Influenza is an RNA virus that belongs to the Orthomyxoviridae family. There are three influenza virus genera; A [Medina, R.A. and A. Garcia-Sastre, Nat Rev Microbiol, 2011. 9(8): p. 590-603.], B [Paul Glezen, W., et al., Am J Public Health, 2013. 103(3): p. e43-51] and C. These types of influenza are categorized based on antigenic differences between the

matrix and nucleoproteins. In the US, influenza A and B are responsible for seasonal epidemics during the winter months. Influenza C is not known to cause epidemics and is associated with mild respiratory disease.

Human-to-human transmission of influenza A or B typically occurs as a result of aerosol or fine droplets that are spread through sneezing or coughing of the infected subject. The influenza virus typically enters the nasal airway passages. There the hemagglutinin (HA) binds to the sialic acid receptors present on respiratory epithelial cells and the viral envelope fuses with the host cell membrane. Subsequently the viral RNA enters the cytosol and eventually the host cell nucleus where it replicates. Following viral replication, the host cell then lyses and several thousand viruses are released. This cycle continues, the virus replicates and eventually disseminates to the lower airways where it causes severe disease that may be fatal in certain high-risk subject groups [Medina, cited above].

While influenza vaccine coverage in the US has increased in the last decade, studies have demonstrated low efficacy of seasonal influenza vaccines in elderly patients and immunocompromised patients [Ljungman, P., Clin Microbiol Infect, 2012. 18 Suppl 5: p. 93-9]. Several aspects of the influenza virus and the immune response of the human host to an influenza infection conspire against a simple prophylaxis remedy. Key targets of the adaptive immune response such as the HA protein of the virus evolve rapidly rendering immune memory responses only partially protective to new infections [Medina, cited above]. The response of humans to a natural infection or an influenza vaccine is usually limited in breadth, providing protection only against closely related influenza subtypes. This has led to annual vaccination of subjects aged 6 months and over against seasonal strains of influenza viruses that are predicted to emerge during the upcoming season.

Influenza A viruses can infect humans and various other mammals including pigs, horses, dogs as well as birds [Medina,cited above]. These viruses are divided into two subtypes based on the structure of the two surface proteins hemagglutinin (HA) and neuraminidase (NA). There are 18 known HA subtypes and 11 known NA subtypes [CDC. Types of Influenza Viruses. 2014; Available from: <http://www.cdc.gov/flu/about/viruses/-types.htm>]. Influenza A has been linked to large epidemics and pandemics, notably the 1918 pandemic that have been associated with significant morbidity and loss of life. Epidemic influenza, also known as seasonal influenza, is typically uncomplicated and remains confined to the upper respiratory tract of only humans [Molinari, cited above; Glezen, cited

above]. Viral pneumonia rarely occurs but susceptible subject populations, including pregnant women, the elderly, patients with pre-existing cardiovascular and lung disease, are considered high risk groups for complications from influenza B. Typically seasonal influenza is considered to result in mild disease.

5 The emergence of a new influenza pandemic remains a threat that could result in substantial loss of life and worldwide economic disruption. It is believed that the repertoire of immune memory generated from previous influenza infections and vaccinations helps to blunt the sequelae of a new infection and augments the efficacy of a vaccine. This is not the case when an influenza virus residing in animal reservoirs acquires a human respiratory
10 tropism and is transmitted to humans [Juno, J., et al., *Clin Dev Immunol*, 2012. 2012: p. 797180]. These zoonotic strains are quite distinct from those that normally circulate in the human population and can lead to pandemics with lethal consequences as they are not effectively controlled by vaccines developed to human strains of the virus [Juno, cited above]. As was learned from the 2009 H1N1 pandemic [Shapshak, P., et al., *Mol Diagn Ther*, 2011. 15(2): p. 63-81], the vaccine development time is not fast enough to support population vaccination in response to an emerging pandemic. Unlike epidemics, influenza pandemics are infrequent but can result in significant loss of life. Pandemic influenza viruses arise from genetic assortment between nonhuman (i.e., avian) and human viruses.
15 Reassortment of segmented viruses is a key mechanism for rapid novel virus creation. These “antigenic shift” events introduce an immunologically novel influenza virus into the human population, to which there is no pre-existing immunity. Two human influenza pandemics in the last century were linked to lineages that arose from reassortment of genomic segments with a genome of nonhuman origin. During the most recent pandemic (2009), younger people were disproportionately affected by lower respiratory tract disease requiring
20 hospitalization, relative to inter-pandemic years [Dawood, F.S., et al., *Lancet Infect Dis*, 2012. 12(9): p. 687-95.].

 Influenza vaccines are not always effective at protecting from influenza. In early 2013, an influenza outbreak was reported in a vaccinated US Navy minesweeper population of 102 young (21-44 years of age) healthy men. Almost 25% of these vaccinated subjects
30 presented with influenza symptoms that required medical care. PCR analysis showed that the influenza strain was H3N2 and shared 99% homology to the strains circulating in the 2013-2014 influenza season, similar to the H3N2 antigenic component of the 2013-2014 influenza

vaccine [T. L. Aquino, et al., Influenza Outbreak in a Vaccinated Population — USS Ardent. *mmwr* 63(42);947-949 2014].

Two monoclonal antibodies have emerged as candidates as prophylactics for influenza. For influenza A, FI6 [Oliveira, et al, cited above] that was discovered by the Lanzavecchia group [US2010/0080813] and for influenza B, CR8033 [Dreyfus, C., et al., Science, 2012. 337(6100): p. 1343-8; US Patent 8,852,595] an antibody discovered by the Wilson (The Scripps Research Institute) and Friesen (Crucell) groups. FI6 has been reported to be highly effective against several strains of influenza A (including pandemic strains A/H1N1/1918 and A/H1N1/2009) and has a broad and potent reactivity and neutralizing profile across all Group 1 and Group 2 HAs [Corti, D., et al., Science, 2011. 333(6044): p. 850-6]. Importantly, these Abs have been shown not to generate escape mutants even after multiple serial passages [Oliveira, cited above; Paul Glezen cited above]. It has been reported that when this antibody was delivered via a vector it protected mice and ferrets from lethal challenge from multiple influenza A strains [Limberis, M.P., et al., *Sci Transl Med*, 2013. 5(187): p. 187ra72; Limberis, M.P., et al., *Clin Vaccine Immunol*, 2013. 20(12): p. 1836-7; Adam et al. *Clin Vaccine Immunol*. 2014 Nov;21(11):1528-33;].

There is one universal antibody, CR9114 [Dreyfus., C. et al, Science, 2012, 337(6100): p1343-8], that binds to both Group 1 and Group 2 influenza A and influenza B viruses. However, the utility of this antibody is hampered by the relatively high antibody concentrations required for neutralization of the various influenza A and B strains. The IC₅₀ for Group 1 viruses ranged from 0.1 to 100 µg/ml, with only two strains falling between 0.1 and 1 µg/ml, and the remaining between 1 and 100 µg/ml. The IC₅₀ for Group 2 viruses was even less favorable, with just one strain falling below 1 µg/ml [Dreyfus, cited above]. Relatively high concentrations of CR8071 were required for *in vivo* protection against influenza B and suboptimal doses resulted in higher weight loss compared to non-immunized mice (Figure S2, and escape mutants were generated after just 15 passages [Dreyfus, cited above]. Another Influenza B antibody CR8020 resulted in generation of escape mutants after just four passages [Dreyfus, cited above].

There is currently one approved therapy (FluMist Quadrivalent) which is delivered intranasally. However, it is not suitable for subjects that have a severe allergy to eggs and subjects who are 2 through 17 and take aspirin or medicines taking aspirin [Shapiro RJ, S.K., et al. "The potential American market for generic biological treatments and the associated

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cost savings". 2008; Available from: http://www.sonecon.com/docs/studies-/0208_Generic-BiologicsStudy.pdf].

Adeno-associated viruses (AAVs) are members of the family parvoviridae. These small DNA viruses have shown substantial promise as vectors for achieving stable transgene
5 expression following *in vivo* delivery. AAVs were initially discovered as contaminants in laboratory preparations of adenovirus [Melnick, J.L., et al., Association of 20-Millimicron Particles with Adenoviruses. J Bacteriol, 1965. 90(1): p. 271-4]. Immunological characterization of these isolates suggested the existence of six serotypes of AAV. Sero-epidemiologic studies indicate a broad exposure of humans to the various AAV serotypes,
10 with greater than 60% of the population demonstrating NABs to most of the six AAV serotypes by the age of 10 [Calcedo, R., et al., J Infect Dis, 2009. 199(3): p. 381-90]. In the early 2000s, the repertoire of AAV vectors was expanded through the isolation of several hundred new AAV viruses from human and non-human primates [Gao, G., et al., J Virol, 2004. 78(12): p. 6381-8]. More than 120 genotypes of AAV vectors, including the original
15 six serotypes from human and NHP tissue sources were isolated, characterized phylogenetically and organized in six different clades [Gao, G., et al., J Virol, 2004. 78(12): p. 6381-8].

There remains a need in the art for anti-influenza therapies effective for therapeutic and/or prophylactic use.

20

SUMMARY OF THE INVENTION

In one aspect the invention provides a composition useful for passive immunization against influenza infection. The composition comprises an anti-influenza A antibody expressed from an AAV vector and an anti-influenza B antibody expressed from a second
25 AAV vector. In one embodiment, a first non-replicating recombinant AAV has an AAV9 capsid (rAAV9) and a vector genome which comprises nucleic acid sequences encoding: (a) an AAV inverted terminal repeat (ITR) (b) an enhancer, (b) a chicken beta-actin promoter, (c) an intron (d) a 5' UTR, (e) a leader peptide operably linked to a F16v3 heavy chain, (f) a F16v3 heavy variable chain; (g) human IgG1 Fc chain (CH2-3); (h) a leader peptide operably
30 linked to a immunoglobulin kappa light variable chain; (i) an immunoglobulin constant light chain; (j) a furin recognition site; (k) an F2A linker; (l) a polyadenylation signal; and (m) an AAV inverted terminal repeat. In certain embodiments, the composition contains a second

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non-replicating rAAV9 which has a vector genome which comprises nucleic acid sequences encoding: (a) an AAV inverted terminal repeat (ITR) (b) an enhancer, (b) a chicken beta-actin promoter, (c) an intron (d) a 5' UTR, (e) a leader peptide operably linked to a CR8033 heavy chain, (f) a CR8033 heavy variable chain; (g) a human IgG1 Fc chain (CH2-3); (h) a leader peptide operably linked to an immunoglobulin light variable kappa chain; (i) a constant light chain cDNA; (j) a furin recognition site; (k) an F2A linker; (l) a polyadenylation signal; and (m) an AAV inverted terminal repeat, and an aqueous liquid suspension base.

In another aspect, a method for protecting human patients against influenza is provided which involves administering an effective amount of an anti-influenza composition as provided herein. Suitably, the patient is administered a dose in an amount of about 1×10^{10} to about 3×10^{13} genome copies. In certain embodiments, the composition is administered intranasally. In other embodiments, the composition is administered intramuscularly or intravenously.

In a further aspect, a product is provided which comprises a container comprising an anti-influenza composition as described here in, an optional diluent, and instructions for administration.

In still a further aspect, the invention provides a method of preventing influenza comprising administering a vector expressing a synthetic anti-influenza antibody as described herein. Such a therapy may be in combination with other vectors expressing different antibodies, or other anti-viral compositions. Optionally, the method may be used as a vaccine, i.e., prior to influenza exposure.

Still other aspects and advantages of the invention will be readily apparent from the following detailed description of the invention.

25

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs 1A-1B provide as a schematic representation of the GTP101 vector components. FIG 1A is a schematic representation of AAV-FI6 mAb and FIG 1B is a representation of AAV-CR8033 mAb.

FIG 2 is a flow diagram providing an overview of the manufacturing process.

FIGs 3A and 3B provide the determination of the minimum effective dose (MED) of AAV2/9.CB7.FI6 required for protection against challenge with PR8. (A) Weights of mice

treated intranasal (IN or i.n.) with varying doses of AAV2/9.CB7.FI6 following challenge with 5LD₅₀ PR8. (B) Survival of challenged mice. Mice were euthanized when they appeared in distress or their body weight declined $\geq 30\%$.

FIGs 4A and 4B show the determination of the FI6 expression at the lung (BALF) and nasal (NLF) surfaces. Mice were given IN various doses of AAV2/9.CB7.FI6 and the level of antibody expressed in the surface liquid lining the lung and nose harvested at necropsy was assessed. FIG 4A provides results for FI6 expression in BALF and FIG 4B provides results for FI6 expression in NLF surfaces.

FIG 5 shows quantification of viral load in lung of naïve and mice vaccinated with AAV2/9.CB7.FI6. Three mice from the non-vaccinated (naïve) and the AAV2/9.CB7.FI6 vaccinated groups were necropsied at day 6 to quantify viral load in the lung.

FIG 6A and FIG 6B show rapid onset of protection against influenza challenge. 1×10^{11} GC of AAV2/9.CB7.FI6 was delivered IN in BALB/c mice and 8 hours later mice were challenged with 5LD₅₀ of PR8. FIG 6A shows weights of mice following challenge at day 0. FIG 6B shows survival of challenged mice. Mice were euthanized when they appeared in distress or their body weight declined $\geq 30\%$.

FIG 7A and FIG 7B show the determination of the MED of AAV2/9.CB7.CR8033 required for protection against challenge with B/Lee/40. FIG 7A shows weights of mice pretreated IN with varying doses of AAV2/9.CB7.CR8033 following challenge with 5LD₅₀ B/Lee/40. FIG 7B shows survival of challenged mice. Mice were euthanized when they appeared in distress or their body weight declined $\geq 30\%$.

FIG 8 shows the effect of mixing two AAV vectors on protection against challenge with influenza A (PR8). Mice were dosed with either 1×10^9 GC of AAV2/9.CB7.FI6 (triangles) or with a mixture of 1×10^9 GC of AAV2/9.CB7.FI6 and 1×10^9 GC of AAV2/9.CB7.CR8033 (squares). Mice were challenged 14 days later with 5LD₅₀ PR8 (influenza A). Mice were euthanized when they appeared in distress or their body weight declined $\geq 30\%$.

FIG 9 shows the effect of mixing two AAV vectors on protection against challenge with influenza B. Mice were dosed with either 1×10^9 GC of AAV2/9.CB7.CR8033 (triangles) or with a mixture of 1×10^9 GC of AAV2/9.CB7.CR8033 and 1×10^9 GC AAV2/9.CB7.FI6 (squares). Mice were challenged 14 days later with 5LD₅₀ B/Lee/40

(influenza B). Mice were euthanized when they appeared in distress or their body weight declined $\geq 30\%$.

FIG 10 shows levels of serum-circulating AAV2/9-specific NAb. Mice were given intranasal (i.n.) AAV2/9 vector at doses ranging from 1×10^9 to 1×10^{11} GC/mouse and
5 AAV2/9 neutralizing antibody in serum assayed at day 28 following AAV2/9 delivery.

FIGS 11A-11G shows vector-mediated prophylaxis against challenge with influenza A (PR8).. 6 week old female BALB/c mice (n=5/group) were given i.n. a mixture of 1×10^9 GC of AAV2/9.CB7.CR8033 and 1×10^9 GC of AAV2/9.CB7.FI6 in a total volume of 50 μ l PBS. The vector was formulated in three different solutions: PBS-pH 6.8, PBS-pH 7.2 or
10 PBS-pH 7.4. Mice were challenged with 5LD₅₀ of PR8 7 days after vector administration. The weights of the animals were recorded daily and mice were euthanized when they appeared in distress or when they lost $\leq 30\%$ of their pre-challenge body weight. Mice were given i.n. 1×10^9 GC AAV2/9.CB7.CR8033 and 1×10^9 GC of AAV2/9.CB7.FI6 in PBS-pH 6.8 (FIG11A), PBS-pH 7.2 (FIG 11D or PBS-pH 7.4 (FIG 11G and challenged with PR8
15 seven days later. FIGS 11A, 11D and 11G show the weights of mice overtime; black circles represent mice that received the vector via a pipette (liquid delivery), dark grey squares represent mice that received vector that was processed through the IMAD and light grey triangles represent mice that did not receive vector (naïve mice) and served as controls for the PR8 challenge. The naïve mice were euthanized by day 8 due to severe weight loss. At
20 the conclusion of the experiment (day 21), the survivors were sacrificed, BALF harvested for antibody expression and lungs processed for quantification of AAV9 genomes by Taqman PCR. FIGS 11B, 11E, and 11H show antibody expression by protein A ELISA was quantified in the BALF of mice given a mixture of 10^9 GC each of AAV2/9.CB7.CR8033 and AAV2/9.CB7.FI6 formulated in PBS-pH 6.8, PBS-pH 7.2. and PBS-pH 7.4,
25 respectively. Results are shown in ng/mL for the device (IMAD) and pipette control (liquid). FIG 11C, FIG 11F, and FIG 11-I show AAV9 genomes quantified by Taqman PCR in the lung of mice given a mixture of 10^9 GC each of AAV2/9.CB7.CR8033 and AAV2/9.CB7.FI6 formulated in PBS-pH 6.8, PBS-pH 7.2. and PBS-pH 7.4, respectively.

FIGS 12A-12G show vector-mediated prophylaxis against challenge with PR8 and
30 influenza B (B/Lee/40) virus. 6 week old female BALB/c mice (n=5/group) were given i.n. a mixture of 1×10^9 genome copies (GC) of AAV2/9.CB7.CR8033 and 1×10^9 GC of AAV2/9.CB7.FI6 in a total volume of 50 μ l PBS. The vector was formulated in PBS-pH 7.4.

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Mice were challenged with 5LD₅₀ of PR8 (FIG 12A) or B/Lee/40 (FIG 12D) virus seven days later. The weights of the animals were recorded daily and mice were euthanized when they appeared in distress or when they lost $\leq 30\%$ of their pre-challenge body weight. Shown are the weights of mice challenged with PR8 (FIG 12A) or B/Lee/40 (FIG 12D) overtime;

5 black circles represent mice that received the vector via a pipette (liquid delivery), dark grey squares represent mice that received vector that was processed through the IMAD and light grey triangles represent mice that did not receive vector (naïve mice) and served as controls for the PR8 challenge. With the exception of one survivor, naïve mice (4 of 5) were euthanized by day 8. At the conclusion of the experiment (day 21), the survivors were

10 sacrificed, BALF harvested for antibody expression and lungs processed for quantification of AAV9 genomes by Taqman PCR. FIG 12B shows antibody expression by protein A ELISA quantified in the BALF and FIG 12C shows AAV9 genomes present in the lung quantified by Taqman PCR in mice given a mixture of 10^9 GC each of AAV2/9.CB7.CR8033 and AAV2/9.CB7.FI6 formulated in PBS-pH 7.4 and challenged with PR8. FIG 12E shows

15 antibody expression in the BALF quantified by protein A ELISA and FIG 12F shows AAV9 genomes present in the lung quantified by Taqman PCR in mice given a mixture of 1×10^9 GC each of AAV2/9.CB7.CR8033 and AAV2/9.CB7.FI6 formulated in PBS-pH 7.4 and challenged with B/Lee/40. FIG 12G shows the amount of vector suspension (0.5mL) plunged through the IMAD to evaluate physical loss of vector solution. As shown, a physical

20 liquid loss of 4 - 6.6% of initial volume was observed when the vector solution was plunged through the IMAD.

DETAILED DESCRIPTION OF THE INVENTION

A composition is provided herein which is a bipartite drug product composed of two

25 non-replicating recombinant adeno-associated virus (AAV) vectors of serotype 9 expressing recombinant antibodies which confer passive immunization against influenza A and influenza B, respectively.

The composition provided herein has several advantages over currently available influenza vaccines. The anti-influenza A and anti-influenza B antibody constructs co-

30 expressed in vivo provide passive immunization against influenza A and influenza B infection. In other words, in contrast to a traditional vaccine which delivers an influenza antigen and relies upon the patient's immune system to mount an immune response and

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generate antibodies, the composition provided herein delivers the anti-influenza antibodies to the patient. Thus, the vaccine is useful for patients who have immune systems which are not capable of generating a satisfactory protective immune response following immunization with an influenza antigen. Further, the composition provided herein may provide a more rapid onset of protection post-administration than an antigen-based vaccine approach. Other advantages include the fact that the composition may be delivered intranasally; thus, the approach is minimally invasive and is without the risk of mild infection or other side effects associated with delivery of current intranasal vaccines having an attenuated virus. Still another advantage is that the composition is useful for patients having egg allergies. Nor is the composition contraindicated for patients taking aspirin or medicines containing aspirin. Further, the rAAV compositions provided herein substantially reduce the number of repeated parental administrations which are required for protein therapeutics to be effective. In certain embodiments, readministration is required only about 1 time per year following delivery to nasal epithelial cells. In still another advantage, the compositions provided herein may be produced in a shorter time frame than many traditional viral-based influenza vaccines. Thus, the composition of the invention provides a more practical method for protecting at-risk populations.

