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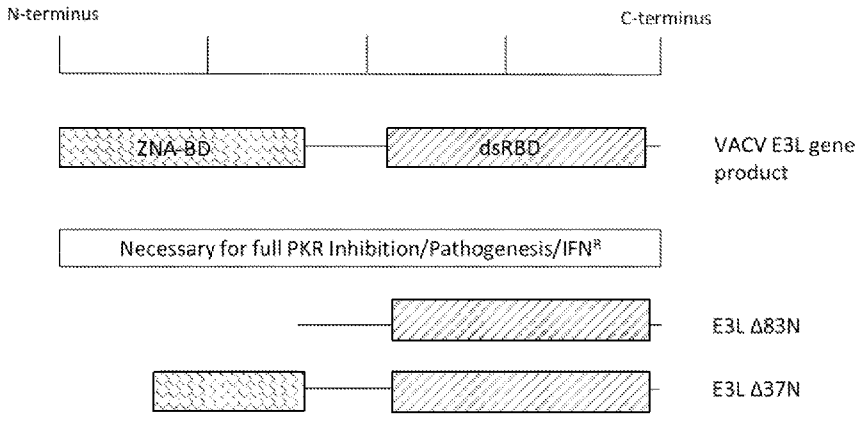
(54) Title: ATTENUATED VACCINIA VIRUS VACCINES FOR MONKEYPOX

FIG. 1



(57) Abstract: This disclosure provides compositions and methods for treating and/or preventing monkeypox infection and/or symptoms post-infection, the method including administering a composition including at least one attenuated vaccinia virus, the at least one attenuated vaccinia virus including a modification to the coding sequence of E3L.

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ATTENUATED VACCINIA VIRUS VACCINES FOR MONKEYPOX

Related Applications

[001] This application claims priority to Ser. No. 63/535,964 filed on August 31, 2023 and Ser. No. 63/536,538 filed on September 5, 2023, which are hereby incorporated into this disclosure in their entireties.

Field of the Disclosure

[002] This disclosure relates to compositions and methods for treating and/or preventing monkeypox infection and/or symptoms post-infection, the method including administering a composition including at least one attenuated vaccinia virus, the at least one attenuated vaccinia virus including a modification to the coding sequence of E3L.

Statement of Support

[003] This invention was made with government support under R01 AI095394 awarded by the National Institutes of Health. The government has certain rights in the invention.

Sequence Listing

[004] This application contains a Sequence Listing which has been submitted electronically in HTML ST26 format via EFS-Web and hereby incorporated by reference in its entirety. Said HTML copy, created on 30 August 2024, is named "M24-004LWO1-a SEQ LIST v2" and is 47 kilobytes in size.

Background of the Disclosure

[005] The monkeypox (mpox (or MPXV as may be used herein)) virus is an Orthopoxvirus discovered in 1958 in crab-eating monkeys with skin lesions. The first human infection was identified in 1970 in the Democratic Republic of Congo (DRC) and has since been reported in several other African countries. The Clade I and I mpox strains have been endemic in Africa for decades, with Clade II variants have evolved to become more virulent in recent years, with a fatality rate of 3% to 4%. The first mpox cases outside of Africa were reported in the United States in 2003 and since that time sporadic mpox outbreaks have occurred throughout the world, the population with apparently increased susceptibility being males with a median age of 31 years and male gender. Mpox infection can be transmitted by direct contact with contaminated body fluids, respiratory droplets with prolonged face-to-face contact, skin injuries, and fomites,

ingestion of inadequately cooked animal products. The infected animal (e.g., human being) does not have clinical signs during the seven to 21-day incubation period but is subsequently associated with nonspecific clinical signs such as fever, myalgia, headache, chills, fatigue, mouth ulcers, throat ulcers, and lymphadenopathy, skin rashes on the face, extremities, trunk and abdomen (e.g., vesiculopustular rashes that become transformed into scabs), and secondary bacterial infections. Diagnosis of mpox infection can be by various assays, typically DNA-based tests as serological tests suffer from cross-reactivity with other viruses. Since no effective mpox treatments are available, approaches for preventing and/or treating the disease have been adapted those used with smallpox. Approved mpox vaccines include the modified vaccinia Ankara virus (known as MVA, JYNNEOS, and/or IMVAMUNE (see, e.g., Peterson, et al. Vaccinating against monkeypox in the Democratic Republic of the Congo. Antiviral Res., 162: 171-177 (2019); and, Preyamvada, et al. Serological responses to the MVA-based JYNNEOS monkeypox vaccine in a cohort of participants from the Democratic Republic of Congo. Vaccine, 40: 7321-7327 (2022)) and the replication-competent vaccine ACAM2000 (containing a live vaccinia virus (see, e.g., Nalca, et al. ACAM2000™: The new smallpox vaccine for United States Strategic National Stockpile (Drug Des. Devel. Ther. 4: 71-79 (2010); and, Kandeel, et al. Efficacy of the modified vaccinia Ankara virus vaccine and the replication-competent vaccine ACAM2000 in monkeypox prevention. Int. Immunopharmacol. 119: 110206 (2023)).

