(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
15 December 2016 (15.12.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/200951 A1

(72) Inventors: TRAGER, George; 385 Oyster Point Blvd
#9a, South San Francisco, California 94080 (US). TUCK-
ER, Sean; 385 Oyster Point Blvd #9a, South San Fran-
cisco, California 94080 (US). KIM, Leesun; 385 Oyster
Point Blvd #9a, South San Francisco, California 94080
(US). SCALLAN, Ciaran; 385 Oyster Point Blvd #9a,
South San Francisco, California 94080 (US).

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(54) Title: FORMULATIONS FOR SMALL INTESTINAL DELIVERY OF RSV AND NOROVIRUS ANTIGENS
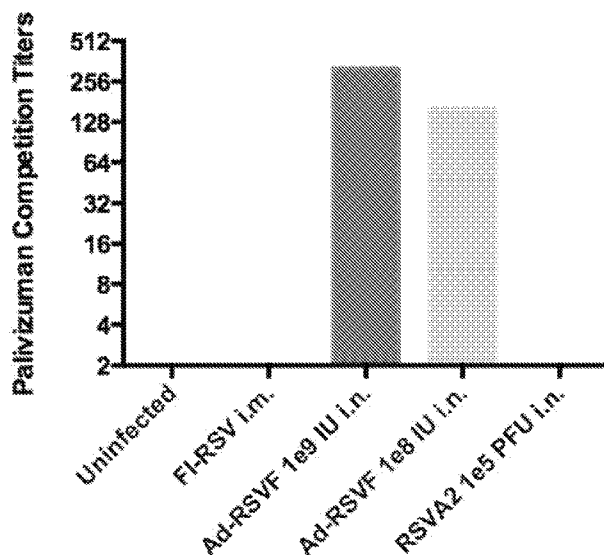
(57) Abstract: Provided herein are compositions
and methods for generating an immunogenic re-
sponse in humans. Further provided are methods for
designing such compositions, e.g., for vaccines.

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FORMULATIONS FOR SMALL INTESTINAL DELIVERY OF RSV AND NOROVIRUS ANTIGENS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001]   The present application claims priority to US Provisional Application No. 62/175,081, filed June 12, 2015, the disclosure of which is incorporated herein in its entirety.

REFERENCE TO SUBMISSION OF A SEQUENCE LISTING AS A TEXT FILE

[0002]   The Sequence Listing written in file 1011223_ST25.txt, created on May 13, 2016, 62,317 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0003]   Vaccines are an important means for preventing and/or treating a number of diseases and disorders (*e.g.*, viral infection, bacterial infection, and cancer). Vaccinization is typically carried out using injection, which reduces participation due to inconvenience of traveling to a vaccination site and aversion to injections. Furthermore, injection of vaccines requires use of sterile kit, such as syringes and needles, and a skilled practitioner to administer.

[0004]   For the influenza vaccine, large-scale yearly campaigns are conducted to collect enough fertilized eggs to harvest and process sufficient virus to meet the needs of the market. Cell culture or plant derived hemagglutinin (HA) may reduce the burden of egg acquisition and processing, but these approaches still require expensive sterile fill and finish to produce individual syringe needles, that need to be disposed of as a biohazard. During a pandemic, schools can be closed and social distancing mandated, yet mass influenza immunization typically requires lining up subjects at health clinics for injections. Oral vaccines, for influenza or other pathogens, could be sent through the mail thus avoiding most human to human contact. Further, tableting is a rapid, sanitary process that does not require the expensive sterile fill and finishing process that injected vaccines require.

[0005]   Vaccines that can be delivered in a non-parenteral manner, *e.g.*, orally or mucosally, are described in US Patent No. 8,222,224.

BRIEF SUMMARY OF THE INVENTION

[0006]   Provided herein are methods and compostions for more effective vaccination of a subject (human or non-human) involving delivery of an immunogenic biological agent specifically to the ileum of the subject. The present disclosure thus provides more efficient and effective vaccines, and demonstrates their effectiveness in humans.

[0007]   Provided herein are immunogenic compositions for eliciting an immune response in a subject comprising: an immunogenic biological agent encompassed by a delivery agent that directs delivery of the immunogenic biological agent to the ileum of the subject. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human animal, *e.g.*, primate, mouse, rat, rabbit, horse, dog, cat, or poultry. In some embodiments, the immunogenic biological agent is selected from an immunogenic polypeptide (*e.g.*, virus like particle, glycoprotein, phosphoprotein), carbohydrate, and lipid. In some embodiments, the immunologenic biological agent is an adenoviral vector encoding the viral protein 1 of norovirus or the fusion protein (F) of Respiratory syncytial virus (RSV). In some embodiments, the viral protein 1 of norovirus is SEQ ID NO: 2 or SEQ ID NO: 4. In some embodiments, the fusion protein (F) of RSV is SEQ ID NO: 6. In some embodiments, the adenoviral vector comprises a nucleotide sequence of SEQ ID NO: 7.

[0008]   In some embodiments, the immunogenic biological agent is an expression vector encoding an immunogenic polypeptide. In some embodiments, the expression vector is a viral vector (*e.g.*, adenoviral, AAV, retroviral, or lentiviral). In some embodiments, the viral vector is attenuated or replication incompetent. In some embodiments, the expression vector comprises a promoter (*e.g.*, CMV, SV40 early or late, β-actin, etc.) operably linked to the sequence encoding the immunogenic polypeptide. In some embodiments, the expression vector further encodes double stranded (dsRNA). In some embodiments, the dsRNA encoding sequence is operably linked to a promoter, *e.g.*, either the same promoter (using an Internal Ribosomal Entry Site (IRES)) or a different promoter as the promoter operably linked to the immunogenic polypeptide encoding sequence.

[0009] In some embodiments, the immunogenic composition further comprises at least one adjuvant, *e.g.*, a TLR3 agonist. In some embodiments, the TLR3 agonist is dsRNA or a dsRNA mimetic.

[0010] In some embodiments, at least 50% of the immunogenic biological agent is delivered (released) in the ileum, *e.g.*, at least 60%, 70%, 75%, 80%, 90%, 95%, or more of the immunogenic biological agent present in the administered composition. In some embodiments, the enteric coating or matrix begins to dissolve before the immunogenic composition reaches the ileum, but retains at least 50% of the immunogenic biological agent until the immunogenic composition reaches the ileum. In some embodiments, the enteric coating retains the immunogenic biological agent through the stomach, duodenum, and jejunum, but releases the immunogenic biological agent in the ileum.

[0011] In some embodiments the immunogenic biological agent is covered by an enteric coating. In some embodiments, the enteric coating disintegrates at pH≥5, *e.g.*, 5.2, 5.5, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 7.0, 5.5-6.8, 5.8-6.8, etc. In some embodiments, the enteric coating is selected from the group consisting of methacrylic acid-ethyl acrylate copolymer (*e.g.*, 1:1), type A; methacrylic acid copolymer, type C; a mixture of methacrylic copolymer types A and C; and Time Clock®. In some embodiments, the enteric coating does not include cellulose acetate phthalate (CAP). In some embodiments, the enteric coating is of a thickness that results in release of the immunogenic biological agent in the ileum. In some embodiments, the enteric coating is methacrylic acid copolymer-based with a coverage of 5.5-10 milligram per square centimeter. In some embodiments, the delivery agent is a radio-controlled capsule.

[0012] In some embodiments, the enteric coating comprises Poly(methylacylic acid-co-methyl methacrylate) 1:1. In some embodiments, the enteric coating comprises Eudragit® L-100. In some embodiments, the enteric coating comprises Eudragit® L-100, triethyl citrate, and talc, *e.g.*, 1, 2, 3, 4 or 1-4 parts Eudragit® L-100, 1-2 parts triethyl citrate, and 1-2 parts talc. In some embodiments, enteric coating comprises a mixture of poly(methacylic acid-co-methyl methacrylate) 1:1 and poly(methacylic acid-co-ethyl acrylate) 1:1. In some embodiments, the ratio of of poly(methacylic acid-co-methyl methacrylate) 1:1 to poly(methacylic acid-co-ethyl acrylate) 1:1 is 1:4 to 4:1, *e.g.*, 1:3, 1:2, 1:1, 2:1, 3:1. In some embodiments, the enteric coating comprises a mixture of Eudragit® L-100 and Eudragit®L100-55. In some embodiments, the enteric coating comprises Eudragit® L-100

and Eudragit®L100-55, triethyl citrate, and talc, *e.g.*, 1-4 parts Eudragit® L-100 and

Eudragit®L100-55, 1-2 parts triethyl citrate, and 1-2 parts talc. In some embodiments, the

enteric coating comprises poly(methacylic acid-co-methyl methacrylate) 1:1 and

poly(methacylic acid-co-methyl methacrylate) 1:2. In some embodiments, the ratio of

5   poly(methacylic acid-co-methyl methacrylate) 1:1 to poly(methacylic acid-co-methyl

acrylate) 1:2 is 1:2 to 2:1. In some embodiments, the enteric coating comprises a mixture of

Eudragit® L-100 and Eudragit®S100. In some embodiments, the enteric coating comprises

Eudragit® L-100 and Eudragit®S100, triethyl citrate, and talc, *e.g.*, 1-4 parts Eudragit® L-

100 and Eudragit®S100, 1-2 parts triethyl citrate, and 1-2 parts talc. In some embodiments,

10   the enteric coating comprises a mixture of poly(methacylic acid-co-methyl methacrylate) 1:2

and poly(methacylic acid-co-ethyl acrylate) 1:1. In some embodiments, the ratio of

poly(methacylic acid-co-methyl methacrylate) 1:2 and poly(methacylic acid-co-ethyl

acrylate) 1:1 is 1:4 to 4:1, *e.g.*, 1:3, 1:2, 1:1, 2:1, or 3:1. In some embodiments, the enteric

coating comprises a mixture of Eudragit® L-100-55 and Eudragit®S100. In some

15   embodiments, the enteric coating comprises Eudragit® L-100-55 and Eudragit®S100,

triethyl citrate, and talc, *e.g.*, 1-4 parts Eudragit® L-100-55 and Eudragit®S100, 1-2 parts

triethyl citrate, and 1-2 parts talc.

[0013]   In some embodiments, the immunogenic composition is in the form of a tablet or

capsule, *e.g.*, in the form of a compressed tablet covered by enteric coating. In some

20   embodiments, the immunogenic composition is encapsulated in a polymeric capsule

comprising gelatin, hydroxypropylmethylcellulose, starch, or pullulan. In some

embodiments, the immunogenic composition is in the form of microparticles less than 2mm

in diameter, *e.g.*, each microparticle covered with enteric coating as described herein.

[0014]   Further provided is a method of delivering an immunogenic composition to the

25   ileum of a subject comprising orally administering the immunogenic composition as

described above (*i.e.*, an immunogenic biological agent encompassed by a delivery agent that

directs delivery of the immunogenic biological agent to the ileum, optionally including an

adjuvant) to the subject. In some embodiments, the subject is a human. In some

embodiments, the subject is a non-human animal. In some embodiments, the method results

30   in an immune response in the subject that is at least 10% higher, *e.g.*, at least 20%, 30%,

40%, 50%, 60%, 75%, 80%, 100% or more, than the immune response in a subject (either the

same subject at a different time, or a different subject) receiving the same immunogenic

composition not directed to the ileum. In some embodiments, the immune response in the

subject is at least 1.5-fold higher (e.g., 2-fold, 2.5-fold, 5-fold, or more) than the immune response in a subject (either the same subject at a different time, or a different subject) receiving the same immunogenic composition not directed to the ileum. In some embodiments, the immune response is an increase in antibodies specific for the immunogenic biological agent. In some embodiments, the immune response is a cellular immune response, e.g., an increase in cytokines such as IFN-γ. In some embodiments, the immune response is immunization (e.g., the subject is resistant to infection by the virus, bacteria, etc. from which the immunogenic biological agent was derived).

[0015]   Further provided are methods of eliciting an increased immune response in a subject comprising orally administering the immunogenic composition as described above (i.e., an immunogenic biological agent encompassed by a delivery agent that directs delivery of the immunogenic biological agent to the ileum, optionally including an adjuvant) to the subject, e.g., human subject. In some embodiments, the immune response is increased by at least 10%, e.g., at least 20%, 30%, 40%, 50%, 60%, 75%, 80%, 100% or more, compared to the immune response in a subject (either the same subject at a different time, or a different subject) receiving the same immunogenic composition not directed to the ileum. In some embodiments, the immune response in the subject is increased at least 1.5-fold (e.g., 2-fold, 2.5-fold, 5-fold, or more) compared the immune response in a subject (either the same subject at a different time, or a different subject) receiving the same immunogenic composition not directed to the ileum. In some embodiments, the immune response is an increase in antibodies specific for the immunogenic biological agent. In some embodiments, the immune response is a cellular immune response, e.g., an increase in cytokines such as IFN-γ. In some embodiments, the immune response is immunization (e.g., the subject is resistant to infection by the virus, bacteria, etc. from which the immunogenic biological agent was derived).

BRIEF DESCRIPTION OF THE DRAWINGS

[0016]   FIG. 1. Antibody Secreting Cells (ASCs) specific for HA were measured in the peripheral blood 7 days after subjects were given a radio-controlled capsule containing rAd-HA-dsRNA. Subjects were randomized to have the vaccine released in either the ileum or jejunum. (N=12 per group). Results show that 12 of 12 subjects with vaccine delivered to the ileum were able to generate antibody secreting B cells that recognize HA, whereas only 9 of 12 subjects given the vaccine to the jejunum were able to generate antigen specific B cells. The average number of IgA and IgG ASCs was significantly higher for the ileum than the jejunum.

[0017]   FIG. 2. T cell response to rAd-HA-dsRNA was determined by detecting IFN-γlevels 7 days post-administration. All of the individuals in the ileum-delivery group showed higher levels of IFN-γ, compared to 75% of the jejunum-delivery group. The average IFN-γ level was also significantly higher in the ileum-delivery group.

[0018]   FIG. 3. Microneutralizing antibody (MN) responses to influenza A/CA/07/2009 were measured at day 0 and day 28 after immunization. The fold increase in MN titers was plotted for individual subjects that had an initial MN titer less than or equal to 40. Results showed that ileum delivery resulted in a high proportion of subjects (9 of 10) with increased MN titers following immunization compared to jejunum delivery (6 of 10).

[0019]   FIG. 4. Tablets were made using microcrystalline cellulose and starch, with 10% barium sulfate as a radiopaque material. These tablets were enteric coated with Eudragit L100® and given to female cynomolgus macaques by oral gastric tube. X-rays were taken over time post administration. A. Tablet in the stomach with the arrow pointing toward the tablet. B. One hour later, the tablet can be seen in the intestine, the white spot to the left of the spinal column with an arrow point toward it. It dissolved in the intestine within the next two hours, and cannot be seen.

[0020]   FIG. 5. The numbers of ASCs are reported on days 7 and 35, 7 days after each immunization. Background ASCs at days 0 and 28 were miniscule, and not plotted. Average responses for day 7 are shown for each treated group with a horizontal line.

[0021]   FIG. 6. Fold increase in MN titers for individual subjects. The dark shaded columns indicate where the titers rose between days 28 and 56, whereas the light shaded columns shows the response after the initial immunization. A line was drawn at two fold increases in MN to show which subjects had a detectable neutralizing antibody response. No subject in the placebo group responded, whereas 3 subjects in the low dose and 7 subjects in the high dose group had a 2 fold or greater neutralizing antibody response to influenza after immunization. Placebo N=10, Low Dose and High Dose N=11.

[0022]   FIG. 7. Antibody responses following a single oral immunization. A. Hemagglutination Inhibition ("HAI") antibody titers pre and post immunization (days 0 and 28 respectively) are shown for individual subjects. B. HAI Geometric Mean Titers (GMT) vs Time. HAI titers were measured at 0, 1, and 6 months post immunization to evaluate the durability of the antibody response. C. MN titers, pre and post immunization are shown for

individual subjects. D. ASC responses following immunization. The numbers of IgG and IgA ASCs are reported (per 10^6 PMBCs) 7 days after immunization.

[0023]   FIG. 8. Serum ELISA IgG Titers Versus Dose of VXA-G1.1-NN in mice. Mice were immunized with VXA-G1.1-NN of 1x108, 5x108 and 1x109. VXA-G1.1-NN was delivered orally by gavage on days 0 and 28. The serum IgG responses against Norwalk VP1 were measure by ELISA at week 8. N=6. Each icon represents an individual mouse. The top of the bar for each study indicates the GMT. As the dose was increased from 1x108 to 5x108 to 1x109, the serum VP1 IgG titer showed a dose-dependent increase from 2x103 to 1x104 to 5x105.

[0024]   FIG. 9. Fecal SIgA ELISA Titers Versus Dose of VXA-G1.1-NN in mice. Mice were immunized with VXA-G1.1-NN of 1x108, 5x108 and 1x109. VXA-G1.1-NN was delivered orally by gavage on days 0 and 28. The fecal SIgA responses against Norwalk VP1 were measure by ELISA at week 8. Each study has a total of 6 mice. Each icon represents an individual mouse. The top of the bar for each study indicates the GMT. As the dose was increased from 1x108 to 5x108 to 1x109, the fecal VP1 sIgA titer showed a dose-dependent increase from 1x103 to 2x103 to 3x104.

[0025]   FIG. 10. Serum ELISA IgG Titers of VXA-G1.1-NN Versus VP1 protein in mice. Mice were immunized with VXA-G1.1-NN of 1x108 orally or Norwalk virus VP1 protein (1ug) intramuscularly on days 0 and 28. The serum IgG responses against Norwalk VP1 were measured by ELISA at weeks 4 and 8. Each study has a total of 6 mice. Each icon represents an individual mouse. The top of the bar for each study indicates the geomean titer. Oral vaccine generated slightly higher serum IgG titer values than the i.m. protein vaccine.

[0026]   FIG. 11. Fecal SIgA ELISA Titers Versus Dose of VXA-G1.1-NN in mice. Mice were immunized with VXA-G1.1-NN of 1x108 orally or Norwalk virus VP1 protein (1ug) intramuscularly on days 0 and 28. The fecal IgA responses against Norwalk VP1 were measure by ELISA at weeks 4 and 8. Each study has a total of 6 mice. Each icon represents an individual mouse. The top of the bar for each study indicates the geomean titer. The oral vaccine generated a dramatically higher fecal IgA immune response than the i.m. protein vaccine.

[0027]   FIG. 12. Oral immunization of VXA-G1.1-NN compared to VP1 protein immunization for Serum ELISA Titers. Mice were immunized with VXA-G1.1-NN of 1x108 orally or Norwalk virus VP1 protein (1ug) in the presence of adjuvant, aluminium hydroxide

intramuscularly on days 0 and 28. The serum IgG responses against Norwalk VP1 were measure by ELISA at weeks 4 and 8. Each study has a total of 6 mice. Each icon represents an individual mouse. The top of the bar for each study indicates the geomean titer. Intramuscular injection with the VP1 protein together with alum generated much higher

5   serum titer.

[0028]   FIG. 13. Oral immunization of VXA-G1.1-NN compared to VP1 protein immunization for Fecal SIgA ELISA Titers Mice were immunized with VXA-G1.1-NN of 1x108 orally or Norwalk virus VP1 protein (1ug) in the presence of adjuvant, aluminium hydroxide intramuscularly on days 0 and 28. The fecal IgA responses against Norwalk VP1

10   were measure by ELISA at weeks 4 and 8. Each study has a total of 6 mice. Each icon represents an individual mouse. The top of the bar for each study indicates the geomean titer. Even in the presence of adjuvant, aluminium hydroxide, the oral vaccine generated a higher fecal SIgA immune response than the i.m. protein vaccine.

[0029]   FIG. 14. Serum and Fecal IgA Titers following Oral Immunization of VXA-G2.4-

15   NS in mice. Mice were immunized with VXA-G2.4-NS of 1x108 orally on days 0 and 28. For comparison, oral delivered groups from study#3 were presented again. The serum IgG responses against Sydney VP1 were measure by ELISA at weeks 4 and 8 (A). The fecal SIgA response against the Sydney VP1 was measured by ELISA at week 8 (B). At 4 weeks, the Sydney strain vaccine generated better IgG titer values than the Norwalk vaccine. In addition,

20   even at 4 weeks the titer values from the Sydney strain were slightly higher than the Norwalk values at 8 weeks (A). The Sydney vaccine generated slightly higher fecal VP1 IgA titer values than the Norwalk vaccine.

[0030]   FIG. 15. Serum and Fecal IgA Titers following Oral Immunization of VXA-G2.4-
NS in ferrets. Ferrets were endoscopically administered VXA-G2.4-NS on days 0 and 2

25   (group1) or days 0 and 28 (group2). Ferrets in group3 were intramuscularly administered the recombinant VP1 protein from the Sydney strain norovirus on days 0 and 28. Whereas group1 generated higher IgG titer values than group2, group2 generated higher SIgA titer values than group1. Group3 failed to generate fecal SIgA response although serum IgG responses were generated.

30   [0031]   FIG. 16. Serum IgG following Oral Immunization of VXA-G2.4-NS in Non human primates (NHP, Cynomolgous macaque). NHPs were endoscopically administered VXA-

G2.4-NS on days 0 and 56 (the "Noro" group). Serum IgG titers were measured by ELISA at day 0, 7, 28, 56, and 72.

[0032]   FIG. 17. Anti-RSVF antibody titers in cotton rats at 4weeks post vaccination with Ad-RSVF vaccine vectors or control formalin inactivated RSV (FIRSV) or live wild-type RSVA2. Titers were determined using an anti-RSVF IgG ELISA.

[0033]   FIG. 18. Palivizumab Competition using sera from cotton rats vaccinated with Ad-RSVF vaccine. ELISA plates were coated with an RSVA2 lysate at 1 ug/mL. Biotinylated Palivizumab (10 ng/mL) was mixed with serial 2-fold dilutions of control and test sera. Naïve sera was used to determine 100% Palivizumab binding. Unlabelled Palivizumab was used as positive control. HRP-Strepavidin with TMB substrate was used to detect biotinylated Palivizumab. Inhibition was scored relative to 100% biotinyaled Paliziumab binding. The maximum dilution that gave 50% or greater neutralization activity was assigned as the competition titer.

[0034]   FIG. 19. Enrollment criteria for the study of "High Titer Neutralizing Antibodies to Influenza Following Oral Tablet Immunization: A Randomized, Placebo-controlled Trial." The major exclusion criteria are:

- positive for H1 influenza by HAI;
- has had an influenza vaccine in the past 2 years;
- current history of chronic alcohol consumption and/or illicit and/or recreational drug use;
- history of any confirmed or suspected immunodeficient or immunosuppressive condition;
- positive serology for HIV, HCV, or HBV;
- previous serious reactions to vaccination such as anaphylaxis, respiratory problems, hives, or abdominal pain;
- history of irritable bowel disease or other inflammatory digestive or gastrointestinal conditions that could affect the intended distribution of the vaccine targeting the mucosa of the small intestine;
- use of proton pump inhibitors (Nexium, Prilosec); and
- stool sample with occult blood at baseline exam.

[0035]   FIGs. 20A-20D. Antibody responses following a single oral immunization of a recombinant Ad serotype 5 (rAd5) based oral vaccine against H1 seasonal influenza. FIG. 20A shows HAI antibody titers pre and post immunization (days 0 and 28 respective) are shown for individual subjects. FIG. 20B shows HAI GMT v. time. HAI titers were measured at 0, 1, and 6 months post immunization to evaluate the durability of the antivody response. FIG. 20C shows MN titers pre and post immunization for individual subjects. FIG 20D shows ASC response following immunization. The numbers of IgG and IgA ASCs are reported (per 1e6 PMBCs) 7 days after immunization.

[0036]   FIGs. 21A-21B. Anti-Ad5 immunity and effects on neutralizing antibody responses. FIG. 21A shows Fold change in MN versus starting anti-Ad5 titer for vaccine treated subjects. Subjects were not pre-screened for anti-Ad5 titers, but retrospectively measured. FIG. 21B shows fold change in HAI responses that were plotted for etiher Ad5 titer positive or negative, analogous as performed in FIG. 2. No trend was observed for Ad5 starting titers on the ability to elicit a neutralizing antibody response (by MN or HAI) to influenza virus.

[0037]   FIG. 22 shows mice elicit robust antibody titers against RSV when given VXA-RSV-f. Study No. WCB254: Balb/c mice were immunized with VXA-RSV-f at 0 and 3 weeks using three different routes of delivery. ELISA IgG antibody titers were measured at week 7. All routes of delivery generated significant immune responses against RSV; however, i.n. and i.m. were more effective at producing higher titers than oral (p=0.04, or 0.02 by Mann-Whitney). N=6 per group. As negative controls, mouse sera from animals given a norovirus vaccine were used.

[0038]   FIGs. 23A-23C shows immunization of cotton rats with VXA-RSV-f vaccine induces antibody responses to RSV. (Experiment XV-95). FIG. 23A shows female cotton rats were immunized on week 0 and 4, and antibody titers against RSV-f were measured on week 8. VXA-RSV-f immunization was significantly better than using RSV2 virus or FI-RSV for inducing IgG ELISA titers to RSV. (p<0.0022 by Mann-Whitney) FIG. 23B shows palivizumab competitive ELISA titers were evaluated using pooled serum samples from each of the previously described groups. FI-RSV vaccine was not able to induce epitope specific antibodies that compete for the binding of palivizumab, but the VXA-RSV-f vaccine and RSV2 treated groups were able to induce these titers. FIG. 23C shows the VXA-RSV-f vaccine induced neutralizing antibody responses to RSV following immunization of cotton

rats. VXA-RSV-f and RSV2 groups were superior to the FI-RSV, and the no vaccine controls (no infection and buffer groups) at inducing neutralizing titers against RSV. N=6 per group, except the "no infection" control (N=3).

[0039]   FIGs. 24A-24B show oral immunization induces potent antibodies to RSV in cotton rats. (Experiment XV-112). In FIG. 24A, female cotton rats were immunized with VXA-RSV-f on week 0 and 4, and the total IgG antibody ELISA titers were measured on weeks 4 and 8. Dose dependent antibody responses were observed with 1×10^9 and 1×10^{10} trending better than the 1×10^8 IU dose group. In FIG. 24B, Palivizumab competitive ELISA titers were evaluated for the same groups as described before, but at week 8. Higher vaccine doses trended with higher Palivizumab competitive antibody titers, but the results were not significantly different. FIG. 24C shows oral immunization of VXA-RSV-f also induced dose dependent neutralizing antibody titers to RSV, with the 1x1010 group performing significantly better than the 1x108 group (p=0.018 by Mann-Whitney). N=8 per oral group, N=6 for buffer control group.

[0040]   FIGs. 25A-25C shows VXA-RSV-f protects against RSV replication and adaptive immune enhancements of RSV disease. (Experiment XV-95) FIG. 25A shows animals immunized with VXA-RSV-f before RSV challenge were completely protected against RSV replication in lung and nasal samples. Animals immunized with either buffer, adjuvant alone, or FI-RSV were not protected. Two different doses of vaccine were given for the i.n. treated animals (1×10^8=low). N=6, except the no infection control animals (N=3). In FIG 25B, inflammation in the lungs was scored by immunohistology. The vaccine groups (VXA-RSV-f and RSV2) did not cause adaptive immune enhanced inflammation (PB, PA, A, IP) as did the FI-RSV group. In FIG. 25C, cytokine abundance levels were measured by qRT-PCR. Only the FI-RSV immunized group had substantial increases in the relative abundance of IL-4 and IL-13, the Th2 cytokines measured.

[0041]   FIGs. 26A-26C shows oral immunization induces protection against RSV replication and disease. (Experiment XV-112). In FIG. 26A, animals immunized orally show dose dependent immune responses to RSV infection, with the highest dose leading to complete protection in the lungs and almost complete protection in the nose. In contrast, there was no protection in the buffer control group. In FIG. 26B, inflammation was compared between vaccine doses and the buffer control group. In FIG. 26C, cytokine abundance levels were measured by qRT-PCR. No group had substantial increases in the

relative abundance of either IL-4 or IL-13, the Th2 cytokines measured. (N=8 with the exception of buffer control N=6).

DETAILED DESCRIPTION OF THE INVENTION

[0042]   The inventors have discovered that delivery of an immunogenic biological agent to a particular part of the small intestine, *i.e.*, the ileum, results in a much greater therapeutic response than when the agent is not targeted, or is targeted to a different site. This allows for design of more effective vaccines, reduced costs for materials, and reduced side effects for the recipient.

I.   Definitions

[0043]   The term "immunogenic" refers to the ability of an agent to give rise to an immune response in a host, either humoral or cell-mediated. Immunogenic agents are typically "foreign" to the host, *e.g.*, from a different species, or from a bacteria, virus, or fungus. A non-foreign agent can be immunogenic, *e.g.*, in the case of an autoimmune response. Certain cancer cell-specific agents can be exploited as immunogenic agents, allowing the host's immune system to attack the cancer.

[0044]   The term "biological agent" refers to a nucleic acid, polypeptide, glycoprotein, carbohydrate, lipid, or modified form thereof (*e.g.* methylated, glycosylated, detectably labeled). Biological agents are distinguished from small molecule drugs in that they can be created by biological processes (including recombinant techniques) instead of chemical synthesis. Biological agents can, however, be chemically modified or include non-natural nucleotides or amino acids. Biological agents can also be non-naturally occurring, *e.g.*, recombinant or chimeric entities.