In certain embodiments, the vectors provided herein express effective levels of functional antibody when delivered intranasally a dose of about 3 mL or less, 2 mL or less, or 1 mL or less, 0.5 mL, or less, e.g., in the range of about 100 μ L to 250 μ L. In general, the compositions are formulated at a pH of about 5.5 to about 8.5, 6 to 8, or 6.5 to 7.5, or 6.8, 7.2, or 7.4. Thus, the vectors provided herein are highly efficient at providing therapeutic levels of antibody at doses which are convenient for metered doses, or in products or kits containing pre-measured doses. In certain embodiments, the compositions are formulated for intranasal delivery in a manner that the volume and droplet size of the spray preferentially targets the cells of the intranasal epithelium. Expression in the nasal epithelium has been shown to confer protective passive immunity. The compositions may also be formulated for intranasal delivery which targets the lung. The lung may be targeted in addition to the nasal epithelium, or preferentially to the nasal epithelium, e.g., by adjusting the volume and/or droplet size delivered intranasally. When compared to conventional flu vaccine delivered intramuscularly, AAV delivered intranasally results in faster onset of Ab expression (within hrs or a couple of days). However, in certain embodiments, the

compositions described herein may be formulated for delivery by other routes, e.g., intramuscular injection, intravenous injection, or other suitable routes. In such instances, suitable formulations and volumes may be determined by one of skill in the art.

5 Antibody Constructs

In certain embodiments, a composition provided herein delivers two influenza antibodies which are expressed in a cell *in vivo* via a non-replicating viral vector and secreted therefrom to provide protective immunity. As illustrated in the examples herein, the viral vector is an rAAV and the composition contains two different rAAV stocks, each of which has a different vector genome. In a first stock, the vector genome contains the coding sequences for an influenza A antibody. In a second stock, the vector genome contains the coding sequences for an influenza B antibody.

The influenza A antibody is a synthetic FI6 construct. See, e.g., US Patent Publication No. 2010/0080813, for amino acid sequences of the FI6v3 heavy chain, light chain variable chain, variable regions, and complementarity determining regions. See, also, US Patent 8,124,092. In certain embodiments, the FI6v3 expression cassette is constructed encoding the FI6 light variable regions of FI6 (GenBank Accession Numbers PDB: AEL31310.1, GI: 342674599 and 342674581), linked to the constant (CH1, CH2 and CH3) domains of human IgG1 and light chain germline kappa sequence linked to human CL domain, generating a monoclonal antibody architecture. In another embodiment, the FI6 expression cassette contains the full-length heavy chain of FI6v3 (see, nt 2440-2760 (CH1), nt2761-3426 (CH2-CH3) of SEQ ID NO:1). The cDNA encoding an illustrative light chain is provided at nt 3511 - 3909 of SEQ ID NO: 1. (leader nt 3511-3510, kappa chain nt 3571-3909) In still another embodiment, the synthetic FI6 construct contains, at a minimum, the FI6v3 heavy chain variable region, heavy chain constant regions CH1, CH2 and CH3), and an immunoglobulin light chain.

In certain embodiments, the nucleic acid sequence encoding the immunoglobulin domains which compose the anti-influenza A antibody are designed for expression in human cells. Such sequences may include, e.g., FI6v3 heavy chain variable region (nt 2053-2439 of SEQ ID NO:1) and constant regions CH2 and CH3 (nt 2761-3426 of SEQ ID NO:1). However, other coding sequences for the FI6v3 amino acid sequences provided herein may be engineered and are encompassed by the present invention. See, e.g., other sequences

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encoding the heavy chain variable region (e.g., amino acids of SEQ ID NO:2), the CH1 (e.g., amino acids of SEQ ID NO:3), and/or the CH2-CH3 (e.g., amino acids of SEQ ID NO:4). See, also, SEQ ID NO: 14, 15, 16, 17, 18, 19, 20, 21 22, or 23.

In certain embodiments, the “variable” light chain is a kappa chain derived from a
5 germ line source. For example, the examples provided herein utilize IGHV4-1*01
germline gene [Homo sapiens] (see, e.g., nt 3571-3909 of SEQ ID NO:1 or nt 3265-3606 of
SEQ ID NO:8). Optionally, another nucleic acid sequence encoding the amino acid sequence
of the kappa light chain (SEQ ID NO: 5) and/or constant light chain (SEQ ID NO: 7) may be
selected. In another alternative, another suitable germline sequence may be selected. In
10 another embodiment, the light chain is a lambda chain. Preferably, a germline sequence
which does not alter antigenic specificity of the heavy chain partner is selected as the source
of the light chain. Sources of such immunoglobulin germline sequences are provided, e.g.,
in Kabat database, www.ncbi.nlm.nih.gov/ and
[http://www.imgt.org/genedb/table?mode=3d&selectGenes=IGKV4-](http://www.imgt.org/genedb/table?mode=3d&selectGenes=IGKV4-1&selectSpecies=Homo%20sapiens)
15 [1&selectSpecies=Homo%20sapiens](http://www.imgt.org/genedb/table?mode=3d&selectGenes=IGKV4-1&selectSpecies=Homo%20sapiens).

The influenza B antibody is a synthetic CR8033 antibody. See, e.g., US Patent
8,852,595, for amino acid sequences of the CR8033 heavy chain, light chain, variable
regions, and complementarity determining regions. The antibody has a heavy chain which is
an engineered sequence derived from the CR8033 anti-influenza antibody. In one
20 embodiment, the engineered antibody provided herein contains a codon optimized heavy
chain variable (e.g., nt 1753-2133 of SEQ ID NO:8), CH1 (e.g., nt 2134-2454 of SEQ ID
NO:8), and CH2-CH3 (e.g., nt 2455-3120 of SEQ ID NO:8). In certain embodiments, the
CH1 region may be omitted. In other embodiments, different nucleic acid sequences
encoding the heavy chain variable region (e.g., SEQ ID NO:9), CH1 (e.g., SEQ ID NO:10),
25 and/or CH2-3 (e.g., SEQ ID NO: 11) may be selected.

In still other embodiments, the light chains may be derived from the F16 antibody.
See, e.g., Corti et al, Science, 2011 Aug 12; 333(6044):860-6, Epub 2011 Jul 28.

In certain embodiments, a synthetic F16 antibody construct provided herein may be
expressed *in vitro* and used, e.g., a protein therapy or for generating anti-idiotypic antibodies.
30 This antibody construct is composed of, at a minimum, an F16 heavy chain combination
with a light chain from a germline source.

In other embodiments, a synthetic CR8033 antibody construct provided herein may be expressed in vitro and used, e.g., in a protein therapy or for generating anti-idiotypic antibodies. This antibody construct is composed of, at a minimum, a CR8033 heavy chain in combination with a light chain from a germline source.

5 Such expression methods and uses are known in the art.

Vector Genomes

In order to express a selected immunoglobulin domain, a nucleic acid molecule may be designed which contains codons which have been selected for optimal expression of the immunoglobulin polypeptides in a selected mammalian species, e.g., humans. Further, the
10 nucleic acid molecule may include a heterologous leader sequence for each heavy chain and light chain of the selected antibody, which encodes the IL-2 signal leader peptide fused upstream of the heavy and chain polypeptides composed of the variable and constant regions. However, another heterologous leader sequence may be substituted for one or both of the IL-
15 2 signal/leader peptide. Signal/leader peptides may be the same or different for each the heavy chain and light chain immunoglobulin constructs. These may be signal sequences which are natively found in an immunoglobulin (e.g., IgG), or may be from a heterologous source. Such heterologous sources may be a cytokine (e.g., IL-2, IL12, IL18, or the like), insulin, albumin, β -glucuronidase, alkaline protease or the fibronectin secretory signal
20 peptides, or sequences from tissue specific secreted proteins, amongst others.

As used herein, an "expression cassette" refers to a nucleic acid sequence which comprises at least a first open reading frame (ORF) and optionally a second ORF. An ORF may contain two, three, or four antibody domains. For example, the ORF may contain a full-length heavy chain. Alternatively, an ORF may contain one or two antibody domains. For
25 example, the ORF may contain a heavy chain variable domain and a single heavy chain constant domain. In another example, the ORF may contain a light chain variable and a light chain constant region. Thus, an expression cassette may be designed to be bicistronic, i.e., to contain regulatory sequences which direct expression of the ORFs thereon from shared regulatory sequences. In this instance, the two ORFs are typically separated by a linker.
30 Suitable linkers, such as an internal ribozyme binding site (IRES) and/or a furin-2a self-cleaving peptide linker (F2a) [see, e.g., Radcliffe and Mitrophanous, Gene Therapy (2004), 11, 1673-1674] are known in the art. Suitably, the ORF are operably linked to regulatory

control sequences which direct expression in a target cell. Such regulatory control sequences may include a polyA, a promoter, and an enhancer. In order to facilitate co-expression from an AAV vector, at least one of the enhancer and/or polyA sequence may be shared by the first and second expression cassettes.

5 In one embodiment, the rAAV has packaged within the selected AAV capsid, a nucleic acid molecule comprising: an expression cassette comprising: a 5' AAV inverted terminal repeat sequence (ITR), a promoter, a 5' UTR, an optional Kozak sequence, a first signal peptide operably linked to a first immunoglobulin chain comprising a heavy chain, a linker sequence, a second signal peptide operably linked to a second immunoglobulin chain,
10 and a 3' AAV ITR wherein one of and the second immunoglobulin is an immunoglobulin light chain, wherein said expression cassette co-expresses the immunoglobulin chains in a host cell under conditions which permit the chains to assemble into a functional antibody construct having the specificity of the antibody providing the heavy chain. In one embodiment, the heavy chain is a synthetic anti-influenza FI6v3 heavy chain
15 immunoglobulin and the light chain is a kappa light chain from a germline.

 In one embodiment, the expression cassette comprises AAV ITRs from a source different than the AAV capsid to form a pseudotyped AAV. In one embodiment, the expression cassette further comprises a constitutive promoter and an RGB polyA. Other suitable vector elements such as promoters and polyA sequences may be selected. For
20 example, a minimal promoter and/or a minimal polyA may be selected in order to conserve space. Typically, in this embodiment, each promoter is located either adjacent (either to the left or the right (or 5' or 3')) to the enhancer sequence and the polyA sequences are located adjacent to the ITRs, with the ORFs there between. While it is preferred to express the heavy chain sequences first, the order of the ORFs may be varied, as may the
25 immunoglobulin domains encoded thereby. For example, the light chain constant and variable sequences may be located to the left of the enhancer and the heavy chain may be encoded by ORFs located to the right of the enhancer. Alternatively, the heavy chain may be located to the left of the enhancer and the ORFs to the right of the enhancer by encode a light chain. Alternatively, the opposite configuration is possible.

30 In another embodiment, the rAAV has packaged within the selected AAV capsid, a nucleic acid molecule comprising: a 5' AAV inverted terminal repeat sequence (ITR), a promoter, a 5' UTR, an optional Kozak sequence, an IL-2 signal peptide operably linked to

an Fc6 immunoglobulin heavy chain, an F2a, an IL-2 signal peptide operably linked to a germline kappa light chain, and a 3' AAV ITR. In one embodiment, the AAV capsid is AAV9 or AAV8. In a further embodiment, the ITRs are from AAV2, or a different source which is different from the AAV capsid source.

5 Suitable regulatory control sequences may be selected and obtained from a variety of sources. In one embodiment, a minimal promoter and/or a minimal polyA may be utilized to conserve size.

 As used herein, the term “minimal promoter” means a short DNA sequence comprised of a TATA-box and other sequences that serve to specify the site of transcription
10 initiation, to which regulatory elements are added for control of expression. In one embodiment, a promoter refers to a nucleotide sequence that includes a minimal promoter plus regulatory elements that is capable of controlling the expression of a coding sequence or functional RNA. This type of promoter sequence consists of proximal and more
15 distal upstream elements, the latter elements often referred to as enhancers. In one embodiment, the minimal promoter is a Cytomegalovirus (CMV) minimal promoter. In another embodiment, the minimal promoter is derived from human CMV (hCMV) such as the hCMV immediate early promoter derived minimal promoter (see, US 20140127749, and Gossen and Bujard (Proc. Natl. Acad. Sci. USA, 1992, 89: 5547-5551), which are
20 incorporated herein by reference). In another embodiment, the minimal promoter is derived from a viral source such as, for example: SV40 early or late promoters, cytomegalovirus (CMV) immediate early promoters, or Rous Sarcoma Virus (RSV) early promoters; or from eukaryotic cell promoters, for example, beta actin promoter (Ng, Nuc. Acid Res. 17:601-615, 1989; Quitsche et al., J. Biol. Chem. 264:9539-9545, 1989), GAPDH promoter (Alexander, M. C. et al., Proc. Nat. Acad. Sci. USA 85:5092-5096, 1988, Ercolani, L. et al., J. Biol.
25 Chem. 263:15335-15341, 1988), TK-1 (thymidine kinase) promoter, HSP (heat shock protein) promoters, UbB or UbC promoter, PGK, Efl1-alpha promoter or any eukaryotic promoter containing a TATA box (US Published Application No. 2014/0094392). In another embodiment, the minimal promoter includes a mini-promoter, such as the CLDN5 mini-promoter described in US Published Application No. 2014/0065666. In another
30 embodiment, the minimal promoter is the Thymidine Kinase (TK) promoter. In one embodiment, the minimal promoter is tissue specific, such as one of the muscle-cell specific promoters minimal TnISlow promoter, a minimal TnIFast promoter or a muscle creatine

kinase promoter (US Published Application No. 2012/0282695). Each of these documents is incorporated herein by reference.

5 A recombinant AAV vector (AAV viral particle) may comprise, packaged within an AAV capsid, a nucleic acid molecule expressing a functional antibody as described in this specification. An expression cassette may contain regulatory elements for an open reading frame(s) within each expression cassette and the nucleic acid molecule may optionally contain additional regulatory elements.

10 The AAV vector may contain a full-length AAV 5' inverted terminal repeat (ITR) and a full-length 3' ITR. A shortened version of the 5' ITR, termed Δ ITR, has been described in which the D-sequence and terminal resolution site (*trs*) are deleted. The abbreviation "sc" refers to self-complementary. "Self-complementary AAV" refers a construct in which a coding region carried by a recombinant AAV nucleic acid sequence has been designed to form an intra-molecular double-stranded DNA template. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary
15 halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. *See, e.g.,* D M McCarty *et al.*, "Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis", *Gene Therapy*, (August 2001), Vol 8, Number 16, Pages 1248-1254. Self-complementary AAVs are described in, *e.g.,* U.S. Patent
20 Nos. 6,596,535; 7,125,717; and 7,456,683, each of which is incorporated herein by reference in its entirety.

Where a pseudotyped AAV is to be produced, the ITRs are selected from a source which differs from the AAV source of the capsid. For example, AAV2 ITRs may be selected for use with an AAV capsid having a particular efficiency for a selected cellular
25 receptor, target tissue or viral target. In one embodiment, the ITR sequences from AAV2, or the deleted version thereof (Δ ITR), are used for convenience and to accelerate regulatory approval. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. However, other sources of AAV ITRs may be utilized.

30 A variety of AAV capsids have been described. Methods of generating AAV vectors have been described extensively in the literature and patent documents, including, *e.g.,* WO 2003/042397; WO 2005/033321, WO 2006/110689; US 7588772 B2. The source of AAV

capsids may be selected from an AAV which targets a desired tissue. For example, suitable AAV may include, e.g., AAV9 [US 7,906,111; US 2011-0236353-A1], rh10 [WO 2003/042397] and/or hu37 [see, e.g., US 7,906,111; US 2011-0236353-A1]. However, other AAV, including, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, 5 [US Patent 7790449; US Patent 7282199] and others. However, other sources of AAV capsids and other viral elements may be selected, as may other immunoglobulin constructs and other vector elements.

Methods for generating and isolating AAV viral vectors suitable for delivery to a subject are known in the art. See, e.g., US Patent 7790449; US Patent 7282199; WO 10 2003/042397; WO 2005/033321, WO 2006/110689; and US 7588772 B2]. In one system, a producer cell line is transiently transfected with a construct that encodes the transgene flanked by ITRs and a construct(s) that encodes rep and cap. In a second system, a packaging cell line that stably supplies rep and cap is transiently transfected with a construct encoding the transgene flanked by ITRs. In each of these systems, AAV virions are produced in 15 response to infection with helper adenovirus or herpesvirus, requiring the separation of the rAAVs from contaminating virus. More recently, systems have been developed that do not require infection with helper virus to recover the AAV - the required helper functions (*i.e.*, adenovirus E1, E2a, VA, and E4 or herpesvirus UL5, UL8, UL52, and UL29, and herpesvirus polymerase) are also supplied, *in trans*, by the system. In these newer systems, 20 the helper functions can be supplied by transient transfection of the cells with constructs that encode the required helper functions, or the cells can be engineered to stably contain genes encoding the helper functions, the expression of which can be controlled at the transcriptional or posttranscriptional level. In yet another system, the transgene flanked by ITRs and rep/cap genes are introduced into insect cells by infection with baculovirus-based 25 vectors. For reviews on these production systems, see generally, e.g., Zhang *et al.*, 2009, "Adenovirus-Adeno-associated virus hybrid for large-scale recombinant adeno-associated virus production," Human Gene Therapy 20:922-929, the contents of each of which is incorporated herein by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following U.S. patents, the contents of 30 which is incorporated herein by reference in its entirety: 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065.

In one embodiment, the polyadenylation (poly(A)) signal is a minimal poly(A) signal, i.e., the minimum sequence required for efficient polyadenylation. In one embodiment, the minimal poly(A) is a synthetic poly(A), such as that described in Levitt et al, *Genes Dev.*, 1989 Jul, 3(7):1019-25; and Xia et al, *Nat Biotechnol.* 2002 Oct; 20(10):1006-10. Epub 2002 Sep 16. In another embodiment, the poly(A) is derived from the rabbit beta-globin poly(A). In one embodiment, the polyA acts bidirectionally (An et al, 2006, *PNAS*, 103(49): 18662–18667. In one embodiment, the poly(A) is derived from the SV40 early poly A signal sequence. Each of these documents is incorporated herein by reference.

10 Optionally, a single enhancer, or the same enhancer, may regulate the transcription of multiple heterologous genes in the plasmid construct. Various enhancers suitable for use in the invention are known in the art and include, for example, the CMV early enhancer, Hoxc8 enhancer, nPE1 and nPE2. Additional enhancers useful herein are described in Andersson et al, *Nature*, 2014 March, 507(7493):455-61, which is incorporated herein by reference. Still
15 other enhancer elements may include, *e.g.*, an apolipoprotein enhancer, a zebrafish enhancer, a GFAP enhancer element, and tissue specific enhancers such as described in WO 2013/1555222, woodchuck post hepatitis post-transcriptional regulatory element. Additionally, or alternatively, other, *e.g.*, the hybrid human cytomegalovirus (HCMV)-immediate early (IE)-PDGR promoter or other promoter - enhancer elements may be
20 selected. To enhance expression the other elements can be introns (like promega intron or chimeric chicken globin-human immunoglobulin intron). Other enhancers useful herein can be found in the Mammalian Promoter/Enhancer Database found at <http://promoter.cdb.riken.jp/>.

The constructs described herein may further contain other expression control or
25 regulatory sequences such as, *e.g.*, include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence; sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product.
30 In the working examples below, the Kozak sequence used is: CCACCATG (nt (1988)..(1992) of SEQ ID NO:1); however, other suitable sequences may be selected. A promoter may be selected from amongst a constitutive promoter, a tissue-specific promoter,

a cell-specific promoter, a promoter responsive to physiologic cues, or a regulatable promoter [see, e.g., WO 2011/126868 and WO 2013/049492].

These control sequences are “operably linked” to the immunoglobulin construct gene sequences. As used herein, the term “operably linked” refers to both expression control
5 sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

Examples of constitutive promoters suitable for controlling expression of the antibody domains include, but are not limited to chicken β -actin (CB) or beta actin promoters from other species, human cytomegalovirus (CMV) promoter, the early and late
10 promoters of simian virus 40 (SV40), U6 promoter, metallothionein promoters, EFla promoter, ubiquitin promoter, hypoxanthine phosphoribosyl transferase (HPRT) promoter, dihydrofolate reductase (DHFR) promoter (Scharfmann *et al.*, Proc. Natl. Acad. Sci. USA 88:4626-4630 (1991), adenosine deaminase promoter, phosphoglycerol kinase (PGK) promoter, pyruvate kinase promoter phosphoglycerol mutase promoter, the β -actin promoter
15 (Lai *et al.*, Proc. Natl. Acad. Sci. USA 86: 10006-10010 (1989), UbB, UbC, the long terminal repeats (LTR) of Moloney Leukemia Virus and other retroviruses, the thymidine kinase promoter of Herpes Simplex Virus and other constitutive promoters known to those of skill in the art. Examples of tissue- or cell-specific promoters suitable for use in the present invention include, but are not limited to, endothelin-I (ET -I) and Flt-I, which are specific for
20 endothelial cells, FoxJ1 (that targets ciliated cells).