[006] Vaccinia virus (VACV) was used to vaccinate the world against smallpox so successfully that smallpox in nature has been eradicated. The current version of that vaccine is the previously mentioned ACAM2000 vaccine, which is a tissue culture purified New York City Board of Health vaccinia strain and has exhibited fewer side effects than the original but has been found pathogenic in patients with compromised immune systems. The JYNNEOS vaccine was against Mpox because it is replication-deficient and believed to less inclined to cause undesirable side effects. While both of the vaccines were found to induce neutralizing antibodies, with a higher significance following administration of JYNNEOS than ACAM2000, with the level observed for ACAM2000 lacking statistical significance (Kandeel, et al.). There are also issues of limited supply of the JYNNEOS vaccine. There are currently perhaps 200,000 mpox vaccines available, while some estimates suggest a potential need to 10 million or more doses.

[007] Thus, there is a need in the art for monkeypox vaccines. This disclosure provides solutions to this problem. For instance, in some embodiments, this disclosure provides

compositions including a vaccinia virus having a genome in which the E3L gene is at least partially deleted. This disclosure thereby providing solutions to these and other art-recognized, and unrecognized, problems.

Brief Description of the Drawings

[008] **Figure 1** illustrates the vaccinia virus E3L gene product as well as the E3LΔ37 and /or E3LΔ83 modifications.

[009] **Figs. 2A**, **2B** and **2C**, respectively, show that the clinical score of mice vaccinated with wild-type ACAM2000 or attenuated ACAM2000 after challenge with MPXV showed improved clinical scores over mock vaccinated animals after challenge with MPXV.

[0010] **Figure 3** presents the clinical scores of mice vaccinated with one of NYVAC-KCΔB8RΔB19R-E3LΔ37N, attenuated ACAM2000-E3LΔ83N, or wild-type ACAM2000 showed improved clinical scores over mock vaccinated animals following challenge with MPXV.

[0011] **Figure 4** presents the clinical score of mice vaccinated with one of NYVAC-KC-E3LΔ37N, where the NYVAC-KC-E3LΔ37N prime and prime-boost groups each showed improved and similar clinical scores over mock vaccinated animals following challenge with MPXV.

Summary of the Disclosure

[0012] This disclosure provides compositions and methods for treating and/or preventing monkeypox infection and/or symptoms post-infection, the method including administering a composition including at least one attenuated vaccinia virus, the attenuated vaccinia virus preferably being derived from an ACAM2000 virus or a modified NYVAC virus, the at least one attenuated vaccinia virus including a modification to the coding sequence of E3L. In preferred embodiments, the coding sequence of E3L encodes SEQ ID NO: 1 or SEQ ID NO: 3, or a polypeptide having at least about 85% identity therewith. In preferred embodiments, the coding sequence encoding SEQ ID NO: 1 or SEQ ID NO: 3 is SEQ ID NO: 2 or SEQ ID NO: 4, respectively, or a polynucleotide having at least about 85% identity therewith. Others aspects of this disclosure will be apparent to those of ordinary skill in the art.

Detailed Description of the Invention

[0013] As mentioned above, ACAM2000, a tissue culture purified New York City Board of Health vaccinia strain, is licensed in the United States as a smallpox vaccine that has shown fewer

side effects than that the original vaccine but remains pathogenic in patients with compromised immune systems. ACAM2000 has also been recommended for vaccination of people aged one year and older who have been determined to be at high risk for infection to prevent mpox (see, e.g., CDC Interim Guidance at https://www.cdc.gov/poxvirus/mpox/interim-considerations/acam2000-vaccine.html accessed on August 31, 2023). And a new vaccine, JYNNEOS, was used during the recent monkeypox outbreak because it is replication-deficient but there are reports this vaccine is ineffective, and there are limited supplies available (see, e.g., CDC Interim guidance and package insert available at the website shown above). This disclosure relates to the use of modified vaccinia virus-containing compositions for use as a vaccine against monkeypox (mpox as defined under the World Health Organization International Classification of Diseases (ICD) (or MPXV as used herein)).