[0045]   As used herein, an "immunogenic biological agent" refers to an agent that acts directly as an antigen (*e.g.*, is recognized by a T cell receptor or antibody), or an agent that, once expressed in a cell, acts as an antigen. For example, an immunogenic biological agent can include an expression vector encoding an immunogenic polypeptide.

[0046]   The term "antigen" refers to a polypeptide, glycoprotein, lipoprotein, lipid, carbohydrate, or other agent that is bound (*e.g.*, recognized as "foreign") by a T cell receptor and/or antibody. Antigens are commonly derived from bacterial, viral, or fungal sources. The term "derived from" indicates that the antigen is essentially as it exists in its natural antigenic context, or that it has been modified to be expressed under certain conditions, to

include only the most immunogenic portion, or to remove other potentially harmful associated components, etc.

[0047]   An "immunogenically effective dose or amount" of a composition as described herein is an amount that elicits or modulates an immune response specific for an antigen selected for vaccination. Immune responses include humoral immune responses and cell-mediated immune responses. An immunogenic composition can be used therapeutically or prophylactically to treat or prevent disease at any stage.

[0048]   "Humoral immune responses" are mediated by cell free components of the blood, *e.g.*, plasma or serum; transfer of the serum or plasma from one individual to another transfers humoral immunity. Humoral immune responses are typically B cell-mediated, *e.g.*, antibody production.

[0049]   "Cell mediated immune responses" are mediated by antigen specific lymphocytes; transfer of the antigen specific lymphocytes from one individual to another transfers immunity. Cell-mediated immune responses are mediated at least in part by T cells, and can be detected, *e.g.*, by detecting T cell-specific cytokines or increase in T cell proliferation.

[0050]   The "ileum" is the longest of the three segments that form the small intestine, along with the duodenum and jejunum. It is makes up the terminal portion, between the jejunum and cecum.

[0051]   An enteric coating is a barrier applied to oral medications that prevents the therapeutic agent inside from being digested in the low pH environment of the stomach and duodenum (~pH 3).

[0052]   A delivery agent, such as an enteric coating, matrix, or capsule, is said to retain an encompassed or embedded therapeutic agent when at least 60%, *e.g.*, at least about 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the original administered amount of therapeutic agent remains encompassed or embedded within the agent. The delivery agent, *e.g.*, enteric coating or matrix, is typically designed to disintegrate under certain conditions and release the therapeutic agent. Disintegration can be gradual, *e.g.*, in the case of a thicker or more chemically complex coating. The enteric coating is said to "disintegrate" once the coating thickness is reduced at least 10%, *e.g.*, at least 25%, 50%, or 75% compared to the original administered thickness. Disintegration is not an absolute term, as it can occur over a different time course depending on conditions. For example, a coating that is designed to disintegrate

in 5 minutes at pH 6.5 may disintegrate, albeit slowly, at pH 6 (e.g., in 1 hour). Disintegration does not necessarily indicate that the encompassed or embedded therapeutic agent is released. The therapeutic agent can, however, begin to be released before the enteric coating or matrix is entirely disintegrated.

[0053]   The term "chimeric" or "recombinant" as used herein with reference, *e.g.*, to a nucleic acid, protein, or vector, indicates that the nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein. Thus, for example, chimeric and recombinant vectors include nucleic acid sequences that are not found within the native (non-chimeric or non-recombinant) form of the vector. A chimeric viral expression vector refers to a viral expression vector comprising a nucleic acid sequence encoding a heterologous (*e.g.*, immunogenic) polypeptide.

[0054]   An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter. Viral expression vectors are typically rendered replication incompetent or attenuated. A virally-derived vector can include the components of the expression vector required for expression of a desired sequence, but omit those involved in, *e.g.*, replication or other pathogenic effects.

[0055]   The terms "promoter" and "expression control sequence" are used herein to refer to a nucleic acid control sequence that directs transcription of a nucleic acid. Promoter sequences are typically near the start site of transcription, such as a TATA element in the case of a polymerase II type promoter. A promoter can also include distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. Promoters include constitutive and inducible promoters. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression

control sequence directs transcription of the nucleic acid corresponding to the second sequence.

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

[0057]   The terms "nucleic acid" and "polynucleotide" are used interchangeably herein to refer to polymers of deoxyribonucleotides or ribonucleotides in either single- or double-stranded form. The terms encompass genes, cDNA, RNA, and oligonucleotides (short polynucleotides). The terms encompass nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). The term "nucleotide" typically refers to a nucleic acid monomer.

[0058]   Unless otherwise indicated, a particular nucleic acid sequence also encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[0059]   A "therapeutic dose" or "therapeutically effective amount" or "effective amount" of a composition as described herein is an amount that prevents, alleviates, abates, or reduces

the severity of symptoms of diseases and disorders associated with the source of the antigen selected for vaccination (e.g., a virus, bacteria, a parasite, or a cancer).

[0060]   The term "antibody" refers to a polypeptide encoded by an immunoglobulin gene or fragments thereof that specifically bind and recognizes an antigen. Immunoglobulin sequences include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region sequences, as well as myriad immunoglobulin variable region sequences. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0061]   T cells refer to a particular class of lymphocytes that express a specific receptor (T cell receptor) encoded by a family of genes. The recognized T cell receptor genes include alpha, beta, delta, and gamma loci, and the T cell receptors typically (but not universally) recognize a combination of MHC plus a short peptide. T cells are typically broadly classified as T helper cells (CD4+) and cytotoxic T cells (CD8+). Antibodies are naturally produced by B cells, e.g., Antibody Secreting Cells (ASCs). Mature B cells can be naïve, plasma B cells (activated and antibody-producing), memory, B-1, marginal-zone B cells, follicular B cells, and regulatory B cells.

[0062]   An adaptive immune response refers to T cell and/or B cell and/or antibody recognition of antigen.

[0063]   Antigen presenting cells (APCs) are cells that are able to present immunogenic peptides or fragments thereof to T cells to activate or enhance an immune response. APCs include dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects per se and/or to be immunologically compatible with the receiver (i.e., matched HLA haplotype). APCs may be isolated from any of a variety of biological fluids and organs including bone marrow, peripheral blood, tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells. APCs typically utilize a receptor from the major histocompatability (MHC) locus to present short polypeptides to T cells.

[0064]   An adjuvant is a non-specific immune response enhancer. Suitable adjuvants include, for example, cholera toxin, monophosphoryl lipid A (MPL), Freund's Complete

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Adjuvant, Freund's Incomplete Adjuvant, Quil A, and Al(OH). Adjuvants can also be those substances that cause APC activation and enhanced presentation of T cells through secondary signaling molecules like Toll-like receptors, e.g., double-stranded RNA (dsRNA), dsRNA mimetics, bacterial flagella, LPS, CpG DNA, and bacterial lipopeptide (Reviewed recently in [Abreu et al., J Immunol, 174(8), 4453-4460 (2005)]).

[0065]   The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0066]   The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0067]   Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0068]   "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the

codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations.

5 Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a

10 polypeptide is implicit in each described sequence.

[0069] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution

15 of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0070] The following eight groups each contain amino acids that are conservative

20 substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine I, Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

25 [0071] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of complementary (or largely complementary) nucleotides in a complex mixture (e.g., total cellular or library DNA or RNA).

[0072] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an individual polypeptide or dsRNA or a portion thereof) or may comprise a variant

30 of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that at least one biological activity of the encoded polypeptide (e.g., immunogenicity) is not diminished, relative to a polypeptide comprising

native antigens. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the adjuvant activity of an encoded dsRNA is not diminished, relative to a dsRNA that does not contain the substitutions, additions, deletions and/or insertions. Variants preferably exhibit at least about 50%, 55%, 60%, 65%, 70%, 75%,

5   80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a polynucleotide sequence that encodes a native polypeptide or a portion thereof or a dsRNA.

[0073]   The terms "identical" or percent "identity," in the context of two or more polynucleotide 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

10   the same (*i.e.*, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical."

15   This definition also refers to the compliment of a test polynucleotide sequence. Optionally, the identity exists over a region that is at least about 10 to about 100, about 20 to about 75, about 30 to about 50 amino acids or nucleotides in length.

[0074]   A "control" sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken

20   from a test condition, *e.g.*, in the presence of a test compound or treatment, and compared to samples from known conditions, *e.g.*, in the absence of the test compound (negative control), or in the presence of a known compound (positive control). In the context of the present disclosure, an example of a negative control would be a biological sample from a known healthy (non-infected) individual, and an example of a positive control would be a biological

25   sample from a known infected patient. A control can also represent an average value or a range gathered from a number of tests or results. One of skill in the art will recognize that controls can be designed for assessment of any number of parameters. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (*e.g.*, half-life) or therapeutic measures (*e.g.*, comparison of benefit and/or side effects). Controls can be

30   designed for *in vitro* applications. One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if

values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0075]   The term "diagnosis" refers to a relative probability that a subject has a disorder such as an infection or cancer. Similarly, the term "prognosis" refers to a relative probability that a certain future outcome may occur in the subject. The terms are not intended to be absolute, as will be appreciated by any one of skill in the field of medical diagnostics.

[0076]   The terms "therapy," "treatment," and "amelioration" refer to any reduction in the severity of symptoms. In the context of infection, treatment can refer to a reduction of infectious agent, reduced symptoms, etc. In the case of treating cancer, treatment can refer to, e.g., reducing tumor size, number of cancer cells, growth rate, metastatic activity, reducing cell death of non-cancer cells, etc. The terms "treat" and "prevent" are not intended to be absolute terms. Treatment and prevention can refer to any comparative reduction or apparent absence of infectious agent, delay in onset, amelioration of symptoms, improvement in patient survival, increase in survival time or rate, etc. Treatment and prevention can be complete (undetectable levels of infectious agent or neoplastic cells) or partial, such that fewer infectious agent or neoplastic cells are found in a patient than would have occurred without the presently described immunogenic biological agents. The effect of treatment can be compared to an individual or pool of individuals not receiving the treatment, or to the same patient prior to treatment or at a different time during treatment. In some aspects, the severity of infection or disease is reduced by at least 10%, as compared, e.g., to the individual before administration or to a control individual not undergoing treatment. In some aspects the severity of infection or disease is reduced by at least 25%, 50%, 75%, 80%, or 90%, or in some cases, no longer detectable using standard diagnostic techniques.

[0077]   "Subject," "patient," "individual" and like terms are used interchangeably and refer to, except where indicated, mammals such as humans and non-human primates, as well as rabbits, rats, mice, goats, pigs, and other mammalian species. The term does not necessarily indicate that the subject has been diagnosed with a particular disease, but typically refers to an individual under medical supervision. A patient can be an individual that is seeking treatment, monitoring, adjustment or modification of an existing therapeutic regimen, etc.

II.   Immunogenic biological agents

[0078]   An immunogenic biological agent is any biological agent that causes an immune response in the host, e.g., human host. The immunogenic biological agent can thus be a

polypeptide (*e.g.*, glycoprotein, phosphoprotein, or other modified form), carbohydrate, lipid, polynucleotide (*e.g.*, chromatin, methylated polynucleotide, or other modified form). In some embodiments, the immunogenic biological agent directly causes an immune response, *e.g.*, is itself a target immunogen (antigen). In some embodiments, the immunogenic

5   biological agent is a polynucleotide encoding the target immunogen. For example, when a polynucleotide encoding a target antigen is expressed in an antigen presenting cell (APC), an immune response is mounted against the expressed antigen. The immunogenic biological agent can be administered alone, in combination with a second, third, and/or fourth immunogenic biological agent (*e.g.*, in the case of a multi-target preventative vaccine), and/or

10   in combination with an adjuvant to increase the immune response.

A. Expression vectors

[0079]   Expression vectors for use as described herein can include virally-derived vectors, *e.g.*, recombinant adeno-associated virus (AAV) vectors, retroviral vectors, adenoviral vectors, modified vaccinia Ankara (MVA) vectors, and lentiviral (*e.g.*, HSV-1-derived)

15   vectors (*see, e.g.*, Brouard *et al.* (2009) *British J. Pharm.* 157:153). Virally-derived vectors for therapeutic use are typically rendered replication incompetent or attenuated. For example, in the case of an adenoviral vector, the adenoviral genome can be modified to remove the E1 and E3 genes. For production, the replication deficient vector can be administered to a cell that expresses the E1 gene such that recombinant adenovirus (rAd) is produced by the cell.

20   This rAd can be harvested and used for a single round of infection to deliver the transgenic composition to another cell within a mammal in order to elicit immune responses to an encoded polypeptide antigen.

[0080]   Examples of suitable viral vectors include adenovirus 5, including, for example, Ad5 with deletions of the E1/E3 regions and Ad5 with a deletion of the E4 region as

25   described in US Patent No. 8,222,224 and Scallan *et al.* Clinical and Vaccine Immunology 2013; 20(1): 85-94. An exemplary Ad5 viral vector backbone is provided in SEQ ID NO: 7. Other suitable adenoviral vectors include strains 2, orally tested strains 4 and 7, enteric adenoviruses 40 and 41, and other strains (*e.g.* Ad34, Ad26, or Ad35) that are sufficient for delivering an antigen and eliciting an adaptive immune response to the transgene antigen

30   [Lubeck et al., Proc Natl Acad Sci USA, 86(17), 6763-6767 (1989); Shen et al., J Virol, 75(9), 4297-4307 (2001); Bailey et al., Virology, 202(2), 695-706 (1994)]. The viral vector does not need to have been isolated from humans, but can come from a non-human such as

chimpanzee adenovirus 3 (ChAd3) (see, e.g., Colloca et al. (2012) Sci. Transl. Med. 4:115; Stanley et al. (2014) Nat. Med. doi:10.1038/nm.3702). In some embodiments, the adenoviral vector is a live, replication incompetent adenoviral vector (such as E1 and E3 deleted rAd5), live and attenuated adenoviral vector (such as the E1B55K deletion viruses), or a live adenoviral vector with wild-type replication.

[0081] Transcriptional and translational control sequences in expression vectors to be used as described herein can be provided by viral sources. For example, commonly used promoters and enhancers are derived, e.g., from beta actin, adenovirus, simian virus (SV40), and human cytomegalovirus (CMV). For example, vectors allowing expression of proteins under the direction of the CMV promoter, SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, transducer promoter, or other promoters shown effective for expression in mammalian cells are suitable. Additional viral and non-viral promoter, control and/or signal sequences may be used, provided such control sequences are compatible with the host cells to be transfected.

B. Immunogens

[0082] Immunogens for use as described herein can be derived from antigens, such as, for example, viral antigens, bacterial antigens, cancer antigens, fungal antigens, or parasite antigens (see, e.g., US Patent No. 8,222,224 for a list of antigens that can be used as described herein).

[0083] Particular examples of antigens that can be used as described herein are those derived from norovirus (e.g., VP1) and Respiratory syncytial virus (RSV) (e.g.,). Other suitable antigens include those from the influenza virus (e.g., HA, NA, M1, NP), human immunodeficiency virus (HIV, e.g., gag, pol, env, etc.), human papilloma virus (HPV, e.g., capsid proteins such as L1), Venezuelan Equine Encephalomyelitis (VEE) virus, Epstein Barr virus, herpes simplex virus (HSV), human herpes virus, rhinoviruses, cocksackieviruses, enteroviruses, hepatitis A, B, C, E, and G (HAV, HBV, HCV, HEV, HGV e.g., surface antigen), mumps virus, rubella virus, measles virus, poliovirus, smallpox virus, rabies virus, and Varicella-zoster virus.

[0084] Suitable viral antigens also include viral nonstructural proteins, e.g., proteins encoded by viral nucleic acid that do not encode for structural polypeptides, in contrast to those that make capsid or the protein surrounding a virus. Non-structural proteins include

those proteins that promote viral nucleic acid replication, viral gene expression, or post-translationsal processing, such as, for example, Nonstructural proteins 1, 2, 3, and 4 (NS1, NS2, NS3, and NS4, respectively) from Venezuelan Equine encephalitis (VEE), Eastern Equine Encephalitis (EEE), or Semliki Forest.

[0085]   Bacterial antigens can be derived from, for example, Staphylococcus aureus, Staphylococcus epidermis, Helicobacter pylori, Streptococcus bovis, Streptococcus pyogenes, Streptococcus pneumoniae, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium leprae, Corynebacterium diphtheriae, Borrelia burgdorferi, Bacillus anthracis, Bacillus cereus, Clostridium botulinum, Clostridium difficile, Salmonella typhi, Vibrio chloerae, Haemophilus influenzae, Bordetella pertussis, Yersinia pestis, Neisseria gonorrhoeae, Treponema pallidum, Mycoplasm sp., Legionella pneumophila, Rickettsia typhi, Chlamydia trachomatis, and Shigella dysenteriae, Vibrio cholera (*e.g.*, Cholera toxin subunit B, cholera toxin-coregulated pilus (TCP)); Helicobacter pylorii (*e.g.*, VacA, CagA, NAP, Hsp, catalase, urease); E. coli (*e.g.*, heat-labile enterotoxin, fimbrial antigens).

[0086]   Parasite antigens can be derived from, for example, Giardia lamblia, Leishmania sp., Trypanosoma sp., Trichomonas sp., Plasmodium sp. (*e.g.*, P. falciparum surface protein antigens such as pfs25, pfs28, pfs45, pfs84, pfs 48/45, pfs 230, Pvs25, and Pvs28); Schistosoma sp.; Mycobacterium tuberculosis (*e.g.*, Ag85, MPT64, ESAT-6, CFP10, R8307, MTB-32 MTB-39, CSP, LSA-1, LSA-3, EXP1, SSP-2, SALSA, STARP, GLURP, MSP-1, MSP-2, MSP-3, MSP-4, MSP-5, MSP-8, MSP-9, AMA-1, Type 1 integral membrane protein, RESA, EBA-175, and DBA).

[0087]   Fungal antigens can be derived from, for example, Tinea pedis, Tinea corporus, Tinea cruris, Tinea unguium, Cladosporium carionii, Coccidioides immitis, Candida sp., Aspergillus fumigatus, and Pneumocystis carinii.

[0088]   Cancer antigens include, for example, antigens expressed or over-expressed in colon cancer, stomach cancer, pancreatic cancer, lung cancer, ovarian cancer, prostate cancer, breast cancer, skin cancer (*e.g.*, melanoma), leukemia, or lymphoma. Exemplary cancer antigens include, for example, HPV L1, HPV L2, HPV E1, HPV E2, placental alkaline phosphatase, AFP, BRCA1, Her2/neu, CA 15-3, CA 19-9, CA-125, CEA, Hcg, urokinase-type plasminogen activator (Upa), plasminogen activator inhibitor, CD53, CD30, CD25, C5, CD11a, CD33, CD20, ErbB2, CTLA-4. *See* Sliwkowski & Mellman (2013) *Science* 341:6151 for additional cancer targets.

C. Adjuvants

[0089]   In some embodiments, the compositions further comprise at least one adjuvant. Suitable adjuvants include, for example, the lipids and non-lipid compounds, cholera toxin (CT), CT subunit B, CT derivative CTK63, E. coli heat labile enterotoxin (LT), LT derivative LTK63, Al(OH)$_3$, and polyionic organic acids as described in e.g., WO2004/020592, Anderson and Crowle, Infect. Immun. 31(1):413-418 (1981), Roterman et al., J. Physiol. Pharmacol., 44(3):213-32 (1993), Arora and Crowle, J. Reticuloendothel. 24(3):271-86 (1978), and Crowle and May, Infect. Immun. 38(3):932-7 (1982)). Suitable polyionic organic acids include for example, 6,6'-[3,3'-demithyl[1,1'-biphenyl]-4,4'-diyl]bis(azo)bis[4-amino-5-hydrox- y-1,3-naphthalene-disulfonic acid] (Evans Blue) and 3,3'-[1,1' biphenyl]-4,4'-diylbis(azo)bis[4-amino-1-naphthalenesulfonic acid] (Congo Red). It will be appreciated by those of skill in the art that the polyionic organic acids may be used for any nucleic acid-based vaccination method in conjunction with any type of administration.

[0090]   TLR-3 agonists (e.g., dsRNA, and mimetics thereof such as polyI:C, poly A:U, and polyI:polyC) can also be used. TLR-3 agonists include, for example, short hairpin RNA, virally derived RNA, short segments of RNA that can form double-strands or short hairpin RNA, and short interfering RNA (siRNA). In some embodiments, the TLR-3 agonist is virally derived dsRNA, e.g., dsRNA derived from a Sindbis virus or dsRNA viral intermediates (Alexopoulou et al. (2001) Nature 413:732). In some embodiments, the TLR-3 agonist is a short hairpin RNA. Short hairpin RNA sequences typically comprise two complementary sequences joined by a linker sequence. The particular linker sequence is not a critical aspect of the invention. Any appropriate linker sequence can be used so long as it does not interfere with the binding of the two complementary sequences to form a dsRNA. TLR-3 agonists can result in pro-inflammatory cytokine release (e.g. IL-6, IL-8, TNF-alpha, IFN-alpha, IFN-beta) when contacted with a responder cell (e.g., a dendritic cell, a peripheral blood mononuclear cell, or a macrophage) in vitro or in-vivo.

[0091]   Other suitable adjuvants include topical immunomodulators such as, members of the imidazoquinoline family such as, for example, imiquimod and resiquimod (see, e.g., Hengge et al., Lancet Infect. Dis. 1(3):189-98 (2001).

[0092]   Additional suitable adjuvants are commercially available as, for example, additional alum-based adjuvants (e.g., Alhydrogel, Rehydragel, aluminum phosphate, Algammulin); oil based adjuvants (Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories,

Detroit, Mich.), Specol, RIBI, TiterMax, Montanide ISA50 or Seppic MONTANIDE ISA 720); nonionic block copolymer-based adjuvants, cytokines (*e.g.*, GM-CSF or Flat3-ligand); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); AS-2 (SmithKline Beecham, Philadelphia, Pa.); salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and Quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, are also suitable adjuvants. Hemocyanins (*e.g.*, keyhole limpet hemocyanin) and hemoerythrins can also be used as adjuvants. Polysaccharide adjuvants such as, for example, chitin, chitosan, and deacetylated chitin are also suitable as adjuvants. Other suitable adjuvants include muramyl dipeptide (MDP, N acetylmuramyl L alanyl D isoglutamine) bacterial peptidoglycans and their derivatives (*e.g.*, threonyl-MDP, and MTPPE). BCG and BCG cell wall skeleton (CWS) can be used as adjuvants, with or without trehalose dimycolate. Trehalose dimycolate can be used itself (*see, e.g.*, US Patent No. 4,579,945). Detoxified endotoxins are also useful as adjuvants alone or in combination with other adjuvants (*see, e.g.*, US Patent Nos. 4,866,034; 4,435,386; 4,505,899; 4,436,727; 4,436,728; 4,505,900; and 4,520,019). The saponins QS21, QS17, QS7 are also useful as adjuvants (*see, e.g.*, US Patent No. 5,057,540; EP 0362 279; WO 96/33739; and WO 96/11711). Other suitable adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, Calif., United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2, SBAS-4 or SBAS-6 or variants thereof, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, Mont.), and RC-529 (Corixa, Hamilton, Mont.).

[0093] Superantigens are also contemplated for use as adjuvants in the present invention. Superantigens include Staphylococcus exoproteins, such as the alpha, beta, gamma, and delta enterotoxins from S. aureus and S. epidermidis, and the alpha, beta, gamma, and delta E. coli exotoxins. Common Staphylococcus enterotoxins are known as staphylococcal enterotoxin A (SEA) and staphylococcal enterotoxin B (SEB), with enterotoxins through E (SEE) being described (Rott et al., 1992). Streptococcus pyogenes B (SEB), Clostridium perfringens enterotoxin (Bowness et al., 1992), cytoplasmic membrane-associated protein (CAP) from S. pyogenes (Sato et al., 1994) and toxic shock syndrome toxin 1 (TSST 1) from S. aureus (Schwab et al., 1993) can also be used.

[0094] For the pharmaceutical compositions provided herein, the adjuvant(s) can be designed to induce, *e.g.*, an immune response predominantly of the Th1 or Th2 type. High

levels of Th1-type cytokines (*e.g.*, IFN-gamma, TNF-alpha, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following oral delivery of a composition comprising an immunogenic polypeptide as provided herein, an immune response that includes Th1- and Th2-type responses will typically be elicited.

III.   Targeted delivery systems

[0095]   The presently described compositions and methods for ileal delivery can rely on appropriate coatings, matrices, and devices such as those described below.

A. Enteric coatings, matrices, and devices

[0096]   Enteric coatings are used to shield substances from the low pH environment of the stomach and delay release of the enclosed substance until it reaches a desired target later in the digestive tract. Enteric coatings are known, and commercially available. Examples include pH-sensitive polymers, bio-degradable polymers, hydrogels, time-release systems, and osmotic delivery systems (*see, e.g.*, Chourasia & Jain (2003) *J. Pharm. Pharmaceutical Sci.* 6:33).

[0097]   The pH of the gastrointestinal tract (GIT) progresses from very acidic in the stomach (pH ~2), to more neutral in the ileum (pH ~ 5.8-7.0). pH sensitive coatings can be used that dissolve in the ileum or just before the ileum. Examples include Eudragit® L and S polymers (threshold pH's ranging from 5.5-7.0); polyvinyl acetate phthalate (pH 5.0), hydroxypropyl methylcellulose phthalate 50 and 55 (pH 5.2 and 5.4, respectively), and cellulose acetate phthalate (pH 5.0). Thakral *et al.* (2013) *Expert Opin. Drug Deliv.* 10:131 review Euragit® formulations for ileal delivery, in particular, combinations of L and S that ensure delivery at pH≤7.0. Crotts *et al.* (2001) *Eur. J Pharm. Biol.* 51:71 describe Eudragit® formulations with appropriate disintegration properties. Vijay *et al.* (2010) *J. Mater. Sci. Mater. Med.* 21:2583 review acrylic acid (AA)-methyl methacrylate (MMA) based copolymers for ileal delivery at pH 6.8.

[0098]   For ileal delivery, the polymer coating typically dissolves at about pH 6.8 and allows complete release within about 40 min (*see, e.g.*, Huyghebaert *et al.* (2005) *Int. J. Pharm.* 298:26). To accomplish this, a therapeutic substance can be covered in layers of different coatings, *e.g.*, so that the outermost layer protects the substance through low pH

conditions and is dissolved when the tablet leaves the stomach, and at least one inner layer that dissolves as the tablet passes into increasing pH. Examples of layered coatings for delivery to the distal ileum are described, *e.g.*, in WO2013148258.

[0099]   Biodegradable polymers (*e.g.*, pectin, azo polymers) typically rely on the enzymatic activity of microflora living in the GIT. The ileum harbors larger numbers of bacteria than earlier stages, including lactobacilli and enterobacteria.

[0100]   Osmotic-controlled Release Oral delivery Systems (OROS®; Alza) is an example of an osmotic system that degrades over time in aqueous conditions. Such materials can be manipulated with other coatings, or in varying thicknesses, to deliver specifically to the ileum (*see, e.g.*, Conley *et al.* (2006) *Curr. Med. Res. Opin.* 22:1879).

[0101]   Combination polymers for delivery to the ileum are reported in WO2000062820. Examples include Eudragit® L100-55 (25 mg/ capsule) with triethyl citrate (2.4 mg/ capsule), and Povidone K-25 (20 mg/ tablet) followed by Eudragit® FS30D (30 mg/ tablet). pH sensitive polymers can be applied to effect delivery to the ileum, as described above and, *e.g.*, methacrylic acid copolymers (*e.g.*, poly(methacylic acid-co-methyl methacrylate) 1:1), cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, cellulose acetate trimellitate, carboxymethyl ethyl- cellulose, shellac or other suitable polymer(s). The coating layer can also be composed of film-forming polymers being sensitive to other luminal components than pH, such as bacterial degradation or a component that has such a sensitivity when it is mixed with another film-forming polymer. Examples of such components providing delayed release to the ileum are polymers comprising azo bond(s), polysaccharides such as pectin and its salts, galactomannans, amylose and chondroitin, disulphide polymers and glycosides.

[0102]   Components with varying pH, water, and enzymatic sensitivities can be used in combination to target a therapeutic composition to the ileum. The thickness of the coating can also be used to control release. The components can also be used to form a matrix, in which the therapeutic composition is embedded. See generally, Frontiers in Drug Design & Discovery (Bentham Science Pub. 2009) vol. 4.

B. Frequency or radio-controlled capsules

[0103]   As an alternative to dissolving coatings and matrices, site-specific delivery can be via capsules that release upon an externally generated signal. Early models released for a

high-frequency (HF) signal, as disclosed in Digenis *et al.* (1998) *Pharm. Sci. Tech. Today* 1:160. The original HF capsule concept has since been updated and the result marketed as InteliSite®. The updated capsule is a radio-frequency activated, non-disintegrating delivery system. Radiolabeling of the capsule permits the determination of the capsule location within a specific region of the GI tract via gamma scintigraphy. When the capsule reaches the desired location in the GI tract, external activation opens a series of windows to the capsule drug reservoir.

[0104]   In some embodiments, the immunogenic biological agent can be enclosed in a radio-controlled capsule, so that the capsule is tracked and signaled once it reaches the ileum. In some embodiments, the capsule is signaled at a given time after administration that corresponds to when the capsule is expected to arrive in the ileum, with or without detecting.