Although less desired, inducible promoters suitable for controlling expression of the antibody domains include promoters responsive to exogenous agents (*e.g.*, pharmacological agents) or to physiological cues may be utilized. These response elements include, but are not limited to a hypoxia response element (HRE) that binds HIF-1 α and β , a metal-ion
25 response element such as described by Mayo *et al.* (1982, Cell 29:99-108); Brinster *et al.* (1982, Nature 296:39-42) and Searle *et al.* (1985, Mol. Cell. Biol. 5:1480-1489); or a heat shock response element such as described by Nouer *et al.* (in: Heat Shock Response, ed. Nouer, L., CRC, Boca Raton, Fla., pp167-220, 1991)

In one embodiment, expression of an open reading frame is controlled by a
30 regulatable promoter that provides tight control over the transcription of the ORF (gene), *e.g.*, a pharmacological agent, or transcription factors activated by a pharmacological agent or in alternative embodiments, physiological cues. Examples of regulatable promoters which

are ligand-dependent transcription factor complexes that may be used include, without limitation, members of the nuclear receptor superfamily activated by their respective ligands (e.g., glucocorticoid, estrogen, progestin, retinoid, ecdysone, and analogs and mimetics thereof) and rTTA activated by tetracycline. Examples of such systems, include, without
5 limitation, the ARGENT™ Transcriptional Technology (ARIAD Pharmaceuticals, Cambridge, Mass.). Examples of such promoter systems are described, e.g., in WO 2012/145572, which is incorporated by reference herein. In other embodiments, small RNA based switches are described in <http://www.ncbi.nlm.nih.gov/pubmed/25605380>.

Still other promoters may include, e.g., human cytomegalovirus (CMV) immediate-
10 early enhancer/promoter, the SV40 early enhancer/promoter, the JC polymovirus promoter, myelin basic protein (MBP) or glial fibrillary acidic protein (GFAP) promoters, herpes simplex virus (HSV-1) latency associated promoter (LAP), rouse sarcoma virus (RSV) long terminal repeat (LTR) promoter, neuron-specific promoter (NSE), platelet derived growth factor (PDGF) promoter, hSYN, melanin-concentrating hormone (MCH) promoter, CBA,
15 glial fibrillary acidic protein (GFAP) promoter, matrix metalloprotein promoter (MPP), and the chicken beta-actin promoter. The promoters may be the same or different for each expression cassette.

In certain embodiments, the vector genomes each contain an immunoglobulin expression cassette flanked by inverted terminal repeats (ITRs). The expression cassettes
20 contain coding sequences driven by a CB7 promoter, a hybrid between a cytomegalovirus (CMV) immediate early enhancer (C4) and the chicken beta actin promoter. Transcription from this promoter is enhanced by the presence of the chicken beta actin intron (CI). The 5' untranslated region (UTR) of the human c-myc gene is positioned upstream of the mAb coding region as a translational enhancer. The polyadenylation signal for the expression
25 cassette is the rabbit beta-globin (RBG) polyA. Leader (signal) peptides precede both the heavy and light chains of the antibody and mediate the secretion of the antibody from the transduced cell. The heavy and light chains are separated by a linker containing the furin cleavage site and the FMDV 2A sequence. This linker mediates the post-translational cleavage of heavy and light chains and with the exception of a single amino acid substitution
30 (the terminal lysine in the heavy chain is substituted with an arginine residue), is completely removed from the final mAb product.

15-7484PCT

In one embodiment, the vector comprises a constitutive promoter. In another embodiment, the 5' UTR is a truncated UTR from human c-myc gene. The linker sequence may be F2A or an IRES. The heavy signal peptide and the light signal peptide may be the same or different. In one embodiment the leader sequence is an interleukin (IL) IL-2 leader sequence, which may be the wild-type IL2 (MYRMQLLSICIALSLALVTNS, SEQ ID NO:24),
5 such as used in the FI6v3 heavy chain, or a synthetic leader, such as the mutated IL2 used in the germline kappa light chain (MYRMQLLLLIALLSLALVTNS, SEQ ID NO:5) or the mutated IL2 leader used for the CR8033 heavy chain (MRMQLLLLIALLSLALVTNS, SEQ ID NO: 25). Other leader sequences including innate antibody signal peptides can be used, or other
10 heterologous leaders. In one embodiment, a wild-type or synthetic polyA may be selected. As used herein a vector may be any suitable genetic element which transfects, transduces or infects a host cell and expresses the immunoglobulins which assemble into a functional antibody. Such vectors may be selected from a lentiviral vector, a baculovirus vector, a parvovirus vector, a plasmid, modified RNA, and a DNA molecule where mRNA and DNA
15 may be in a form of nanoparticles.

In one embodiment, the vector genome for an AAV vector carrying the influenza A antibody comprises:

Inverted terminal repeats (ITR): AAV2 ITRs (GenBank # NC001401) represent the only cis sequences required for vector genome replication and packaging. An
20 AAV having ITRs from a different source than its capsid are termed "pseudotyped". In certain embodiments, ITRs from a source other than AAV2 may be selected for this construct to generate another pseudotyped AAV. Alternatively, ITRs from the same source as the capsid may be selected.

Cytomegalovirus (CMV) immediate early enhancer (260 bp, C4; GenBank # K03104.1). In another embodiment, another enhancer may be selected. See, discussion of
25 enhancers below.

Chicken beta-actin promoter (281 bp; CB; GenBank # X00182.1). In certain embodiments, another promoter may be selected. See, discussion of promoters below. In still other embodiments, multiple enhancers and/or promoters may be included.

30 Chicken beta-actin intron: The 875 bp intron from the chicken beta actin gene (GenBank # X00182.1) is present in the vector expression cassette.

C-myc 5' UTR: Nucleotides 381-428 of Homo sapiens v-myc avian myelocytomatosis viral oncogene homolog (MYC) mRNA GenBank Accession # NM002467).

5 Leader (signal) peptide cDNA: A codon optimized cDNA sequence (<http://www.uniprot.org/uniprot/P60568>) preceding and in frame with the FI6 heavy chain. FI6v3 heavy variable chain cDNA: A codon optimized human cDNA sequence encoding a human heavy chain variable region anti-influenza variable region (GenBank Accession # AEL31303.1).

10 Constant heavy 1 (CH1) chain: A codon optimized cDNA encoding the protein sequence of human immunoglobulin heavy chain GenBank Accession # BAF64540.1 was synthesized.

15 FI6 Fc chain (CH2-3): A codon optimized cDNA encoding the human immunoglobulin fraction of GenBank Accession # 2J6E_A with the exception of C-terminal lysine which was replaced with arginine and position 57, where aspartic acid was replaced glutamic acid).

Leader (signal) peptide cDNA: A codon optimized cDNA sequence which precedes the universal light chain (ILhy1 described in [Zhang et al, cited above) was synthesized.

20 Universal light chain cDNA: A codon optimized human cDNA sequence (GenBank Accession # ACJ71709.1) was synthesized.

Constant light chain cDNA: A codon human sequence (GenBank Accession # AGH70219.1) was synthesized.

Furin recognition site: An Arginine-Lysine-Arginine-Arginine and codon optimized cDNA was synthesized.

25 F2A linker: A 24 amino acid peptide derived from FMDV (GenBank # CAA2436.1) and codon optimized cDNA was synthesized.

Polyadenylation Signal: The 127 bp rabbit beta-globin polyadenylation signal (GenBank # V00882.1) provides cis sequences for efficient polyadenylation of the antibody mRNA.

30 In certain embodiments, the vector genome for an AAV vector carrying the influenza B antibody comprises: inverted terminal repeats (ITR): AAV2 ITRs; cytomegalovirus (CMV) immediate early enhancer; chicken beta-actin promoter; chicken beta-actin intron;

truncated c-myc 5' UTR; leader (signal) peptide cDNA; CR8033 heavy variable chain cDNA; constant heavy 1 (CH1) chain: A codon optimized cDNA; CR8033 Fc chain (CH2-3); leader (signal) peptide cDNA; universal light chain cDNA; constant light chain cDNA; furin recognition site; F2A linker; polyadenylation signal.

5 In certain embodiments, the vector elements of the influenza A and/or influenza B may be varied. For example, the vector genome as illustrated above, but without the AAV ITRs, may be used in a different expression system (e.g., baculovirus, lentivirus, plasmid, naked DNA). Other suitable vector elements, including, e.g., promoters, enhancers, linkers, polyA, introns, for use in an AAV or non-AAV expression system are described herein.

10 The two or more ORF(s) carried by the nucleic acid molecule packaged within the vector may be expressed from two expression cassettes, one or both of which may be bicistronic.

 The coding sequences for the selected immunoglobulin (e.g., heavy and/or light chain(s)) or other elements (e.g., leader sequences) may be obtained and/or synthesized.

15 Methods for sequencing a nucleic acid (e.g., RNA and DNA) are known to those of skill in the art. Once the sequence of a nucleic acid is known, there are web-based and commercially available computer programs, as well as service based companies which back translate the amino acids sequences to nucleic acid coding sequences. See, e.g., backtranseq by EMBOSS, <http://www.ebi.ac.uk/Tools/st/>; Gene Infinity (http://www.geneinfinity.org/sms/sms_backtranslation.html); ExPasy (<http://www.expasy.org/tools/>). In one embodiment, the RNA and/or cDNA coding sequences are designed for optimal expression in human cells. Methods for synthesizing nucleic acids are known to those of skill in the art and may be utilized for all, or portions, of the nucleic acid constructs described herein.

25 Codon-optimized coding regions can be designed by various different methods. This optimization may be performed using methods which are available on-line (e.g., GeneArt), published methods, or a company which provides codon optimizing services, e.g., as DNA2.0 (Menlo Park, CA). One codon optimizing algorithm is described, e.g., in US Patent Application No. WO 2015/012924, which is incorporated by reference herein. See also, e.g.,
30 US Patent Publication No. 2014/0032186 and US Patent Publication No. 2006/0136184. Suitably, the entire length of the open reading frame (ORF) for the product is modified. However, in some embodiments, only a fragment of the ORF may be altered. By using one

of these methods, one can apply the frequencies to any given polypeptide sequence, and produce a nucleic acid fragment of a codon-optimized coding region which encodes the polypeptide.

For use in producing an AAV viral vector (*e.g.*, a recombinant (r) AAV), the expression cassettes can be carried on any suitable vector, *e.g.*, a plasmid, which is delivered to a packaging host cell. The plasmids useful in this invention may be engineered such that they are suitable for replication and packaging in prokaryotic cells, mammalian cells, or both. Suitable transfection techniques and packaging host cells are known and/or can be readily designed by one of skill in the art.

Methods for generating and isolating AAVs suitable for use as vectors are known in the art. *See generally, e.g.*, Grieger & Samulski, 2005, "Adeno-associated virus as a gene therapy vector: Vector development, production and clinical applications," *Adv. Biochem. Engin/Biotechnol.* 99: 119-145; Buning *et al.*, 2008, "Recent developments in adeno-associated virus vector technology," *J. Gene Med.* 10:717-733; and the references cited below, each of which is incorporated herein by reference in its entirety. For packaging a transgene into virions, the ITRs are the only AAV components required in *cis* in the same construct as the nucleic acid molecule containing the expression cassettes. The cap and rep genes can be supplied in *trans*.

In one embodiment, the expression cassettes described herein are engineered into a genetic element (*e.g.*, a shuttle plasmid) which transfers the immunoglobulin construct sequences carried thereon into a packaging host cell for production a viral vector. In one embodiment, the selected genetic element may be delivered to an AAV packaging cell by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Stable AAV packaging cells can also be made. Alternatively, the expression cassettes may be used to generate a viral vector other than AAV, or for production of mixtures of antibodies *in vitro*. The methods used to make such constructs are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. *See, e.g.*, *Molecular Cloning: A Laboratory Manual*, ed. Green and Sambrook, Cold Spring Harbor Press, Cold Spring Harbor, NY (2012).

AAV Vectors

As used herein, "AAV9 capsid" refers to the AAV9 having the amino acid sequence of GenBank accession:AAS99264, which is incorporated by reference herein. Some variation from this encoded sequence is encompassed by the present invention, which may include sequences having about 99% identity to the referenced amino acid sequence in
5 GenBank accession:AAS99264 and US7906111 (also WO 2005/033321) (*i.e.*, less than about 1% variation from the referenced sequence. However, in other embodiments, other variants of AAV9, or AAV9 capsids having at least about 95% identity to the above-referenced sequences may be selected. Methods of generating the capsid, coding sequences therefore, and methods for production of rAAV viral vectors have been described. *See, e.g.*,
10 Gao, et al, Proc. Natl. Acad. Sci. U.S.A. 100 (10), 6081-6086 (2003) and US 2013/0045186A1.

The term "AAV9 intermediate" or "AAV9 vector intermediate" refers to an assembled rAAV capsid which lacks the desired genomic sequences packaged therein. These may also be termed an "empty" capsid. Such a capsid may contain no detectable
15 genomic sequences of an expression cassette, or only partially packaged genomic sequences which are insufficient to achieve expression of the gene product. These empty capsids are non-functional to transfer the gene of interest to a host cell.

The recombinant adeno-associated virus (AAV) described herein may be generated using techniques which are known. *See, e.g.*, WO 2003/042397; WO 2005/033321, WO
20 2006/110689; US 7588772 B2. Such a method involves culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid; a functional rep gene; a expression cassette composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the expression cassette into the AAV capsid protein.

25 Briefly, cells are manufactured in a suitable cell culture (e.g., HEK 293) cells. Methods for manufacturing the gene therapy vectors described herein include methods well known in the art such as generation of plasmid DNA used for production of the gene therapy vectors, generation of the vectors, and purification of the vectors. In some embodiments, the gene therapy vector is an AAV vector and the plasmids generated are an AAV cis-plasmid
30 encoding the AAV genome and the gene of interest, an AAV trans-plasmid containing AAV rep and cap genes, and an adenovirus helper plasmid. The vector generation process can include method steps such as initiation of cell culture, passage of cells, seeding of cells,

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transfection of cells with the plasmid DNA, post-transfection medium exchange to serum free medium, and the harvest of vector-containing cells and culture media. The harvested vector-containing cells and culture media are referred to herein as crude cell harvest.

5 The crude cell harvest may thereafter be subject method steps such as concentration of the vector harvest, diafiltration of the vector harvest, microfluidization of the vector harvest, nuclease digestion of the vector harvest, filtration of microfluidized intermediate, crude purification by chromatography, crude purification by ultracentrifugation, buffer exchange by tangential flow filtration, and/or formulation and filtration to prepare bulk vector.

10 A two-step affinity chromatography purification at high salt concentration followed anion exchange resin chromatography are used to purify the vector drug product and to remove empty capsids. These methods are described in more detail in US Patent Application No. 62/322,071, filed April 13, 2016 and US Patent Application No. 62/226,357, filed December 11, 2015 and entitled "Scalable Purification Method for AAV9", which is
15 incorporated by reference herein. Purification methods for AAV8 are US Patent Application No. 62/322,098, filed April 13, 2016 and and US Patent Application No. 62/266,341, filed December 11, 2015, and rh10, US Patent Application No. 62/322,055, filed April 13, 2016 and US Patent Application No. 62/266,347, entitled "Scalable Purification Method for AAVrh10", filed December 11, 2015, and for AAV1, US Patent Application No.
20 62/322,083, filed April 13, 2016 and US Patent Application No 62/26,351, for "Scalable Purification Method for AAV1", filed December 11, 2015, are all incorporated by reference herein.

To calculate empty and full particle content, VP3 band volumes for a selected sample (*e.g.*, in examples herein an iodixanol gradient-purified preparation where # of GC = # of
25 particles) are plotted against GC particles loaded. The resulting linear equation ($y = mx+c$) is used to calculate the number of particles in the band volumes of the test article peaks. The number of particles (pt) per 20 μ L loaded is then multiplied by 50 to give particles (pt) /mL. Pt/mL divided by GC/mL gives the ratio of particles to genome copies (pt/GC). Pt/mL–GC/mL gives empty pt/mL. Empty pt/mL divided by pt/mL and x 100
30 gives the percentage of empty particles.

Generally, methods for assaying for empty capsids and AAV vector particles with packaged genomes have been known in the art. See, *e.g.*, Grimm et al., *Gene Therapy* (1999)

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6:1322-1330; Sommer et al., *Molec. Ther.* (2003) 7:122-128. To test for denatured capsid, the methods include subjecting the treated AAV stock to SDS-polyacrylamide gel electrophoresis, consisting of any gel capable of separating the three capsid proteins, for example, a gradient gel containing 3-8% Tris-acetate in the buffer, then running the gel until
5 sample material is separated, and blotting the gel onto nylon or nitrocellulose membranes, preferably nylon. Anti-AAV capsid antibodies are then used as the primary antibodies that bind to denatured capsid proteins, preferably an anti-AAV capsid monoclonal antibody, most preferably the B1 anti-AAV-2 monoclonal antibody (Wobus et al., *J. Virol.* (2000) 74:9281-9293). A secondary antibody is then used, one that binds to the primary antibody and
10 contains a means for detecting binding with the primary antibody, more preferably an anti-IgG antibody containing a detection molecule covalently bound to it, most preferably a sheep anti-mouse IgG antibody covalently linked to horseradish peroxidase. A method for detecting binding is used to semi-quantitatively determine binding between the primary and secondary antibodies, preferably a detection method capable of detecting radioactive isotope
15 emissions, electromagnetic radiation, or colorimetric changes, most preferably a chemiluminescence detection kit. For example, for SDS-PAGE, samples from column fractions can be taken and heated in SDS-PAGE loading buffer containing reducing agent (e.g., DTT), and capsid proteins were resolved on pre-cast gradient polyacrylamide gels (e.g., Novex). Silver staining may be performed using SilverXpress (Invitrogen, CA)
20 according to the manufacturer's instructions or other suitable staining method, i.e. SYPRO ruby or coomassie stains. In one embodiment, the concentration of AAV vector genomes (vg) in column fractions can be measured by quantitative real time PCR (Q-PCR). Samples are diluted and digested with DNase I (or another suitable nuclease) to remove exogenous DNA. After inactivation of the nuclease, the samples are further diluted and amplified using
25 primers and a TaqMan™ fluorogenic probe specific for the DNA sequence between the primers. The number of cycles required to reach a defined level of fluorescence (threshold cycle, Ct) is measured for each sample on an Applied Biosystems Prism 7700 Sequence Detection System. Plasmid DNA containing identical sequences to that contained in the AAV vector is employed to generate a standard curve in the Q-PCR reaction. The cycle
30 threshold (Ct) values obtained from the samples are used to determine vector genome titer by normalizing it to the Ct value of the plasmid standard curve. End-point assays based on the digital PCR can also be used.

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In one aspect, an optimized q-PCR method is used which utilizes a broad spectrum serine protease, e.g., proteinase K (such as is commercially available from Qiagen). More particularly, the optimized qPCR genome titer assay is similar to a standard assay, except that after the DNase I digestion, samples are diluted with proteinase K buffer and treated
5 with proteinase K followed by heat inactivation. Suitably samples are diluted with proteinase K buffer in an amount equal to the sample size. The proteinase K buffer may be concentrated to 2 fold or higher. Typically, proteinase K treatment is about 0.2 mg/mL, but may be varied from 0.1 mg/mL to about 1 mg/mL. The treatment step is generally conducted at about 55 °C for about 15 minutes, but may be performed at a lower temperature
10 (e.g., about 37 °C to about 50 °C) over a longer time period (e.g., about 20 minutes to about 30 minutes), or a higher temperature (e.g., up to about 60 °C) for a shorter time period (e.g., about 5 to 10 minutes). Similarly, heat inactivation is generally at about 95 °C for about 15 minutes, but the temperature may be lowered (e.g., about 70 to about 90 °C) and the time extended (e.g., about 20 minutes to about 30 minutes). Samples are then diluted (e.g., 1000
15 fold) and subjected to TaqMan analysis as described in the standard assay.

Additionally, or alternatively, droplet digital PCR (ddPCR) may be used. For example, methods for determining single-stranded and self-complementary AAV vector genome titers by ddPCR have been described. See, e.g., M. Lock et al, *Hu Gene Therapy Methods*, *Hum Gene Ther Methods*. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131.
20 Epub 2014 Feb 14.

In brief, the method for separating rAAV9 particles having packaged genomic sequences from genome-deficient AAV9 intermediates involves subjecting a suspension comprising recombinant AAV9 viral particles and AAV 9 capsid intermediates to fast performance liquid chromatography, wherein the AAV9 viral particles and AAV9
25 intermediates are bound to a strong anion exchange resin equilibrated at a pH of 10.2, and subjected to a salt gradient while monitoring eluate for ultraviolet absorbance at about 260 and about 280. Although less optimal for rAAV9, the pH may be in the range of about 10.0 to 10.4. In this method, the AAV9 full capsids are collected from a fraction which is eluted when the ratio of A260/A280 reaches an inflection point. In one example, for the Affinity
30 Chromatography step, the diafiltered product may be applied to a Capture Select™ Poros-AAV2/9 affinity resin (Life Technologies) that efficiently captures the AAV2/9 serotype.