[0014] This disclosure relates to compositions including a vaccinia virus having a genome in which the E3L gene is at least partially deleted. In one embodiment, this disclosure provides an attenuated ACAM2000 or an attenuated NYVAC virus (preferably NYVAC-KC-ΔB8R-ΔB19R) as a vaccine against monkeypox virus, determined using a mouse model (e.g., CAST/EiJ mice as shown in the examples) provides protection equal to that of the wild type vaccine strain (e.g., ACAM2000 or NYVAC). Other vaccinia viruses may also be suitable for attenuation in this same manner and used as a mpox vaccine. In preferred embodiments, the attenuation is achieved through the deletion of the amino terminus of E3L, a host range and pathogenesis protein expressed by vaccinia virus (all strains). The N-terminal deletion of E3L is preferably of either 37 amino acids or 83 amino acids, most preferably from the genome of a parental vaccinia virus, most preferably ACAM2000, JYNNEOS (an MVA virus), or NYVAC (preferably NYVAC-KC-ΔB8R-ΔB19R) (see **Fig. 1**). Either deletion is shown herein to prevent the virus from escaping the cellular programmed death process of necroptosis, reducing the ability of the virus to spread and cause disease while yet remaining replication competent, and therefore, able to introduce the immune system to a full complement of virus proteins.

[0015] In some preferred embodiments, this disclosure also provides attenuated vaccinia virus that includes a deletion of the N-terminal 37 amino acids of E3L (E3LΔ37) (see **Fig. 1**). An attenuated vaccinia virus having a deletion of 37 N-terminal amino acids from E3L was shown herein, surprisingly and unexpectedly, particularly in attenuated ACAM2000 and NYVAC-KC-ΔB8R-ΔB19R, to act as a vaccine against MPXV.

[0016] Vaccinia virus (strain WR) having a deletion of 83 N-terminal amino acids from E3L (E3LΔ83-WR) has been previously described by Brandt, et al. (The N-terminal domain of the vaccinia virus E3L-protein is required for neurovirulence, but not induction of a protective immune response. Virology, 333: 263-270 (2005)). Brandt showed E3LΔ83-WR protected against challenge with a high dose of wild-type vaccinia virus, suggesting that this replication competent, but attenuated strain of vaccinia virus may have promise as an improved vaccine for protecting against smallpox, and as a vector for inducing mucosal immunity to heterologous pathogenic organisms. Vijayrsi et al. (Vaccinia viruses with mutations in the E3L gene as potential replication-competent, attenuated vaccines: Intra-nasal vaccination. Vaccine, 26(5): 664-676 (2008) (see, e.g., Fig. 1 thereof)) subsequently showed E3LΔ83-WR could be used a to induce an immune response against wild-type vaccinia. Vijayrsi also showed that similar constructs in a Copenhagen or NYCBH backgrounds could also be used to protect against vaccinia infection. However, there has not been any suggestion that such attenuated vaccinia viruses could or should be used as a monkeypox vaccine. An attenuated vaccinia virus having a deletion of 83 N-terminal amino acids from E3L (E3LΔ83) (see **Fig. 1**) was shown herein, surprisingly and unexpectedly, to act as a vaccine against MPXV.

[0017] Each of these preferred attenuated vaccinia viruses disclosed herein (E3LΔ83 and E3LΔ37 (**Fig. 1**)) were found to prevent MPXV from escaping the cellular programmed death process of necroptosis, reducing the ability of MPXV to spread and cause disease while yet remaining replication competent, and therefore, able to introduce the immune system to a full complement of virus proteins. Thus, this disclosure provides vaccine compositions including the attenuated vaccinia viruses having the E3LΔ37 (also referred to as E3LΔ37N) and /or E3LΔ83 (also referred to as E3LΔ83N) modifications that are effective against MPXV. To be effective, a vaccine of this disclosure induces MPXV neutralizing antibodies, an anti-MPXV cellular response (e.g., $CD4^+$ and/or $CD8^+$ T cells), and/or to reduce the ability of MPXV to spread to other cells and/or organisms (e.g., mouse, human being) as can be determined using the methods disclosed herein (e.g., in the Examples) or as may be otherwise available to those of ordinary skill in the art.