C. Formulations

[0105]   Pharmaceutical compositions can be used for prophylactic and therapeutic purposes as described herein. As explained above, pharmaceutical compositions can be prepared to protect against stomach degradation such that the administered immunogenic biological agent reach the desired location. Methods for microencapsulation of DNA and drugs for oral delivery are described, *e.g.*, in US2004043952.

[0106]   An immunogenic pharmaceutical composition can contain pharmaceutically acceptable salts of the immunogenic biological agent (*e.g.*, immunogenic polypeptide, or polynucleotide encoding an immunogenic polypeptide). Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.*, salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.*, sodium, potassium, lithium, ammonium, calcium and magnesium salts). Some particular examples of salts include phosphate buffered saline and saline (*e.g.*, for ingestion, nasal delivery, or injection).

[0107]   A delayed release coating or an additional coating of the formulation can contain other film-forming polymers being non-sensitive to luminal conditions for technical reasons or chronographic control of the drug release. Materials to be used for such purpose includes, but are not limited to; sugar, polyethylene glycol, polyvinylpyrrolidone, polyvinyl alcohol, polyvinyl acetate, hydroxypropyl cellulose, methylcellulose, ethylcellulose, hydroxypropyl methylcellulose, carboxymethylcellulose sodium and others, used alone or in mixtures.

[0108]   Additives such as dispersants, colorants, pigments, additional polymers, *e.g.*, poly(ethylacrylat, methylmethacrylat), anti-tacking and anti-foaming agents can be included into a coating layer. Other compounds may be added to increase film thickness and to decrease diffusion of acidic gastric juices into the core material. The coating layers can also contain pharmaceutically acceptable plasticizers to obtain desired mechanical properties. Such plasticizers are for instance, but not restricted to, triacetin, citric acid esters, phthalic acid esters, dibutyl sebacate, cetyl alcohol, polyethylene glycols, glycerol monoesters, polysorbates or other plasticizers and mixtures thereof. The amount of plasticizer can be optimised for each formula, and in relation to the selected polymer(s), selected plasticizer(s) and the applied amount of said polymer(s).

[0109]   Other suitable pharmaceutical ingredients known in the art can be employed in the pharmaceutical compositions of this invention. Suitable carriers include, for example, water, saline, alcohol, a fat, a wax, a buffer, a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, or biodegradable microspheres (*e.g.*, polylactate polyglycolate). Suitable biodegradable microspheres are disclosed, for example, in US Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883. The immunogenic polypeptide and/or carrier expression vector can be encapsulated within the biodegradable microsphere or associated with the surface of the microsphere.

[0110]   Such compositions may also comprise non-immunogenic buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilate. Compounds may also be encapsulated within liposomes using well known technology.

IV.   Immune responses and vaccines

[0111]   The pharmaceutical compositions for ileum delivery as described herein are designed to elicit an immune response from an individual that is specific for an immunogenic biological agent included in the pharmaceutical composition. The pharmaceutical composition can be used prophylactically or therapeutically as a vaccine to avoid or reduce a viral infection, bacterial infection, parasitic infection, fungal infection, or cancer. The

pharmaceutical compositions can be used to treat at any stage, *e.g.*, at the pre-cancer, cancer, or metastatic stages, or to prevent disease or infection.

[0112]   For example, the compositions described herein may be used to prevent or treat infection, such as influenza, hepatitis, or HIV, or for prevention or treatment of cancer. Within such methods, pharmaceutical compositions are typically administered to an individual that may or may not be afflicted with the disease, disorder, or infection. In some embodiments, a disease, disorder, or infection is diagnosed prior to administration, *e.g.*, using criteria generally accepted in the art. For example, viral infection may be diagnosed by the measurement of viral titer in a sample from the patient, bacterial infection may be diagnosed by detecting the bacteria in a sample from the patient, and cancer may be diagnosed by detecting the presence of a malignant tumor. Pharmaceutical compositions can be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs.

[0113]   Immunotherapy is typically active immunotherapy, in which treatment relies on the in vivo stimulation of the endogenous host immune system to react against, *e.g.*, tumors or bacterially or virally infected cells, with the administration of immune response-modifying agents (*e.g.*, immunogenic biological agents).

[0114]   Frequency of administration of the prophylactic or therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and can be readily established using standard techniques. Typically, between 1 and 52 doses can be administered over a 52 week period. In some embodiments, 3 doses are administered, at intervals of 1 month, or 2-3 doses are administered every 2-3 months. In some embodiments, a combination of more than one antigen can be administered simultaneously or sequentially, *e.g.*, an annual influenza vaccine that contains individual components directed at each subtype of influenza or multiple clades within a subtype. In some embodiments, the intervals are more like once a year, *e.g.*, an annual flu vaccine based on the particular current strain. Booster vaccinations can be given periodically thereafter. Alternate protocols may be appropriate for individual patients and particular diseases and disorders.

[0115]   A suitable dose is an amount of an immunogenic biological agent that, when administered as described above, is capable of promoting, *e.g.*, an anti-tumor, an anti-viral, or an antibacterial, immune response, and is at least 15-50% above the basal (untreated) level, or at least 5-50% (*e.g.*, 5%, 10%, 20%, 30%, 50%, 1.5-fold, 2-fold, or higher) above the level

from non-ileum targeted treatment. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic T cells capable of killing, *e.g.*, the patient's tumor cells, the patient's virally infected cells, or the patient's bacterially infected cells *in vitro*. Such vaccines can also generate an immune response that leads to an improved clinical outcome (*e.g.*, complete or partial or longer disease-free survival, reduced viral titers) in vaccinated patients as compared to non-vaccinated patients, or patients receiving non-ileum targeted treatment.

[0116]   In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, reduced or negative viral titer, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to patients treated with non-ileum targeted treatment, or non-treated patients. Such immune responses can generally be evaluated using standard proliferation, cytotoxicity or cytokine assays described above, which can be performed using samples obtained from a patient before and after treatment.

[0117]   For example, detection of immunocomplexes formed between an immunogenic polypeptide and antibodies in body fluid that are specific for the immunogenic polypeptide can be used to monitor the effectiveness of therapy, *e.g.*, for a disease or disorder in which the immunogenic polypeptide is associated. Samples of body fluid taken from an individual prior to and subsequent to initiation of therapy (*e.g.*, ileum-targeted therapy) may be analyzed for the immunocomplexes using known methods. Briefly, the number of immunocomplexes detected in both samples is compared. A significant change in the number of immunocomplexes in the second sample (post-targeted therapy) relative to the first sample (pre-targeted therapy) reflects successful therapy.

V.   Examples

[0118]   Pharmaceutical methods for delivering small molecules to the intestine are known, but the ability to deliver a large biological to the intestine for proper immune recognition is poorly understood. Mice are not able to swallow pills, so it is difficult to perform studies with tablets in animal models. Further, the location of the best place to deliver the vaccine vector in order to elicit a response to transgene antigen has not been characterized in humans. In sheep, the jejunum was shown to be the most effective target for eliciting an immune response to an adenovirally-encoded transgene antigen (Mutwari *et al.* (1999) *Immunology*

97:455). Here we show the result of several human or non-human primate studies with improved human oral dosage forms for delivery of biological agents.

Example 1

[0119]   In order to determine which region of the small intestine is most active for inducing an immune response to antigen, tests were performed in humans. Radio-controlled capsules were given to healthy normal volunteers, with the vaccine either released early in the small intestine (jejunum) or later in the small intestine (ileum). The use of the radio-controlled capsules for delivery of small molecule drugs has been described, but not for vaccine delivery (Digenis *et al.* (1991) *Crit. Rev. Ther. Drug Carrier Syst.* 7:309).

[0120]   The vaccine was composed of recombinant adenovirus expressing the influenza antigen HA from A/CA/04/2009 (rAd-HA-dsRNA) (*see*, *e.g.*, US2012/0244185). A total of 10^{11} infectious units (IU) were given to each subject on day 0. The numbers of circulating pre-plasma B cells in peripheral blood were measured by Antibody Secreting Cell (ASC) assay on days 0 and 7 after the administration of the vaccine. Results only measure the numbers of ASCs that recognize the antigen HA.

[0121]   Results show that ASCs could be measured 7 days after immunization in each of the treated groups (Figure 1). Average responses were higher in the ileum dosed group than the jejunum dosed group. Background ASCs on day 0 were negligible. For the ileum, an average of 340 +/- 111 (standard error) IgG and 74 +/- 18 IgA ASCs were observed on day 7. For the jejunum, the average and standard error responses were 118 +/- 30 IgG and 28 +/- 8 IgA ASCs. The ileum group was significantly different than placebo (P=0.03 on day 7 for IgA ASC, and trended higher for IgG ASC p=0.07). Contrary to the results in sheep, the results in humans indicate that ileum delivery is more potent at eliciting an IgG or an IgA antibody response than jejunum delivery.

[0122]   T cell responses were also determined by detecting interferon-γ release (IFN-γ) using the ELISPOT® assay. Figure 2 shows that 12/12 of the ileum-dosed group had increased levels of IFN-γ, compared to 8/12 of the jejunum-dosed group 7 days post-administration. In addition, IFN-γ levels were significantly higher in the ileum-dosed group than in the jejunum-dosed group.

[0123]   Microneutralizing (MN) antibody titers to influenza A/CA/07/2009 were measured. Increased MN antibody levels are indicative of a neutralizing antibody response. After

excluding subjects that had an initial neutralizing antibody response greater than 40 (Faix *et al.* (2012) *PloS One 7:e34581*), the fold increases in MN titers were plotted for individual subjects. The number of subjects with a positive increase was 9 out of 10 for the ileum delivered vaccine versus 6 out of 10 for jejunum delivered vaccine (Figure 3). The

5   geometric mean titers (GMT) were similar between the two groups, with ileum GMT rising from 22 to 92 versus the jejunum GMT rising from 18 to 90. The results indicate that ileum release is more reliable at inducing neutralizing antibody responses to influenza, possibly leading to a greater percentage of subjects protected against influenza.

Example 2

10 **[0124]**   Tablets were hand made using microcrystalline cellulose (PH-101, FMC) and starch (Starch 1500, Colorcon) incorporating 10% barium sulfate as a radiopaque material containing fumed silica as a flow aid and magnesium stearate as a tablet lubricant. The tablets of 7.14 mm diameter and 150 mg weight were coated with Eudragit® L-100 in a pan coater using 10% coating solids weight gain as a guide to whether the enteric coating was

15 added; coating solids contained 4 parts Eudragit® polymer to one part triethyl citrate and 1 part talc. As an initial test of enteric coating performance, four cynomolgus macaques were given tablets using an oral gastric tube. The oral gastric tube is solid and rigid, but hollow down the middle for instilling liquids. It has a flexible silicone tube on the leading end of the rigid tube that can hold a small tablet in place. The tube and pill apparatus were threaded

20 down the esophagus of restrained monkeys until the leading end passed through the cardiac sphincter and into the stomach. A flush of orange juice was used to dislodge the pill into the stomach. X-rays were taken at set time points, and examined for location and dissolution of the tablet. Table 1 summarizes the results.

Table 1: L-100 coating performance

Animal	Time and Pill Location			
	1 hr	2 hr	3 hr	4hr
1	stomach	stomach	stomach	intestine
2	stomach	intestine	intestine	dissolved
3	intestine	intestine	dissolved	dissolved
4	stomach	stomach	intestine	dissolved

25

[0125]   Figure 4 shows that the tablets were completely intact in the low pH environment of the stomach; there was no evidence of premature dissolution of the tablets. While large for a monkey, the tablets were able to pass through the stomach intact into the intestine. In the

intestine, they dissolved at a reasonable rate and were completely dissolved in 3 out of 4 monkeys. In the 4th monkey, the pill left the stomach sometime after 3 hours and the pill had not dissolved at the time of the last x-ray. Overall, the tablets performed in an acceptable manner and the Eudragit® L-100 coating was selected for future human studies.

5   Example 3

[0126]   A phase 1, sequentially enrolled clinical study, with a randomized and placebo-controlled cohort to evaluate safety, and immunogenicity of a recombinant Ad serotype 5 (rAd5) based oral vaccine against H1 seasonal influenza was completed. The rAd5 vector (rAd-HA-dsRNA with HA from A/CA/04/2009) was described in Example 1. The study had

10   an active phase of approximately 3 months, and was conducted in accordance with applicable Good Clinical Practice guidelines, the United States Code of Federal Regulations, and the International Conference on Harmonization guidelines. Informed consent was obtained from all subjects after discussion of the risks. IRB approval was given before dosing of subjects.

[0127]   Good manufacturing practice (GMP)-grade rAd-HA-dsRNA was produced in

15   Wave® bags (GE Healthcare, Waukesha, WI) at Lonza Biologicals (Houston, TX). Purification was performed by ion exchange chromatography, followed by buffer exchange. Purified vector was mixed with excipients, lyophilized, and then tableted at Lonza using microcrystalline cellulose and starch as tableting bulk. Tablets were enteric coated with Eudragit® L 100 (Evonik Industries, Darmstadt, Germany) using a Vector HiCoater® LDCS-

20   5 coater (Vector Freund, Cedar Rapids, IA). The final product was released in one lot, and titered by standard IU assay. Placebo was prepared as similarly sized and shaped tablets containing 150 mg of microcrystalline cellulose, without enteric coating. The study compared 10^9 IU, 10^{10} IU, and placebo treated subjects for the ability to elicit an immune response to transgene. Subjects were given tablets on days 0 and 28.

25   [0128]   The numbers of circulating pre-plasma B cells in peripheral blood were measured by ASC assays on days 0 and 7 after the initial dose, and at days 28 and 35 after the second dose (the second dose was delivered at day 28). Results show that ASC counts could be measured 7 days after each immunization in the treated groups, but not the placebo group (Figure 5). Average responses were higher on day 7, and higher in the high dose group than

30   the low dose group. Background ASCs on days 0 and 28 were negligible, and negligible for the placebo group at all time points. For the high dose group, an average of 105 +/- 33 and 27 +/- 12 ASCs were found for days 7 and 35 respectively. For the low dose group, average

ASCs were 41 +/- 32 and 14 +/- 8 for days 7 and 35 respectively. The placebo group had an average of 0.3 +/- 0.3 and 0, for days 7 and 35 respectively. The high dose group was significantly higher than placebo (P=0.01 and 0.05 for days 7 and 35 respectively.)

[0129]   Neutralizing antibody responses to influenza were measured by MN assay. Results show a dose dependent increase in the MN titers in the treated groups versus the placebo control (Figure 6). The frequency of MN responders with at least a 2-fold increase in the high dose group was significantly different than the placebo group (P=0.003 by Fisher's exact test), whereas the low dose trended higher, but was not significantly higher than placebo (P=0.2). After removing subjects that had MN titers greater than 40, the geometric mean titers (GMT) were calculated in the remaining subjects (Table 2). Day 56 Geometric Fold Titer Response (GMFR) was also calculated (Table 2). These results show that neutralizing antibody titers to influenza are being generated by oral immunization, with a greater than 3 fold increase in the GMT after immunization in the high dose group. These results show that L 100 coated tablets can be used for vaccine delivery to the intestine.

Table 2: GMT changes in MN titers for subjects with MN≤ 40

Group	N	GMT D0	GMT D28	GMT D56	GMFR
Placebo	8	14.1	14.1	14.1	1
Low Dose	10	12.3	14.1	16.2	1.3
High Dose	7	15.6	36.2	53.8	3.4

Example 4

[0130]   We tested parameters for enteric coatings *in vitro* to determine dissolution times with varying pH and coating percentage. The data provide guidelines for ileal delivery following gastric exposure at low pH (as in the stomach) and subsequent transit through an increasing pH gradient (as is found in the duodenum and jejunum) prior to reaching the ileum.

[0131]   Tablet disintegration was tested with 150 mg tablets prepared as described above, and coated with 8, 10, or 12% total solids weight gain, utilizing Eudragit® L100, Eudragit® L100-55, or 1:1 (w/w) mixture of L100 and L100-55 polymers, applied as an organic solvent suspension. In duplicate, tablets prepared with each coating polymer, and at each level of coating application, were pre-exposed to USP simulated gastric fluid (SGF, pH 1.6, no pepsin) for 120 minutes in a VanKel Bio-Dis III reciprocating cylinder dissolution test apparatus at 37 °C at a reciprocation rate of 10 dips per minute (DPM). The tablets were then

transferred to USP simulated intestinal fluid (SIF, pH 6.8, no pancreatin). Tablets were observed for disintegration and the time to complete disintegration of both tablets was recorded to the nearest 5 minutes. The data indicate that disintegration time is influenced by both polymer composition and thickness and provide guidance with regard to proper selection

5   of coating composition to influence the behavior of the coatings after tablets exit the stomach.

Coating Polymer	Time to disintegrate at indicated coating level (minutes)		
	8%	10%	12%
L100	20	30	45
L100/L100-55	15	20	30
L100-55	10	20	25

[0132]   The effect of pH on disintegration time was tested with 150 mg tablets, coated to 10% total solids weight gain with either Eudragit® L100 or Eudragit® L100-55. A series of buffers were prepared by adjusting the pH of USP SIF (no pancreatin) to values

10   encompassing the USP specification of 6.8. Tablets were pre-exposed to USP SGF (no pepsin) for 120 minutes 37 °C and 10 DPM, then transferred to the pH-modified USP SIF solutions. The tablets were observed for disintegration and the time to complete disintegration was recorded to the nearest 5 minutes. The data indicate that the rate of disintegration is influenced by the environmental pH and differs between the two polymers.

15   Again, the results can be used for proper selection of a coating composition to accomplish drug retention through the stomach and upper small intestine.

pH of SIF	Time to disintegrate for polymer at pH (minutes)	
	Eudragit L100	Eudragit L100-55
5.4	250	145
6	110	60
6.4	55	45
7	30	20

Example 5

[0133]   We carried out a Phase 1, sequentially enrolled study, with a randomized and

20   placebo-controlled cohort to evaluate safety, and immunogenicity of a recombinant Ad serotype 5 (rAd5) based oral vaccine against H1 seasonal influenza. Tablets containing the vaccine were coated as described herein to dissolve in the ileum. The data show that an oral tablet vaccine would be competitive with existing vaccines in terms of eliciting neutralizing antibody responses to influenza.

[0134] Hemagglutination Inhibition (HAI) responses were measured on days 0 and 28 (Figure 7A). No placebo treated subject seroconverted, but one placebo subject slipped through screening and had a high day 0 value. None of the vaccine subjects had a starting HAI titer > 20. After immunization, nine subjects in the vaccine group reached seroprotective levels (HAI ≥40) (Figure 7A). The Geometric Mean Titer (GMT) for the group was 61·1 (95% CI: 30 - 124), a 7.7-fold geometric mean fold rise (GMFR) over the initial GMT of 7·9 (95% CI: 6 - 11). Of the eleven 4-fold risers (92%), nine seroconverted (SC) with the other 2 subjects showing a 4-fold increase in HAI titer from 5 to 20. The vaccine group had a statistically significant increase in the number of 4-fold responders versus placebo (11 versus 0, with P<0·0000 by Fisher's Exact Test). The placebo subjects had a GMT of 11.9 (95% CI: 6 - 25) on day 28 versus a GMT of 11.0 on day 0 (95% CI: 5 - 23).

[0135] Durability of the antibody response was measured by examining HAI responses 180 days after immunization. In the vaccine-immunized group, 75% (9 of 12) of the subjects were seroprotected on day 28 and 75% (9 of 12) were still seroprotected on day 180. The HAI GMT were plotted (Figure 7B), and the decrease in the GMT was found to be 28% between 28 and 180 days post immunization.

[0136] Neutralizing antibody responses to influenza were measured by MN assay. Significant increases in the MN titers in the treated group versus the placebo control were observed (Figure 7C). The frequency of 4-fold MN responders in the vaccine treated group was significantly different than the placebo group, with 11 subjects responding in the vaccine treated group versus 0 in the placebo group (P<0·0000 by Fisher's exact test).

[0137] After removing subjects that had baseline MN titers (and HAI titers) greater than 40, the geometric mean titers (GMT) were calculated in the remaining subjects on days 0 and 28 as shown in the following table. The GMT for the vaccine group rose to 247 (95 CI: 89-685) versus no rise in the placebo for a day 28 GMT of 9·6 (95 CI: 5-18). These calculations had no impact on the vaccine group, as none of the subjects had high initial MN or HAI titers. These results show that neutralizing antibody titers to influenza are generated by oral immunization, with a greater than 20-fold increase in the GMT after immunization in the vaccine-treated group.

Table 3. GMT changes in HAI and MN titers for subjects with MN≤ 40.

ASSAY	GROUP	N	GMT D0	GMT D28	GMFR	SC
HAI	Placebo	11	8.3	8.8	1.1	0%

	Vaccine	12	7.9	61.1	7.7	75%
MN	Placebo	9	9.3	9.6	1.0	N/A
	Vaccine	12	8.6	247	29	N/A

[0138]   In order to measure total antibody responses to HA, the numbers of circulating pre-plasma B cells in peripheral blood were measured by ASC assay on days 0 and 7 after immunization. Results show that ASCs could be reliably measured on day 7 in the vaccine-treated group (Figure 7D). Background ASCs were generally negligible on day 0. For the vaccine treated group, an average of 992 (+/- std err 209, 95% CI: 532-1452) IgG ASCs and 337 IgA ASCs (+/- std err 104, 95% CI: 117-580) each per 1 x 10^6 PBMC were found for day 7, with only one subject out of 12 having no detectable ASC response. The placebo group had no IgA spots on day 7, but one subject had a high background smear and a measurable IgG ASC response with smaller spots than normally observed. The treated group was significantly different than placebo in terms of the ability to elicit an IgG or an IgA ASC response at day 7 (P=0.0007 and P=0.008 respectively by T Test).

[0139]   Subjects were retrospectively measured for their anti-vector titers pre- and post-immunization. Following oral immunization, a few vaccine-treated subjects had an increase in neutralizing antibody responses to Ad5, which led to a 2.6-fold increase in the GM neutralizing antibody titers, compared to 1.0-fold GM fold rise in the placebo treated subjects. In the vaccine group, HAI and MN responses trended similarly for individual subjects. Eight subjects were Ad5 negative before immunization, and four were Ad5 positive before immunization. One subject that was Ad5 positive did not HAI seroconvert, however, one subject that was Ad5 positive had the highest increase in HAI titers (64 fold) of any of the subjects in the study. This same subject had a gain in MN titers of 362 fold without any increase in the Ad5 neutralizing antibody titers pre and post immunization. There was no observed correlation between starting Ad5 titers versus fold MN response (or HAI response) for the subjects immunized with the tablet vaccine.

[0140]   Moreover, the presently disclosed tablet vaccine is stable at room temperature for greater than 270 days and can tolerate short-term excursions at higher temperatures, which makes this approach technically feasible.

Example 5 Discussion

[0141]   The US military conducted an independent study to measure the effects of their seasonal vaccine campaigns on neutralizing antibody responses in military personnel, and reported a MN Titer GMFR of 5.6 after trivalent inactivated vaccine (TIV) injection and a GMFR of 2.2 following live-attenuated influenza vaccine (LAIV) intranasal administration, after accounting for subjects that had MN titers above 40 to start (Faix *et al.* (2012) *PloS one* 7:e34581). In another study, the SC rate to H1N1 was found to be 45% for one injection of 45 ug of HA protein (without adjuvant) (Gordon *et al.* (2012) *Vaccine* 30:5407), while in another, the H1N1 vaccine was highly immunogenic with a 78% SC rate observed after 1 dose of a split vaccine (Greenberg *et al.* (2009) 361:2405).

[0142]   In contrast to the variable results observed with injected vaccines, in the present study, MN GMFR was calculated at 29 for the 12 vaccine treated subjects with 92% of subjects showing a greater than 4-fold rise in MN titers. In the present tablet study, the HAI SC rate among vaccine treated subjects was 75% with over 92% of subjects having a 4-fold rise in HAI titers (Figure 7A). MN titers were higher than the HAI titers. It is possible that the MN assay is more sensitive or that the oral rAd based vaccine elicits stronger neutralizing responses outside the head region than protein injected vaccines.

[0143]   HAI responses are elicited with injected commercial vaccines, but HAI titers are known to wane. For example, non-HIV infected volunteers had a 67% drop in GMT HAI titers between 1 and 6 months post immunization (Crum-Cianflone *et al.* (2011) *Vaccine* 29:3183). Similarly, the percentage of seroprotected subjects dropped from 75% to 56% for HIV negative subjects that enrolled with seronegative HAI titers (≤1:10). Studies with pandemic influenza vaccines have also shown decreases in durability. In the AS03 avian influenza vaccine study, the GMT reached 563 after 2 vaccine doses, but at 6 months post immunization, the GMT had dropped to 18, a 96% decrease (Leroux-Roels *et al.*(2010) *Vaccine* 28:849). In the present tablet vaccine study, the percentage of seroprotected subjects remained constant at 75% at 1 and 6 months post immunization, and the HAI GMT titer drop was less dramatic showing only a 28% decrease (Figure 7B). One possibility is that the durability is better for vector-based vaccines because of enhanced T cell responses.

Example 5 Materials and methods

[0144]   *Clinical protocol and enrollment.* Subjects were pre-screened for Hemagglutination Inhibition (HAI) titers within 45 days of enrollment. In order to be eligible for study

participation subjects had to have an initial HAI titer of $\leq$ 1:20, be between 18-49 years of age, and be in good health. The active phase of the trial was through day 28, with the follow-up phase for monitoring safety to continue for 1 year.

[0145]   24 subjects were enrolled. All subjects that enrolled completed safety and immunogenicity assessments through the active phase, and through day 180 of the monitoring phase.

[0146]   *Randomization and Masking.* The study was designed to evaluate the vaccine (VXA-A1·1) in 12 subjects at a single dose of 1 x 10^{11} infectious units (IU) with 12 subjects given a placebo control. There were 3 sequentially enrolled sentinel vaccine-treated subjects, with each subject dosed no more frequently than one every 24 h. After a week of monitoring for vaccine-related toxicities, the remaining subjects in the treated cohort (9) were randomized along with 12 placebo controls. Randomization was performed by computer generated assignment, and study drug was distributed with concealed identity to the blinded staff by the unblinded pharmacist. All investigative site staff as well as persons directly involved with immunological assays or the assessment of clinical safety remained blind to treatment assignments. All subjects were blinded in the study.

[0147]   *Vaccine.* The rAd vector (non-replicating Ad5) carries DNA which encodes the HA (A/CA/04/2009) transgene whose expression is driven by a CMV promoter and a molecular dsRNA hairpin driven by a separate promoter. GMP drug substance was produced in Wave bags (GE Healthcare, Waukesha, WI) at Lonza Biologicals (Houston, TX). Purification was performed by ion exchange chromatography, followed by buffer exchange. Purified vector was mixed with excipients, lyophilized, and then tableted at Lonza using microcrystalline cellulose and starch as tableting bulk. Tablets were enteric coated with Eudragit L100® (Evonik Industries, Darmstadt, Germany) using a Vector Hi-Coater system (Vector Freund, Cedar Rapids, IA). The final product was released in one lot, and titered by standard IU assay at Lonza. Placebo was prepared as similarly sized and shaped tablets containing 150 mg of microcrystalline cellulose, without enteric coating.

[0148]   *Endpoints.* The primary endpoint for this study is safety and the secondary endpoint is immunogenicity through the active phase, primarily by HAI titers and HAI seroconversions. Additional immunological endpoints include MN titers and ASCs. There were 5 adverse events in the placebo group and 4 in the vaccine group, all of which were grade 1 in severity. There were no serious adverse events reported in the study.

[0149] *PBMC isolation and cryopreservation.* Blood was collected in K$_3$ EDTA Vacutainer® tubes (BD, Franklin Lakes, NJ) and PBMCs were isolated the same day using Lymphoprep™ tubes (Axis-Shield, Norway). PBMCs were frozen and thawed using serum free reagents according to the manufacturer's instructions (Cellular Technology Ltd [CTL],
5   Shaker Heights, OH).

[0150] *Antibody Secreting cells (ASCs).* Enzyme linked immunosorbent (ELISpot) kits for IgG and IgA secreting B cells were performed according to manufacturer's instructions (Mabtech, Mariemont, OH). Cells were cultured (between $1 \cdot 5 \times 10^4$ to 5×10^5 cells per well) in triplicate wells, in CTL-Test medium to optimize spots. HA protein (Protein Sciences
10 Corp, Meriden, CT) was biotinylated and quantitated using a biotinylation kit (Pierce, Rockford, IL).

[0151] *Antibody assays.* HAI and Microneutralizing (MN) Titers were performed and were measured against MDCK derived A/CA/07/2009 and egg derived A/CA/07/2009 respectively. HAI and MN titers less than 10 were marked as 5 as suggested by regulatory
15 advice.

[0152] *Statistical analysis.* Unpaired Students "t" tests were performed to test for significant differences between groups. A two-tailed Fisher's Exact test was used to determine if the observed frequencies were different for some analyses, as stated in the text. For both tests, p values of ≤ 0.05 were considered significant. 95 percent confidence intervals
20 (95 CI) were provided for measured values.

Example 6

Nonclinical Studies of a norovirus vaccine.