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Under these ionic conditions, a significant percentage of residual cellular DNA and proteins flow through the column, while AAV particles are efficiently captured.

A suitable formulation is an aqueous suspension buffered to a physiologically compatible pH and salt concentration. Optionally, one or more surfactants are present in the
5 formulation.

A suitable surfactant, or combination of surfactants, may be selected from among non-ionic surfactants that are nontoxic. In one embodiment, a difunctional block copolymer surfactant terminating in primary hydroxyl groups is selected, e.g., such as Pluronic® F68 [BASF], also known as Poloxamer 188, which has a neutral pH, has an average molecular
10 weight of 8400. Other surfactants and other Poloxamers may be selected, i.e., nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)), SOLUTOL HS 15 (Macrogol-15 Hydroxystearate), LABRASOL (Polyoxy caprylic glyceride), polyoxy 10 oleyl ether, TWEEN (polyoxyethylene sorbitan
15 fatty acid esters), ethanol and polyethylene glycol. In one embodiment, the formulation contains a poloxamer. These copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits: the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content. In one embodiment Poloxamer 188 is selected. The surfactant may
20 be present in an amount up to about 0.0005 % to about 0.001% of the suspension.

USES AND REGIMENS

In one embodiment, a method of treating influenza and/or preventing infection with influenza virus comprising co-administering the synthetic FI6v3 antibody and synthetic
25 CR8033 antibody provided herein. Optionally, such a passive immunization regimen may be combined with a traditional vaccine, with an additional anti-influenza antibody, or with other anti-viral ingredients may be selected.

In certain embodiments, the two antibodies are co-expressed from different rAAV9 vectors. In another embodiment, an rAAV-mediated artificial antibody delivery is combined
30 with a different antibody expression system.

The vectors are preferably suspended in a physiologically compatible carrier, for administration to a human or non-human mammalian patient. Suitable carriers may be

readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (*e.g.*, phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, maltose, and water. The selection of the carrier is not
5 a limitation of the present invention. Optionally, the compositions of the invention may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers.

In certain embodiments, the two different vector stocks (*e.g.*, rAAV9.FI6v3 + rAAV9.CR8033) are formulated separately. In such an embodiment, they may be delivered
10 separately, but substantially simultaneously with each other, *e.g.*, within minutes to about 1 hour. In certain embodiments, the two different vector stocks are admixed and combined into a single composition for administration. Whether delivered separately or in a combination suspension, the two vector stocks are admixed in a ratio of about 1:1 based on genome copies. In other embodiments, this ratio may be altered, *e.g.*, from about 3:1, about
15 2:1, to about 1:2, based on total genome copies.

In certain embodiments, the rAAV9 formulation is a suspension containing an effective amount of AAV vector suspended in phosphate-buffered saline (PBS) with total concentration of about 200 mM, 0.001% (w/v) Pluronic F68 and 5% glycerol. Suitably, the formulation is adjusted to a physiologically acceptable pH, *e.g.*, in the range of pH 6 to 9, or
20 pH 6.5 to 8, pH 7.0 to 7.7, or pH 7.2 to 7.8. However, other pHs within the broadest ranges and these subranges may be selected for other route of delivery.

In one embodiment, the formulation may contain, *e.g.*, a concentration of at least about 1×10^9 GC/mL to 3×10^{13} GC/mL as measured by oqPCR or digital droplet PCR (ddPCR) as described in, *e.g.*, M. Lock et al, *Hu Gene Therapy Methods*, *Hum Gene Ther Methods*. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14, which is
25 incorporated herein by reference.

In certain embodiments, a composition as provided herein is formulated for intranasal delivery. The invention encompasses products including kits which include an intranasal delivery device and a container for the composition of the invention, optionally
30 with other components, *e.g.*, diluents, etc.

In certain embodiments, the intranasal delivery device provides an spray atomizer which delivers a mist of particles having an average size range of about 30 microns to about

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100 microns in size. In certain embodiments, the average size range is about 10 microns to about 50 microns. Suitable devices have been described in the literature and some are commercially available, e.g., the LMA MAD NASAL™ (Teleflex Medical; Ireland); Teleflex VaxINator™ (Teleflex Medical; Ireland); Controlled Particle Dispersion® (CPD) from Kurve Technologies. See, also,, PG Djupesland, Drug Deliv and Transl. Res (2013) 3: 42-62. In certain embodiments, the particle size and volume of delivery is controlled in order to preferentially target nasal epithelial cells and minimize targeting to the lung. In other embodiments, the mist of particles is about 0.1 micron to about 20 microns, or less, in order to deliver to lung cells. Such smaller particle sizes may minimize retention in the nasal epithelium.

Any suitable method or route can be used to administer an AAV-containing composition as described herein, and optionally, to co-administer other active drugs or therapies in conjunction with the AAV-mediated antibodies described herein. Routes of administration include, for example, systemic, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration.

In one embodiment, a frozen composition is provided which contains an rAAV in a buffer solution as described herein, in frozen form. Optionally, one or more surfactants (e.g., Pluronic F68), stabilizers or preservatives is present in this composition. Suitably, for use, a composition is thawed and titrated to the desired dose with a suitable diluent, e.g., sterile saline or a buffered saline.

Optionally, a composition described herein may be used in combination with other anti-viral medications and/or vaccines against other viral targets, including other influenza vaccines, including: Influenza A, Influenza B, and Influenza C. The type A viruses are the most virulent human pathogens. The serotypes of influenza A which have been associated with pandemics include, H1N1, which caused Spanish Flu in 1918, and Swine Flu in 2009; H2N2, which caused Asian Flu in 1957; H3N2, which caused Hong Kong Flu in 1968; H5N1, which caused Bird Flu in 2004; H7N7; H1N2; H9N2; H7N2; H7N3; and H10N7. Broadly neutralizing antibodies against influenza A have been described. As used herein, a “broadly neutralizing antibody” refers to a neutralizing antibody which can neutralize multiple strains from multiple subtypes. For example, CR6261 [The Scripps Institute/ Crucell] has been described as a monoclonal antibody that binds to a broad range of the influenza virus including the 1918 "Spanish flu" (SC1918/H1) and to a virus of the H5N1

class of avian influenza that jumped from chickens to a human in Vietnam in 2004 (Viet04/H5). CR6261 recognizes a highly conserved helical region in the membrane-proximal stem of hemagglutinin, the predominant protein on the surface of the influenza virus. This antibody is described in WO 2010/130636, incorporated by reference herein.

5 Another neutralizing antibody, F10 [XOMA Ltd] has been described as being useful against H1N1 and H5N1. [Sui *et al*, Nature Structural and Molecular Biology (Sui, et al. 2009, 16(3):265-73)] Other antibodies against influenza, *e.g.*, Fab28 and Fab49, may be selected. See, *e.g.*, WO 2010/140114 and WO 2009/115972, which are incorporated by reference. Still other antibodies, such as those described in WO 2010/010466, US Published Patent
10 Publication US/2011/076265, and WO 2008/156763, may be readily selected.

Methods for using these rAAV, *e.g.*, for passive immunization are described, *e.g.*, in WO 2012/145572. Other methods of delivery and uses will be apparent to one of skill in the art. For example, a regimen as described herein may comprise, in addition to one or more of the combinations described herein, further combination with one or more of a biological
15 drug, a small molecule drug, a chemotherapeutic agent, immune enhancers, radiation, surgery, and the like. A biological drug as described herein, is based on a peptide, polypeptide, protein, enzyme, nucleic acid molecule, vector (including viral vectors), or the like.

In a combination therapy, the AAV-delivered immunoglobulin construct described
20 herein is administered before, during, or after commencing therapy with another agent, *e.g.*, anti-viral therapy, antibiotics, as well as any combination thereof, *i.e.*, before and during, before and after, during and after, or before, during and after commencing the therapy. For example, the AAV can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing therapy.

25 As described above, the term “about” when used to modify a numerical value means a variation of $\pm 10\%$, unless otherwise specified.

As used throughout this specification and the claims, the terms “comprise” and “contain” and its variants including, “comprises”, “comprising”, “contains” and
30 “containing”, among other variants, is inclusive of other components, elements, integers, steps and the like. The term “consists of” or “consisting of” are exclusive of other components, elements, integers, steps and the like.

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 70% identity, preferably 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 5 96%, 97%, 98%, 99%, or higher identity over a specified region (*e.g.*, any one of the modified ORFs provided herein 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). As another example, 10 polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, 15 herein incorporated by reference. Generally, these programs are used at default settings, although one skilled in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program that provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. This definition also refers to, or can be applied to, the complement of a sequence. The definition 20 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, 50, 75, 100, 150, 200 amino acids or nucleotides in length, and oftentimes over a region that is 225, 250, 300, 350, 400, 450, 500 amino acids or nucleotides in length or over the full-length of an amino acid or 25 nucleic acid sequences.

Typically, when an alignment is prepared based upon an amino acid sequence, the alignment contains insertions and deletions which are so identified with respect to a reference AAV sequence and the numbering of the amino acid residues is based upon a reference scale provided for the alignment. However, any given AAV sequence may have 30 fewer amino acid residues than the reference scale. In the present invention, when discussing the parental sequence, the term "the same position" or the "corresponding position" refers to the amino acid located at the same residue number in each of the

sequences, with respect to the reference scale for the aligned sequences. However, when taken out of the alignment, each of the proteins may have these amino acids located at different residue numbers. Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Sequence alignment
5 programs are available for amino acid sequences, e.g., the “Clustal X”, “MAP”, “PIMA”, “MSA”, “BLOCKMAKER”, “MEME”, and “Match-Box” programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided
10 by the referenced algorithms and programs. See, e.g., J. D. Thomson *et al*, *Nucl. Acids. Res.*, “A comprehensive comparison of multiple sequence alignments”, 27(13):2682-2690 (1999).

As used herein, an “effective amount” refers to the amount of the rAAV9 composition which delivers and expresses in the target cells an amount of anti-influenza antibodies sufficient to reduce or prevent influenza infection. An effective amount may be
15 determined based on an animal model, rather than a human patient. Examples of a suitable murine model are described herein.

As used herein, the term “patient” generally refers to a human diagnosed or at-risk for infection with influenza. Such a patient may also be referred to herein as a “subject”. The term “subject” may also refer to non-human animals, e.g., cats, dogs, and non-human
20 primates.

A “functional antibody” may be an antibody or immunoglobulin which binds to a selected target (e.g., an antigen on a cancer cell or a pathogen, such as a virus, bacteria, or parasite) with sufficient binding affinity to effect a desired physiologic result, which may be protective (e.g., passive immunization) or therapeutic.

As used herein, an “immunoglobulin domain” refers to a domain of an antibody heavy chain or light chain as defined with reference to a conventional, full-length antibody. More particularly, a full-length antibody contains a heavy (H) chain polypeptide which contains four domains: one N-terminal variable (VH) region and three C-terminal constant (CH1, CH2 and CH3) regions and a light (L) chain polypeptide which contains two domains:
25 one N-terminal variable (VL) region and one C-terminal constant (CL) region. An Fc region may contain two domains (CH2 - CH3). A Fab region may contain one constant and
30

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one variable domain for each the heavy and light chains. Constant domain allotypes suitable for those constructs may include, e.g., G1m17.1 and G1m3.

As used herein, "different specificities" indicates that the referenced immunoglobulin constructs (e.g., a full-length antibody, a heavy chain, or other construct capable of binding a specific target) bind to different target sites. These may refer to different targets on the same antigen, different strains of the same pathogen (e.g., different viral strains) or to different antigens.

The "same specificity" refers to the ability of the immunoglobulin to bind to specific target site which may be present on multiple strains of a pathogen (e.g., influenza virus) or for a single, or subset of strains, of the virus or other pathogen. Suitably, these specificities are such that there is no significant or measurable binding to non-target sites.

The term "heterologous" when used with reference to a protein or a nucleic acid indicates that the protein or the nucleic acid comprises two or more sequences or subsequences which 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. For example, in one embodiment, the nucleic acid has a promoter from one gene arranged to direct the expression of a coding sequence from a different gene. Thus, with reference to the coding sequence, the promoter is heterologous. The term "heterologous light chain" is a light chain containing a variable domain and/or constant domain from an antibody which has a different target specificity from the specificity of the heavy chain.

As used throughout the specification, the term "a" or "an" refers to one or more. As such, the terms "a" (or "an"), "one or more," and "at least one" are used interchangeably herein.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

The following examples illustrate methods for production and use of a mixture of AAV2/9 vectors in which one expresses a broadly neutralizing antibody to influenza A, called FI6; the other expresses a broadly neutralizing antibody to influenza B, called

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CR8033. In one embodiment, the composition termed herein GTP101 is formulated in phosphate-buffered saline (PBS) with total salt concentration brought to 200 mM, 0.001% (w/v) pluronic F68 and 5% glycerol. AAV2/9 will be administered intranasally (IN) after dilution into sterile buffer solution at 4 dose levels. The initial dose levels have been chosen
5 based on preclinical efficacy data, and will be refined based on nonclinical safety assessments, with the highest dose limited by the concentration that can be achieved without AAV vector particle aggregating. Doses range from 3×10^{12} to 2.4×10^{13} genome copies (GC) administered as 4×0.2 ml per nostril via an intranasal mucosal atomization device (IMAD). It will be understood that the invention encompasses variations on these illustrative studies.
10 For example, other means for intranasal administration may be used, other doses, volumes, and other regimens. Additionally, the composition may be adapted for other routes of administration.

The following examples are illustrative only and are not a limitation on the invention
15 described herein.

EXAMPLE 1: AAV vectors

20 AAV9 vectors expressing the heavy and light chains of FI6v3 and CR8033 monoclonal antibodies under the control of a hybrid cytomegalovirus enhancer chicken β -actin promoter were designed, cloned and packaged. The cis-plasmids used to construct each vector used in the following studies are provided in SEQ ID NO: 1 (FI6v3) and SEQ ID NO: 8 (CR8033), which are incorporated by reference with their features. The AAV9
25 vectors were generated using triple transfection of human HEK 293 MCB cells using techniques which were previously described, e.g., in US Patent Published Application No. US 2009/0275107; see, also, M. Lock et al, Hum Gene Ther, 2010; 21(10):1259-1271

The human HEK 293 MCB cells are transfected with: (i) the vector genome plasmids, (ii) an AAV helper plasmid termed pAAV2/9.KanR containing the AAV rep2 and
30 cap 9 wild-type genes and (iii) a helper adenovirus plasmid termed pAd Δ F6(Kan). The size of the GTP101 packaged vector genomes are 1) AAV-FI6: 4650nt. and 2) AAV-CR8033: 4585nt. Other methods can also be used, utilizing producer cell lines of baculovirus systems.

Alternative cis-plasmids and vectors may be generated using the other coding sequences provided herein.

A. GTP101 AAV vector genome plasmid 1: pAAV.CB7.CI.FI6.RBG

The AAV-FI6 mAb vector genome plasmid pAAV.CB7.CI.FI6.RBG (p3193) is 7,471 bp in size (Figure 7.2). The vector genome derived from this plasmid is a single-stranded DNA genome with AAV2 derived ITRs flanking the mAb expression cassette. The FI6 coding sequences encode a mAb specific for influenza A. Expression from the transgene cassettes is driven by a CB7 promoter, a hybrid between a cytomegalovirus (CMV) immediate early enhancer (C4) and the chicken beta actin promoter, while transcription from this promoter is enhanced by the presence of the chicken beta actin intron (CI). The 5' untranslated region (UTR) of the human c-myc gene is positioned upstream of the mAb coding region as a translational enhancer. The polyA signal for the expression cassette is the rabbit beta-globin (RBG) polyA. Leader (signal) peptides precede both the heavy and light chains of the antibody and mediate the secretion of the antibody from the transduced cell. The heavy and light chains are separated by a linker containing the furin cleavage site and the FMDV 2A sequence. This linker mediates the post-translational cleavage of heavy and light chains and with the exception of a single amino acid substitution (the terminal lysine in the heavy chain is substituted with an arginine residue), is completely removed from the final mAb product. The antibody translation cassette including 5' UTR and codon optimized mAb coding sequence was de novo synthesized and subcloned into pZac2.1 to make p3191. The gene cassette was subsequently shuttled into CB7 backbone, pN406 using NheI/NotI enzymes to replace the polylinker with the antibody translation cassette to give pAAV.CB7.CI.FI6.RBG (p3193). All component parts of the plasmid have been verified by direct sequencing by Qiagen Genomic Services.

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B. GTP101 AAV vector genome plasmid 2: pAAV.CB7.CI.CR8033.RBG

The AAV-CR8033 mAb vector genome plasmid pAAV.CB7.CI.CR8033.RBG (p3523) will be 7,462 bp in size. The vector genome derived from this plasmid is a single-stranded DNA genome with AAV2 derived inverted terminal repeats (ITR) flanking the mAb expression cassette. The CR8033 coding sequences encode a mAb specific for influenza B. Expression from the transgene cassettes is driven by a CB7 promoter, a hybrid between a cytomegalovirus (CMV) immediate early enhancer (C4) and

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the modified chicken beta actin promoter, while transcription from this promoter is enhanced by the presence of the chicken beta actin intron (CI). The truncated 5' UTR of the human c-myc gene is positioned upstream of the mAb coding region as a translational enhancer. The polyA signal for the expression cassette is the rabbit beta-globin (RBG) polyA. Leader (signal) peptides precede both the heavy and light chains of the antibody and mediate the secretion of the antibody from the transduced cell. The heavy and light chains are separated by a linker containing the furin cleavage site and the FMDV 2A sequence. This linker mediates the post-translational cleavage of heavy and light chains and with the exception of a single amino acid substitution (the terminal lysine in the heavy chain is substituted with an arginine residue), is completely removed from the final mAb product. The antibody translation cassette including the 5' untranslated region and codon optimized mAb coding sequence encoding the TCN032 mAb was de novo synthesized, and subcloned into pZac2.1 to make p3187. The light chain of TCN032 was replaced with the "universal" light chain sourced from pAAV.CB7.CI.FI6.RBG (p3193) described above using EcoRV/BsiWI enzyme sites. The CR8033 heavy variable chain coding sequence has been codon optimized, de-novo synthesized and will be used to replace the TCN032 heavy variable chain via SphI/SalI restriction sites. The entire translational cassette will be shuttled into a CB7 promoter backbone plasmid, pN437 using SphI/NotI enzymes to replace the TCN032 translational cassette with CR8033 translation cassette and generate the plasmid pAAV.CB7.CI.CR8033.RBG (p3523). All component parts of the plasmid were verified by direct sequencing by Qiagen Genomic Services.

C. Kanamycin backbone cloning

The transgene cassettes from both (pAAV.CB7.CI.FI6.RBG (p3193) and pAAV.CB7.CI.CR8033.RBG (p3523) will be cloned into an AAV2 ITR-flanked construct. The plasmid backbone in this construct is derived from, pZac2.1, a pKSS-based plasmid and contains the kanamycin resistance gene. The final vector genome plasmids will be pAAV.CB7.CI.FI6.RBG.KanR and pAAV.CB7.CI.CR8033.RBG.Kan. All component parts of the final vector genome plasmids will be verified by sequencing and the sequences confirmed as part of the GMP manufacturing process.

D. Description of the sequence elements

1. *Inverted terminal repeats (ITR)*: AAV ITRs (GenBank # NC001401) are sequences that are identical on both ends, but in opposite orientation. The AAV2 ITR sequences function as both the origin of vector DNA replication and the packaging signal of the vector genome, when AAV and adenovirus helper functions are provided in trans. As such, the ITR sequences represent the only cis sequences required for vector genome replication and packaging.
2. *Cytomegalovirus (CMV) immediate early enhancer* (260 bp, C4; GenBank # K03104.1). This element is present in both vector genome plasmids.
3. *Chicken beta-actin promoter* (281 bp; CB; GenBank # X00182.1) promoter and is used to drive high-level antibody expression. This element is present in both vector genome plasmids.
4. *Chicken beta-actin intron*: The 875 bp intron from the chicken beta actin gene (GenBank # X00182.1) is present in the vector expression cassette. The intron is transcribed, but removed from the mature mRNA by splicing, bringing together the sequences on either side of it. The presence of an intron in an expression cassette has been shown to facilitate the transport of mRNA from the nucleus to the cytoplasm, thus enhancing the accumulation of the steady level of mRNA for translation. This is a common feature in gene vectors intended for increased level of gene expression. This element is present in both vector genome plasmids.
5. *C-myc 5' UTR*: Nucleotides 381-428 of Homo sapiens v-myc avian myelocytomatosis viral oncogene homolog (MYC) mRNA GenBank Accession # NM002467). This element is present in both vector genome plasmids.
6. *Leader (signal) peptide cDNA*: A codon optimized cDNA sequence (GenBank Accession # AAB8686.1) preceding and in frame with the FI6 heavy chain was synthesized.
7. *FI6v3 heavy variable chain cDNA*: A codon optimized human cDNA sequence (GenBank Accession # AEL31303.1) was synthesized.
8. *Leader (signal) peptide cDNA*: A codon optimized cDNA sequence (ILco2 described in [Zhang, L., Q. Leng, and A.J. Mixson, J Gene Med, 2005. 7(3):

- p. 354-6]) preceding and in frame with the CR8033 heavy chain was synthesized.
9. *CR8033 heavy variable chain cDNA*: A codon optimized human cDNA sequence (GenBank Accession # AFP87542.1) was synthesized.
 - 5 10. *Constant heavy 1 (CH1) chain*: A codon optimized cDNA (GenBank Accession # BAF64540.1) was synthesized. This element is present in both vector genome plasmids.
 - 10 11. *CR8033 Fc chain (CH2-3)*: A codon optimized cDNA (GenBank Accession # 226787 with the exception of C-terminal lysine which was replaced with arginine) was synthesized.
 12. *FI6 Fc chain (CH2-3)*: A codon optimized cDNA (GenBank Accession # 2J6E_A with the exception of C-terminal lysine which was replaced with arginine and position 57, where aspartic acid was replaced glutamic acid) was synthesized by GeneArt.
 - 15 13. *Leader (signal) peptide cDNA*: A codon optimized cDNA sequence which precedes the universal light chain (ILhy1 described in [Zhang et al, cited above) was synthesized.
 14. *Universal light chain cDNA*: A codon optimized human cDNA sequence (GenBank Accession # ACJ71709.1) was synthesized. This element is present
20 in both vector genome plasmids.
 15. *Constant light chain cDNA*: A codon human sequence (GenBank Accession # AGH70219.1) was synthesized. This element is present in both vector genome plasmids.
 - 25 16. *Furin recognition site*: An Arginine-Lysine-Arginine-Arginine and codon optimized cDNA was synthesized. This element is present in both vector genome plasmids.
 17. *F2A linker*: A 24 amino acid peptide derived from FMDV (GenBank # CAA2436.1) and codon optimized cDNA was synthesized. This element is present in both vector genome plasmids.
 - 30 18. *Polyadenylation Signal*: The 127 bp rabbit beta-globin polyadenylation signal (GenBank # V00882.1) provides cis sequences for efficient polyadenylation of the antibody mRNA. This element functions as a signal

for transcriptional termination, a specific cleavage event at the 3' end of the nascent transcript and addition of a long polyadenyl tail. This element is present in both vector genome plasmids.

5 E. AAV2/9 helper plasmid pAAV2.9.KanR

This AAV2/9 helper plasmid p5E18.AAV2/9n (p0061; 7330bp) is an AAV helper plasmid that encodes the 4 wild-type AAV2 rep proteins and the 3 wild-type AAV VP capsid proteins from AAV serotype 9 [J Virol. 2004 Ju; 78(12): 6381-8].

To create the chimeric packaging construct, the AAV2 cap gene from
10 plasmid p5E18, containing the wild type AAV2 rep and cap genes, was removed and replaced with a PCR fragment of the AAV2/9 cap gene amplified from liver DNA to give plasmid p5E18VD2/AAV2/9. Note that the AAV p5 promoter which normally drives rep expression is moved in this construct from the 5' end of rep to the 3' end of cap. This arrangement serves to introduce a spacer between the promoter and the rep gene (i.e. the
15 plasmid backbone), down-regulate expression of rep and increase the ability to support vector production. The plasmid backbone in p5E18 is from pBluescript KS. All component parts of the plasmid have been verified by direct sequencing. The ampicillin resistance gene will be replaced by the kanamycin resistance gene to give pAAV2/9n.KanR.

F. pAdDeltaF6(Kan) adenovirus helper plasmid

20 Plasmid pAdDelta (Δ)F6(Kan) is 15,774 bp in size. The plasmid contains the regions of adenovirus genome that are important for AAV replication, namely E2A, E4, and VA RNA (the adenovirus E1 functions are provided by the 293 cells), but does not contain other adenovirus replication or structural genes. The plasmid does not contain the cis elements critical for replication such as the adenoviral inverted terminal repeats and
25 therefore, no infectious adenovirus is expected to be generated. It was derived from an E1, E3 deleted molecular clone of Ad5 (pBHG10, a pBR322 based plasmid). Deletions were introduced in the Ad5 DNA to remove expression of unnecessary adenovirus genes and reduce the amount of adenovirus DNA from 32Kb to 12kb). Finally the ampicillin resistance gene was replaced by the kanamycin resistance gene to give pAd Δ F6(Kan). The E2, E4 and
30 VAI adenoviral genes which remain in this plasmid, along with E1, which is present in HEK293 cells, are necessary for AAV vector production.

Example 2 - Manufacturing Process

Cells are cultivated in Corning 10 layer Cell Stacks (CS-10) and HS-36 and all open manipulations are performed in class II biosafety cabinets in an ISO Class 5 environment. The purification process is performed in a closed system where possible however, column chromatography manipulations are not viewed as a completely closed system. To minimize this risk, single-use disposable flow paths are utilized as part of the GE ReadyMate column chromatography production skid platform. After column chromatography purification, the product is diafiltered with final formulation buffer (1x PBS (200 mM NaCl) 0.001% Pluronic F68, 5% glycerol) and sterile filtered to yield the BDS, which is frozen at $\leq -60^{\circ}\text{C}$. After BDS testing, the BDS is thawed, pooled, and diluted as necessary with sterile final formulation buffer and filled at SAFC in their Fill Suite. In the case where the two vector components of GTP101 are manufactured separately, the components are mixed at a 1:1 ratio prior to fill.

An overview of the manufacturing process is given in FIG 2.

A. **Cell Seeding:** HEK 293 cells express the E1a and E1b gene products required for high-titer rAAV production. HEK293 cells are adherent and highly transfectable yielding high-titers of rAAV upon DNA plasmid transfection. Cells will be expanded to $5 \times 10^9 - 5 \times 10^{10}$ cells using Corning T-flasks and CS-10, which will allow sufficient cell mass to be generated for seeding twenty HS-36 for vector production per BDS lot. Cells will be cultivated in medium composed of Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% gamma irradiated, New Zealand-sourced, Fetal Bovine Serum (FBS). The cells are anchorage dependent and cell disassociation will be accomplished using TrypLE Select, a non-animal cell dissociation reagent. Cell seeding is accomplished using sterile, single-use disposable bioprocess bags and tubing sets. The cells will be maintained at $37^{\circ}\text{C} (\pm 1^{\circ}\text{C})$, in 5% ($\pm 0.5\%$) CO_2 atmosphere.

B. **Transient Transfection:** Following 3 days of growth (DMEM media + 10% FBS), HS-36 cell culture media will be replaced with fresh, serum free DMEM media and transfected with the three production plasmids using an optimized PEI-based transfection method. All plasmids used in the production process will be produced by Aldevron Inc. under its GMP-Source™ quality system and infrastructure utilizing the most salient features of cGMP manufacturing; traceability, document control, and materials segregation.

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Sufficient DNA plasmid transfection complex will be prepared in the BSC to transfect twenty HS-36 (per BDS lot). Initially a DNA/PEI mixture will be prepared containing 3.0 mg of pAAV.CB7.CI.FI6.RBG.KanR or pAAV.CB7.CI.CR8033.RBG.KanR vector genome plasmid, 60mg of pAdDeltaF6(Kan), 30mg of pAAV2.rh10.KanR AAV helper plasmid and GMP grade PEI (PEIPro, PolyPlus Transfection SA). In the alternate case where mixed vector genome plasmid transfections are employed, 1.5mg of each of the two cis plasmids will be used. After mixing well, the solution is allowed to sit at room temperature for 25 min. and then added to serum-free media to quench the reaction and then added to the HS-36's. The transfection mixture is equalized between all 36 layers of the HS-36 and the cells are incubated at 37°C ($\pm 2^\circ\text{C}$) in a 5% ($\pm 0.5\%$) CO₂ atmosphere for 5 days.

C. **Cell Media Harvesting:** Transfected cells and media will be harvested from each HS-36 using disposable bioprocess bags by aseptically draining the medium out of the units. Following the harvest of media, the ~ 80 liter volume will be supplemented with MgCl₂ to a final concentration of 2 mM (co-factor for Benzonase) and Benzonase nuclease (Cat#: 1.016797.0001, Merck Group) added to a final concentration of 25 units/ml. The product (in a disposable bioprocess bag) will be incubated at 37°C for 2hr in an incubator to provide sufficient time for enzymatic digestion of residual cellular and plasmid DNA present in the harvest as a result of the transfection procedure. This step is performed to minimize the amount of residual DNA in the final vector FDP. After the incubation period, NaCl will be added to a final concentration of 500 mM to aid in the recovery of the product during filtration and downstream tangential flow filtration (see below steps 4 and 5).

D. **Clarification:** Cells and cellular debris will be removed from the product using a depth filter capsule (1.2 μm /0.22 μm) connected in series as a sterile, closed tubing and bag set that is driven by a peristaltic pump. Clarification assures that downstream filters and chromatography columns will be protected from fouling and bioburden reduction. Filtration ensures that at the end of the filter train, any bioburden potentially introduced during the upstream production process will be removed before downstream purification. The media will be passed through a Sartorius Sartoguard PES capsule filter (1.2/0.22 μm) (Sartorius Stedim Biotech Inc.).

E. **Large-scale Tangential Flow Filtration:** Volume reduction (10-fold) of the clarified product will be achieved using Tangential Flow Filtration (TFF) using a custom sterile, closed bioprocessing tubing, bag and membrane set produced by Spectrum Labs. The

principle of TFF is to flow a solution under pressure parallel to a membrane of suitable porosity (100 kDa). The pressure differential drives molecules of smaller size through the membrane and effectively into the waste stream while retaining molecules larger than the membrane pores. By recirculating the solution, the parallel flow sweeps the membrane surface preventing membrane pore fouling. By choosing an appropriate membrane pore size and surface area, a liquid sample may be rapidly reduced in volume while retaining and concentrating the desired molecule. Diafiltration in TFF applications involves addition of a fresh buffer to the recirculating sample at the same rate that liquid is passing through the membrane and to the waste stream. With increasing volumes of diafiltration, increasing amounts of the small molecules are removed from the recirculating sample. This results in a modest purification of the clarified product, but also achieves buffer exchange compatible with the subsequent affinity column chromatography step. Accordingly, a 100 kDa, PES membrane (Spectrum Labs) is used for concentration that is then diafiltrated with 4 volumes of a buffer composed of: 20 mM Tris pH 7.5 and 400 mM NaCl. The diafiltered product will be stored overnight at 4°C and then further clarified with a 1.2 µm/0.22 µm depth filter capsule to remove any precipitated material.

F. **Affinity Chromatography:** The diafiltered product will be applied to a Capture Select™ Poros- AAV2/9 affinity resin (Life Technologies) that efficiently captures the AAV2/9 serotype. Under these ionic conditions, a significant percentage of residual cellular DNA and proteins flow through the column, while AAV particles are efficiently captured. Following application, the column is washed to remove additional feed impurities followed by a low pH step elution (400 mM NaCl, 20 mM Sodium Citrate; pH 2.5) that is immediately neutralized by collection into a 1/10th volume of a neutralization buffer (Bis Tris Propane, 200 mM, pH 10.2).

G. **Anion Exchange Chromatography:** To achieve further reduction of in-process impurities including empty AAV particles, the Poros-AAV2/9 elution pool is diluted 50-fold (20 mM Bis Tris Propane, 0.001% Pluronic F68; pH 10.2) to reduce ionic strength to enable binding to a CIMultus Q monolith matrix (BIA Separations). Following a low-salt wash, vector product is eluted using a 60 CV NaCl linear salt gradient 10-180 mM NaCl). This shallow salt gradient effectively separates capsid particles without a vector genome (empty particles) from particles containing vector genome (full particles) and results in a preparation largely devoid of empty capsids. Fractions will be collected into tubes containing

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1/100th volume of 0.1% pluronic F68 and 1/27th volume of Bis Tris pH 6.3 to minimize non-specific binding to tubes and the length of exposure to high pH respectively. Peak purity and yield elution fractions are identified by qPCR, pooled and stored at 4°C until final formulation using TFF. AEX pool qPCR titer data will be used to estimate the target final volume to achieve a titer of 2×10^{13} GC/ ml for final formulation (step immediately below).

H. Final Formulation and Sterile Filtration to yield the BDS: TFF will be used to achieve final formulation on the pooled AEX fractions with a 100 kDa membrane (Spectrum Labs, Inc.). This will be achieved by diafiltration with 4 volumes of formulation buffer (1x PBS (200 mM NaCl) 0.001% Pluronic F68, 5% glycerol) and concentrated to the calculated volume to achieve the target concentration. Following diafiltration, the product will be terminally filtered through a 0.22 um filter (Millipore, Billerica, MA) to yield the BDS. Samples will be removed for BDS testing (described in the section below). The filtered Purified Bulk will be stored in sterile polypropylene tubes and frozen at $\leq -60^\circ\text{C}$ in a quarantine location until release for Final Fill.

I. Final Fill: The frozen BDS will be thawed (and pooled as required) and filled into West Pharmaceutical's "Ready-to-Use" (pre-sterilized) 2 ml glass vials and 13 mm stoppers and seals at a fill volume $\geq 0.6\text{ml}$ to $\leq 2.0\text{ ml}$ per vial. Fill operations will occur according to standardized Fill operations supported by technician and process qualification at SAFC. At the completion of Final Fill, all operators working in the Fill suite will be monitored with RODAC plates (to detect viable particulates) on their sterile gloves as they leave the BSC. The BSC surface will also be monitored for viable organisms at the conclusion of Fill. SAFC QC will inspect the vials for appearance, particulates, volume and integrity. Individually labeled vials will be labeled to include protocol number, product name, lot number, allocation number and stored in labeled boxes. Labeled vials are transferred to quarantine $\leq -60^\circ\text{C}$ until release.

J. ddPCR GC Titer: The accuracy and reliability of the quantitative PCR (qPCR) genome copy (GC) titration is paramount for AAV quality control and vector dosing. Genome titers will be measured by a new qPCR technique based on droplet digital PCR [Lock, M., et al., Absolute determination of single-stranded and self-complementary adeno-associated viral vector genome titers by droplet digital PCR. Hum Gene Ther Methods, 2014. 25(2): p. 115-25] which gives unparalleled accuracy and reproducibility. DNA detection will be accomplished using sequence specific primers and fluorescently

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labelled probes targeting the FI6 and CR8033 coding regions to allow for quantification of component genomes in the FDP. In addition primers and probes targeting the RBG polyA region will be used to determine the total genome content of the FDP. A number of standards, validation samples and controls (for background and DNA contamination) have
5 been introduced into the assay. The assay will be qualified by establishing and defining assay parameters including sensitivity, limit of detection, range of qualification and intra and inter assay precision. An internal AAV2/9 reference lot produced using the newly developed production process will be established and used to perform the qualification studies. Note that our previous experience suggests that the titer obtained by droplet digital PCR is
10 generally 2-fold higher than that achieved by our standard pPCR technique. Once this new method of titering is qualified, all previous test articles used in pilot studies described in sections 6 and 8 will be re-assayed and the dosing will be adjusted. This may impact on the actual dosing used in the nonclinical and clinical studies although the expectation is that the difference will be no greater than 2-fold.

15 L. **Infectious Titer:** The infectious unit (IU) assay is used to determine the productive uptake and replication of GTP101 vector in RC32 cells (rep2 expressing HeLa cells). A 96-well end-point format has been employed similar to that previously published. Briefly, RC32 cells are co-infected by serial dilutions of GTP101 BDS and a uniform dilution of Ad5 with 12 replicates at each dilution of rAAV. Seventy-two hours after
20 infection the cells are lysed, and qPCR performed to detect rAAV vector amplification over input. An end-point dilution TCID₅₀ calculation (Spearman-Kärber) is performed to determine a replicative titer expressed as IU/ml. Since “infectivity” values are dependent on particles coming into contact with cells, receptor binding, internalization, transport to the nucleus and genome replication, they are influenced by assay geometry and the presence of
25 appropriate receptors and post-binding pathways in the cell line used. Receptors and post-binding pathways are not usually maintained in immortalized cell lines and thus infectivity assay titers are not an absolute measure of the number of “infectious” particles present. However, the ratio of encapsidated GC to “infectious units” (described as GC/IU ratio) can be used as a measure of product consistency from lot to lot.

30 M. **Host Cell (human) DNA**

A qPCR assay will be used to detect residual human 293 DNA in the BDS. After spiking with a “non-relevant DNA”, total DNA (non-relevant, vector and residual

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genomic) is extracted from ~1 ml of product. The Host Cell DNA is quantified using qPCR targeting the 18S rDNA gene. The quantities of DNA detected are normalized based on the recovery of the spiked non-relevant DNA.

N. Host Cell (human) Protein

5 An ELISA will be performed on a BDS sample to measure levels of contaminating host HEK293 cell proteins. The Cygnus Technologies HEK 293 Host Cell Proteins 2nd Generation ELISA kit will be used. Samples and pre-diluted HEK 293 HCP standards are added to microtiter wells pre-coated with an affinity purified anti-HEK 293 HCP capture antibody, along with a peroxidase conjugated polyclonal anti-HEK 293 HCP
10 detection antibody. Following incubation, the wells are washed to remove unbound reactants, and TMB, a peroxidase substrate, is added. After development, the reaction will be stopped using a sulfuric acid solution. The absorbance of the resulting colored product will be measured using a microplate reader, and the amount of HEK 293 HCP in each sample calculated from the standard curve.

15 O. Replication-competent AAV (rcAAV)

As part of the BDS test plan, a sample of GTP101 FDP will be analyzed for the presence of replication competent AAV2/9 (rcAAV) that can potentially arise during the production process. A three passage assay has been developed consisting of cell-based amplification and passage followed by detection of rcAAV DNA by real-time qPCR (cap 9
20 target). The cell-based component consists of inoculating monolayers of HEK293 cells (P1) with dilutions of the test sample and wild-type human adenovirus type 5 (Ad5). 10^{10} DRP of the vector product will be the maximal amount of the product tested. Due to the presence of adenovirus, replication competent AAV will amplify in the cell culture. After two days, a cell lysate is generated and Ad5 heat inactivated. The clarified lysate is then passed onto a
25 second round of cells (P2) to maximize sensitivity (again the presence of Ad5). After 2 days, a cell lysate is generated and Ad5 heat inactivated. The clarified lysate is then passed onto a third round of cells (P3) to maximize sensitivity (again the presence of Ad5). After 2 days, cells are lysed to release DNA which is then subjected to qPCR to detect AAV2/9 cap sequences. Amplification of AAV2/9 cap sequences in an Ad5 dependent manner indicates
30 the presence of rcAAV. The use of a AAV2/9 surrogate positive control containing AAV2 rep and AAV2/9cap genes enables the Limit of Detection (LOD) of the assay to be determined (0.1, 1 10 and 100 IU) and using a serial dilution of GTP101 vector (1×10^{10} ,

1×10^9 , 1×10^8 , 1×10^7 DRP) the approximate level of rcAAV present in the test sample can be quantitated.

P. In vitro BioPotency

To relate the qPCR GC titer to gene expression, an *in vitro* bioassay will be performed by transducing HEK293 cells with a known multiplicity of GCs per cell and measuring secreted FI6 and CR8033 expression 72 hours post transduction using anti-idiotypic ELISA's specific for the two mAbs. Comparison to highly active pre-clinical and tox vector preparations will enable interpretation of product activity.

Q. Total Protein, Capsid protein and Protein Purity Determination

The vector samples are first quantified for total protein against a Bovine Serum Albumin (BSA) protein standard curve using a bicinchoninic acid assay. The determination is made by mixing equal parts of sample with a Micro-BCA reagent provided in the kit. The same procedure is applied to dilutions of a BSA Standard. The mixtures are incubated at 60°C and absorbance measured at 562 nm. A standard curve is generated from the standard absorbance of the known concentrations using a 4-Parameter fit. Unknown samples are quantified according to the 4-Parameter regression.

To provide a semi-quantitative determination of AAV purity, the samples will then be normalized for genome titer and 5×10^9 GC separated on an SDS-polyacrylamide (SDS-PAGE) gel. The gel is then stained with SYPRO Ruby dye. Any impurity bands are quantified by comparison to co-electrophoresed BSA standards of 25, 50, and 100 ng of protein per lane. These quantities represent 1%, 2% and 4% of the total AAV protein sample. Stained bands that appear in addition to the three AAV specific proteins VP1, VP2 and VP3 are considered protein impurities. All impurity bands are compared to the reference proteins and the impurity mass percent as well as approximate molecular weight are reported. The SDS-PAGE gels will also be used to quantify the VP1, VP2 and VP3 proteins and determine their ratio.