Introduction

[0153] The norovirus VP1 vaccines (VXA-G2.4-NS and VXA-G1.1-NN) of the invention
25 has the same replication-defective viral vector backbone and adjuvant RNA sequence as described in US Patent No. 8,222,224 and Scallan *et al.* Clinical and Vaccine Immunology 2013; 20(1): 85-94. The sequence of the vector backbone is provided in SEQ ID NO: 7.

[0154] VXA-G2.4-NS is an E1/E3-deleted replication-incompetent serotype 5 adenovirus vector designed for use as a vaccine for the prevention of norovirus disease (NVD). The
30 recombinant adenovirus (rAd) vector codes for a 1.6 kb gene from the viral protein 1 (VP1)

of norovirus (G2.4 Sydney strain) and an adjuvant dsRNA sequence that enhances the immunogenicity of the expressed antigen in the gut mucosa via its TLR3 agonistic activity. The VP1 gene has been codon-optimized for expression in mammalian cells and is expressed using a human cytomegalovirus intermediate early region (hCMVIE) enhancer/promoter and a bovine growth hormone polyadenylation (pA) signal. This expression cassette also includes the first intron of human β-globin to enhance transgene expression. A second hCMVIE promoter is used to express the adjuvant RNA sequence. The adjuvant sequence is derived from a luciferase sequence and has been reported to stimulate the induction of type I interferons in vitro (2). The adjuvant is expressed as a short hairpin RNA, comprising a 21-nucleotide sequence (GAAACGATATGGGCTGAATAC) as a tandem sequence in forward and reverse orientations separated by six nucleotides that comprise the loop of the RNA. The 21-nucleotide forward and reverse RNA sequences anneal to form the stem of the loop. This adjuvant cassette utilizes a synthetic poly A (SPA).

[0155] As described below, preclinical studies in mice and ferrets show that vaccination with VXA-G2.4-NS (and the related VXA-G1.1-NN) elicited substantial and reliable antibody responses in both systemic serum IgG and intestinal (fecal) IgA response in test animals. A unique immune response elicited by oral vaccination is the induction of antibodies derived from local sources of B cells. After oral vaccination or enteric infection, B cells residing in the lamina propria (underneath the gut epithelial cells) predominantly produce dimeric antibodies of the IgA isotype . IgA antibodies pass across the gut epithelial cells into the lumen through facilitated transport, leaving a secretory component attached to dimeric IgA. The resulting molecule is known as secretory IgA or SIgA. SIgA serve as an additional external barrier to block enteric infection. SIgA are eventually flushed out of the system, and can be detected in fecal samples.

Nonclinical Pharmacology

Introduction

[0156] The primary objective of the immunogenicity studies was to demonstrate that oral adenoviral constructs that express VP1 from two different norovirus species could elicit intestinal specific SIgA as well as serum IgG immune responses against norovirus antigen (VP1) following oral immunization of norovirus vaccine.

[0157] The secondary objective of the immunogenicity studies was to compare two routes of immunization (oral with VXA-G2.4-NS vs. i.m. with VP1 protein). The VP1 protein was

compared since VP1 VLP based injectable vaccine is one of the few norovirus vaccines under development. Generally, SIgA are induced by mucosal immunization but a much lesser degree by parenteral immunization. Therefore, we investigated intestinal SIgA response induced by oral VXA-G2.4-NS delivery compared to injectable recombinant VP1 protein based vaccines.

Table 4: Nonclinical Pharmacology Studies

Study Title (Study No.)	Animal Model (Species, Gender, No.)	Therapy (Treatment)	VXA-G2.4-NS (Immunization schedule)	Endpoints (Sample collection time points)	Endpoints (Assays)
IMMUNOGENICITY STUDY					
Immunogenicity study of Ad-dsRNA-CMV-VP1 (Norwalk) vaccine Study No.					
(1)	Balb/c mice. 6 F per group	Group 1: oral VXA-G1.1-NN (1×10^8 IU[a]) Group 2: oral VXA-G1.1-NN (5×10^8 IU[a])	Immunization on days 0 and 28	8 weeks	VP1 ELISA (serum IgG and fecal IgA)
(2)	Balb/c mice. 6 F per group	VXA-G1.1-NN: oral (1×10^9 IU[a])	Immunization on days 0 and 28	8 weeks	VP1 ELISA (serum IgG and fecal IgA)
(3)	Balb/c mice. 6 F per group	Group 1: oral VXA-G1.1-NN (1×10^8 IU[a]) Group 2: i.m. VP1 protein from Norwalk virus (1ug)	Immunization on days 0 and 28	4 and 8 weeks	VP1 ELISA (serum IgG and fecal IgA)
(4)	Balb/c mice. 6 F per group	Group 1: oral VXA-G1.1-NN (1×10^8 IU[a]) Group 2: i.m. VP1 protein from Norwalk virus (1ug) +Alum	Immunization on days 0 and 28	4 and 8 weeks	VP1 ELISA (serum IgG and fecal IgA)
Immunogenicity study of VXA-G2.4-NS (Sydney) vaccine					
(5)	Balb/c mice. 6 F per group	VXA-G2.4-NS: oral	Immunization on days 0 and 28	4 and 8 weeks	VP1 ELISA Serum IgG

Study Title (Study No.)	Animal Model (Species, Gender, No.)	Therapy (Treatment)	VXA-G2.4-NS (Immunization schedule)	Endpoints (Sample collection time points)	Endpoints (Assays)
		(1x10^8 IU^a)		8 weeks	Fecal IgA
IMMUNOGENICITY STUDY					
Immunogenicity study of VXA-G2.4-NS (Sydney) vaccine Study	Ferrets 4M/4F per group 1 and 2	Group 1: oral VXA-G2.4-NS (1x10^9 IU^a)	Immunization on days 0 and 28	8 weeks	VP1 ELISA (serum IgG and fecal IgA)
		Group 2: oral VXA-G2.4-NS (1x10^9 IU^a)	Immunization on day 0 and 2	8 weeks	VP1 ELISA (serum IgG and fecal IgA)
	2M/2F per group 3	Group 3: i.m. VP1 protein from Sydney strain NoV (5ug)	Immunization on days 0 and 28	8 weeks	VP1 ELISA (serum IgG and fecal IgA)

a IU = infectious units

Immunogenicity Studies

[0158]   The primary objective of the initial mice immunogenicity studies was to determine if the vector backbone expressing a norovirus VP1 from Norwalk virus or the Sydney strain could induce an antibody response to VP1 from Norwalk virus or the Sydney strain as measured by ELISA.

[0159]   rAd expressing the Norwalk VP2 strain and the dsRNA adjuvant (VXA-G1.1-NN) was delivered orally by gavage on days 0 and 28. A dose titration was conducted (study#1 and study#2). Norwalk virus VP1 specific IgG and sIgA responses were measured from serum and fecal samples, respectively at 8 weeks. As expected, as the dose was increased from 1x10^8 to 5x10^8 to 1x10^9, the serum VP1 IgG titer showed a dose-dependent increase geometric mean titer (GMT) increase from 2x10^3 to 1x10^4 to 5x10^5 (Figure 8). A similar but slightly more gradual dose dependent increase from 1x10^3 to 2x10^3 to 3x10^4 in fecal VP1 IgA response was also observed (Figure 9). The mice studies showed that VXA-G1.1-NN vaccine is immunogenic and generated dose dependent serum and fecal VP1 antibody responses.

[0160]   In the next study (study#3), immune responses via two routes of administration with oral delivery by gavage with VXA-G1.1-NN (group 1) and intramuscular injection with Norwalk VP1 protein (group 2) were compared (Figs 2.2.3 and 2.2.4). Norwalk virus VP1 specific IgG and SIgA responses were measured from serum and fecal samples, respectively at 4 and 8 weeks. The oral vaccine of the invention generated slightly higher serum IgG titer values than the intramuscular protein vaccine (Figure 10). In the fecal study, oral vaccine generated a dramatically higher intestinal SIgA immune response than the intramuscular protein vaccine (Figure 11).

[0161]   A similar study (study#4) was conducted with the Norwalk virus VP1 protein vaccine with a vaccine adjuvant, aluminium hydroxide (Figures 12 and 13). Intramuscular injection with the VP1 protein together with alum generated much higher serum titer (Figure 12). However, the oral vaccine was clearly superior at generating SIgA, as indicated by the higher fecal VP1 specific IgA titer value (Figure 13). With the presence of alum which is a strong adjuvant, a few animals produced fecal VP1 IgA after intramuscular immunization whereas fecal VP1 IgA from samples without alum was almost non detectable. The data suggest that the oral vaccine of the invention has an immunological advantage over injectable vaccine to produce intestinal SIgA immune response.

[0162]   The currently circulating norovirus variant, the Sydney strain vaccine (VXA-G2.4-NS), was tested (study#5 Fig 2.2.7). At 4 weeks, the Sydney strain vaccine generated far better titer values than the Norwalk virus vaccine. In addition, even at 4 weeks the titer value from the Sydney strain vaccine was slightly higher than the Norwalk value at 8 weeks (Figure 14A). The Sydney strain vaccine generated slightly higher fecal VP1 SIgA titer values than the Norwalk vaccine did (Figure 14B). The Sydney strain vaccine appears to be more immunogenic. Accordingly, we chose the Sydney strain for the following ferret study.

[0163]   The objective of the ferret study (study#6) was to (1) determine whether the oral vaccine of the invention could induce an intestinal SIgA as well as systemic serum IgG response compared to recombinant Sydney VP1 protein and (2) compare SIgA and IgG response with two different immunization schedules. Twenty ferrets were randomized into 3 groups (4 males and 4 females in each of groups 1 and 2 and 2 males and 2 females in Group 3). On day 0 and 2, animals in group 1 were endoscopically administered VXA-G2.4-NS. On days 0 and 28 animals in group 2 were endoscopically administered VXA-G2.4-NS and animals in Group3 were intramuscularly administered the Sydney norovirus recombinant

VP1 protein. Animals in group 1 produced higher IgG titer value than group 2 (3x104 vs

5x103). Whereas animals in group 2 generated higher SIgA titer values than group 1 (247 vs

92). Similarly to the mice study (Fig 4), intramuscular VP1 protein vaccine failed to generate

intestinal SIgA response although serum VP1 IgG response was well generated (8x104)

(Figure 15). Overall, the oral vaccine of the invention generates comparable levels of serum

IgG and superior levels of fecal IgA to VP1 injectable protein vaccine.

Summary of Pharmacology Studies

[0164]   The studies summarized within this section demonstrated that oral administration of

VXA-G2.4-NS could elicit substantial antibody responses to norovirus VP1 by ELISA in

mice and ferrets. Local immunity could play a very important role preventing infection and

disease, and SIgA would likely be the most effective gut immune response to combat a gut

pathogen such as norovirus. These studies demonstrate that VXA-G2.4-NS oral delivery

generated intestinal SIgA response and superior levels of SIgA to VP1 injectable protein

vaccine.

[0165]   The duration of norovirus infection is relatively short. Norovirus has around 2 days

of incubation time, followed by illness with vomiting and diarrhea, which lasts 2 to 4 days. T

and B cell responses start to occur around 4 days after infection. Interestingly, the clearance

of norovirus occurs just after development of the early stage of T and B cells' activation.

However, the oral vaccine of the invention could manipulate the immune response to benefit

the individual. Before norovirus infection, the oral vaccine will induce gut homing B cells to

secrete VP1 specific SIgA antibody. IgA enriched gut homing B cells in PBMC were

detected in previous studies. SIgA will pass across the intestinal epithelial cells to the

alimentary tract (gut lumen). After norovirus infection, SIgA will act as a blocking

(neutralizing) antibody against norovirus infection. In conclusion, a gut pathogen is an

excellent match for the oral platform because the oral delivery method activates immune cells

with the desired gut homing property and intestinal SIgA is the most effective gut immune

response to combat a gut pathogen.

Example 7

Phase 1 VXA-G24-NS study synopsis

[0166] This study is a phase 1, randomized, double-blind, placebo-controlled, dose-ranging

trial to determine the safety and immunogenicity of an adenoviral-vector based norovirus

vaccine (vxa-g2.4-ns) expressing gii.4 vp1 and dsrna adjuvant administered orally to healthy volunteers. The study will be conducted in 1-2 U.S. sites on healthy adult volunteers age 18 to 49 years. Subject participation in the study will last ~1 year following successful screening and enrollment. After vaccination subjects will be followed for one month for efficacy
5   (immunogenicity) and for 12 months for safety.

Investigational product

[0167] VXA-G2.4-NS is an E1/E3-deleted replication-defective Adenovirus serotype 5 vaccine vector for prevention of noroviral gastroenteritis caused by Norovirus GII.4. The vaccine vector encodes for a full-length VP1 (major capsid protein) gene from Norovirus
10   GII.4 Sydney and is described in detail in Example 6.

[0168] The GII.4 VP1 gene is expressed using a human Cytomegalovirus (CMV) intermediate early region (hCMVie) enhancer/promoter. In addition to the transgene cassette, a second hCMVie promoter is used to express a double-stranded RNA (dsRNA) sequence that acts as a TLR3-based adjuvant.

15   **Control product**

[0169] A placebo tablet dosage formulation indistinguishable in appearance and number from the vaccine tablets

Regimen and dosing

[0170] Single administration of VXA-G2.4-NS at a low dose of 1×1010 IU, a high dose of
20   1×1011 IU or placebo. The number of tablets per dose will be calculated based on the release assay results for the drug product. Two sentinel groups will enroll three subjects each in an open-label manner (Cohorts 1 and 3) to receive VXA-G2.4-NS prior to enrolling either of the randomized, controlled cohorts (Cohorts 2 and 4). Within the double-blinded groups (Cohorts 2 and 4), placebo subjects will receive the same number of tablets as vaccine
25   subjects. Subjects will be enrolled and dosed in the low dose group prior to initiation of dosing in the high dose group.

Cohort 1: 1×1010 IU ± 0.5 logs (n=3)

Cohort 2: 1×1010 IU ± 0.5 logs (n=20) or placebo (n=10)

Cohort 3: 1×1011 IU ± 0.5 logs (n=3)
30   Cohort 4: 1×1011 IU ± 0.5 logs (n=20) or placebo (n=10)

[0171] Subjects in Cohorts 2 and 4 will be randomized in a 2:1 ratio to VXA-G2.4-NS at 1×1010 IU (low dose) or 1×1011 IU (high dose), respectively, or placebo.

Objectives

[0172] The primary objective is to determine the safety of a VXA-G2.4-NS norovirus vaccine candidate. The secondary objective is to determine the immunogenicity of a VXA-G2.4-NS norovirus vaccine candidate at two dose levels

Study design

[0173] This is a Phase 1, randomized, placebo-controlled, double-blind (after initial open-label lead-in), dose-ranging study to assess the safety, reactogenicity and immunogenicity of an adenoviral-vector based oral Norovirus GII.4 vaccine and dsRNA adjuvant. All subjects will receive a single vaccine administration.

[0174] The study will be enrolled in four cohorts:
Cohort 1: VXA-G2.4-NS at 1×1010 IU (low dose) sentinel
Cohort 2: VXA-G2.4-NS at 1×1010 IU (low dose) or placebo
Cohort 3: VXA-G2.4-NS at 1×1011 IU (high dose) sentinel
Cohort 4: VXA-G2.4-NS at 1×1011 IU (high dose) or placebo

[0175] Cohort 1 (low dose sentinel group) will enroll 3 subjects to receive a single dose of VXA-G2.4-NS at 1×1010 IU on Day 0. The 3 subjects will be enrolled sequentially (one per day), in an open-label manner. Upon completion of the Day 7 Visit in all 3 subjects, if no dose-limited toxicities are observed in these sentinel subjects (see Halting Rules below), enrollment will begin in Cohort 2.

[0176] Cohort 2 (low dose randomized group) will randomize 30 subjects in a 2:1 ratio to receive either VXA-G2.4-NS at 1×1010 IU (low dose) (n=20) or placebo (n=10) in a double-blinded manner.

[0177] The study will enroll continuously during this phase unless the criteria for pre-established stopping rules are met (see below). Should this happen, enrollment of subsequent subjects will not be initiated until the study Safety Monitoring Committee (SMC) has completed review of safety data and offered the recommendation to proceed. The safety assessment will be performed with the treatment assignments coded in Cohort 2. If the SMC needs treatment information to assess an AE/SAE, the code will be revealed for that subject.

[0178] Upon completion of the Day 7 Visit in all 30 subjects, if no dose-limited toxicities are observed in these subjects (see Halting Rules below), enrollment will begin in Cohort 3. Cohort 3 (high dose sentinel group) will enroll 3 subjects to receive a single dose of VXA-G2.4-NS at 1x1011 IU on Day 0. The 3 subjects will be enrolled sequentially (one per day), in an open-label manner. Upon completion of the Day 7 Visit in all 3 subjects, if no dose-limited toxicities are observed in these sentinel subjects (see Halting Rules below), enrollment will in Cohort 4.

[0179] Cohort 4 (high dose randomized group) will randomize 30 subjects in a 2:1 ratio to receive either a single dose of VXA-G2.4-NS at 1x1011 IU (high dose) (n=20) or placebo (n=10) in a double-blinded manner.

[0180] The study will enroll continuously during this phase unless the criteria for pre-established stopping rules are met (see Halting Rules below). Should this happen, enrollment of subsequent subjects will not be initiated until the SMC has completed review of safety data and offered the recommendation to proceed. The safety assessment will be performed with the treatment assignments coded in Cohort 4. If the SMC needs treatment information to assess an AE/SAE, the code will be revealed for that subject.

[0181] All subjects receiving study drug (vaccine or placebo) will have safety and immunogenicity assessments completed for one month following vaccination. Immunogenicity evaluations will be obtained at baseline prior to vaccination, and at Days 7 and 28 following vaccination. Subjects will also be evaluated for persistent immunogenicity at Day 180 and be followed for safety for 12 months following vaccination.

Sample size

[0182] The planned enrollment in this study is 66 subjects as follows:
Cohort 1: VXA-G2.4-NS (1x1010 IU ± 0.5 logs): n=3, sentinel
Cohort 2: VXA-G2.4-NS (1x1010 IU ± 0.5 logs): n=20, or placebo: n=10; total 30 subjects, 2:1 ratio
Cohort 3: VXA-G2.4-NS (1x1011 IU ± 0.5 logs): n=3, sentinel
Cohort 4: VXA-G2.4-NS (1x1011 IU ± 0.5 logs): n=20, or placebo: n=10; total 30 subjects, 2:1 ratio

[0183] Additional subjects may be enrolled to replace dropouts.

Study population

[0184] Male or female healthy volunteers, 18 to 49 years of age.

Inclusion/exclusion criteria

[0185] Inclusion criteria include:

1.   Male or female volunteers aged 18 – 49 years, inclusive

2.   Able to give written informed consent

3.   Healthy (no clinically significant health concerns), as determined by medical history, physical examination, 12-lead ECG, and vital signs at screening

4.   Safety laboratory values within the following range criteria at baseline:

        a.   Normal range for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, phosphorous (hypophosphatemia), neutrophils, occult blood, white blood cells (WBC), and urine protein;

        b.   Normal or grade 1 abnormality with no clinical significance (NCS) for albumin, amylase, blood urea nitrogen (BUN), calcium, creatine phosphokinase (CPK), creatinine, glucose, magnesium, potassium, sodium, total protein, eosinophils (increase), hemoglobin, lymphocytes (decrease), and platelets;

        c.   Negative or positive with NCS for blood urine

5.   Body mass index between 17 and 35 at screening

6.   Comprehension of the study requirements with ability and willingness to complete all assessments and comply with scheduled visits and contacts

7.   Female participants must have a negative pregnancy test at baseline and fulfill one of the following criteria:

        a.   At least one year post-menopausal;

        b.   Surgically sterile;

        c.   Willing to use oral, implantable, transdermal or injectable contraceptives for 30 days prior to and until 60 days after vaccination;

                i.   A reliable form of contraception must be approved by the Investigator (e.g., double barrier method, Depo-Provera, intrauterine device, Norplant, oral contraceptives, contraceptive patches, abstinence)

[0186] **Exclusion criteria include**:

1.   Receipt of any norovirus vaccine within two years prior to study vaccination

2.   Administration of any investigational vaccine, drug or device within 8 weeks preceding vaccination, or planned use of the above stated during the study through the 12-month safety follow-up

3.   Administration of any licensed vaccine within 30 days prior to vaccination

4.   Presence of significant uncontrolled medical or psychiatric illness (acute or chronic) including institution of new medical/surgical treatment or significant dose alteration for uncontrolled symptoms or drug toxicity within 3 months of screening and reconfirmed at baseline

5.   Any one of the following ECG findings within 30 days prior to vaccination:

a.   QTc (Bazett) interval duration > 450 msec (male) or > 470 msec (female),

b.   QRS interval greater than 120 msec,

c.   PR interval greater than 220 msec,

d.   Clinically significant ST-T wave changes or pathologic Q waves

6.   Positive serology for HIV-1 or HIV-2, or HBsAg or HCV antibodies

7.   Cancer, or treatment for cancer treatment, within past 3 years (excluding basal cell carcinoma or squamous cell carcinoma)

8.   Presence of immunosuppression or medical condition possibly associated with impaired immune responsiveness, including diabetes mellitus

9.   Administration of any medications or treatments that may adversely affect the immune system such as allergy injections, immune globulin, interferon, immunomodulators, cytotoxic drugs or other drugs known to be associated with significant major organ toxicity, or systemic corticosteroids (oral or injectable) during 3 months prior to vaccination. Inhaled and topical corticosteroids allowed

10.   Presence of household members who have received the Ad4 or Ad7 vaccines within 2 months prior to vaccination

11.   Presence of household members who are neonates, pregnant women, or hematopoietic stem cell transplant or solid organ transplant recipients

12.   History of drug, alcohol or chemical abuse within 1 year prior to vaccination

13.   Receipt of blood or blood products 6 months prior to vaccination or planned administration during the follow-up study period

14.   Donation of blood or blood products within 4 weeks prior to vaccination or planned donation during the study period

15.   Acute disease within 72 hours prior to vaccination 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)

16.   Presence of a fever ≥ 38°C measured orally at baseline

17.   Stool sample with occult blood at screening

18.   Positive urine drug screen for drugs of abuse at screening

19.   Consistent/habitual smoking within 2 months prior to vaccination

20.   History of serious reactions to vaccination such as anaphylaxis, respiratory problems, hives or abdominal pain

21.   Diagnosed bleeding disorder or significant bruising or bleeding difficulties that could make blood draws problematic

22.   History of irritable bowel disease or other inflammatory digestive or gastrointestinal condition that could affect the distribution / safety evaluation of an orally administered vaccine targeting the mucosa of the small intestine.

Such conditions may include but are not limited to:

26.   Any condition that, in the opinion of the Investigator, might interfere with ability to assess the primary study objectives

STUDY SCHEDULE The following study visits/calls will be conducted during the study:

*   Screening Period (within 30 days prior to vaccination)

*   Day -2 (Baseline safety laboratory sample collection)

*   Day 0 Visit (Baseline; day of vaccination)

*   Day 2 Visit

*   Day 7 Visit

*   Day 28 Visit

*   Day 180 Visit

*   Day 365 End of Study Contact

[0187] Subjects will be followed via phone call daily on Days 1, 3 to 6, and 14. They will also be contacted monthly between the Day 28 and Day 180 Visits and also following the Day 180 Visit through Day 365 (end of study). See Table 1 for detailed schedule of study procedures.

Safety and immunogenicity assessments

Safety:

[0188] Safety and tolerability will be evaluated by: local (oral, esophageal and gastrointestinal) and systemic reactogenicity (solicited symptoms), and clinical and laboratory assessments. Physical exams, routine urinalysis, complete blood counts and serum chemistries will be collected pre-dose at Screening and Baseline (safety labs at Day -2) and at Study Days 2, 7 and 28. Vital signs will be recorded pre-dose at Screening and Baseline and at Study Days 2, 7, 28 and 180.

[0189] Safety will be evaluated using standard blood chemistry, hematology and urinalysis, and analyses performed per statistical methods below. Any subject who experiences acute symptoms of conjunctivitis, upper respiratory infection, loose stools and/or diarrhea within 14 days following initial vaccination, will be asked to return for a medical evaluation and evaluation for adenoviral 5 infection. In these subjects, adenoviral cultures of throat and rectal swabs will be collected.

Immunogenicity:

[0190] Immunogenicity will be evaluated using cellular and humoral immune function assays from blood samples obtained at baseline (pre-dose) and at Study Days 7 and/or 28 depending on the assay. In addition, a final evaluation for persistent immunogenicity will be performed at Day 180. The following assessments will be performed: Serum IgG VP1; Histo-blood group antigen–blocking antibodies (BT50); IgG ASC VP1; IgA ASC VP1; Flow cytometric B cell immunophenotyping; Pre-plasma B cell culture for IgG VP1 and IgA VP1; Fecal IgA VP1; HAI; and Anti-Ad5.

Halting rules

[0191] The study will be halted (no new enrollments and no further investigational product administered pending a full SMC safety review) if any of the below occur: For Cohorts 1 and 3 (3 subject open-label sentinel lead in groups):

1. One or more subject experiences a vaccine-related SAE of any grade;

2. One or more subject experiences a Grade 3 or higher clinical AE or laboratory abnormality;

3. Two or more subjects experience any vaccine-related Grade 2 clinical AE or laboratory abnormality.

[0192]   For Cohorts 2 and 4 (randomized, placebo-controlled groups):

1.   One or more subject experiences a vaccine-related SAE of any grade;

2.   Two or more subjects experience Grade 3 clinical or laboratory AEs,

3.   One or more subject experiences a Grade 4 clinical AE, or a Grade 4 laboratory abnormality;

4.   Three or more subjects present with symptoms of adenovirus infection and detectable replication competent adenovirus 5 vaccine virus. If three or more subjects present with symptoms of adenovirus infection, enrollment will be halted pending results for detection of adenovirus 5 vaccine virus.

Endpoint parameters

[0193] Safety analyses include: 1) Standard descriptive demography; 2) Proportion of subjects in each treatment group will be tabulated for each local and systemic solicited reactogenicity event and any unsolicited AEs. AE severity will be classified using standardized criteria adapted from the Sept. 2007 FDA Guidance entitled "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" ; 3) Subjects with AEs (including clinical laboratory abnormalities) will be summarized by (1) MedDRA body organ system and preferred term; (2) severity; (3) relatedness; and, separately, (4) seriousness; 4) Proportion of subjects in each treatment group with AE reports within each body organ system will be compared in the same manner. Significant heterogeneity will be probed at the preferred term level

[0194] Immunogenicity analyses include: 1) Serum IgG VP1 and Histo-blood group antigen-blocking antibodies (BT50); 2) Additional exploratory analyses will include IgG ASC VP1; IgA ASC VP1; Flow cytometric B cell immunophenotyping; Pre-plasma B cell culture for IgG VP1 and IgA VP1; Fecal IgA VP1; HAI; Anti-Ad5.

Statistical methods

Sample Size and Power:

[0195] This is the first in human clinical trial with VXA-G2.4-NS that will be conducted by the sponsor. There is currently no clinical information about the study drug. Hence the sample size was determined based on experience of a typical Phase 1 vaccine study. The numbers of volunteers per group in Cohorts 2 and 4 are predicted to yield meaningful immunogenicity results. A sample size of 20 in vaccine group and 10 in placebo group (i.e.

2:1 randomization ratio) will provide approximately 86% power to detect a group difference, assuming the proportion of response (observed in serum IgG VP1) in vaccine group is 50% and in placebo is 0, using two-group Fisher's exact two-sided test at significance level of 0.05.

Data Analysis:

Safety:

[0196] Safety will be summarized by treatment group. Local and systemic reactogenicity, AEs, clinical laboratory results, and vital signs will be summarized descriptively by study visit. Number and percentage of subjects who experience acute symptoms of conjunctivitis, upper respiratory infection, loose stools and/or diarrhea within 14 days following initial vaccination will be compared by treatment group using Fisher's exact test.

Immunogenicity:

[0197] Immunogenicity results evaluated by cellular and humoral immune function assays from blood samples collected at preselected study visits will be summarized descriptively. Analysis of covariance (ANCOVA) will be used in the analysis of the antibody titers, with post baseline log-titer as dependent variable, treatment as a factor, and baseline log-titer as a covariate. Least square (LS) means and 95% CI of the LS means will be obtained from the model. The post baseline Geometric (LS) Mean Titer (GMT) for the VXA-G2.4-NS group and Geometric (LS) Mean Fold Rise (GMFR) over the initial GMT at baseline will be reported.

Example 8

RSV background

[0198]   Respiratory syncytial virus (RSV) is the most important cause of lower respiratory tract infection (LRI) in infants and young children and is a major cause of LRTI in the elderly and immune-compromised patients where it can have devastating effects, causing significant morbidity and mortality. It is estimated to infect 5-10% of nursing home residents per year, with rates of pneumonia or death of 10-20% and 2-5% respectively (Falsey et al. 2000). There is no approved vaccine though there is an approved prophylactic monoclonal antibody, Palivizumab, for disease prevention in high-risk infants.