R. BSA Protein

This assay is performed for Release Testing using the Bovine Albumin ELISA kit obtained from Bethyl Laboratories. This kit is a sandwich ELISA. BSA present in the test sample is captured by anti-Bovine Albumin antibody that has been pre-adsorbed on the surface of microtiter wells. After sample binding, unbound proteins and molecules are washed off, and a biotinylated detection antibody is added to the wells to bind the captured

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albumin. A streptavidin-conjugated horseradish peroxidase (SA-HRP) is then added to catalyze a colorimetric reaction with the chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine). The colorimetric reaction produces a blue product, which turns yellow when the reaction is terminated by addition of dilute sulfuric acid. The absorbance of the yellow product at 450 nm is proportional to the amount of albumin analyte present in the sample and a four-parameter standard curve can be generated. The albumin concentrations in the test samples can then be quantified by interpolating their absorbance from the standard curve generated in parallel with the samples. After factoring sample dilutions, the albumin concentrations in the original sample can finally be calculated.

10 S. Benzonase Endonuclease

Benzonase is used in the production process to degrade nucleic acids to facilitate vector purification and as such represents a process impurity. For Release Testing a commercial ELISA is used to measure the concentration of residual Benzonase. Since the amount of benzonase is likely to be in trace amounts if at all, it is necessary to perform an ELISA with a range of standards that includes concentrations < 1 ng/ml.

15 T. Ratio of GC to IU

The GC/IU ratio is a measure of product consistency. The qPCR titer (GC/ml) is divided by the “infectious unit assay (IU/ml) to give the calculated GC/IU ratio.

U. Osmolality, pH, and Appearance Testing

20 These assays are performed for Release Testing by BREL using test protocols for Osmolality by freezing point depression. Appearance of the product is determined by visual inspection for transparency, color and the absence/presence of foreign particles. The product is inspected against white and black backgrounds. The pH of the FDP is determined using a calibrated pH micro-electrode with temperature compensation.

25

EXAMPLE 3: Assessment of individual components of GTP101

A. Determination of minimum effective dose (MED) of

AAV2/9.CB7.FI6 required to protect against lethal challenge with influenza A

30 AAV2/9.CB7.FI6 was delivered intranasally (IN) in 6 week old female BALB/c mice at doses ranging from 1×10^7 to 1×10^{10} GC/mouse and challenged IN with 5LD₅₀ of mouse adapted H1N1 (PR8) 14 days later. As shown in FIGs 3A-3B, the MED (defined as the dose required for 100% of challenged mice to survive challenge with 5LD₅₀ of PR8) for

AAV2/9.CB7.FI6 in mice was found to be 1×10^9 GC/mouse with partial protection conferred with a half log reduced dose (3×10^8 GC/mouse).

At day 14, 3 mice per treatment group that were not challenged were sacrificed to assess the level of FI6 expression at the airway surfaces, namely the bronchoalveolar lavage fluid (BALF) and nasal lavage fluid (NLF). As shown in Figure 4A and 4B, the amount of FI6 Ab that was measured at the surface of the lung (BALF) or nose (NLF) correlated with AAV2/9 vector dose. The amount of antibody secreted in the NLF is difficult to accurately determine due to the small volume of the mouse airway surface liquid (ASL) layer, that is estimated to be $1 \mu\text{l}$.

The viral load (plaque forming units; PFU) was assessed six days after the challenge with PR8 (FIG 5) and found that for mice treated with 1×10^{10} GC of AAV2/9.CB7.FI6 their lungs were clear of PR8 and significant reduction in viral load was observed in mice treated with 3×10^9 GC and also 1×10^9 GC of AAV2/9.CB7.FI6. Both these doses were found to be protective against the influenza challenge.

B. Onset of AAV2/9-prophylaxis against inhaled influenza

The rapid onset of efficacy is beneficial when dealing with an influenza outbreak that requires fast response times. As such, the interval between AAV2/9.CB7.FI6 IN delivery and challenge with 5LD_{50} PR8 was varied to determine how quickly protection could be achieved. The shortest interval between vector treatment and challenge was 8 hours. Mice that were pre-administered the vaccine and challenged 8 hours later lost some weight but recovered and survived the challenge (FIG 6A and 6B).

C. Determination of the MED of AAV2/9.CB7.CR8033 required to protect against lethal challenge with influenza

In concurrent studies, the potential for AAV2/9 expressing the anti-influenza Ab CR8033 to protect against influenza B challenge was assessed. Six week old female BALB/c mice were given AAV2/9.CB7.CR8033 at doses ranging from 1×10^7 to 1×10^{10} GC/mouse and challenged IN with 5LD_{50} of mouse adapted B/Lee/40. 14 days later we found that likewise the MED was 1×10^9 GC/mouse (FIG 7A and 7B). No mice survived the challenge with B/Lee/40 virus when they were given IN the half log reduced dose of 3×10^8 GC/mouse.

D. AAV2/9 vector dissemination following IN delivery in mice.

An important safety aspect of the AAV-mediated prophylaxis strategy described herein is targeting vector-mediated transduction to the terminally differentiated cells of respiratory surfaces. Potential body wide dissemination of the AAV2/9 vector in mice that survived the PR8 challenge was determined. Mice were given AAV2/9.CB7.FI6 at varying doses and following the challenge mice were necropsied and tissues (lung, liver, spleen, ovaries, brain, heart) harvested to assess vector biodistribution. For vector doses 1×10^9 - 1×10^{10} GC/mouse, all mice (5 per group) survived the challenge. At all doses the highest quantities of vector genomes were found in the lung (e.g., at the highest dose of vector the ratio of lung/liver AAV2/9 genomes was 1783). For the higher dose of 1×10^{10} GC/mouse, no GG/diploid genome was observed in the spleen, brain and heart. Very low levels of AAV2/9 vector were detected in the liver and ovary and as expected the majority of AAV2/9 genome copies were observed in the liver. In mice treated with 3×10^9 GC, no AAV2/9 was detected in the liver, ovaries and spleen, and low levels of capsid were observed in the brain and heart. As expected, there was a decline of vector genomes in the lung. In the mice treated with 1×10^9 GC, vector genome, albeit much reduced, were observed in the lung only. For the survivors of the 3×10^8 GC/mouse treatment group, the level of AAV2/9 genome copies was undetectable (below limit of detection, LD).

	Lung	Liver	Spleen	Ovaries	Brain	Heart	Presence of vector genome/
1×10^{10} GC	0.1783	0.0001	<LD	0.0005	<LD	<LD	
3×10^9 GC	0.0178	<LD	<LD	<LD	0.0043	0.0003	
1×10^9 GC	0.0011	<LD	<LD	<LD	<LD	<LD	
3×10^8 GC	<LD	<LD	<LD	<LD	<LD	<LD	

diploid genome

Example 4: Mixing two AAVs on the protective efficacy against influenza A and influenza B

The impact on prophylaxis against influenza A and influenza B by mixing two different AAV preparations was assessed. Mice were dosed IN with a mixture of 1×10^9 GC

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with AAV2/9.CB7.FI6 and 1×10^9 GC AAV2/9.CB7.CR8033 and challenged with $5LD_{50}$ of either PR8 or B/Lee/40 virus. Should there be interference or receptor binding competition, then protective efficacy would be significantly lower when compared to protection conferred by the administration of the single protective AAV2/9 vector.

5 In the first instance the mixture of 1×10^9 GC AAV2/9.CB7.FI6 and 1×10^9 GC AAV2/9.CB7.CR8033 was assessed for protection against challenge with PR8 and compared to the protection conferred against PR8 when 1×10^9 GC AAV2/9.CB7.FI6 was delivered alone. As shown in FIG 8, no interference was observed.

The mixture of 1×10^9 GC with AAV2/9.CB7.FI6 and 1×10^9 GC
10 AAV2/9.CB7.CR8033 was then assessed for protection against challenge with B/Lee/40 (influenza B) and compared to the protection conferred against influenza B when 1×10^9 GC AAV2/9.CB7.CR8033 was delivered alone. As demonstrated in FIG 9, there was no interference.

15 EXAMPLE 5: Effective vector re-administration and protection against challenge with influenza A

An important feature of the AAV2/9 vector delivery system is that it can be administered more than once. To simulate a setting of preexisting immunity against the AAV2/9 capsid, we delivered IN AAV2/9 vector expressing an irrelevant to influenza
20 transgene product at doses ranging from 1×10^9 to 1×10^{11} GC/mouse. Twenty-eight days later, the level of serum-circulating AAV2/9-specific neutralizing antibody (NAb) was assessed (FIG 10). As expected, the level of AAV2/9 NAb in serum was directly proportional to the dose of AAV2/9 delivered IN.

AAV2/9 pre-immunized mice were then given 1×10^{11} GC of AAV2/9.CB7.FI6. If
25 the NAb against the AAV2/9 capsid prevented effective AAV2/9 re-administration, then the pre-immunized mice would succumb to the PR8 challenge. Despite the high levels of serum-circulating AAV2/9 NAb that reached 1:5,120 serum dilution, AAV2/9.CB7.FI6 was effectively readministered and resulted in full protection against a $5LD_{50}$ challenge with PR8.

30

EXAMPLE 6: Impact of an interim infection on the efficiency of AAV2/9-mediated FI6 prophylaxis against PR8

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Following IN administration of AAV2/9.CB7.FI6, a possibility exists that the patient may experience an interim infection with an airborne infection other than influenza, such as rhinovirus or RSV. As a result of the infection, a subset of respiratory cells that line the surface of the nasal and lung airways will be damaged and lost. The impact of positively-
5 transduced cells being turned over as a result of injury following viral infection, on the efficacy of AAV2/9.CB7.FI6-mediated prophylaxis against influenza was assessed. Briefly, 1×10^{10} GC of AAV2/9.CB7.FI6 was delivered IN in 6 week old female BALB/c mice. To induce damage to the epithelium, two weeks later, one group was challenged with 1×10^6 pfu of RSV and the other group was treated IN with PBS. Four weeks later all mice were
10 challenged IN with $5LD_{50}$ of PR8 and weight loss and survival monitored. There was no impact on the efficacy of AAV2/9-mediated prophylaxis in the event of an interim infection.

EXAMPLE 7: Pilot Toxicity and Expression Studies

A A transitional non-GLP study will be performed in immune competent CD-1
15 mice to evaluate safety, kinetics, and immune responses. Outbred CD-1 mice are expected to respond to the GTP101 (AAV2/9.CB7.FI6 and AAV2/9.CB7.CR8033 vectors; GTP101 investigational product (IP)) in a manner similar to humans due to their genetic heterogeneity. This transitional study is necessary because the majority of the prior work was performed using various inbred strains. Toxicities caused directly by the transgene product
20 can not be assessed in CD-1 mice, and instead will be assessed in immune compromised, inbred mice as described elsewhere in this document.

To model in mice the deposition of IP via the IMAD in the human nasal airways, we will lower the volume of liquid in which the vector is delivered to target the mouse nasal airway. Previously, we conducted studies in which reducing the volume to 20 μ l
25 restricts expression to the nasal airway [Limberis, M.P., et al., Sci Transl Med, 2013. 5(187): p. 187ra72]. In mice this can be further restricted to 5 μ l per nostril. This small volume will still allow for testing in mice the maximal dose of GTP101 proposed in the nonclinical studies.

3 dose groups, 10 animals per dose per time point (5 males and 5 females),
30 control (PBS), 3.2×10^{10} , 3.2×10^{11} , and 3.2×10^{12} GC/kg of IP (see following table). Day 0 is IP administration, day 10 is peak expression, and day 60 is recovery period. Safety assessments include body weights, clinical observations, serum chemistries and hematology

at sacrifice, full necropsy, organ weights and storage of tissues at each sacrifice, histopathology only on selected tissues – lung, brain, liver, kidney, heart on high dose and control. Histopathology on mid and low dose group will be performed only if abnormalities are observed at highest dose. Expression of two transgenes will be assessed by sandwich ELISA at the time of sacrifice in the nasal lavage fluid (NLF), BALF and serum. Immunology studies will evaluate the levels of anti-drug antibody (ADA) by ELISA (transgenes and capsid) and the levels of T-cell responses evaluated in spleen.

Non-GLP pilot toxicology study in immunocompetent CD1 mice

Dose (GC/kg)	Necropsy time point (d)	Number of male CD1 mice per group	Number of female CD1 mice per group
3.2x10 ¹⁰	10	5	5
	60	5	5
3.2x10 ¹¹	10	5	5
	60	5	5
3.2x10 ¹²	10	5	5
	60	5	5
PBS	10	5	5
	60	5	5

10

B. Toxicology Study in Immunodeficient Mice

This toxicology study is performed as the maximal dose proposed for humans, which is roughly 3.2x10¹¹ GC/kg in RAG-1 KO mice and the middle dose group used for previous studies. Group size will be 10 animals per time point, per sex, with sacrifices at days 10 and 60. IP will be administered in a volume of up to 10 µl per nostril.

Nonclinical toxicology study in immunodeficient mice

Dose	Necropsy time point	Number of male Rag-1 KO mice per	Number of female Rag-1 KO mice per
------	---------------------	----------------------------------	------------------------------------

		group	group
3.2x10 ¹¹ GC/kg	10	10	10
	60	10	10
PBS	10	10	10
	60	10	10

One dose group, 20 animals per dose per time point (10 males and 10 females), dose at 3.2x10¹¹ GC per mouse of IP. Day 0 is IP administration, day 10- peak expression, and day 60- recovery period. Safety assessments include body weights, clinical observations, serum chemistries and hematology at sacrifice, full necropsy, organ weights and storage of tissues at each sacrifice, histopathology only on selected tissues (listed in Table 8.8) Histopathology of the low dose group will be performed only if abnormalities are observed at highest dose. Expression of two transgenes will be assessed by ELISA in both, NLF and BALF.

10 C. Toxicity and biodistribution studies in nonhuman primates

This study will be performed at a dose that represents 10-fold higher than the highest dose that will be delivered in the human trial using the same device and volumes used in the human trial when normalized according to total body mass. The highest dose in the human trial will be 3.2x10¹¹ GC/kg (2.4x10¹³ GC total for 75 kg human) administered via 2 x 0.2 ml per nostril. NHPs will receive 3.2x10¹² GC/kg (1.28x10¹³ GC for a 4 kg macaque), which is the highest dose that can be administered with the IMAD device at the volumes to be provided to humans which is 2.4x10¹³ GC administered as 2 x 0.2 ml per nostril.

Toxicity and biodistribution studies will be performed in rhesus macaques. Six males and six females, to a total of 12 rhesus macaques will be used for this study. Three time points will be evaluated, naïve animals will be sacrificed on day 0 prior to IP administration, Day 10 corresponds to peak expression, and day 60 corresponds to long-term recover period..

Two males and two females will be sacrificed at each time point. Toxicity will be evaluated by assessing CBC/chem panels.

25

EXAMPLE 8: Assessment of Intranasal Delivery Device

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In the next set of studies, the ability of AAV9 expressing anti-influenza A and B antibodies (FI6 and CR8033, respectively) coupled with the LMA® MAD Nasal™ intranasal mucosal atomization device (IMAD) to effectively protect mice against challenge with pandemic or seasonal influenza is assessed. First, it was determined that an equimolar combination of AAV9.FI6 and AAV9.CR8033 vectors delivered intranasally (i.n.) at a dose of 5×10^{10} GC/KG (or 1×10^9 GC) per mouse effectively protected mice against lethal challenge with influenza A (mouse adapted H1N1, PR8) or influenza B (mouse adapted B/Lee/40), respectively (Figures 11A, 11D, 11G and 12A, 12D). In an effort to progress AAV9 into the clinic, vector compatibility with the IMAD for loss in volume and vector potency was assessed. For these studies, 0.5 mL of vector suspension was plunged through the IMAD to evaluate physical loss of vector solution as well as to evaluate the impact of the device on vector potency. As indicated in Figure 12G, a physical liquid loss of 4 - 6.6% of initial volume was observed when the vector solution was processed through the IMAD. We also evaluated three different vector formulations: PBS-pH 6.8, PBS-pH 7.2 or PBS-pH 7.4 for vector compatibility. Groups of mice were i.n. administered a mixture of 5×10^{10} GC/KG (or 1×10^9 GC) each of AAV9.FI6 and AAV9.CR8033 as well as a mixture of 5×10^{10} GC/KG (or 1×10^9 GC) each of AAV9.FI6 and AAV9.CR8033 that was processed through the IMAD. Seven days later, the mice were challenged with 5LD50 of PR8 or B/Lee/40 (Figures 11A, 11D, 11G and 12A, 12D). No difference in effectiveness of protection against influenza A or B was observed when the vector mixture was processed through the IMAD (dark grey squares). Additionally the use of the IMAD was compatible with the three different formulations and no diminution of effectiveness was observed when vector formulated with each of the three formulations was processed through the IMAD. Only vector-administered mice survived the challenge. At the conclusion of the experiment (Day 21) mice were sacrificed and the bronchoalveolar lavage fluid (BALF) harvested to evaluate antibody expression. The lungs were also harvested to assess the amount of vector genome present at time of necropsy. No difference was observed in the expression of the antibody at the airway surfaces when vector was delivered as liquid or as liquid following IMAD processing (Figure 11B, 11E, 11H and 12B, 12E). Furthermore, no significant difference in the number of AAV9 vector genomes in the lung was observed.

Next this approach was translated to the nose of macaques, a model more closely related to the human nose that is the target tissue. In this model, the kinetics of the onset of

transgene expression in the nasal lavage fluid (NLF) and stability of expression was assessed when AAV9 vector was delivered via the IMAD compared to the traditional, direct liquid delivery. Immune responses against the human antibody FI6 or CR8033 would have confounded the experiment and interpretation of the results. As such, in these studies a self antigen (rhesus α -fetoprotein, rhAFP) was used as the reporter transgene. Expression of rhesus alpha fetoprotein (rhAFP) here is noted by the presence of a plus (+) sign as accurate quantification of the amount of antibody at the mucosal surfaces is constrained by the accurate sampling of the NLF (Figure 13). At the conclusion of the study, vector biodistribution was also examined. Overall, no significant differences in the kinetics and profile of gene expression or AAV9 vector biodistribution were observed between the two delivery methods.

In conclusion, AAV-mediated prophylaxis against influenza A and B is safe and effective when vectors are delivered via the IMAD, an easy-to-use device that localizes transduction to the site of influenza infection, the nasal mucosa.

15

EXAMPLE 9: Initial Phase 1 Trial Design

The objective of the phase 1 trial is to evaluate preliminary safety and tolerability, to understand the pharmacokinetics of the IP in healthy volunteers, and to select a dose level for further development. The trial will be an open label, dose-escalation trial with the dose levels based on preclinical data. The study will enroll up to five cohorts, with four subjects per cohort. Subjects will be screened 4-12 weeks prior to treatment and will be followed for up for 6 months.

Each volunteer will receive one 0.8 ml dose of IP IN via two sequential applications of 0.2 ml per nostril into each nostril using the approved IMAD. Up to twenty eight (24) volunteers will be enrolled in five cohorts. Cohorts 1-4 will receive increasing dosage levels of IP with the final cohort (cohort 5) expanded to 8 volunteers at the optimal dose level (defined by safety and pharmacokinetic parameters in cohorts 1- 4).

Initial Phase 1 study design in healthy adult volunteers

Cohort	N active	AAV2/9 Product (GC)	Transgene Dosage Level	Total volume to be administered (ml)	Administration Route
1	4	3.0 x 10 ¹²		0.2 ml per each nostril; two sets of applications, 0.8 ml total	IN
2	4	6.0 x 10 ¹²			IN
3	4	1.2 x 10 ¹³			IN
4	4	2.4 x 10 ¹³			IN
5	8	Optimal dose		0.2 ml per each nostril; two sets of applications, 0.8 ml total	IN
Total	24				

A. Target Population Inclusion/Exclusion Criteria

Healthy 18-45 years old adults of any gender with body mass index of 19-30 kg/m² and body weight of 50-100 kg will be enrolled to evaluate safety, immunologic and pharmacokinetic parameters of GTP101.

INCLUSION CRITERIA

- Male or female aged 18 – 45 years inclusive
- Able to give written informed consent to participate
- 10 • Healthy, as determined by medical history, physical examination, vital signs, and clinical safety laboratory examinations @ baseline
- Non-habitual smoker (habitual smokers are persons who smoke more than 4 cigarettes or other tobacco products on a weekly basis) and agree to not use tobacco products during participation
- 15 • Females should fulfill one of the following criteria:
 - At least one year post-menopausal;
 - Surgically sterile;
 - Will use oral, implantable, transdermal or injectable contraceptives for 30 days prior to IP administration and until 60 days after vaccination;

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- Willing to abstain from sexual intercourse or use another reliable form of contraception approved by the Investigator (*e.g.*, intrauterine device (IUD), female condom, diaphragm with spermicide, cervical cap, use of condom by the sexual partner or a sterile sexual partner) from the time of screening through Study Day 28.
- 5
- Women of childbearing potential must have a negative urine pregnancy test within 24 hours preceding IP administration
 - Comprehension of the study requirements, expressed availability for the required study period

10 EXCLUSION CRITERIA

- Significant history of seasonal hay fever or a seasonal allergic rhinitis or perennial allergic rhinitis or chronic or nasal or sinus condition
 - History of asthma requiring treatment for up to 1 year prior to administration of IP
 - Subjects with abnormal nasal structure including septal deviation and nasal polyps, chronic sinusitis, asthma, or COPD
- 15
- Current use of intranasal steroids.
 - Presence of significant uncontrolled medical or psychiatric illness (acute or chronic) as assessed by the Investigator. This includes, but is not limited to, institution of new medical or surgical treatment, or a significant dose alteration for uncontrolled
- 20
- symptoms or drug toxicity within 3 months of screening and reconfirmed on Day 0 prior to challenge.
 - Positive serology for HIV-1 or HIV-2, or HBsAg or HCV antibodies.
 - Cancer, or treatment for cancer, within 3 years, excluding basal cell carcinoma or squamous cell carcinoma, which is allowed.
- 25
- Presence of immunosuppression or any medical condition that may be associated with impaired immune responsiveness, including, but not limited to, diabetes mellitus.
 - Presently receiving (or history of receiving), during the preceding 3-month period, any medications or other treatments that may adversely affect the immune system
- 30
- such as allergy injections, immune globulin, interferon, immunomodulators, cytotoxic drugs or other drugs known to be frequently associated with significant

major organ toxicity, or systemic corticosteroids (oral or injectable). Topical corticosteroids will be allowed.