[0199]   Given the lack of a vaccine Vaxart is addressing this large unmet medical need by
initiating a preclinical program to evaluate a RSV vaccine delivered using it's oral adenovirus
vectored platform. This platform has previously being used to deliver influenza vaccines to
patients where it's efficacy and inducing significant immunity has been demonstrated. An
important strategy in this preclinical path is demonstrating disease protection in a cotton rat
RSV challenge model. To this end we have started such an evaluation and will have a
complete data set shortly. We present preliminary data in this report, which demonstrates that
significant immunity is generated even after a single vaccination. Based on this immune
response we expect the vaccine to be protective.

RSVF Vaccine Evaluation in Cotton rats

[0200]   A vaccine vector (Ad-RSVF) expressing the fusion protein (F) of RSV and a
dsRNA adjuvant was generated as described above. The Ad vaccine vector was produced in
293 cells, purified and a titer determined. The vector was then evaluated first in mice where
significant immune response was elicited to the fusion protein (data not shown) and then in
the relevant RSV animal model, cotton rats. As an oral delivery method has not been
optimized in Cotton rats the intra-nasal route was chosen as an alternate mucosal route of
delivery. Two doses were administered, a low (1e8 IU) and high dose(1e9 IU). Uninfected
animals were used as a negative control and wild-type RSV virus (RSVA2) was used as a
positive control. Formalin inactivated RSV (FIRSV) was used as a control for undesired
negative effects associated with an RSV vaccine developed in the 1960's . After a single
vaccination Ad-RSVF at both low and high doses induced higher titers compared to wild-
type RSV infection (Figure 17) while the FIRSV induced a very weak immune response
(200-300x lower). This data indicates that our vaccine is superior at inducing immunity than
WTRSV virus and more effective than the earlier FIRSVF vaccine.

[0201]   In addition to total anti-Fusion protein antibodies (Fig. 17) we also looked at
antibodies generated by our vaccine that could compete with the approved Palivizumab
antibody. In a competition assay using pooled sera from each of the treatment groups only the
Ad-RSVF vaccine generated detectable antibody titers (Fig 18). These data indicate that the
vaccine is producing RSV protective antibodies.

VXA-RSV-f Nonclinial Studies

8.1   Introduction

[0202]   VXA-RSV-f is an E1/E3-deleted replication-incompetent serotype 5 adenovirus vector designed for use as a vaccine for the prevention of RSV and administered by the oral route. The recombinant adenovirus (rAd) vector codes for 1) the fusion (F) gene from the RSV A2 strain (Genbank# HQ317243.1) and 2) an adjuvant dsRNA sequence that enhances the immunogenicity of the expressed F antigen in the gut mucosa via its TLR3 agonistic activity. The vaccine backbone is identical to that in Vaxart's ND1.1, VXA-A1.1 and VXA-BYW.10 vaccines for pandemic and seasonal influenza A and B, respectively, currently in clinical development; the only change being that VXA-RSV-f has a different surface protein (F protein) being expressed. The RSV F protein gene has been codon-optimized for expression in mammalian cells and is expressed using a human cytomegalovirus intermediate early region (hCMVIE) enhancer/promoter and a bovine growth hormone polyadenylation (pA) signal. This expression cassette also includes the first intron of human β-globin to enhance transgene expression. A second hCMVIE promoter is used to express the adjuvant RNA sequence. The adjuvant sequence is derived from a luciferase sequence and has been reported to stimulate the induction of type I interferons in vitro (1). The adjuvant is expressed as a short hairpin RNA, comprising a 21-nucleotide sequence (GAAACGATATGGGCTGAATAC) as a tandem sequence in forward and reverse orientations separated by six nucleotides that comprise the loop of the RNA. The 21-nucleotide forward and reverse RNA sequences anneal to form the stem of the loop. This adjuvant cassette utilizes a synthetic poly A (SPA).

[0203]   Vaxart has conducted preclinical studies to determine the immunogenic potential of VXA-RSV-f in mice and cotton rats. These studies showed that vaccination with VXA-RSV-f elicited substantial systemic serum IgG responses in test animals.

[0204]   As described above, Vaxart's RSV vaccine (VXA-RSV-f) will use the same replication-defective viral vector backbone and adjuvant RNA sequence as the company's pandemic and seasonal influenza virus programs. The A/Indonesia/05/2005 (H5N1) pandemic influenza vaccine, ND1.1, is presently being studied under BB-INDs 14660 and 15122, and the A/California/04/2009 (H1N1) seasonal influenza vaccine, VXA-A1.1, is presently being studied under BB-INDs 15198 and 15285. The B/Wisconsin/1/2010 (Yamagata) vaccine is presently being studied under BB-IND 16611. Because the only difference between the vaccines is the antigen gene [the F protein in VXA-RSV-f versus HA in VXA-A1.1, ND1.1 and VXA-BYW.10], the preclinical studies from ND1.1 and VXA-

A1.1 are relevant to and support the clinical development of the VXA-RSV-f vaccine for prevention of RSV disease.

8.2   Nonclinical Pharmacology

8.2.1   Introduction

[0205]   The primary objective of the immunogenicity studies of the RSV vaccine candidate VXA-RSV-f was to demonstrate that vector construct could elicit antibody responses in mice and cotton rats, and that the adaptive immune responses generated did not lead to enhanced RSV disease such as that known to occur with the formalin inactivated RSV vaccine. Further, studies were performed in animals to demonstrate the value of the adjuvant for inducing antigen specific immune enhancement.

Table 5 Nonclinical Pharmacology Studies

Study Title (Study No.)	Animal Model (Species, Gender, No.)	Therapy (Treatment)	VXA-RSV-f (Immunization schedule)	Endpoints (Sample collection time points)	Endpoints (Assays)
Immunogenicity Study of Ad-CMV-RSVf-dsRNA Vaccine					
Study No. WCB254	Balb/c mice. 6 F per group	Group 1: VXA-RSV-f i.n. (1×10^8 IU[a]) Group 2: VXA-RSV-f i.m. (1×10^8 IU) Group 3: VXA-RSV-f oral (1×10^8 IU)	Days 0 and 21	7 weeks	RSV-F ELISA
Study No. XV-95[c] **Cotton rat challenge study**	Cotton rats 6 F per group, or 3 F per group for uninfected control group	Group 1: VXA-RSV-f i.n. (1×10^9 IU) Group 2: VXA-RSV-f i.n. (1×10^8 IU) Group 3: VXA-RSV-f i.m. (1×10^9 IU) Group 4: rAd-Adj (1×10^9 IU) Group 5: untreated/unifected Group 6: FI-RSV Group 7: Buffer alone Group 8: RSV/A2 1e5 PFU	Days 0 and 28 Challenge day 56	8 weeks; Day 5 post challenge	RSV-F ELISA (serum IgG), anti-palivizumab competition assay, PRNT antibodies. RSV replication post challenge
Study No. XV-112[c] **Cotton rat immunogen-icity (and challenge)**	Cotton rats 8 F per group, or 6 F for buffer control group	Group 1: Buffer alone Group 2[b]: oral VXA-RSV-f (1×10^{10} IU) Group 3: oral VXA-RSV-f (1×10^9 IU) Group 4: Oral VXA-RSV-f (1×10^8 IU)	Days 0 and 28 Challenge day 56	8 weeks; Day 5 post challenge	RSV-F ELISA (serum IgG), anti-palivizumab competition assay, PRNT antibodies. RSV replication post challenge

[a] IU = infectious units
[b] oral delivery was conducted by gavage by Sigmovir (Rockville, MD)

5

8.2.2   Immunogenicity and Challenge Studies

The primary objective of the initial mouse immunogenicity study (Study No. WCB254) was
to determine if the Vaxart vector backbone expressing the RSV F protein could induce an
antibody response to RSV as measured by ELISA. Following completion of the mouse study,
5   two cotton rat studies were performed. The objectives of the cotton rat studies were to
demonstrate that VXA-RSV-f could elicit potent antibodies to RSV, and that the vaccine
could elicit protective immune responses against RSV. Further, the cotton rat studies were
used to determine that VXA-RSV-f induced adaptive immune responses did not enhance
RSV disease, such as those recorded for the old formalin inactivated RSV vaccine (FI-RSV).

10

Immunogenicity Studies in Mice

Mice (6 females/group) were immunized with VXA-RSV-f using three different routes of
delivery in order to determine whether the construct was immunogenic. Animals were
immunized with 1×10^8 IU on weeks 0 and 3 using three different routes of delivery
15   (intranasal, intramuscular, and oral). Antibody titers to RSV were measured on week 7.
Results show that both the i.n. and i.m. routes of delivery were potent at eliciting antibody
responses, and that oral administration was able to elicit some immunity but was not as potent
in mice as the other two routes of delivery. See Figure 22.

Immunogenicity and Challenge Studies in Cotton Rats

20   [0206]   The objectives of the first cotton rat study (Study No. XV-95) were to determine the
ability of VXA-RSV-f to induce antibody responses and to protect against RSV disease and
viral replication. The cotton rat is considered an important model for preclinical development
of RSV vaccines because of the susceptibility of the animal to RSV infection, and the
reproducibility of the lung inflammation /cytokine skewing phenotype when given formalin
25   inactivated RSV vaccine (FI-RSV) followed by RSV challenge (2). Female cotton rats (N=6
per group) were immunized with VXA-RSV-f by intranasal (i.n.) and intramuscular (i.m.)
delivery on weeks 0 and 4 at 1×10^9 IU. A lower dose i.n. delivery group at 1×10^8 IU of
VXA-RSV-f (VXA-RSV-f low) was also used. The VXA-RSV-f vaccine treated animals
were compared for ELISA IgG titers on week 8 with a no infection/no vaccine control group
30   (No infection), an adenovirus storage buffer alone (buffer) group and a FI-RSV vaccine
group at 1:100 dilution of a stock (Lot #100) made by Pfizer from the RSV-A2 strain (3). To
show that post challenge results were antigen specific, a rAd that expresses the adjuvant
without the antigen at 1×10^9 IU (Ad-Adj) was given i.n. and used as a control. As a positive

control for immunogenicity and adaptive immune mediated protection, a single dose of wild-type RSV strain A2 (RSV2) at 1×10^5 PFU was given on week 0. Refer to **Table 5** for summary of study treatment groups, time points and endpoints.

[0207]   All VXA-RSV-f immunized animals induced significant IgG antibody titers to RSV, with group average antibody titers exceeding 1×10^5 (**Figure 23A**) at week 8. The FI-RSV and the RSV2 immunized animals also induced significant IgG antibody titers, with average titers reaching 1×10^4 on week 8 (**Figure 23A**). The VXA-RSV-f vaccine treated animals were also able to induce antibodies that competed for the palivizumab binding site on the F protein of the RSV virus, as measured by the competitive binding ELISA, whereas the FI-RSV vaccine did not induce antibodies that were able to recognize the palivizumab binding site (**Figure 23B**). There was some ability of the wild-type RSV2 group (positive control) to induce antibodies that compete for the palivizumab binding site. Neutralizing antibodies to RSV were measured by PRNT assay. A similar trend to the palivizumab analysis was seen, with all VXA-RSV-f groups able to induce significant neutralizing antibodies to RSV, whereas the FI-RSV vaccine was not able to induce substantial neutralizing titers (**Figure 23C**). RSV2 control vaccine was able to elicit similar neutralizing titers as the VXA-RSV-f groups statistically speaking, with the VXA-RSV-f (i.m.) having the highest geometric mean titer (p=0.47 by Mann-Whitney).

[0208]   The objectives of the second cotton rat study (Study No. XV-112) were to determine if cotton rats could be immunized orally with the VXA-RSV-f vaccine and induce significant immune responses. Challenge was optional, subsequent to effective oral immunization. Oral immunization can be difficult in animals, and prior documented experience for rAd oral dosing in cotton rats was not available. For this reason, a dose titration of the VXA-RSV-f vaccine was performed with doses at 1×10^8, 1×10^9, and 1×10^{10} IU given by oral gavage to stomach-neutralized cotton rats to approximate human oral tablet delivery. Animals were immunized on weeks 0 and 4 and the antibody titers were measured on weeks 4 and 8. A buffer only group was used as a control, to show the background effects of no immunization.

[0209]   The results show that both 1×10^9 and 1×10^{10} doses could induce significant antibody titers to RSV-F on week 4, with significant boosting at week 8 (**Figure 24A**). The 1×10^8 IU vaccine group trended lower in terms of total IgG antibody responses to RSV. Measurement of the ability of oral VXA-RSV-f to induce antibodies that could compete for the palivizumab

binding site showed a dose dependent effect with higher doses showing higher average competitive titers than the lower 1×10^8 IU dose (**Figure 24B**). Induction of neutralizing antibodies to RSV increased with higher vaccine doses, with the 1×10^9 and 1×10^{10} IU VXA-RSV-f vaccine doses eliciting the higher neutralizing titers (**Figure 24C**).

5   [0210]   Following immunization in the first cotton rat experiment (XV-95, **Figure 23**), cotton rats were given wild-type RSV strain A2 at 1×10^5 PFU at day 56. Lungs and nasal passages were harvested 5 days later and analyzed for the ability of the vaccines to protect against RSV replication and disease in cotton rat tissue. Immunization with the VXA-RSV-f vaccine provided complete protection against RSV replication in both lungs and the nose,
10   whereas the formalin inactivated vaccine (FI-RSV) and adjuvant alone (Ad-Adj) groups provided no protection against RSV replication in the nose and only limited protection in the lungs with the FI-RSV vaccine (**Figure 25A**). Maximal replication (as seen in the buffer control group) had an average of 4.9 $\log_{10}$ PFU RSV/g of lung tissue, and vaccine induced immunity was able to reduce the post challenge RSV titers below the detectable level, a
15   greater than 3 $\log_{10}$ decrease.

[0211]   Lung inflammation was measured by immunohistology and qRT-PCR analysis on day 5 post RSV infection. Four different regions were assessed by immunohistology to determine if the vaccine led to adaptive immune enhancement of disease. The VXA-RSV-f vaccine did not induce significantly increased lung pathology scores for peribroncolitis (PB),
20   perivasculitis (PV), interstitial pneumonia (IP), and alveolitis (A) as compared to the FI-RSV vaccine, the "positive" control for lung inflammation (**Figure 25B**), and did not lead to increases in the relative abundance of IL-4 or IL-13 (**Figure 25C**). Groups given i.n. administration trended higher for PB and PV, including the Buffer, RSV2, and adjuvant control groups, but not necessarily for IP and A (**Figure 25B**). The adjuvant group (without
25   expression of RSV F protein) induced a high level of PB post RSV challenge, but induced only low levels of IP and A. Buffer and adjuvant alone control groups did not induce a significant increase in the relative abundance of IL-4 or IL-13 mRNA as the FI-RSV group did (**Figure 25B**). The FI-RSV vaccine group induced an average relative abundance above 1 for IL-4 and above 3 for IL-13, compared to below 0.1 and 0.3 respectively for the VXA-
30   RSV-f and RSV2 vaccine groups (**Figure 25C**).

[0212] The oral cotton rat immunogenicity study (Study No. XV-112) was challenged with wild-type RSV because of the potent neutralizing antibody titers observed. Following immunization, cotton rats were given wild-type RSV strain A2 at 1×10^5 PFU at day 56. Lungs and nasal passages were harvested 5 days later and analyzed for the ability of the vaccines to protect against RSV replication and disease in cotton rat tissue. Oral immunization with the VXA-RSV-f vaccine induced dose dependent protection against RSV replication in both lungs and the nose, with the highest dose vaccine inducing complete protection in the lungs and nearly complete protection in the nose; 8 out of 8 animals were negative for RSV titers in the lungs and 7 of 8 animals were negative for RSV replication in the nose (**Figure 26A**). Maximal replication (as seen in the buffer control group) had on average of $5.2\log_{10}$ PFU RSV/g of lung tissue, and vaccine induced immunity was able to reduce the post challenge RSV titers greater than 3 $\log_{10}$ for the 1×10^9 and 1×10^{10} IU vaccine groups (**Figure 26A**). The lowest dose group (1×10^8 IU) also demonstrated substantial protection, with 6 of 8 animals having complete protection. Inflammation was assessed as before. The vaccine groups trended higher for inflammation compared to the buffer control group, but were not statistically different (**Figure 26B**). Cytokine analysis of the lungs post challenge saw no substantial increase in the relative abundance of the IL-4 or IL-13 mRNA as measured by qRT-PCR. All vaccine and the buffer control groups had relative abundance levels of IL-4 and IL-13 mRNA below 0.04 and 0.005, respectively (**Figure 26C**). These values are extremely low compared to the relative abundance levels of 1 and 3 for IL-4 and IL-13 respectively induced by the FI-RSV group in Study No. XV-95 (**Figure 25C**), suggesting that no meaningful induction of these cytokines has occurred following oral delivery of VXA-RSV-f.

Example 9

**High Titer Neutralizing Antibodies to Influenza Following Oral Tablet Immunization:
A Randomized, Placebo-controlled Trial**

Introduction

5   [0213]   Seasonal influenza vaccination requires substantial yearly campaigns to collect
enough fertilized eggs and massive machinery to harvest and process each mini-egg
bioreactor. Cell culture or plant derived hemagglutinin (HA) may reduce the burden of egg
acquisition and processing, but these approaches still require expensive sterile fill and finish
to produce individual syringe needles, that need to be disposed of as a biohazard. During a

10 pandemic, schools can be closed and social distancing mandated, yet mass influenza
immunization campaigns typically require lining up subjects at health clinics for injections.
In order to circumvent this dilemma, oral influenza vaccines could be sent through the mail
thus avoiding most human to human contact. Mail delivery is already used for a wide-variety
of oral medications and has already been suggested as a means to deliver critical medicines to

15 veterans during a pandemic. Further, tableting is a rapid, sanitary process that does not
require the expensive sterile fill and finishing process that injected vaccines require.

[0214]   Several adenoviral vector approaches have been attempted to enable oral influenza
immunization. In 2011, a clinical trial using a cell-culture produced oral adenovirus vectored
avian influenza (H5) vaccine was initiated. T cell responses to influenza H5 HA were

20 measured in greater than 75% of subjects, but no neutralizing antibody responses were
observed (Peters *et al.* Vaccine 2013; 31: 1752-8). After additional formulation development
and dose optimization was performed, the clinical trial that is the subject of this report was
initiated for seasonal influenza, using a tablet delivery format instead of a capsule. The same
vector backbone was used as before, but with a new HA sequence from a strain similar to the

25 H1N1 strain of the current commercial seasonal influenza vaccine (A/California/04/2009
(H1N1)) and with a 10 fold higher dose. A single dose of rAd-HA(A/CA/04/2009)-dsRNA
was tested for safety and immunogenicity, in a double-blind, randomized, controlled clinical
study. This report summarizes the findings of this trial.

30

Materials and Methods

Clinical protocol and enrollment

[0215]   This was a phase 1, sequentially enrolled study, with a randomized and placebo-controlled cohort to evaluate safety, and immunogenicity of a recombinant Ad serotype 5 (rAd5) based oral vaccine against H1 seasonal influenza. The study was conducted in accordance with applicable Good Clinical Practice guidelines, the United States Code of Federal Regulations, and the International Conference on Harmonization guidelines. IRB approval was obtained from Aspire IRB (Santee, California; AAHRPP accredited) before enrollment of subjects. Study participants were recruited using the CRO/Phase 1 Unit's existing volunteer database as well as using IRB approved advertising (print ads, radio ads and social media). Informed consent was obtained from all subjects after discussion of the study procedures and potential risks.

[0216]   Subjects were pre-screened for Hemagglutination Inhibition (HAI) titers within 45 days of enrollment. In order to be eligible for study participation subjects had to have an initial HAI titer of < 1:20, be between 18-49 years of age, and be in good health. Additional enrollment criteria are listed at clinicaltrials.gov under NCT01688297. The active phase of the trial was through day 28, with the follow-up phase for monitoring safety to continue for 1 year.

Randomization and Masking

[0217]   The study was designed to evaluate the vaccine (VXA-A1·1) in 12 subjects at a single dose of 1 x 1011 infectious units (IU) with 12 subjects given a placebo control. There were 3 sequentially enrolled sentinel vaccine-treated subjects, with each subject dosed no more frequently than one every 24 h. After a week of monitoring for vaccine-related toxicities, the remaining subjects in the treated cohort (9) were randomized along with 12 placebo controls. Randomization was performed by computer generated assignment, and study drug was distributed with concealed identity to the blinded staff by the unblinded pharmacist. All investigative site staff as well as persons directly involved with immunological assays or the assessment of clinical safety remained blind to treatment assignments. All subjects were blinded in the study.

Sample Size

[0218]   The overall number of volunteers per test group (n=12) was predicted to yield meaningful results. This was defined in a prior study as observing 50% of responders in the vaccine group and none in placebo group. With the sample size of 12 per group, there is 80% power to detect a group difference, assuming the proportion of response (defined as HAI>40) in vaccine group is 50% and in placebo is 0, using two-group Fisher's exact two-sided test at significance level of 0.05.

Vaccine

[0219]   The rAd vector (non-replicating Ad5) carries DNA which encodes the HA (A/CA/04/2009) transgene whose expression is driven by a CMV promoter and a molecular dsRNA hairpin driven by a separate promoter, as described before (Scallan *et al.* Clinical and Vaccine Immunology 2013; 20(1): 85-94). GMP drug substance was produced in Wave Disposable Bioreactors (GE Healthcare, Waukesha, WI) at Lonza Biologicals (Houston, TX). Purification was performed by ion exchange chromatography, followed by buffer exchange. Purified vector was mixed with excipients, lyophilized, and then tableted at Lonza using microcrystalline cellulose and starch as tableting bulk. Tablets were enteric coated with Eudragit L100® (Evonik Industries, Darmstadt, Germany) using a Vector Hi-Coater system (Vector Freund, Cedar Rapids, IA). The final product was released in one lot, and titered by standard IU assay at Lonza. Placebo was prepared as similarly sized and shaped tablets containing 150 mg of microcrystalline cellulose, without enteric coating.

Safety assessments

[0220]   The principal investigator (PI) assessed solicited and unsolicited adverse events (AEs) in a blinded manner. The SMC oversaw the safety of the study but did not participate in the grading of AEs. Solicited AEs (reactogenicity) were collected with the aid of a 7-day solicited symptoms diary card. Unsolicited AEs (all other clinical AEs) were collected with the aid of an unsolicited diary card through Day 28. Investigators used the Center for Biologics Evaluation and Research (CBER) Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials, September 2007 to grade AEs.

[0221]   Because of the novel adjuvant, we collected data on the occurrence of AEs of special interest (AESIs) and new onset of chronic illnesses (NOCIs). These include

neuroinflammatory disorders, musculoskeletal disorders, gastrointestinal disorders, metabolic diseases, skin disorders and other autoimmune disorders. No AESIs or NOCIs have been reported through day 180 following immunization.

Endpoints

[0222] The primary endpoint for this study is safety and the secondary endpoint is immunogenicity through the active phase, primarily by HAI titers and HAI seroconversions. Additional immunological endpoints include MN titers and ASCs.

PBMC isolation and cryopreservation

[0223] Blood was collected in K3 EDTA Vacutainer® tubes (BD, Franklin Lakes, NJ) and PBMCs were isolated the same day using Lymphoprep™ tubes (Axis-Shield, Norway).

[0224] PBMCs were frozen and thawed using serum free reagents according to the manufacturers instructions (Cellular Technology Ltd [CTL], Shaker Heights, OH).

Antibody Secreting cells (ASCs)

[0225] Enzyme linked immunosorbent (ELISpot) kits for IgG and IgA secreting B cells were performed according to manufacturer's instructions (Mabtech, Mariemont, OH). Cells were cultured (between $1·5 \times 104$ to 5×105 cells per well) in triplicate wells, in CTL-Test medium to optimize spots. HA protein (Protein Sciences Corp, Meriden, CT) was biotinylated and quantitated at Vaxart using a biotinylation kit (Pierce, Rockford, IL). Spots were counted at Zellnet Consulting Inc (Fort Lee, NJ).

Antibody assays

[0226] HAI and MN Titers were performed by Focus Diagnostics (Cypress, CA) similarly as described previously (Greenberg *et al.* The New England Journal of Medicine 2009; 361(25): 2405-13). HAI and MN were measured against MDCK derived A/CA/07/2009 and egg derived A/CA/07/2009 respectively. HAI and MN titers less than 10 were marked as 5 as suggested by regulatory advice. Adenovirus neutralizing titers were measured as described before 2.

Statistical analysis

[0227] In general, descriptive statistics for continuous variables included the number of subjects with data to be summarized (n), mean, standard error (std err), and 95% confidence

intervals (95 CI). Titers were reported with geometric means and 95 CI. Categorical variables were presented using frequency counts and percentages. Treatment group differences were compared using two-group t-test in continuous variables and Fisher's Exact test in categorical variables. All statistical tests were two-sided at a significance level of 0.05

5   without adjusting for multiplicity. An analysis of covariance (ANCOVA) model was used for (log transformed) HAI antibody titers, with Day 28 log-titer as dependent variable, treatment as a factor, and Day 0 log-titer as a covariate. Least square (LS) means, 95 CI of the LS means, difference of LS means and the 95 CI of the difference of LS means were obtained from the model. As an exploratory analysis for the HAI antibody titer, another

10   ANCOVA model included age, sex, and body mass index (BMI) as covariates was performed as well.

Results

Demographics

[0228]   365 subjects were screened and 24 subjects were enrolled. All subjects that enrolled

15   completed safety and immunogenicity assessments through the active phase, and through day 180 of the monitoring phase (Figure 19). Demographics are described in Table 8 for both placebo and vaccine treated subjects.

Summary of Adverse Events

[0229]   In the first 7 days following test article administration, there were 8 total solicited

20   adverse events (AEs) reported in the VXA-A1·1 vaccine and placebo groups (Table 9). All of these AEs were grade 1 in severity. The investigator's assessment as to whether the AE was related to treatment is also indicated (Table 9). The most frequent AE was headache (2 in placebo, and 1 in the vaccine group). All other solicited AEs were single events (Table 9). There were a total of 8 unsolicited AEs in vaccine and placebo groups in the 28 days

25   following immunization, with 3 events occurring in the placebo and 5 events occurring in the vaccine group. There were no serious adverse events reported in the study.

[0230]   Clinical laboratory abnormalities were distributed across the vaccine and placebo groups. Of note, there were 6 neutropenic events in the vaccine group and 4 in the placebo group. These events occurred in a total of 8 subjects, 4 of who had pre-treatment neutropenic

30   blood counts. Five of these subjects were also black or Japanese, which are ethnic groups that have a relatively high frequency of benign ethnic neutropenia (BEN). As is the case with

68

(BEN), there were no clinical manifestations that resulted from any of the neutropenic events reported.

Immunogenicity Results

[0231]   HAI responses were measured on days 0 and 28 (Figure 20A). No placebo treated subject seroconverted, but one placebo had a high day 0 value (which would have excluded the subject if measured at screening). None of the vaccine subjects had a starting HAI titer > 20. After immunization, nine subjects in the vaccine group reached seroprotective levels (HAI ≥40) (Figure 20A). Of the eleven 4-fold risers (92%), nine seroconverted (SC) with the other 2 subjects showing a 4-fold increase in HAI titer from 5 to 20. The vaccine group had a statistically significant increase in the number of 4-fold responders versus placebo (11 versus 0, with P<0·0000 by Fisher's Exact Test). Using an ANCOVA model accounting for the Day 0 log-titer as a covariant, the Geometric (LS) Mean Titer (GMT) for the vaccine group was calculated to be 71·5 (95 CI: 45 – 114) on Day 28, a 7·7-fold Geometric (LS) Mean Fold Rise (GMFR) over the initial GMT of 7·9 (95 CI: 4·6 – 13·6) on Day 0. The GMT on Day 28 for placebo group was 10·1 (95 CI: 6·4 – 16·2) on Day 28, a 1·1-fold GMFR over initial GMT of 11·0 (95 CI: 6·4 – 18·9) on Day 0. Comparing to placebo, the vaccine group had a statistically significant increase in GMT on Day 28 (p-value <0.001). The covariate effect of baseline was also statistically significant (p-value <0.001). An exploratory analysis was also carried out using another ANCOVA model, where additional covariates, age, sex and BMI were included. The effects of these covariates were not statistically significant on Day 28 [p-values: 0.993 (for age), 0.696 (for sex), 0.201 (for BMI)].

[0232]   Durability of the antibody response was measured by examining HAI responses 180 days after immunization. In the vaccine-immunized group, 75% (9 of 12) of the subjects were seroprotected on day 28 and 75% (9 of 12) were still seroprotected on day 180. The HAI GMT were plotted (Figure 20B), and the decrease in the GMT was found to be 29% between 28 and 180 days post immunization.

[0233]   Neutralizing antibody responses to influenza were measured by MN assay. Significant increases in the MN titers in the treated group versus the placebo control were observed (Figure 20C). The frequency of 4-fold MN responders in the vaccine treated group was significantly different than the placebo group, with 11 subjects responding in the vaccine treated group versus 0 in the placebo group (P<0·0000 by Fisher's exact test).

[0234]   After removing subjects that had baseline MN titers (and HAI titers) greater than 40, the geometric mean titers (GMT) were calculated in the remaining subjects on days 0 and 28 (Table 10). The GMT for the vaccine group rose to 247 (95 CI: 89-685) versus no rise in the placebo for a day 28 GMT of 9·6 (95 CI: 5-18). These calculations had no impact on the vaccine group, as none of the subjects had high initial MN or HAI titers. These results show that neutralizing antibody titers to influenza are generated by oral immunization, with a greater than 20-fold increase in the GMT after immunization in the vaccine-treated group.

[0235]   In order to measure total antibody responses to HA, the numbers of circulating pre-plasma B cells in peripheral blood were measured by ASC assay on days 0 and 7 after immunization. Results show that ASCs could be reliably measured on day 7 in the vaccine-treated group (Figure 20D). Background ASCs were generally negligible on day 0. For the vaccine treated group, an average of 992 (+/- std err 209, 95 CI: 532-1452) IgG ASCs and 337 IgA ASCs (+/- std err 104, 95 CI: 117-580) each per 1 x 106 PBMC were found for day 7, with only one subject out of 12 having no detectable ASC response. The placebo group had no IgA spots on day 7, but one subject had a high background smear and a measurable IgG ASC response with smaller spots than normally observed. The treated group was significantly different than placebo in terms of the ability to elicit an IgG or an IgA ASC response at day 7 (P=0·0007 and P=0.008 respectively by T Test).

[0236]   Subjects were measured for their anti-vector titers pre- and post-immunization. Following oral immunization, a few vaccine-treated subjects had an increase in neutralizing antibody responses to Ad5, which led to a 2·6-fold increase in the GM neutralizing antibody titers, compared to 1·0-fold GM fold rise in the placebo treated subjects. In the vaccine group, HAI and MN responses trended similarly for individual subjects. Eight subjects were Ad5 negative before immunization, and four were Ad5 positive before immunization. One subject that was Ad5 positive did not HAI seroconvert, however, one subject that was Ad5 positive had the highest increase in HAI titers (64 fold) of any of the subjects in the study (Figure 21B). This same subject had a gain in MN titers of 362 fold (Figure 21A) without any increase in the Ad5 neutralizing antibody titers pre and post immunization. There was no observed correlation between starting Ad5 titers versus fold MN response (or HAI response) for the subjects immunized with the tablet vaccine (Figure 21A and 21B).

Discussion

[0237] When the US military conducted an independent study to measure the effects of their seasonal vaccine campaigns on neutralizing antibody responses in military personnel, they reported a MN Titer GMFR of 5·6 after trivalent inactivated vaccine (TIV) injection and a GMFR of 2·2 following live-attenuated influenza vaccine (LAIV) intranasal administration after accounting for subjects that had MN titers above 40 to start (Faix *et al.* PloS one 2012; 7(4): e34581). In this study, the MN GMFR was calculated at 29 for the 12 vaccine treated subjects (Table 10) with 92% of subjects showing a greater than 4-fold rise in MN titers. In the study by Gordon, et al, the SC rate to H1N1 was found to be 45% for one injection of 45 ug of HA protein (without adjuvant) (Gordon *et al.* Vaccine 2012; 30(36): 5407-16). This contrasts with the results published by Greenberg, et al, (supra) where the H1N1 vaccine was highly immunogenic and a 78% SC rate was observed after 1 dose of a split vaccine. In our tablet study, the HAI SC rate among vaccine treated subjects was 75% with over 92% of subjects having a 4-fold rise in HAI titers (Figure 20A). It is not clear why our MN titers are much higher than the HAI titers. It is possible that the MN assay is just more sensitive or that the oral rAd based vaccine elicits stronger neutralizing responses outside the head region than protein injected vaccines. In either case, our early stage results suggest that an oral tablet vaccine would be competitive with existing vaccines in terms of eliciting neutralizing antibody responses to influenza.

[0238] Individuals with pre-existing immunity to influenza H1N1 were excluded from study participation. This was helpful in analysis of immune responses in this Phase 1 study to better understand effects of the vaccine. In practice, in the "real world", individuals with and without pre-existing immunity to influenza are immunized. Enrollment in Phase 2 and 3 studies will include individuals with and without detectable antibody levels to vaccine antigen at baseline.

[0239] HAI responses are elicited with injected commercial vaccines, but HAI titers are known to wane. In a study by Crum-Cianflone, et al, non-HIV infected volunteers had a 67% drop in GMT HAI titers between 1 and 6 months post immunization (Crum-Cianflone *et al.* Vaccine 2011; 29(17): 3183-91). Similarly, the percentage of seroprotected subjects dropped from 75% to 56% for HIV negative subjects that enrolled with seronegative HAI titers (≤1:10)10. Studies with pandemic influenza vaccines have also shown decreases in durability. In the AS03 avian influenza vaccine study by Leroux-Roels, et al, the GMT reached 563 after 2 vaccine doses, but at 6 months post immunization, the GMT had dropped to 18, a 96% decrease (Leroux-Roels *et al.* Vaccine 2010; 28(3): 849-57). In our tablet

vaccine study that enrolled seronegative subjects (all subjects ≤1:20), the percentage of seroprotected subjects remained constant at 75% at 1 and 6 months post immunization, and the HAI GMT titer drop was less dramatic showing only a 29% decrease (Figure 20B). While unproven, one possibility is that the durability is better for vector-based vaccines because of enhanced T cell responses. These preliminary data are at least encouraging that the tablet vaccine can provide antibody durability.

[0240]   The numbers of clinical reported adverse events were similar to those observed before for other adenoviral vectored based vaccines of the invention. In this study, 17 clinical adverse events were reported though day 28 following immunization and are reasonably evenly distributed between placebo and treated groups. In a published study of an recombinant adenovirus EBOV vaccine injected into humans, the frequency of any adverse event was 55% among vaccine recipients and 25% among placebo recipients, with the most common reported adverse event being headaches (55%), myalgia (46%), and chills (27%) in the high dose vaccine group (Ledgerwood *et al.* Vaccine 2010; 29(2): 304-13). The most frequently reported adverse event in our vaccine tablet study was headache (reported in 1 vaccine and 2 placebo subjects).

[0241]   Although Ad5 immunity can be an issue with injected vaccines, it may not be the case with oral immunization with a non-replicating vector where neutralizing antibody titers do not seem to hinder performance (Xiang *et al.* Journal of virology 2003; 77(20): 10780-9). The ability to elicit a neutralizing immune response to influenza did not appear to be impacted after oral immunization with the vaccine tablet (Figure 21). One of the subjects with the highest anti-Ad5 titer to start had the highest measured increase in neutralizing antibody responses by MN and HAI assay (Figure 21). While i.m. immunization can cause 100% Ad5 seroconversion and GMTs to rise greater than 50 fold (estimated from Fig2b from O'Brien *et al.* Nat Med 2009; 15(8): 873-5), the increase in neutralizing titers with the 1 x 1011 IU tablet were much more modest. Oral immunization appears to lead to a much more selective increase in the immune response to transgene compared to vector with a GMT MN titer rise of 29 compared to Ad5 titer increase of 2·6. In contrast to results with non-replicating Ad5 based vaccine in humans, oral administration of replicating vectors shows the opposite trend where the antibody responses to the vector far exceeds the antibody responses to transgene. As an example, in an oral replicating Ad4-HA study, 80% of subjects seroconverted to Ad4 on the first immunization, but did not have significant neutralizing

antibody responses to HA after 3 immunizations (with a maximum of 19% SC in a vaccine group) (Gurwith *et al.* The Lancet infectious diseases 2013; 13(3): 238-50).

[0242] An oral formulation could greatly facilitate vaccine administration, particularly during a pandemic when rapid distribution is needed. During the 2009 H1N1 pandemic, when vaccine was in short supply, individual county health departments in California had to have a plan for distribution. In Los Angeles County, approximately 60 points of distribution (PODs) were tasked to administer the vaccine. Approximately 247 persons per hour lined up, and the rate of immunization was approximately 239 person per hour at each POD (Saha *et al.* Emerging infectious diseases 2014; 20(4): 590-5). For the PODs in Los Angeles County, this translated to 143,000 people per day. In a city of 9 million, assuming supplies or qualified personnel were not in short supply, it would take more than 60 days to complete an immunization campaign. As an alternative, if the H1N1 pandemic vaccine was delivered by US mail and self-administered by tablet, all 9 million subjects could be immunized within a single day without people having to stand in line, and risking exposure to the growing outbreak. While there are regulatory hurdles to overcome, our tablet vaccine appears to be stable at room temperature for greater than 270 days and can tolerate short-term excursions at higher temperatures (G. Trager, unpublished data), which should make this approach technically feasible.

[0243] In summary, oral influenza vaccine based on rAd administration can elicit antibody responses to influenza in greater than 90% of subjects. While this is an early clinical stage study and several studies will need to be completed that address issues such as interference and repeated seasonal use, these results look encouraging for safety and immunogenicity.

[0244] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, patent applications, websites, and database accession entries cited herein are hereby incorporated by reference in their entirety for all purposes.

REFERENCES:

1.   Kariko K, Bhuyan P, Capodici J, Weissman D. 2004. Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3. J Immunol 172:6545-6549.

2.   Boukhvalova MS, Blanco JC. 2013. The cotton rat Sigmodon hispidus model of respiratory syncytial virus infection. Curr Top Microbiol Immunol 372:347-358.

3.   Prince GA, Curtis SJ, Yim KC, Porter DD. 2001. Vaccine-enhanced respiratory syncytial virus disease in cotton rats following immunization with Lot 100 or a newly prepared reference vaccine. J Gen Virol 82:2881-2888.

4.   Liebowitz D, Lindbloom JD, Brandl JR, Garg SJ, Tucker SN. 2015. High Titer Neutralizing Antibodies to Influenza Following Oral Tablet Immunization: A Randomized, Placebo-controlled Trial Lancet Infect Dis 15:1041-1048.

5.   Schagen FH, Rademaker HJ, Fallaux FJ, Hoeben RC. 2000. Insertion vectors for gene therapy. Gene Ther 7:271-272.

6.   Harui A, Suzuki S, Kochanek S, Mitani K. 1999. Frequency and stability of chromosomal integration of adenovirus vectors. J Virol 73:6141-6146.

7.   Russell WC. 2000. Update on adenovirus and its vectors. J Gen Virol 81:2573-2604.

8.   Paielli DL, Wing MS, Rogulski KR, Gilbert JD, Kolozsvary A, Kim JH, Hughes J, Schnell M, Thompson T, Freytag SO. 2000. Evaluation of the biodistribution, persistence, toxicity, and potential of germ-line transmission of a replication-competent human adenovirus following intraprostatic administration in the mouse. Mol Ther 1:263-274.

9.   Sheets RL, Stein J, Bailer RT, Koup RA, Andrews C, Nason M, He B, Koo E, Trotter H, Duffy C, Manetz TS, Gomez P. 2008. Biodistribution and toxicological safety of adenovirus type 5 and type 35 vectored vaccines against human immunodeficiency virus-1 (HIV-1), Ebola, or Marburg are similar despite differing adenovirus serotype vector, manufacturer's construct, or gene inserts. J Immunotoxicol 5:315-335.

10.   Zhou D, Cun A, Li Y, Xiang Z, Ertl HC. 2006. A chimpanzee-origin adenovirus vector expressing the rabies virus glycoprotein as an oral vaccine against inhalation infection with rabies virus. Mol Ther 14:662-672.

11.   Scallan CD, Tingley DW, Lindbloom JD, Toomey JS, Tucker SN. 2013. An adenovirus-based vaccine with a double-stranded RNA adjuvant protects mice and ferrets against H5N1 avian influenza in oral delivery models. Clin Vaccine Immunol 20:85-94.

WHAT IS CLAIMED IS:

1.   1.   An immunogenic composition for eliciting an immune response in a human
2   comprising an immunogenic biological agent encompassed by an enteric coating that directs
3   delivery of the immunogenic biological agent to the ileum of the human,
4   wherein the immunogenic biological agent is an adenoviral vector encoding the viral protein
5   1 of norovirus or the fusion protein (F) of RSV.

1   2.   The immunogenic composition of claim 1, wherein the enteric coating
2   has a threshold pH of 5.8-6.8.

1   3.   The immunogenic composition of claim 1 or 2, wherein the enteric
2   coating disintegrates at least 75% compared to its original thickness in 110 minutes at pH 5.8-
3   6.8.

1   4.   The immunogenic composition of any one of the foregoing claims,
2   wherein the enteric coating comprises Poly(methylacylic acid-co-methyl methacrylate) 1:1.

1   5.   The immunogenic composition of any one of the foregoing claims,
2   wherein the enteric coating comprises Eudragit® L-100.

1   6.   The immunogenic composition of any one of the foregoing claims,
2   wherein the enteric coating comprises a mixture of Eudragit® L-100 and Eudragit®L100-55.

1   7.   The immunogenic composition of any one of the foregoing claims,
2   wherein the enteric coating comprises Eudragit® L-100, triethyl citrate, and talc.

1   8.   The immunogenic composition of claim 7, comprising 1-4 parts
2   Eudragit® L-100, 1-2 parts triethyl citrate, and 1-2 parts talc.

1   9.   The immunogenic composition of any one of claims 1-6, wherein the
2   enteric coating comprises a mixture of poly(methacylic acid-co-methyl methacrylate) 1:1 and
3   poly(methacylic acid-co-ethyl acrylate) 1:1.

1   10.   The immunogenic composition of claim 9, wherein the ratio of
2   poly(methacylic acid-co-methyl methacrylate) 1:1 to poly(methacylic acid-co-ethyl acrylate)
3   1:1 is 1:4 to 4:1.

1   11.   The immunogenic composition of any one of claims 1-3, wherein the

2   enteric coating comprises poly(methacylic acid-co-methyl methacrylate) 1:1 and

3   poly(methacylic acid-co-methyl methacrylate) 1:2.

1   12.   The immunogenic composition of claim 11, wherein the ratio of

2   poly(methacylic acid-co-methyl methacrylate) 1:1 to poly(methacylic acid-co-methyl

3   acrylate) 1:2 is 1:2 to 2:1.

1   13.   The immunogenic composition of any one of claims 1-3, 11, and 12,

2   wherein the enteric coating comprises a mixture of Eudragit® L-100 and Eudragit®S100.

1   14.   The immunogenic composition of any one of claims 11-13, wherein

2   the enteric coating comprises 1 to 4 parts poly(methacylic acid-co-methyl methacrylate) 1:1

3   and poly(methacylic acid-co-methyl acrylate) 1:2; 1 to 2 parts triethyl citrate; and 1 to 2 parts

4   talc.

1   15.   The immunogenic composition of any one of claims 1-3, wherein the

2   enteric coating comprises a mixture of poly(methacylic acid-co-methyl methacrylate) 1:2 and

3   poly(methacylic acid-co-ethyl acrylate) 1:1.

1   16.   The immunogenic composition of claim 15, wherein the ratio of

2   poly(methacylic acid-co-methyl methacrylate) 1:2 to poly(methacylic acid-co-ethyl acrylate)

3   1:1 is 1:4 to 4:1.

1   17.   The immunogenic composition of any one of claims 1-3, 15, and 16,

2   wherein the enteric coating comprises a mixture of Eudragit® L-100-55 and Eudragit®S100.

1   18.   The immunogenic composition of any one of claims 15-17, wherein

2   the enteric coating comprises 1 to 4 parts poly(methacylic acid-co-methyl methacrylate) 1:2

3   and poly(methacylic acid-co-ethyl acrylate) 1:2; 1 to 2 parts triethyl citrate; and 1 to 2 parts

4   talc.

1   19.   The immunogenic composition of any one of claims 1-18, wherein the

2   adenoviral vector further encodes dsRNA.

1   20.   The immunogenic composition of any one of claims 1-19, wherein

2   adenoviral vector encodes the viral protein 1 of norovirus.

1   21.   The immunogenic composition of any one of claims 1-19, wherein

2   adenoviral vector encodes the fusion protein (F) of RSV.

1   22.   The immunogenic composition of any one of the foregoing claims,

2   wherein the composition is in the form of a compressed tablet.

1   23.   A method for delivering an immunogenic composition to the ileum of

2   a human comprising orally administering the immunogenic composition of any one of the

3   foregoing claims to the human.

1   24.   A method of eliciting an immune response in a human comprising

2   administering the composition of any one of claims 1-23 to the human, wherein the immune

3   response is specific for the immunogenic biological agent.

1

FIG. 1
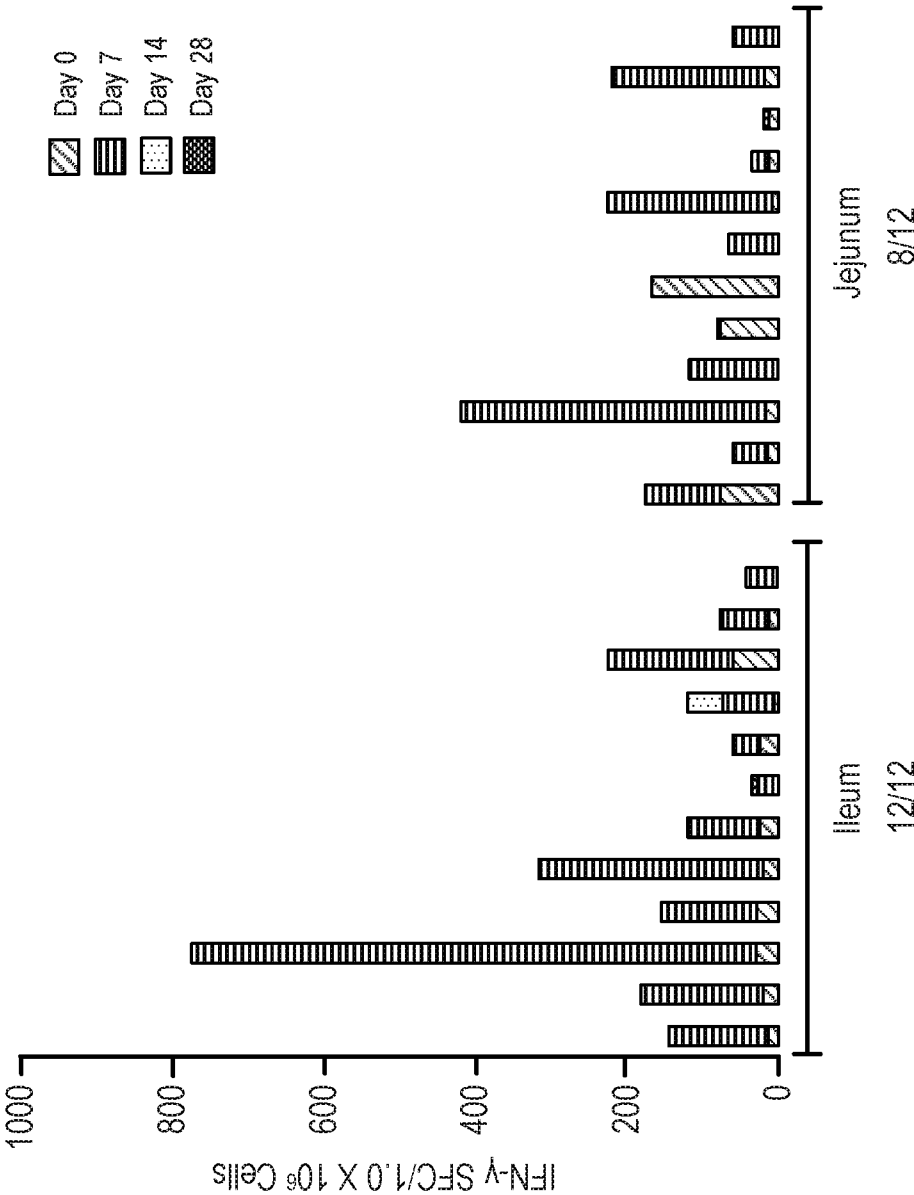
FIG. 2
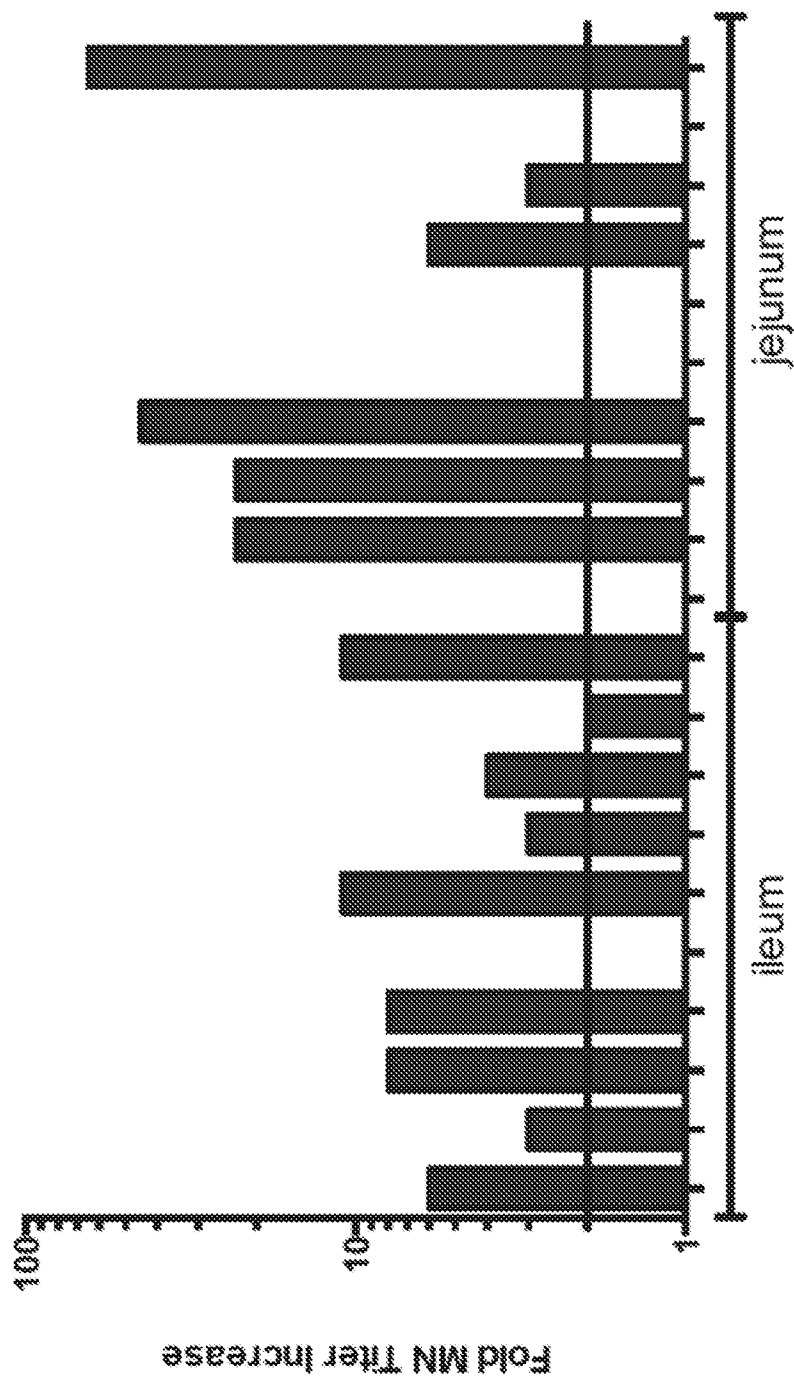
FIG. 3

FIG. 4B

FIG. 4A

FIG. 5

FIG. 6
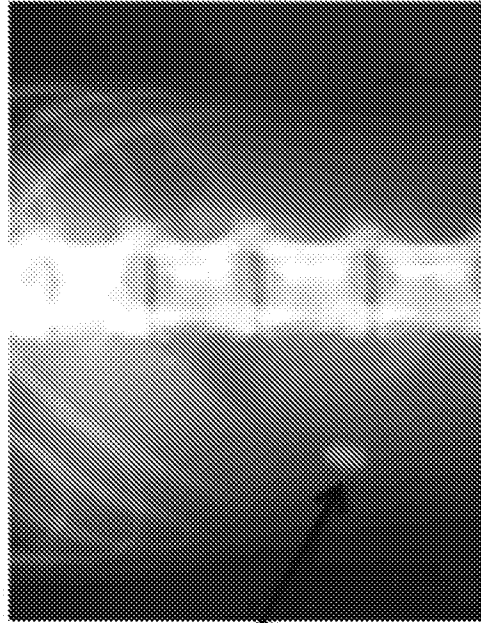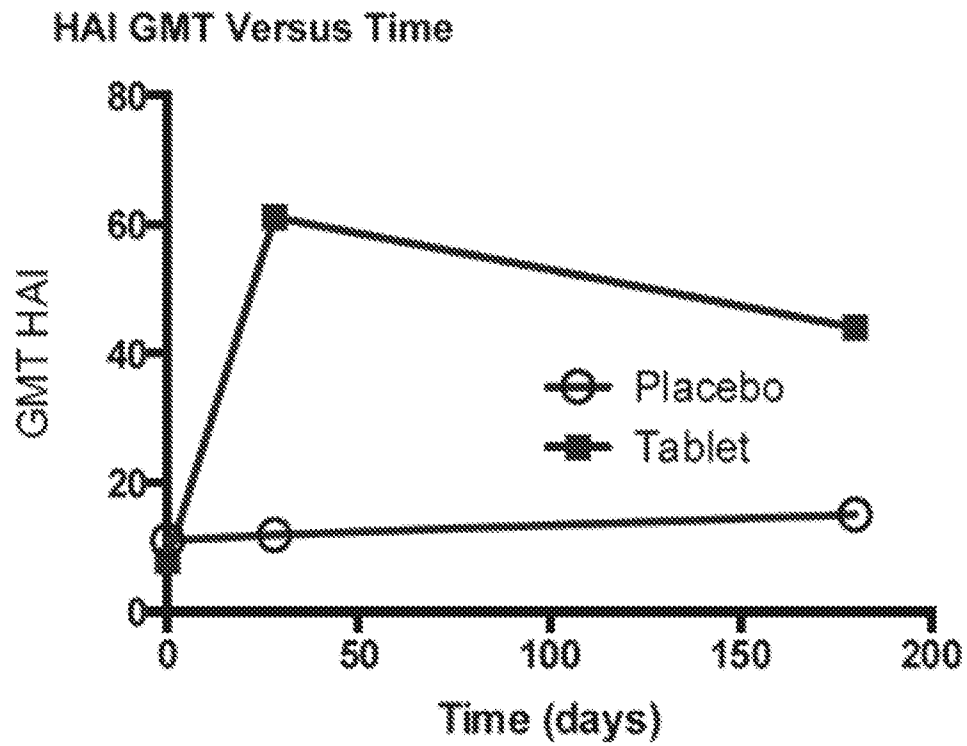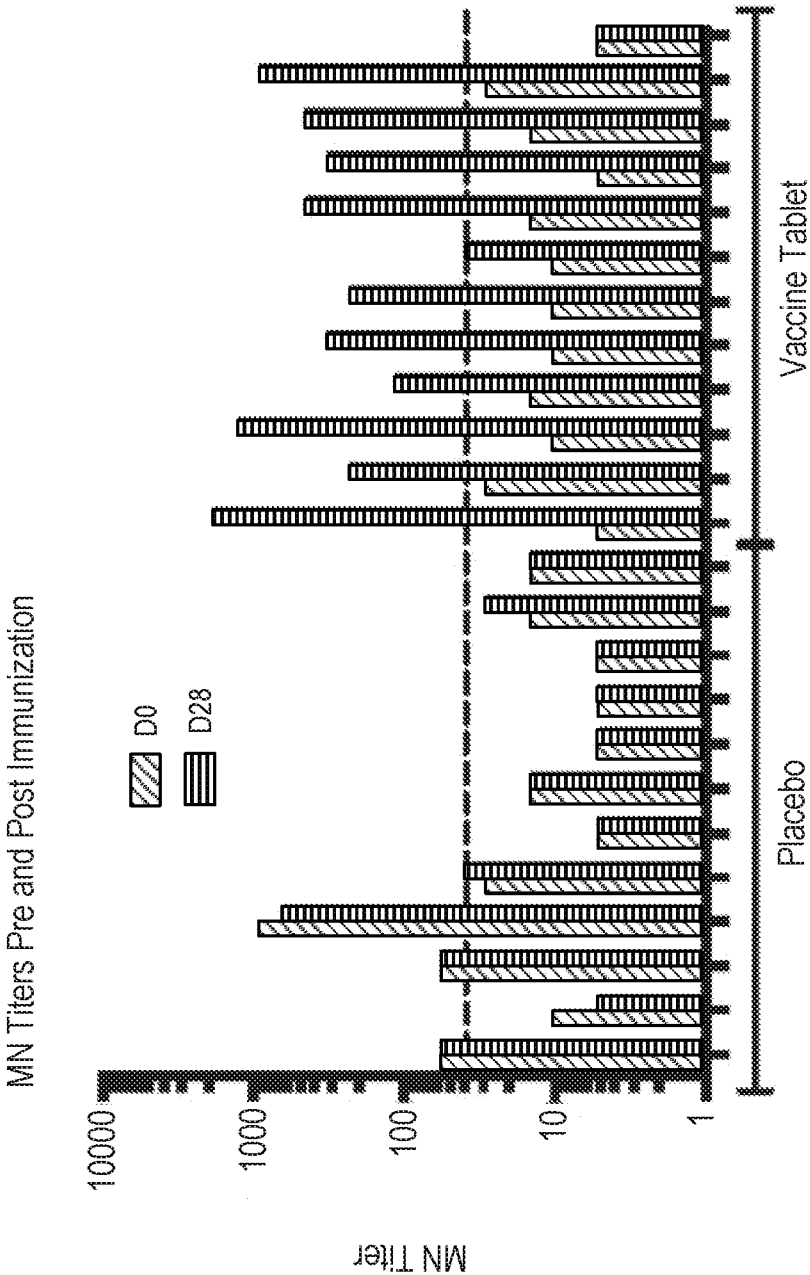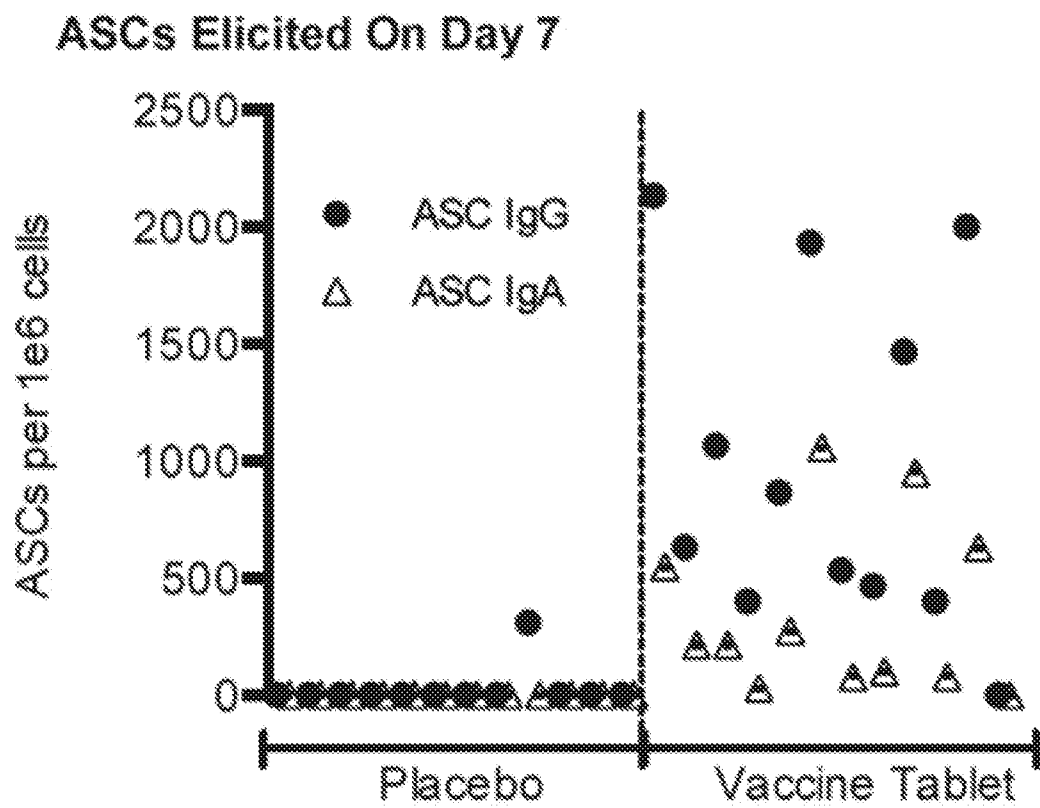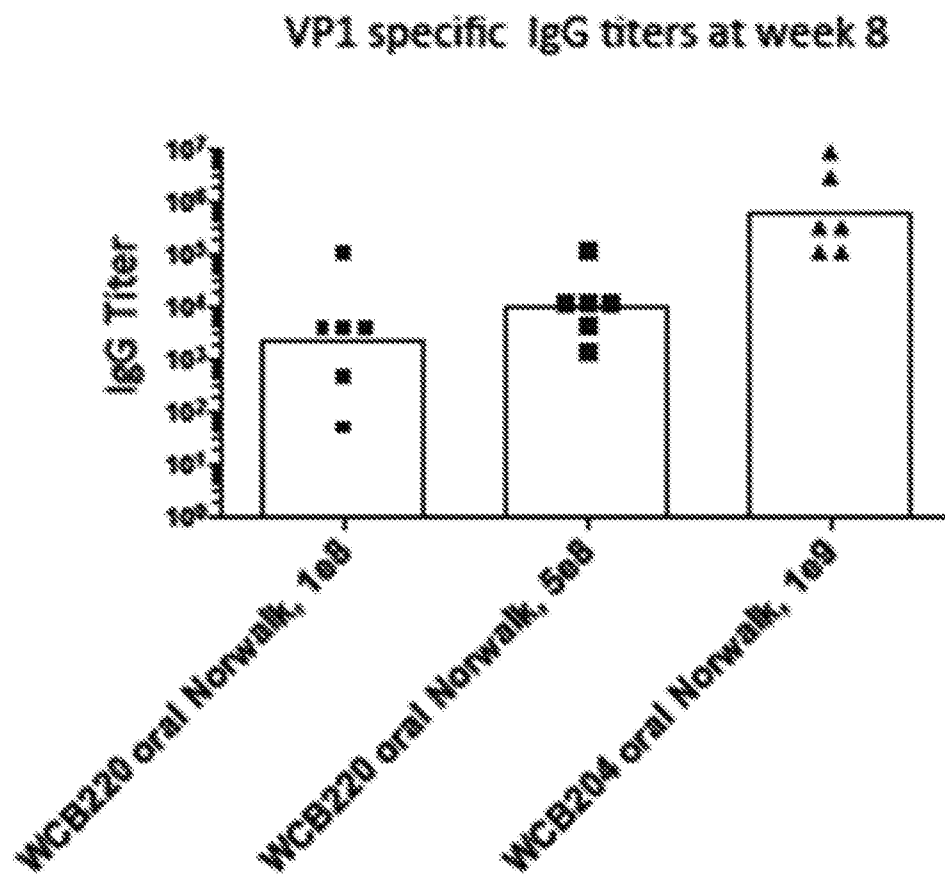
HAI Titers Pre and Post Immunization