- History of drug or chemical abuse in the year before the study.
- Receipt of any investigational product or nonregistered drug within the 30 days prior to administration of IP or currently enrolled in any investigational drug study or intends to enroll in such a study within the ensuing study period.
- Receipt of blood or blood products 6 months prior to IP administration or planned administration during the study period.
- Acute disease within 72 hours prior to IP administration, defined as the presence of a moderate or severe illness with or without fever (as determined by the Investigator through medical history and physical examination), or presence of a fever $\geq 38^{\circ}\text{C}$ orally.
- Pregnant and/or lactating females.
- Serum antibodies to the AAV2/9 of $>1:80$.
- Any condition that, in the opinion of the Investigator, might interfere with the primary study objectives and assessments.

B. Dose Level Determination

Initial dose levels have been chosen based on preclinical efficacy data, and will be refined based on nonclinical safety assessments, with the highest dose limited by the aggregation properties of AAV. Doses will range from 3.0×10^{12} to 2.4×10^{13} GC/dose administered in 4 x 0.2 ml per nostril via IMAD. These doses are substantially lower than those given to monkeys, mice and ferrets when adjusted for total body mass. No substantial toxicity was observed in any nonclinical studies conducted in these species at any dose with the IP or versions of AAV2/9 similar in structure to the IPs planned for the clinical trial.

C. Dosage Administration and Duration

Each dose group will receive IP in 0.4 ml per each nostril via IMAD. Subjects will be dosed one by one. Treatment intervals within the same dose group would be determined by the nonclinical studies but for purposes of presentation will be 1 week.. Once all subjects in one cohort have passed study day 14, preliminary safety data will be reviewed. If no safety issues are identified, dose escalation to the next dose will commence. After completion of cohort 4 and preliminary safety for the highest dose is demonstrated, a fifth

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and final cohort will be enrolled for expansion for a total of 8 subjects (i.e., 4 additional subjects) at the optimal dose which will be the Maximally Tolerated Dose or the highest dose that can be administered.

5 The product administered to patients will be a mixture of two AAV2/9 vectors which have been combined at the time of fill/finish. Administration of the IP will occur in two sequential 0.2 ml doses into each nostril (i.e., total of 0.8 ml of IP). The IP will be administered using a commercially approved device manufactured by Teleflex Medical (Teleflex.com). The device is called the LMA MAD NASAL™ (Intranasal Mucosal
10 Atomization Device). Details as to how best use the device to deliver IP to nasal mucosa are provided in the company's Procedure Guide. Basically, the subject is placed in a recumbent position and the tip of the device is placed at the orifice of each nasal passage (one at a time) after which the IP is delivered by pushing the fluid through the atomizer *via* a syringe.

 The maximum tolerated dose (MTD) will be defined as the dose below the dose at
15 which the subjects demonstrate dose-limiting toxicity (DLT), defined as any treatment related Grade 3 in two subjects or one treatment related Grade 4 event.

20 This application contains sequences and a sequence listing, which is hereby incorporated by reference. Also incorporated by reference herein is US Provisional Patent Application No. 62/323,348, filed April 15, 2016 and US Provisional Application No. 62/161,192, filed May 13, 2015. All publications, patents, and patent applications cited in this application, are hereby incorporated by reference in their entireties as if each individual
25 publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit
30 or scope of the appended claims.

Table
(Sequence Listing Free Text)

The following information is provided for sequences containing free text under numeric identifier <223>.

SEQ ID NO	Free Text under <223>
1	<220> <223> Plasmid carrying Fl6cc HC with germline light chain <220> <221> repeat_region <222> (1)..(130) <223> 5' ITR <220> <221> repeat_region <222> (198)..(579) <223> CMV IE promoter <220> <221> promoter <222> (582)..(862) <220> <221> TATA_signal <222> (836)..(839) <220> <221> Intron <222> (956)..(1928) <223> chicken beta-actin intron <220> <221> misc_feature <222> (1940)..(1987) <223> c-myc 5'UTR <220> <221> misc_feature <222> (1988)..(1992) <223> kozak <220> <221> misc_feature

	<p><222> (1993)..(2052) <223> leader</p>
	<p><220> <221> CDS <222> (2053)..(2439) <223> F16 variable heavy</p>
	<p><220> <221> CDS <222> (2440)..(2760) <223> F16 CH1</p>
	<p><220> <221> CDS <222> (2761)..(3426) <223> F16 CH2-3</p>
	<p><220> <221> misc_feature <222> (3427)..(3438) <223> furin cleavage site</p>
	<p><220> <221> misc_feature <222> (3439)..(3510) <223> F2A</p>
	<p><220> <221> misc_feature <222> (3511)..(3570) <223> leader</p>
	<p><220> <221> CDS <222> (3511)..(3570) <223> leader</p>
	<p><220> <221> CDS <222> (3571)..(3909) <223> kagga germline line</p>
	<p><220> <221> CDS <222> (3910)..(4230)</p>
	<p><220> <221> repeat_region <222> (4308)..(4432)</p>

	<p><223> rabbit globin polyA</p> <p><220></p> <p><221> repeat_region</p> <p><222> (4521)..(4650)</p> <p><223> 3' ITR</p> <p><220></p> <p><221> misc_feature</p> <p><222> (4827)..(5265)</p> <p><223> F1 ori on complementary strand</p> <p><220></p> <p><221> misc_feature</p> <p><222> (5294)..(5936)</p> <p><223> origin of replication</p> <p><220></p> <p><221> misc_feature</p> <p><222> (6611)..(7426)</p> <p><223> CDS for Kan resistance located on complementary strand</p>
8	<p><223> Cls plasmid containing CR8033 heavy chain and light chain from kappa germline</p> <p><220></p> <p><221> misc_feature</p> <p><222> (264)..(287)</p> <p><223> CMV promoter end</p> <p><220></p> <p><221> promoter</p> <p><222> (285)..(565)</p> <p><223> CB promoter</p> <p><220></p> <p><221> misc_feature</p> <p><222> (288)..(318)</p> <p><223> begin promoter</p> <p><220></p> <p><221> TATA_signal</p> <p><222> (539)..(542)</p> <p><220></p> <p><221> Intron</p> <p><222> (659)..(1631)</p> <p><223> chicken beta-actin intron</p>

<220>	<221> misc_feature	<222> (1517)..(1533)	<223> end of intron
<220>	<221> misc_feature	<222> (1643)..(1690)	<223> c-myc 5' UTR
<220>	<221> misc_feature	<222> (1691)..(1698)	<223> kozak
<220>	<221> CDS	<222> (1753)..(2133)	<223> CR8033cc variable heavy
<220>	<221> CDS	<222> (2134)..(2454)	<223> CH1
<220>	<221> CDS	<222> (2455)..(3120)	<223> CH2-3
<220>	<221> misc_feature	<222> (3121)..(3132)	<223> furin cleavage site
<220>	<221> misc_feature	<222> (3133)..(3204)	<223> F2A linker
<220>	<221> CDS	<222> (3265)..(3603)	<223> Kappa germline light
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	<p><220> <221> polyA_signal <222> (4000)..(4126)</p> <p><220> <221> repeat_region <222> (4215)..(4344) <223> 3' ITR (on complement)</p> <p><220> <221> misc_feature <222> (4521)..(4959) <223> F1 ori on complementary strand</p> <p><220> <221> misc_feature <222> (4988)..(5630) <223> pUC origin of replication</p> <p><220> <221> misc_feature <222> (7363)..(7548) <223> Lac promoter</p> <p><220> <221> repeat_region <222> (7603)..(7732) <223> 5' ITR</p> <p><220> <221> misc_feature <222> (7806)..(7835) <223> promoter start</p>
14	<p><223> include leader-entire heavy chain-furin-F2A linker-leader-entire light chain</p>
15	<p><223> IF16 11 eader-entire heavy chain-furin-F2A linker-leader-entire light chain</p>
16	<p><223> F16 26</p>
17	<p><223> F16 28</p>
18	<p><223> F16 30 cDNA</p>
19	<p><223> F16 35 cDNA</p>

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21	<223> F16 cDNA
22	<223> F16 42 cDNA
23	<223> F16 B42
24	<223> mutated IL2
25	<223> mutated IL2

CLAIMS:

1. A composition useful for passive immunization against influenza infection, the composition comprising:

(a) a first non-replicating recombinant adeno-associated virus having an AAV9 capsid (rAAV9) and having a vector genome which comprises nucleic acid sequence encoding: (a) an AAV inverted terminal repeat (ITR) (b) an enhancer, (b) a chicken beta-actin promoter, (c) an intron (d) a 5' UTR, (e) a leader peptide operably linked to a F16v3 heavy variable chain, (f) a F16v3 heavy variable chain; (g) human CH1 domain and human IgG1 Fc chain (CH2-3); (h) a leader peptide operably linked to an immunoglobulin light chain; (i) an immunoglobulin constant light chain; (j) a furin recognition site; (k) an F2A linker; (l) a polyadenylation signal; and (m) an AAV inverted terminal repeat; and

(b) a second non-replicating rAAV9, wherein the second rAAV9 has a vector genome which comprises nucleic acid sequences encoding: (a) an AAV inverted terminal repeat (ITR) (b) an enhancer, (b) a chicken beta-actin promoter, (c) an intron (d) a 5' UTR, (e) a leader peptide operably linked to a CR8033 heavy chain, (f) a CR8033 heavy variable chain; (g) a human CH1 domain and a human IgG1 Fc chain (CH2-3); (h) a leader peptide operably linked to an immunoglobulin light chain; (i) a constant light chain cDNA; (j) a furin recognition site; (k) an F2A linker; (l) a polyadenylation signal; and (m) an AAV inverted terminal repeat,

and an aqueous liquid suspension base.

2. The composition according to claim 1, wherein the vector genome for (a) is selected from: SEQ ID NO: 1, SEQ ID NO: 14, 15, or 16.

3. The composition according to claim 1, wherein the vector genome for (b) comprises SEQ ID NO: 8.

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4. The composition according to claim 1, wherein the total concentration of rAAV9 from (a) and (b) is about 1×10^{11} to about 6×10^{13} genome copies (GC)/mL, wherein GC is as determined using ddPCR.
5. The composition according to claim 1, wherein the ratio of the first rAAV9 of (a) to the second rAAV9 of (b) is about 1:1.
6. The composition according to claim 1, wherein the light chain in (a) and/or (b) is from an immunoglobulin source heterologous to the heavy chain source.
7. The composition according to claim 1, wherein the light chain in (a) and (b) are from the same source.
8. The composition according to claim 1, wherein the variable light chain is a germline antibody sequence with no defined specificity.
9. The composition according to claim 8, wherein the light chain is a kappa chain.
10. The composition according to claim 9, wherein the light chain is a human kappa chain germline IGKV4-1*101.
11. The composition according to claim 1, wherein the 5' UTR of (a) or (b) are independently selected from a human c-myc 5' UTR.
12. The composition according to claim 1, wherein the leader peptide for the F16 heavy chain and F16 light chains in (a) are each heterologous and are different from each other.
13. The composition according to claim 1, wherein the leader peptide for the CR8033 heavy chain and CR8033 light chains in (b) are each and are different from each other.

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14. The composition according to claim 12 or 13, wherein the signal peptide is an IL-2 signal peptide.
15. The composition according to claim 1, wherein the composition is formulated for intranasal administration.
16. The composition according to claim 1, wherein the composition is formulated for intramuscular or intravenous administration.
17. A method for immunizing human patients against influenza, the method comprising administering an effective amount of a composition according to any one of claims 1 to 15.
18. The method according to claim 18, wherein the patient is administered a dose in an amount of about 1×10^{12} to about 3×10^{13} GC.
19. The method according to claim 17, wherein the composition is administered intranasally in a single nostril.
20. The method according to claim 19, wherein the composition is administered intranasally to each nostril.
21. The method according to claim 17, wherein the patient is administered two sequential applications of about 0.4 mL per nostril.
22. The method according to claim 17, wherein the composition is administered intramuscularly or intravenously.
23. A composition according to any one of claims 1 to 15, which is adapted for administration to a patient in an amount of about 1×10^{12} to about 3×10^{13} genome copies.

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24. The composition according to claim 23 which is administrable intranasally in a single nostril.

26. The composition according to claim 23 which is administrable intranasally to each nostril, optionally being administered in two sequential applications of about 0.2 mL per nostril.

27. A product which comprises a container comprising a composition according to any one of claims 1 to 16, optional diluent, and instructions for administration.

FIG 1A

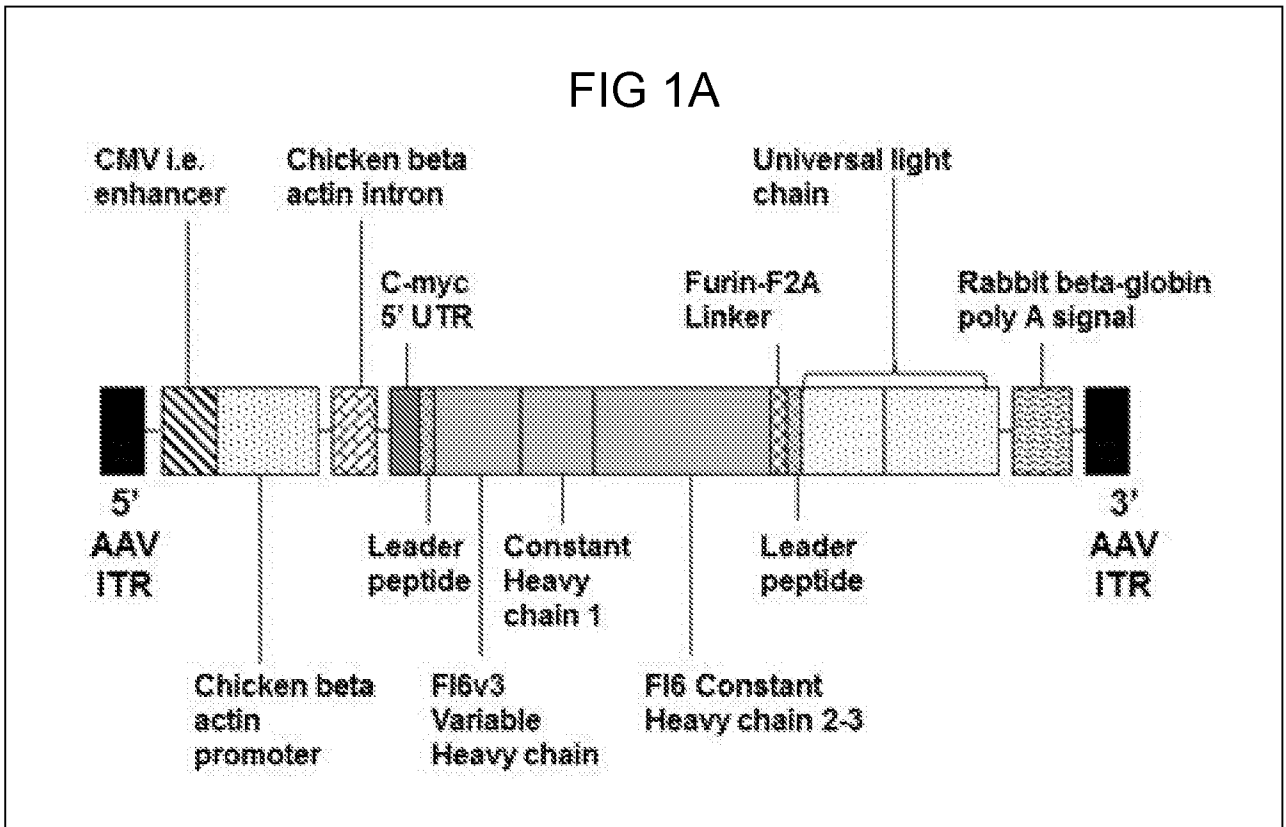


FIG 1B

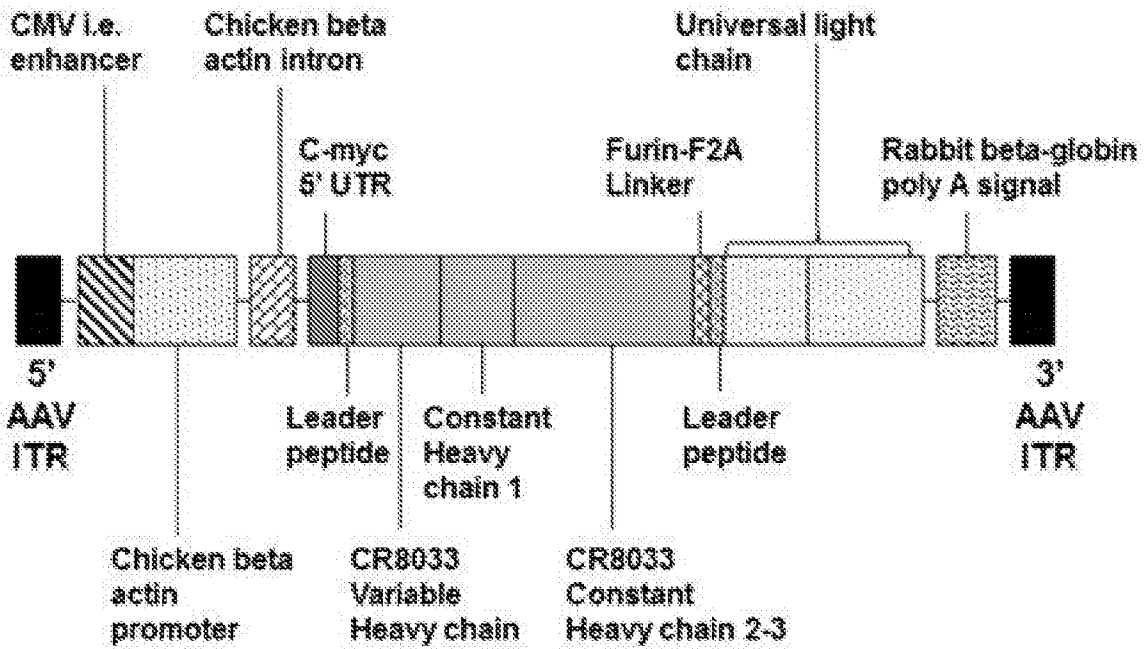
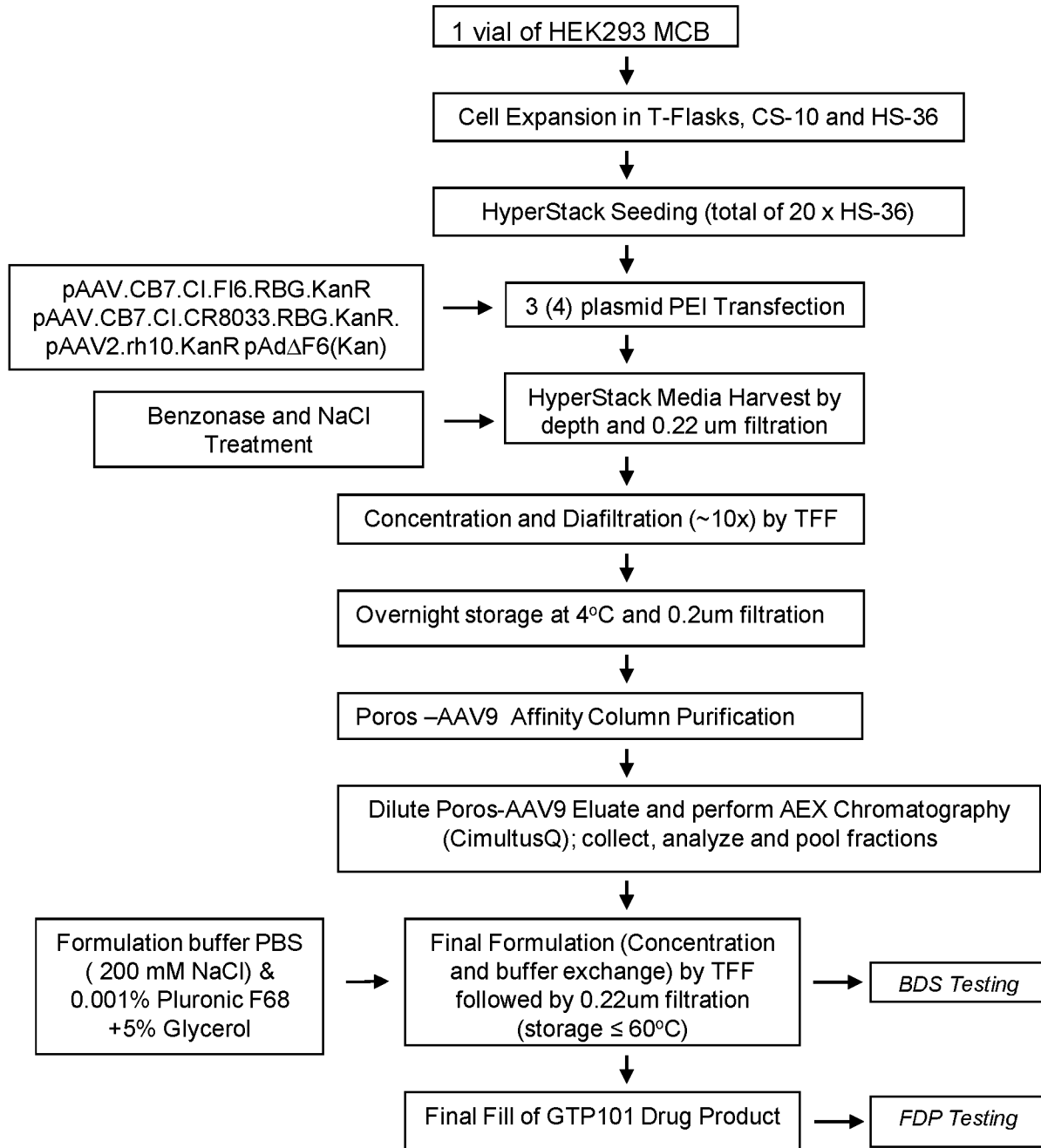