FIG. 7A

FIG. 7B

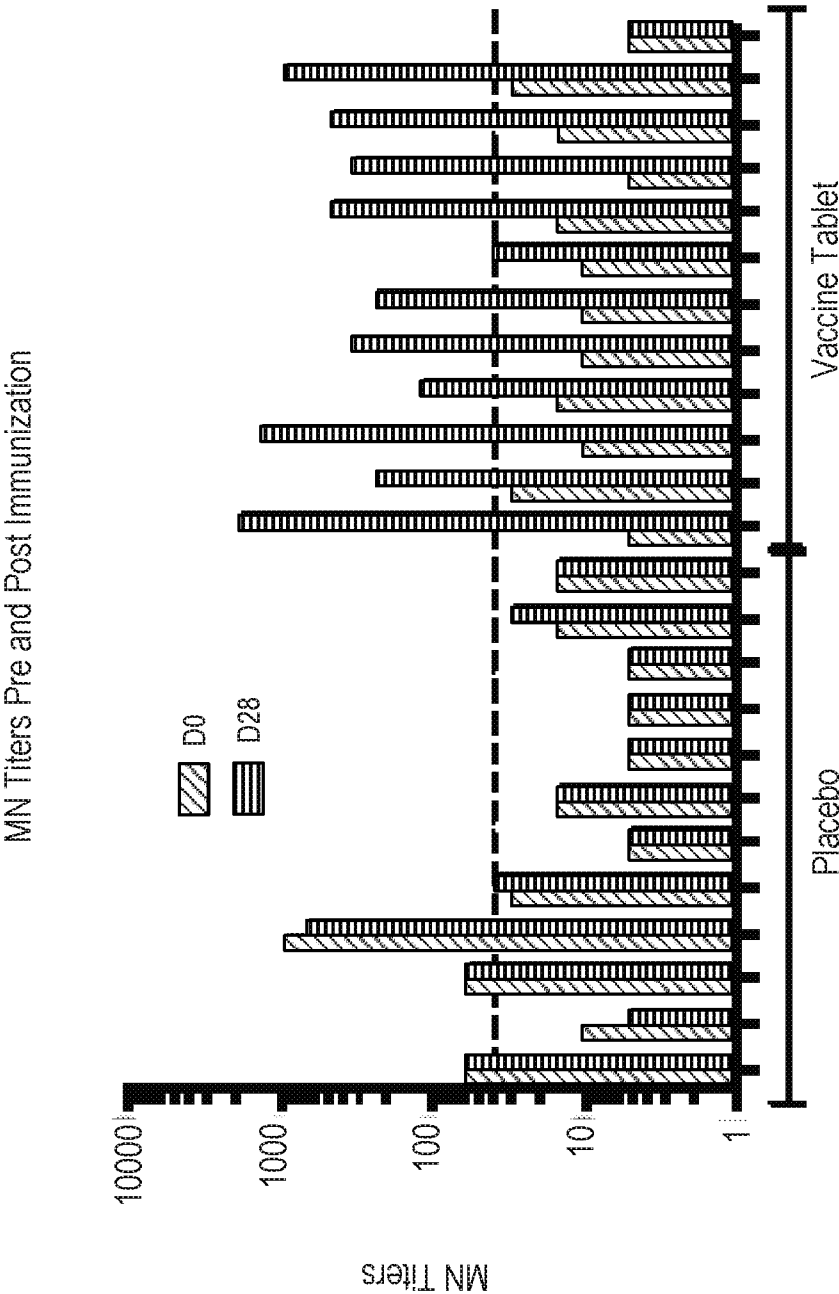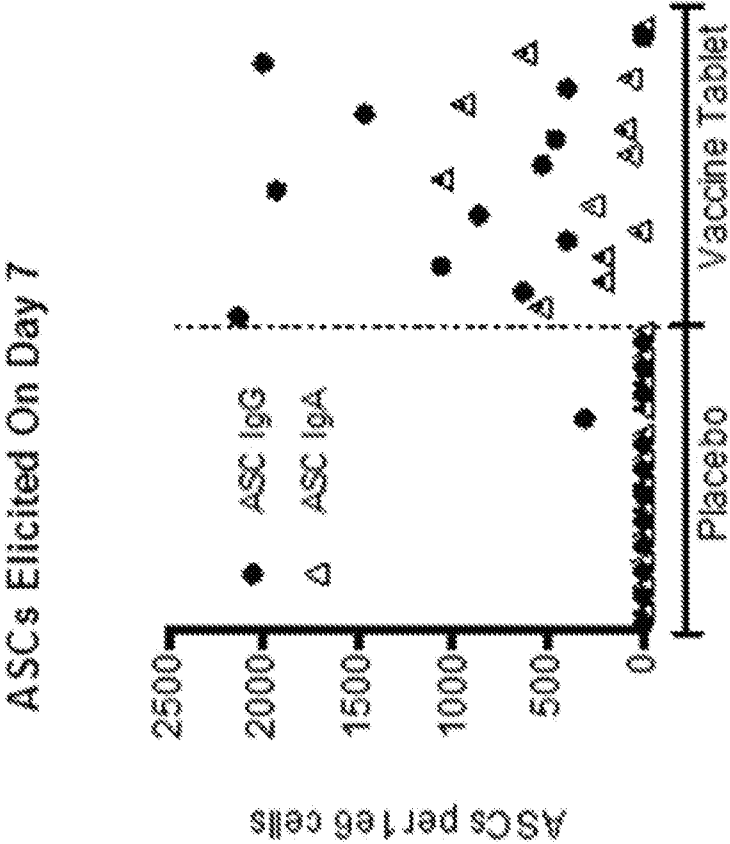FIG. 7C

FIG. 7D

FIG. 8
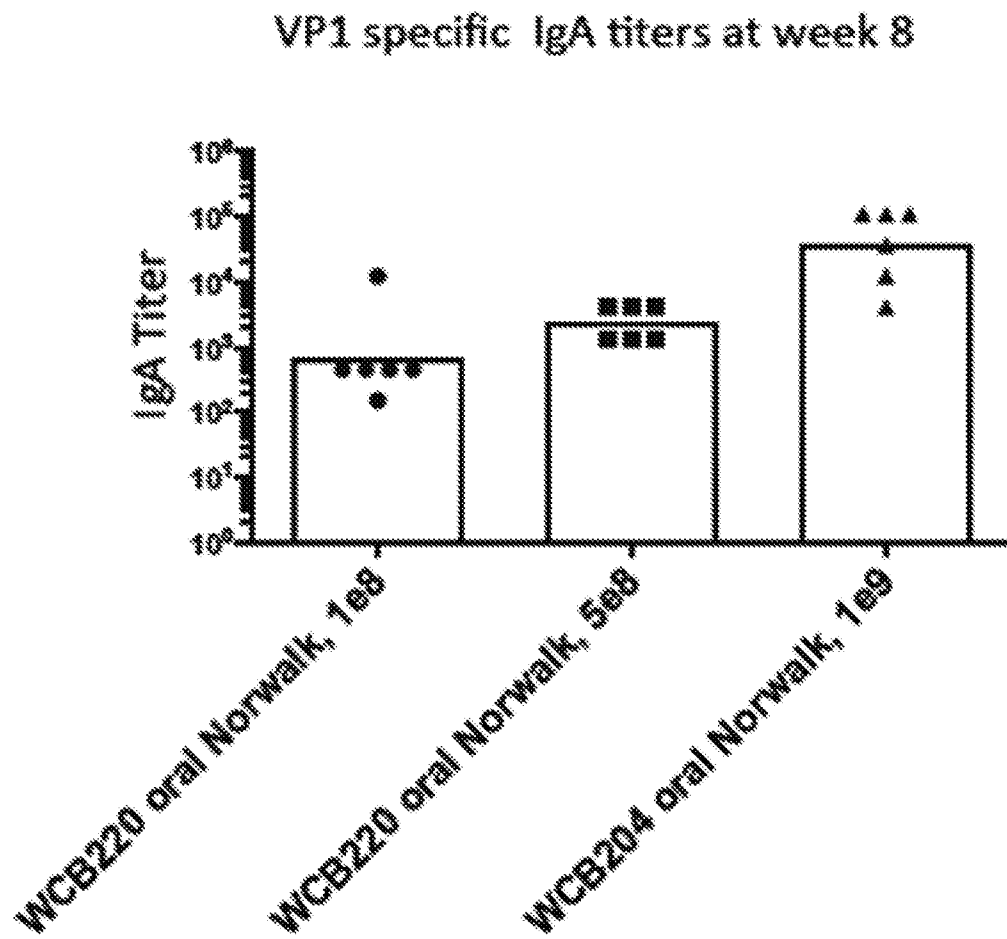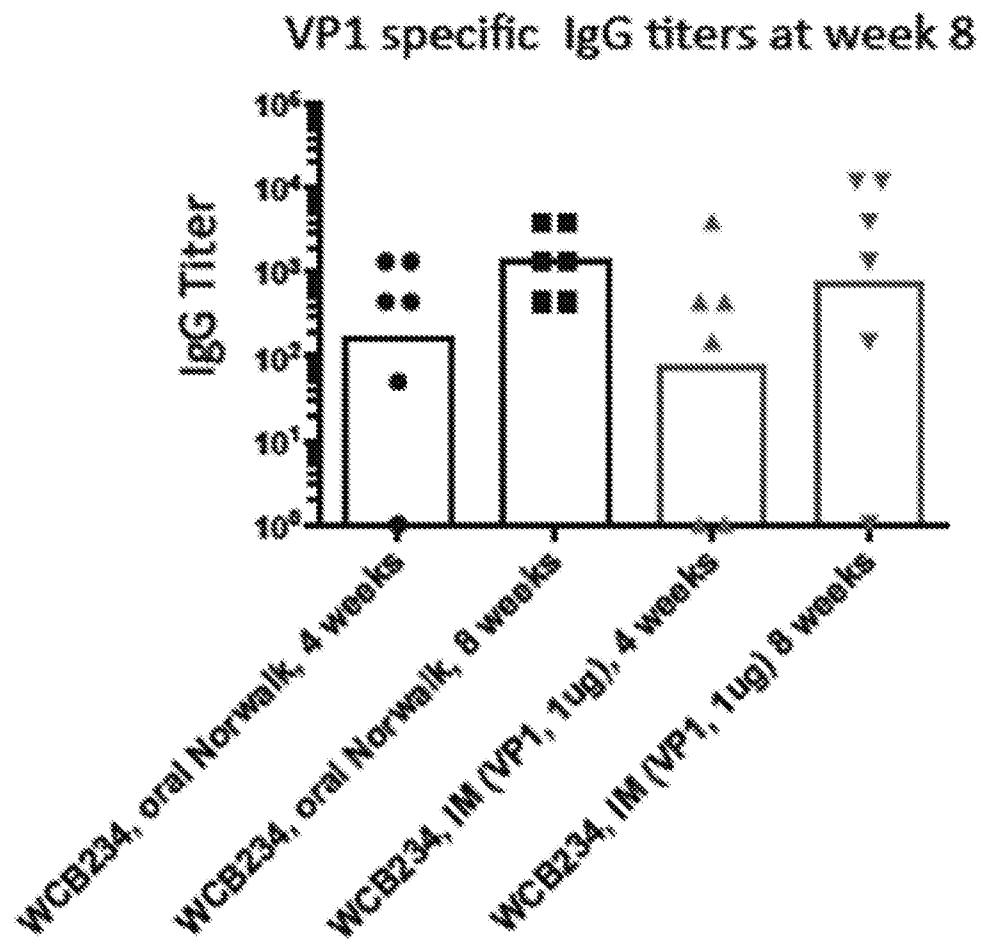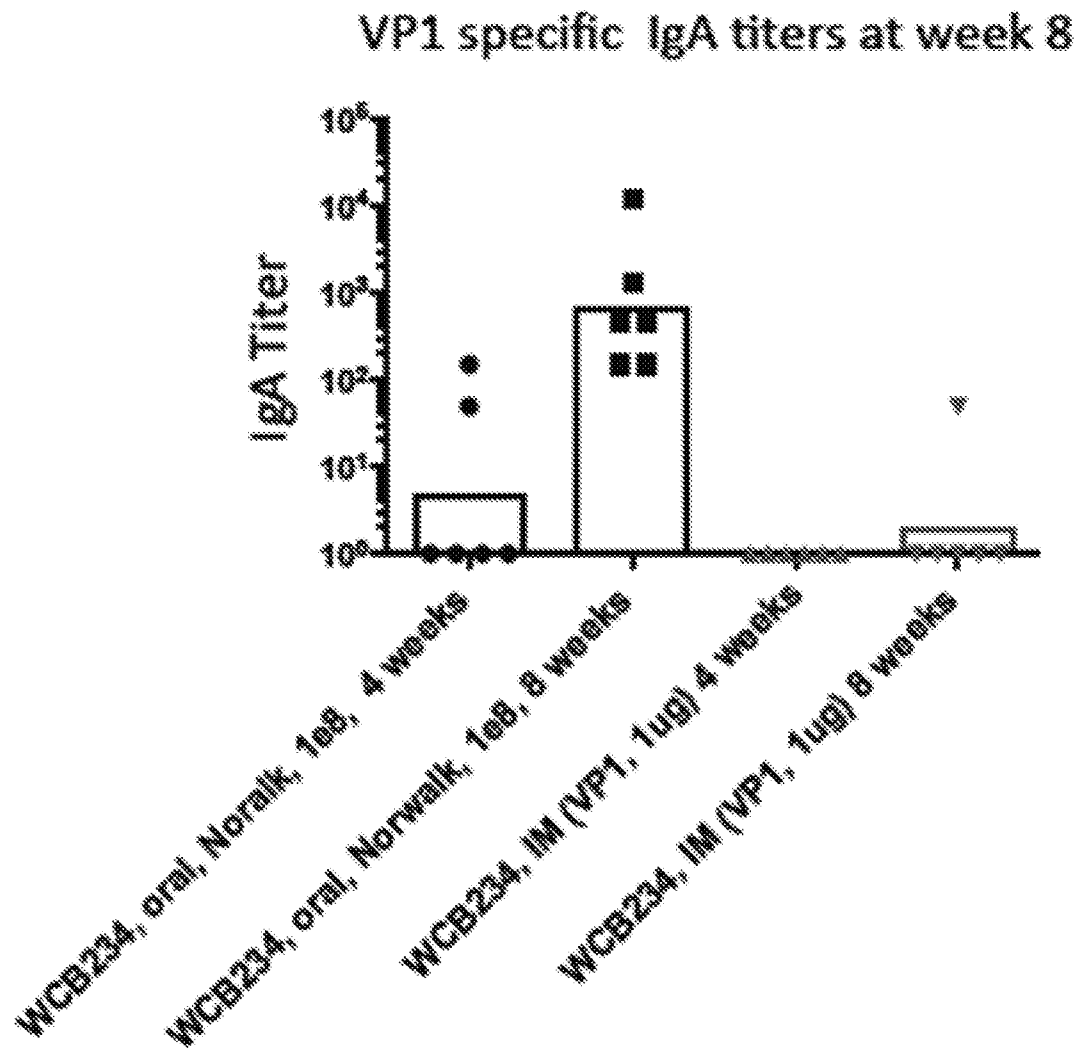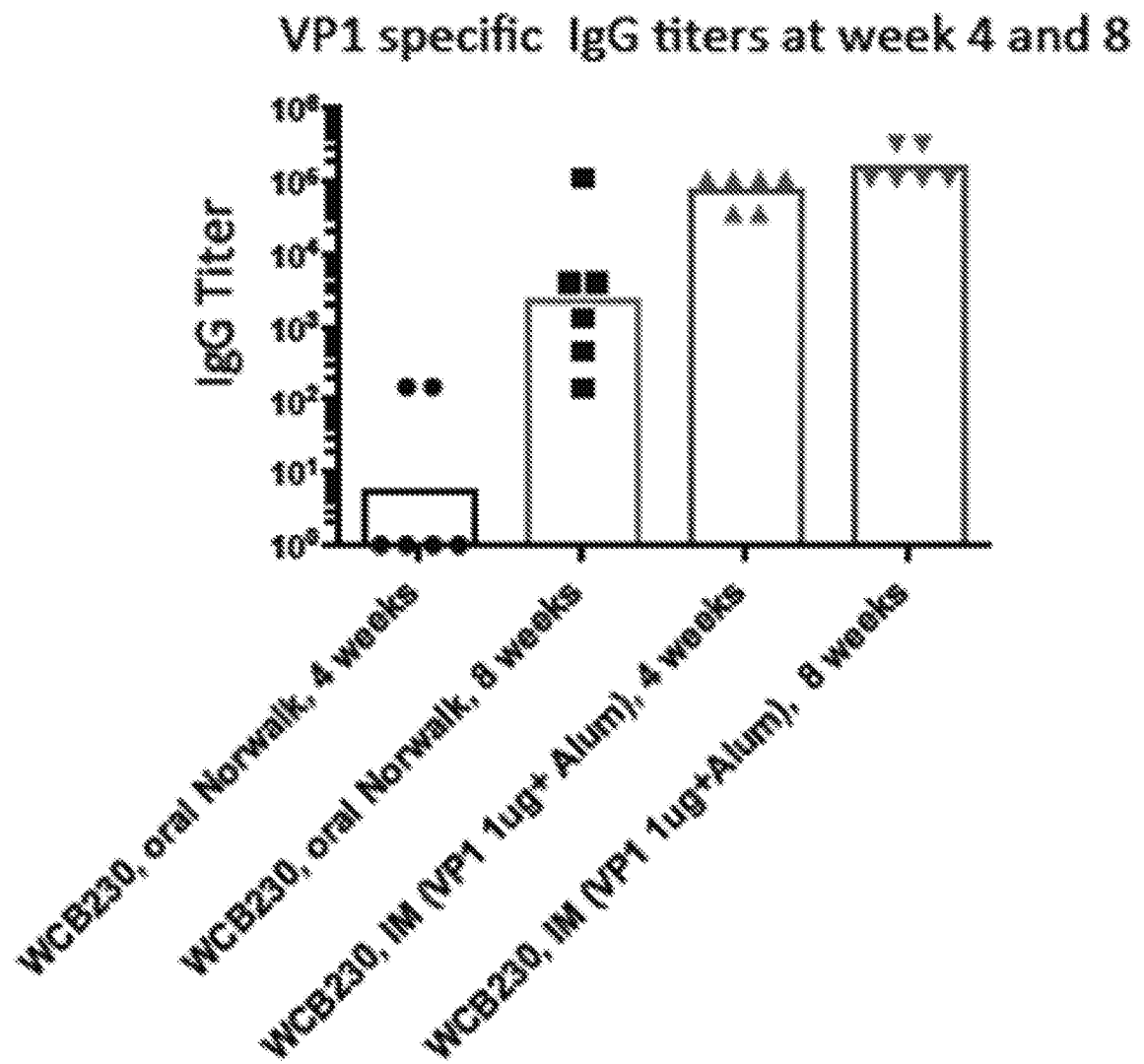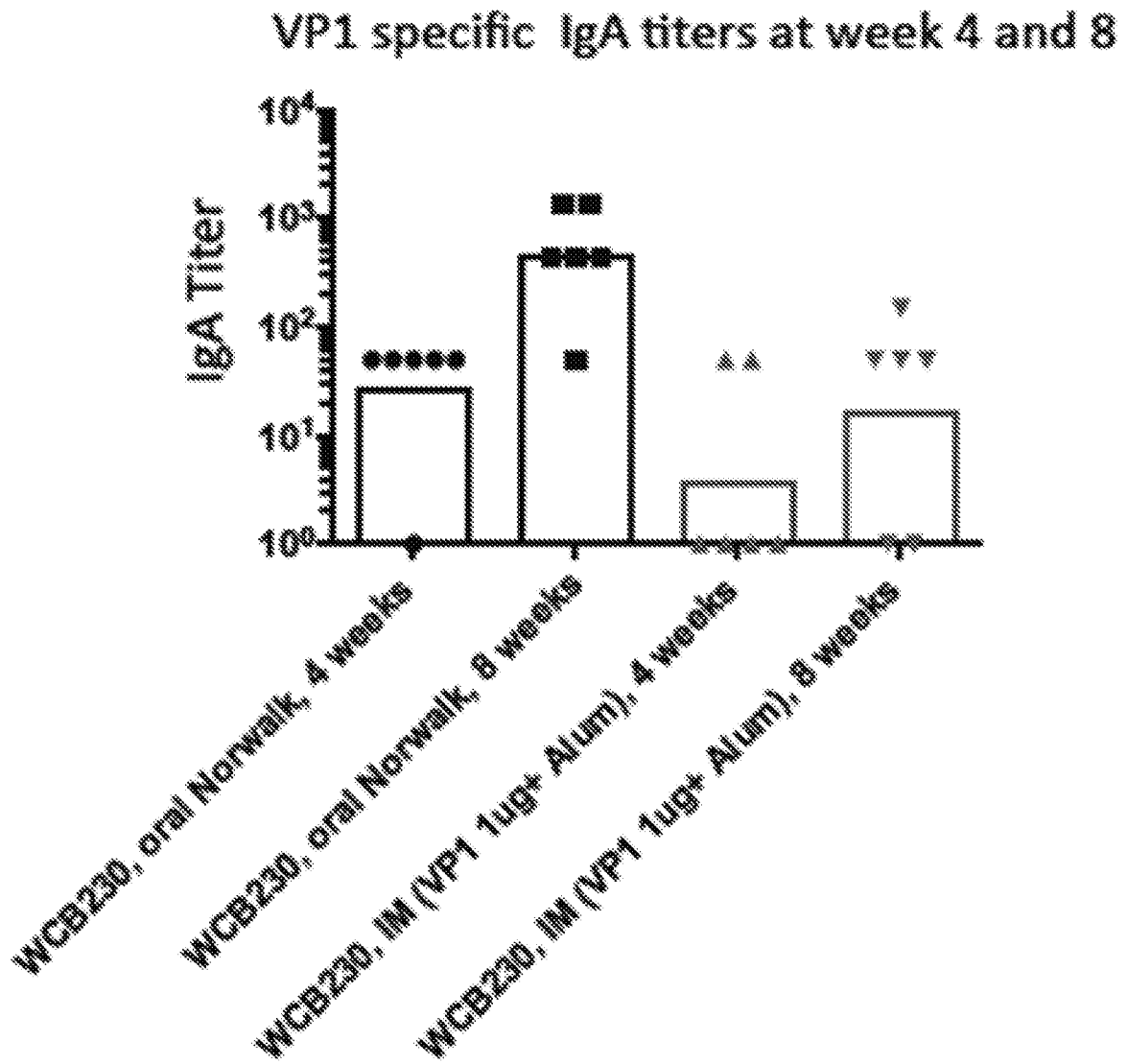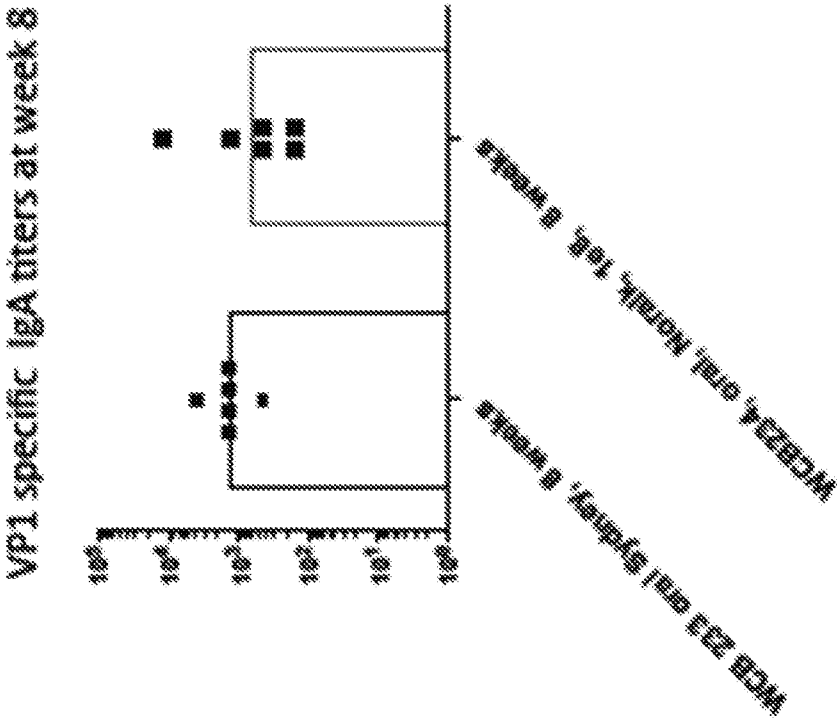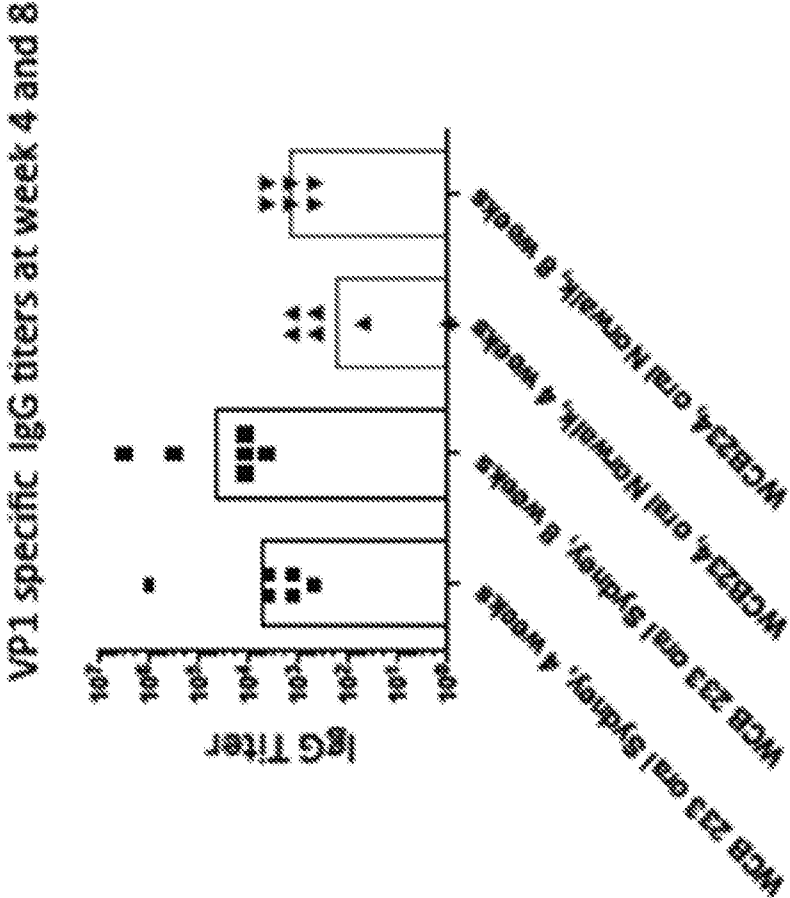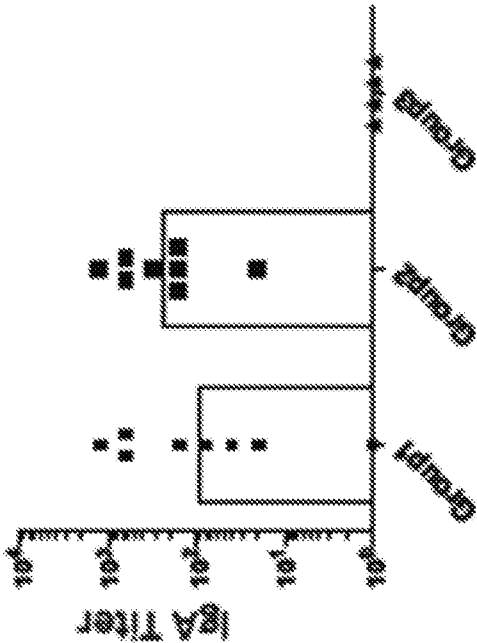
VP1 specific IgA titers at week 8