FIG 2



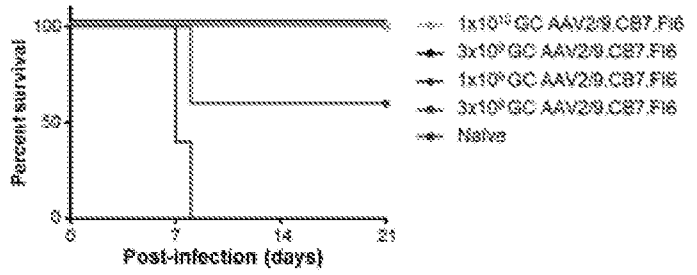
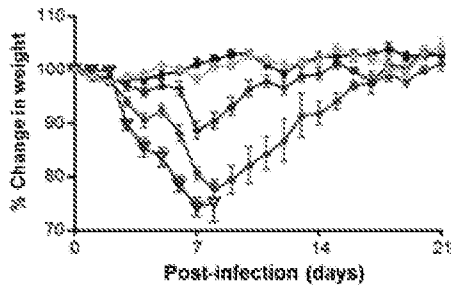


FIG 3A

FIG 3B

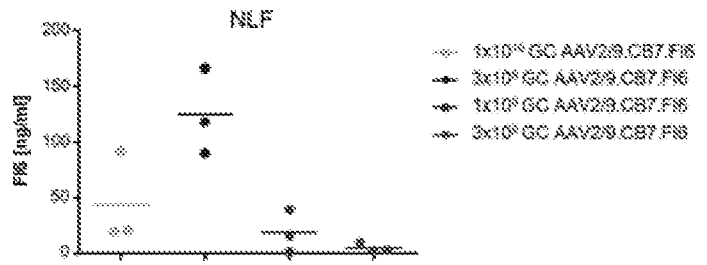
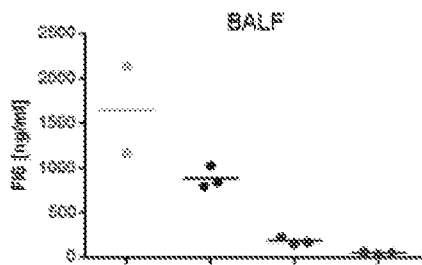


FIG 4A

FIG 4B

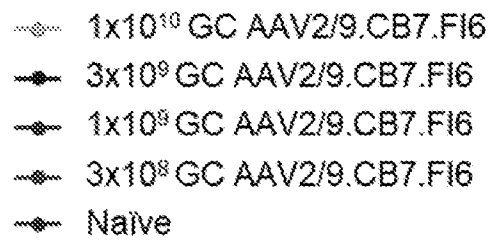
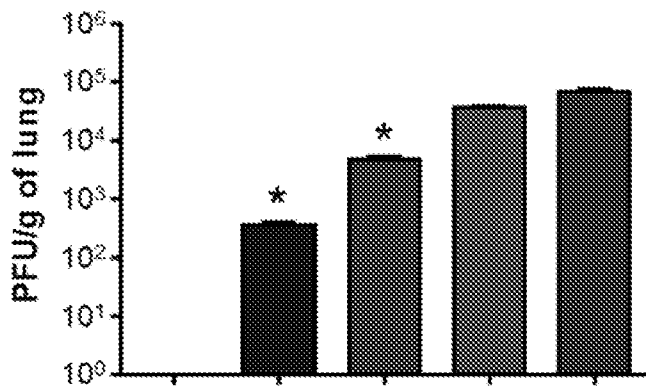
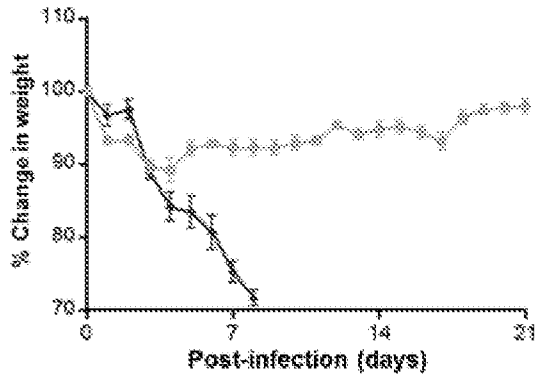


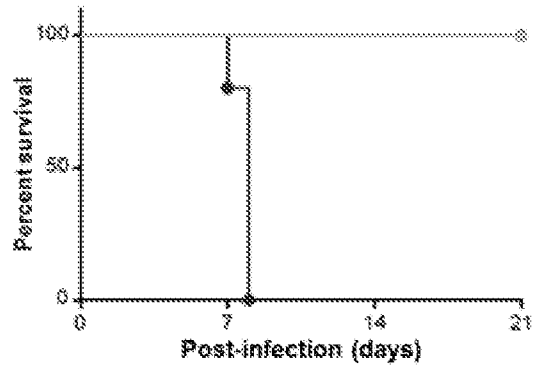
FIG 5

FIG 6A



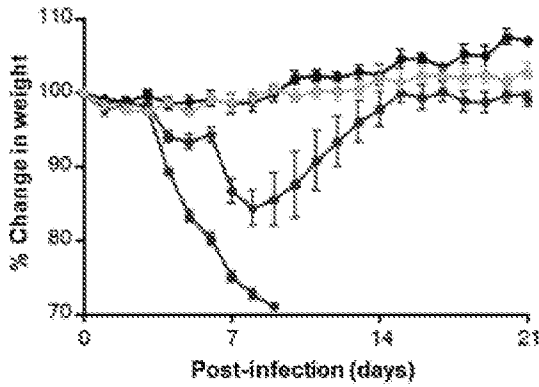
1×10^{11} GC AAV2/9.CB7.F16 (8 hours prior to PR8 challenge)
 Naive

FIG 6B



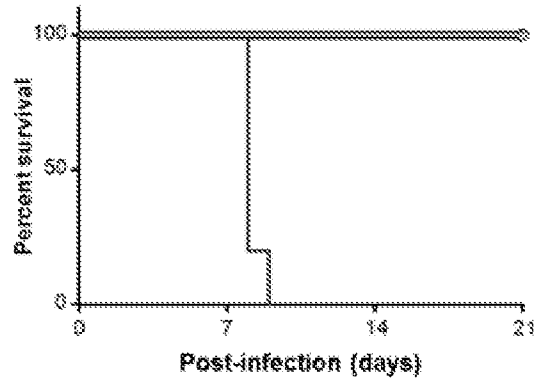
1×10^{11} GC AAV2/9.CB7.F16 (8 hours prior to PR8 challenge)
 Naive

FIG 7A

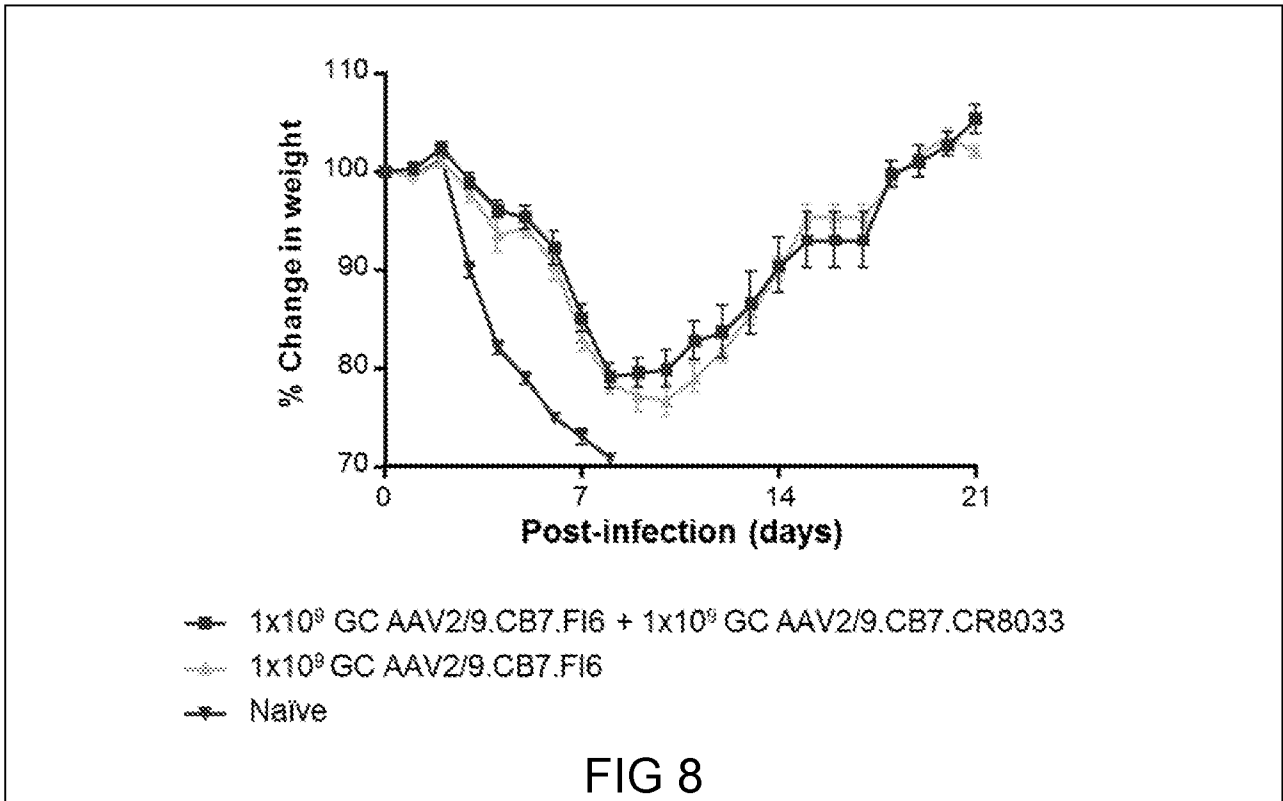


1×10^{10} GC AAV2/9.CB7.CR8033
 3×10^9 GC AAV2/9.CB7.CR8033
 1×10^8 GC AAV2/9.CB7.CR8033
 Naive

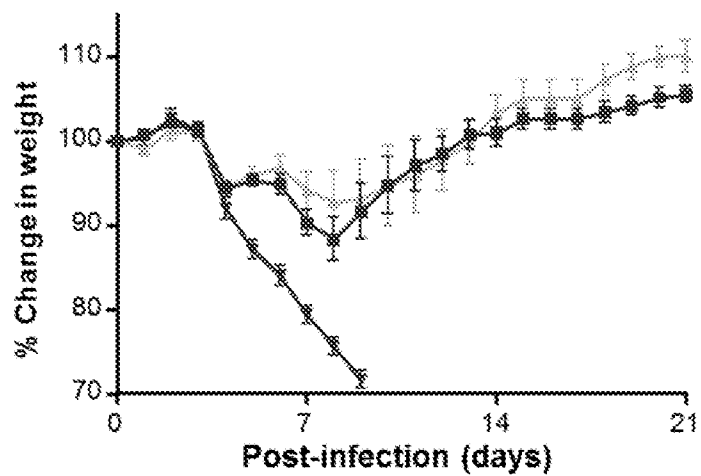
FIG 7B



1×10^{10} GC AAV2/9.CB7.CR8033
 3×10^9 GC AAV2/9.CB7.CR8033
 1×10^8 GC AAV2/9.CB7.CR8033
 Naive



◆ 1x10⁹ GC AAV2/9.CB7.FI6 + 1x10⁹ GC AAV2/9.CB7.CR8033
 ◇ 1x10⁹ GC AAV2/9.CB7.FI6
 ▲ Naive



◆ 1x10⁹ GC AAV2/9.CB7.CR8033 + 1x10⁹ GC AAV2/9.CB7.FI6
 ◇ 1x10⁹ GC AAV2/9.CB7.CR8033
 ▲ Naive

FIG 9

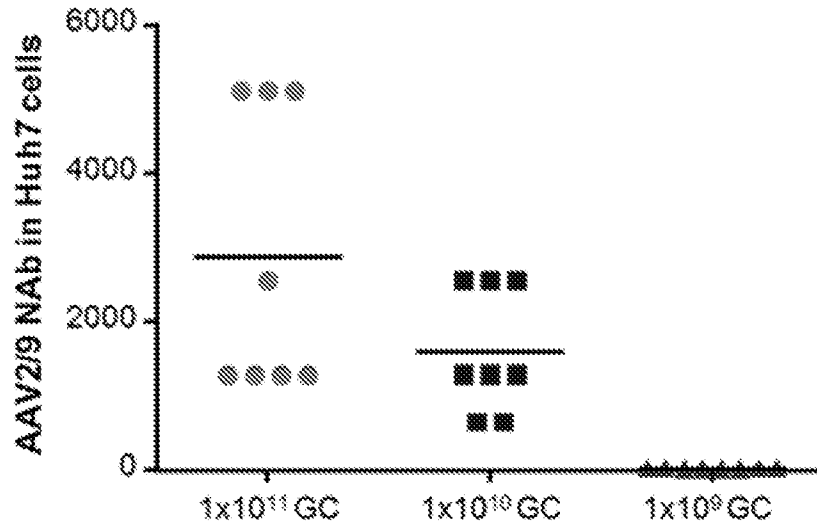


FIG 10

FIG 11A

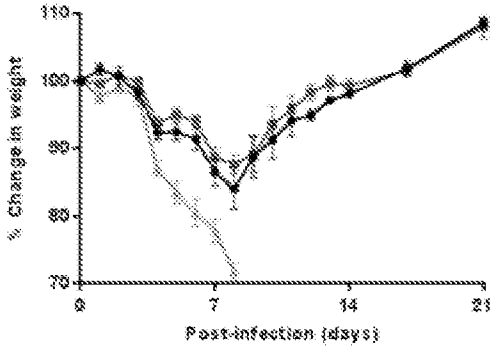


FIG 11B



FIG 11C

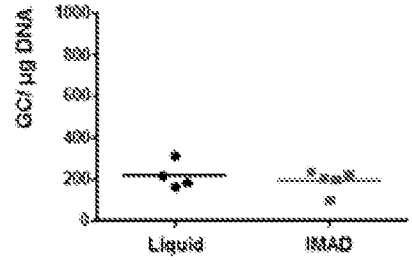


FIG 11D

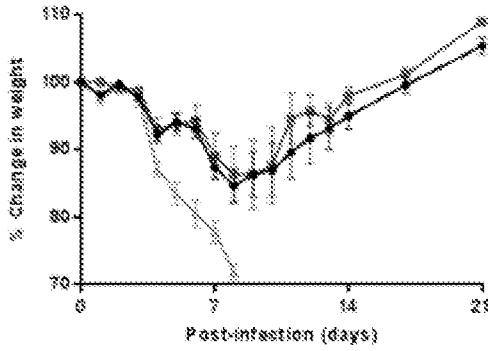


FIG 11E

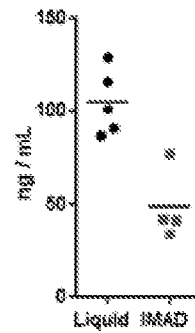


FIG 11F

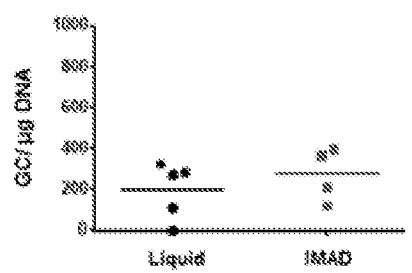


FIG 11G

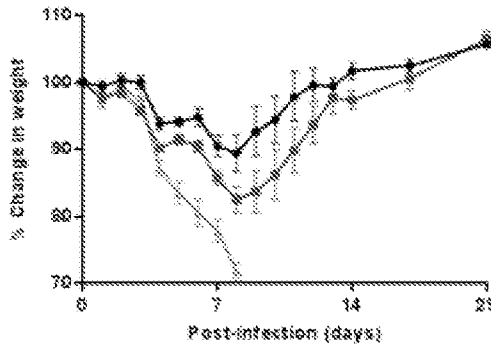


FIG 11H



FIG 11I

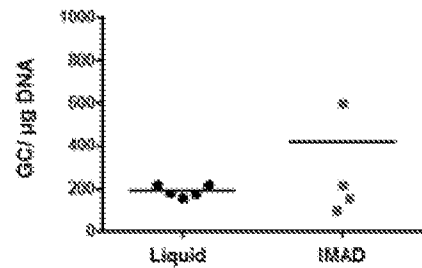


FIG 12A

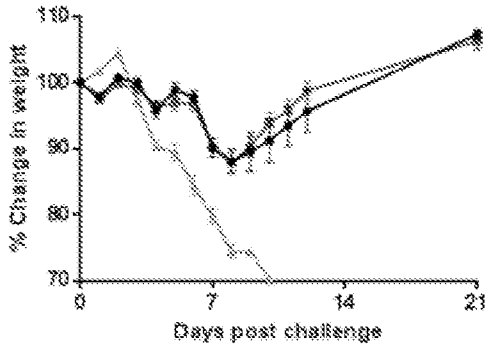


FIG 12B

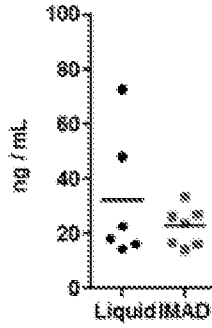


FIG 12C

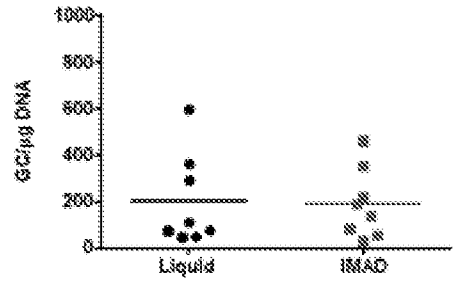


FIG 12D

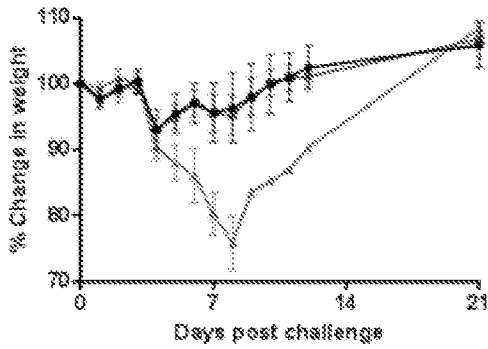


FIG 12E

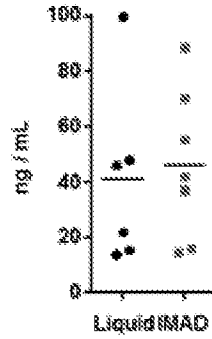


FIG 12F

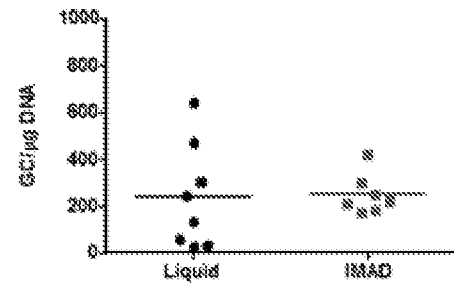


FIG 12G

Starting volume (mL)	Volume post INAD (mL)
0.500	0.467
0.500	0.480