VP1 specific IgG titers at week 8

VP1 specific IgA titers at week 8

11

VP1 specific IgG titers at week 4 and 8

VP1 specific IgA titers at week 4 and 8

FIG. 14B

VP1 specific IgA titers at week 8

FIG. 14A

VP1 specific IgG titers at week 4 and 8

FIG. 15B

FIG. 15A

FIG. 16

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                    ┌──────────────────────┐
                    │ Assessed for eligibility* │
                    │        n=385         │
                    └──────────┬───────────┘
                               │
                               │      ┌──────────────────────────────────────┐
                               ├─────▶│ •  Not meeting eligibility criteria* n=323 │
                               │      │ •  Lost contact or not needed n=18    │
                               │      └──────────────────────────────────────┘
                               ▼
              ┌────────────────┴────────────────┐
              ▼                                  ▼
    ┌──────────────────┐              ┌──────────────────┐
    │  Placebo  n=12   │              │  Vaccine n=12    │
    └────────┬─────────┘              └────────┬─────────┘
             ▼                                 ▼
    ┌──────────────────┐              ┌──────────────────┐
    │ Completed 28 day │              │ Completed 28 day │
    │ (active phase) n=12│            │ (active phase) n=12│
    └────────┬─────────┘              └────────┬─────────┘
             ▼                                 ▼
    ┌──────────────────┐              ┌──────────────────┐
    │ Completed 180 day│              │ Completed 180 day│
    │  follow-up  n=12 │              │  follow-up  n=12 │
    └──────────────────┘              └──────────────────┘
```

HAI Titers Pre and Post Immunization

D0
D28

HAI Titer

1000
100
10
1

Placebo

Vaccine Tablet

FIG. 20A

20B

FIG. 20C

FIG. 20D

FIG. 21B

FIG. 21A

Anti-RSVf ELISA Data at 7 weeks

22

ELISA IgG Antibody Titers to RSV

FIG. 23A

Competition ELISA - Pooled Samples

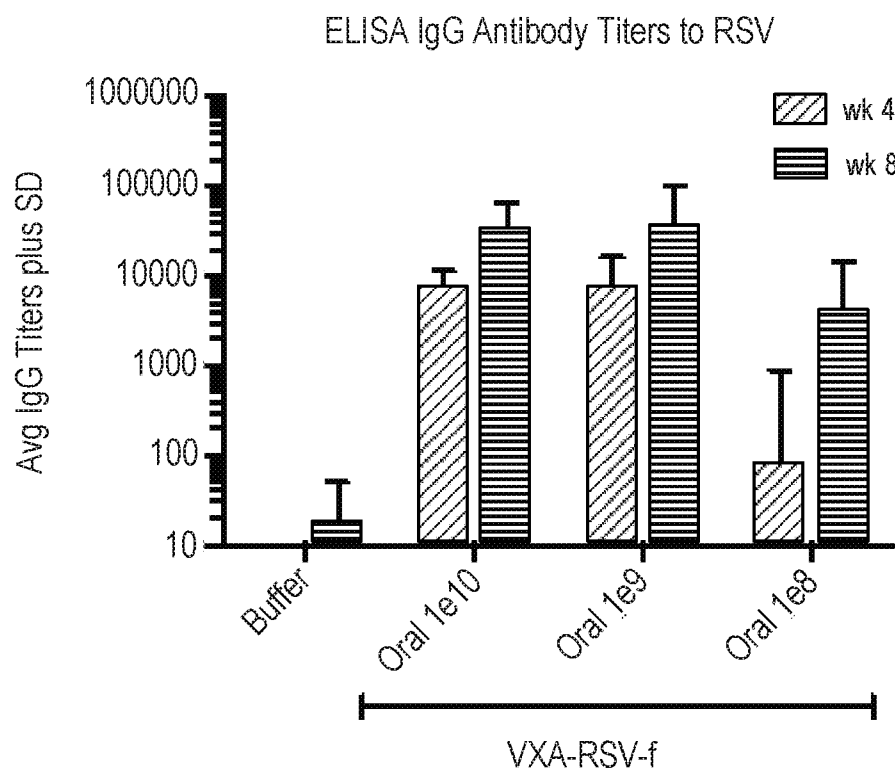*23B*

23C

ELISA IgG Antibody Titers to RSV

FIG. 24A

Competitive Palivizumab Titers

FIG. 24B

24C
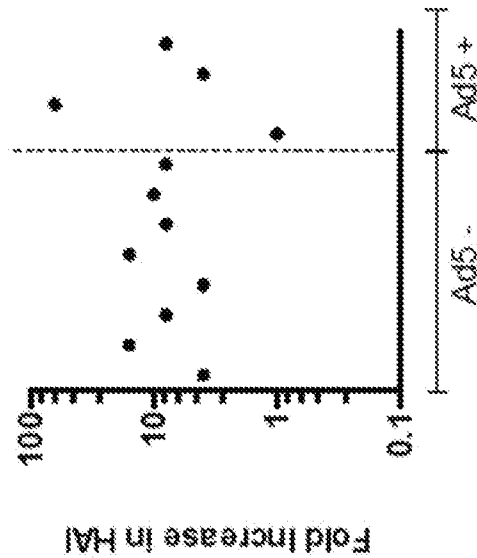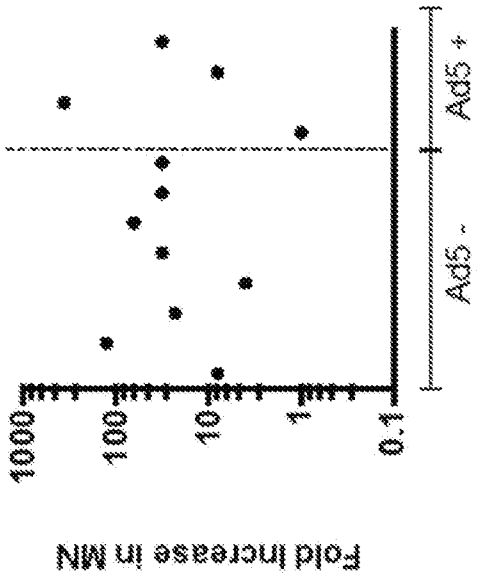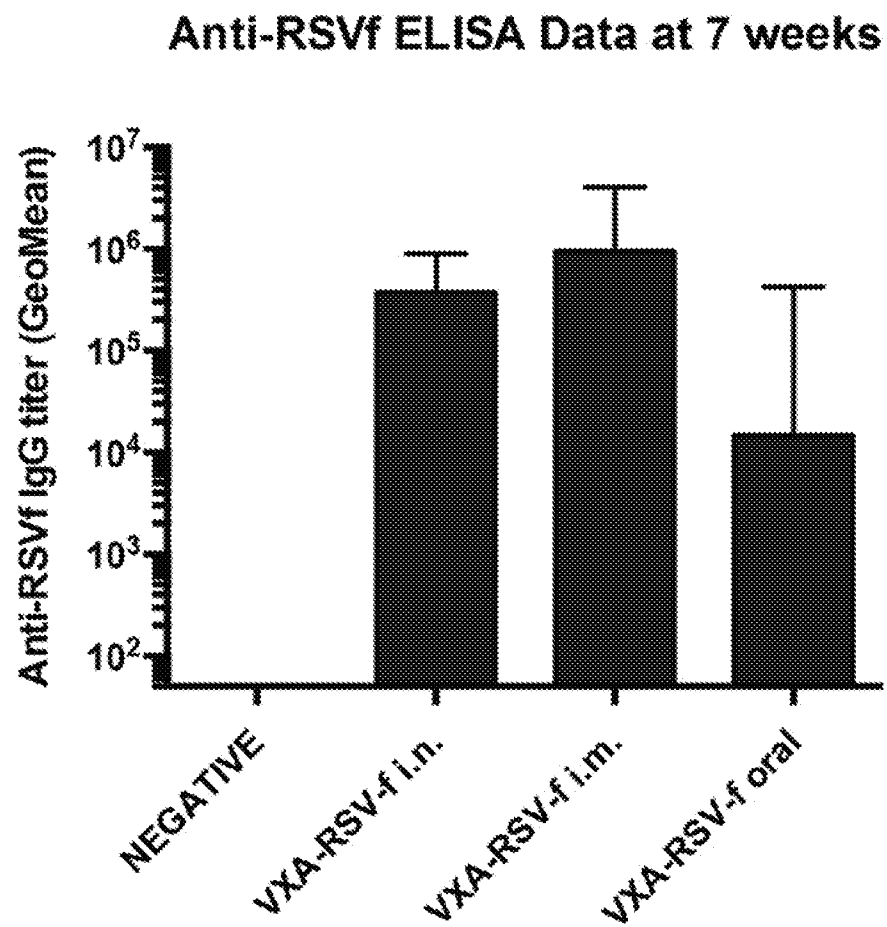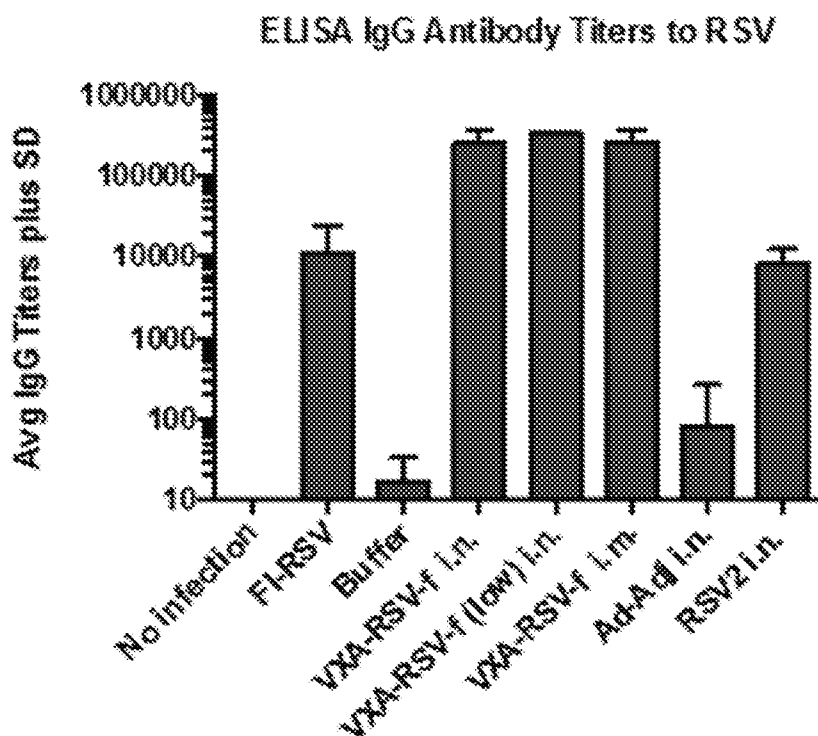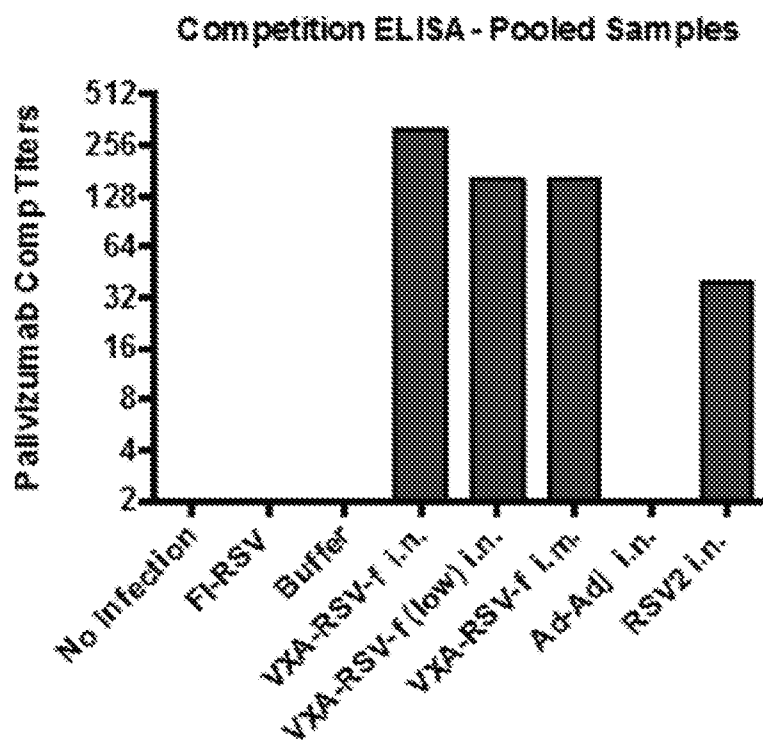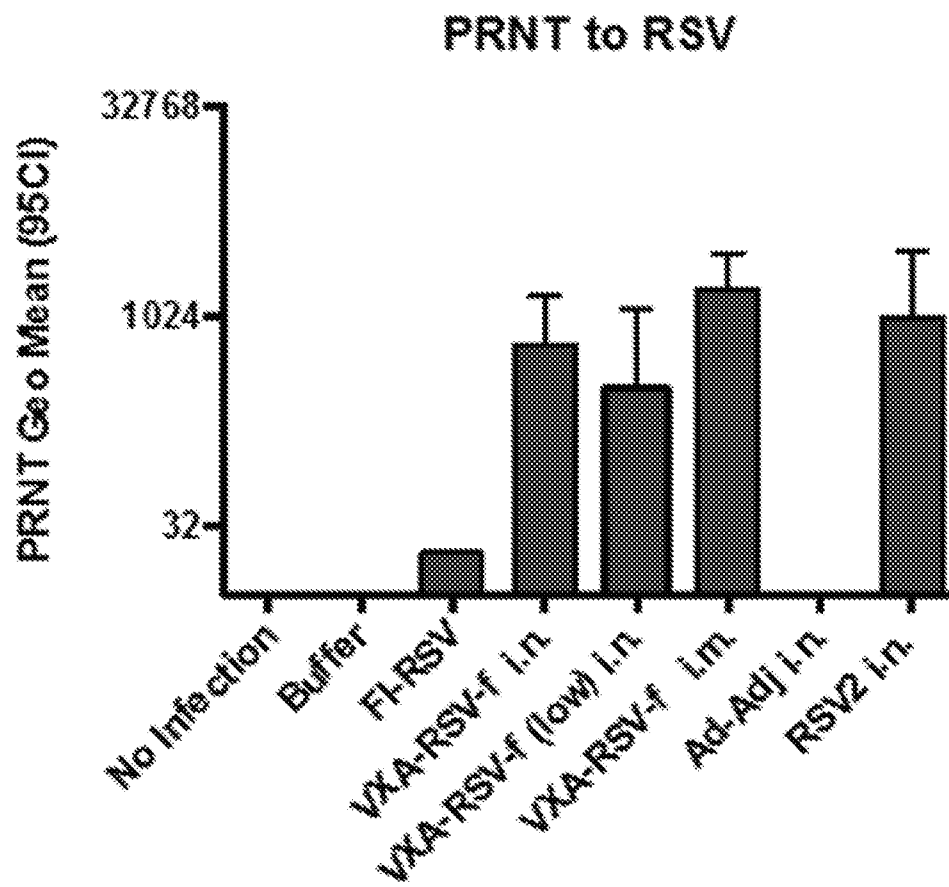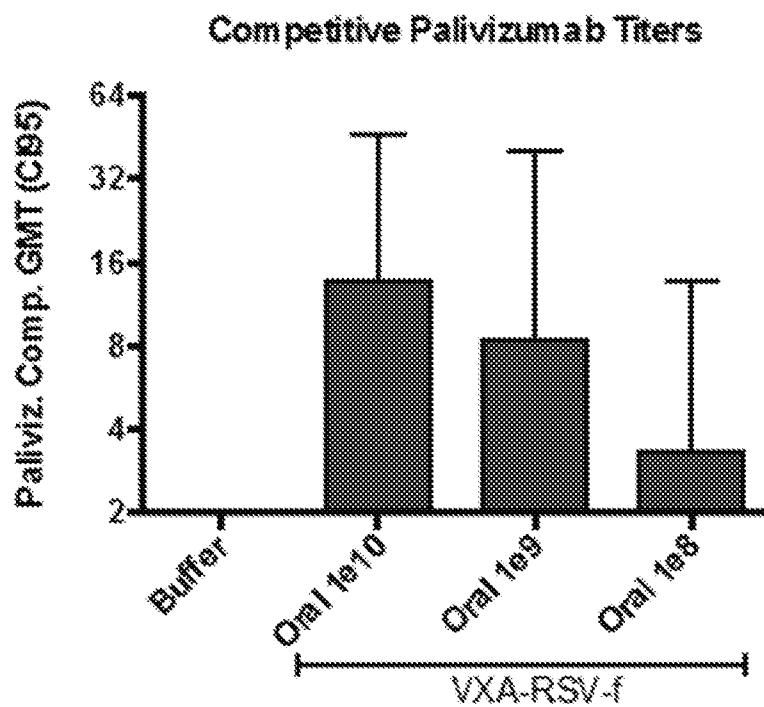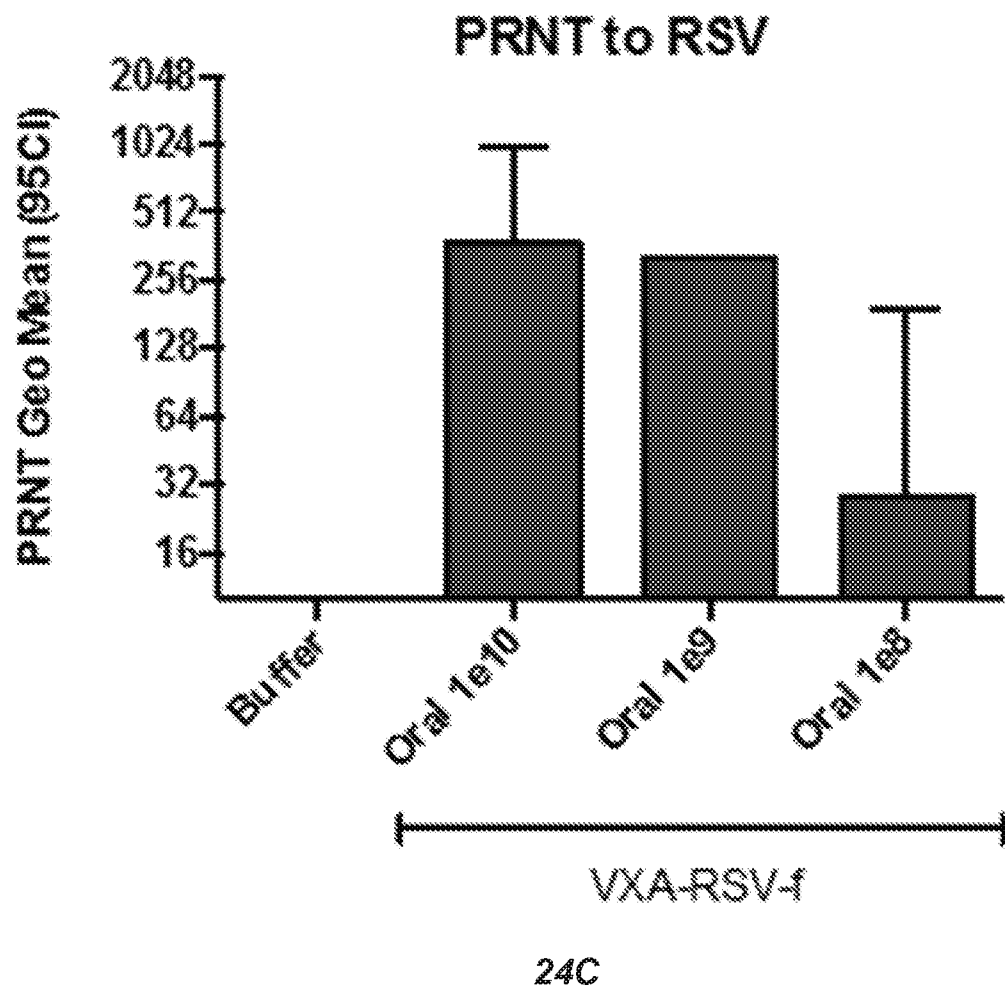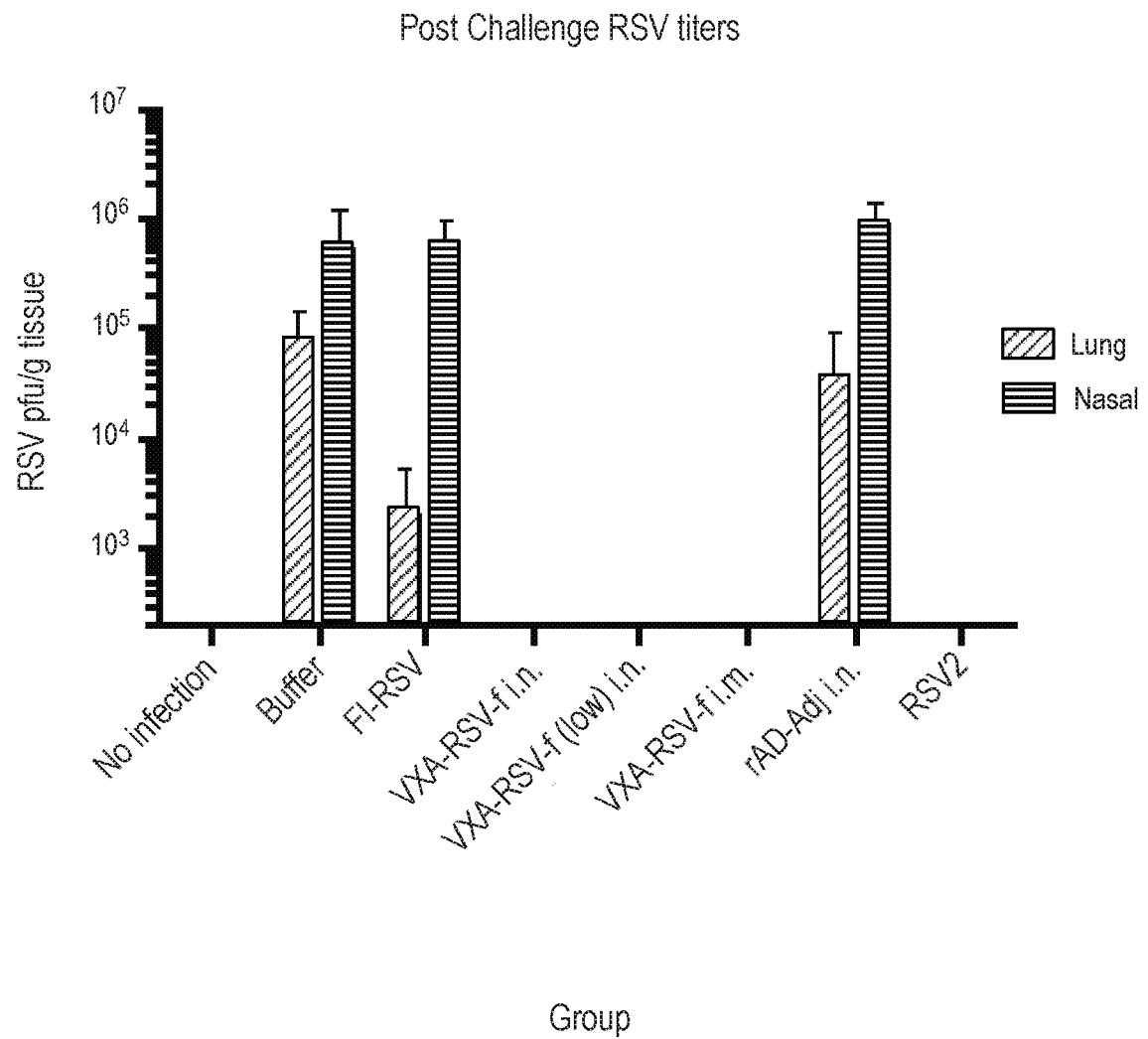
FIG. 25A

FIG. 25B

FIG. 25C

Post Challenge RSV titers

FIG. 26A

Inflammation - Oral Vaccine

FIG. 26B

FIG. 26C

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 9/00, A61K 9/20, A61K 39/00 (2016.01)

CPC - A61K 9/5005, A61K 9/5036, A61K 9/5084

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 9/00, A61K 9/20, A61K 39/00 (2016.01)
CPC: A61K 9/5005, A61K 9/5036, A61K 9/5084

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/452, 424/204.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: immunogenic composition, vaccine, RSV, norovirus, viral protein 1, RSV fusion protein, biological agent, expression vector, adenoviral expression vector, delievry, oral administration, time released, ph sensitive, pH5.8-6.8, threshold, disintegrate, dissolve, release, thickness, coa

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0111989 A1 (GRUNDWALD et al.) 6 May 2010 (06.05.2010) abstract, para [0065], [0069]	1-3
Y	US 2015/0071994 A1 (THERABIOME, LLC) 12 March 2015 (12.03.2015) para [0005], [0012], [0031], [0054], [0056], [0069], [0089], [0097]	1-3

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 August 2016	0 2 SEP 2016

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Lee W. Young
Facsimile No. 571-273-8300	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

Box No. II	Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-24
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III	Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
   ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
   ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
   ☐ No protest accompanied the payment of additional search fees.