[0018] In preferred embodiments, E3LΔ37 of the attenuated vaccinia virus has the amino acid sequence of SEQ ID NO: 1 and can be encoded by the polynucleotide of SEQ ID NO: 2 (and incorporated into the genome of a modified vaccinia virus of this disclosure (see **Fig. 1**)) as

shown below:

MEKREVNKALYDLQRSAMVYSSDDIPPRWFMTTEADEADADAMSDVIIDDVSREKSMREDHKSFD
DVIPAKKIIDWKGANPVTVINEYCQITRRDWSFRIESVGPSNSPTFYACVDIDGRVFDKADGKSK
RDAKNNAAKLAVDKLLGYVIIRF (SEQ ID NO: 1); and,

ATGGAGAAGCGAGAAGTTAATAAAGCTCTGTACGATCTTCAACGTAGTGCTATGGTGTAC
AGCTCCGACGATATTCCTCCTCGTTGGTTTATGACAACGGAGGCGGATGAAGCCGATGCT
GATGCTATGTCGGACGTCATAATAGATGATGTATCCCGCGAAAAATCAATGAGAGAGGAT
CATAAGTCTTTTGATGATGTTATTCCGGCTAAAAAAATTATTGATTGGAAAGGTGCTAAC
CCTGTCACCGTTATTAATGAGTACTGCCAAATTACTAGGAGAGATTGGTCTTTTCGTATT
GAATCAGTGGGGCCTAGTAACTCTCCTACATTTTATGCCTGTGTAGACATCGACGGAAGA
GTATTCGATAAGGCAGATGGAAAATCTAAACGAGATGCTAAAAATAATGCAGCTAAATTG
GCAGTAGATAAACTTCTTGGTTACGTCATCATTAGATTCTGA (SEQ ID NO: 2).

[0019] In preferred embodiments, E3LΔ83 of the attenuated vaccinia virus has the amino acid sequence of SEQ ID NO: 3 and can be encoded by the polynucleotide of SEQ ID NO: 4 (and incorporated into the genome of a modified vaccinia virus of this disclosure (see **Fig. 1**)) as shown below:

MADVIIDDVSREKSMREDHKSFDDVIPAKKIIDWKGANPVTVINEYCQITRRDWSFRIESVGPSN
SPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLLGYVIIRF (SEQ ID NO: 3); and,

ATGGCTGACGTCATAATAGATGATGTATCCCGCGAAAAATCAATGAGAGAGGATCATAAG
TCTTTTGATGATGTTATTCCGGCTAAAAAAATTATTGATTGGAAAGGTGCTAACCCTGTC
ACCGTTATTAATGAGTACTGCCAAATTACTAGGAGAGATTGGTCTTTTCGTATTGAATCA
GTGGGGCCTAGTAACTCTCCTACATTTTATGCCTGTGTAGACATCGACGGAAGAGTATTC
GATAAGGCAGATGGAAAATCTAAACGAGATGCTAAAAATAATGCAGCTAAATTGGCAGTA
GATAAACTTCTTGGTTACGTCATCATTAGATTCTGA(SEQ ID NO: 4).

In some preferred embodiments, the E3 amino acid sequence having the 37 or 83 N-terminal amino acid deletion can exhibit at least about 85%, such as between about any of 85%-99%, 90%, 95%, 95-99%, 99%, or greater amino acid sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3, provide the modified E3 polypeptide retains the ability to bind dsRNA as can be determined by those of ordinary skill in the art using routine methods. In some preferred embodiments, such E3 amino acid sequences can be encoded by a polynucleotide at least about 85%, such as between about any of 85%-99%, 90%, 95%, 95-99%, 99%, or greater, or more nucleotide sequence identity to SEQ ID NO: 2 or SEQ ID NO: 4. Any Other embodiments could also be used as would be understood by those of ordinary skill in the art.

[0020] Preferred attenuated vaccinia viruses including the E3LΔ37 and /or E3LΔ83 modifications (e.g., SEQ ID Nos. 1 and/or 3, preferably encoded by SEQ ID Nos. 2 and 4, respectively) can be based on (or derived from) any suitable vaccinia virus. A preferred suitable vaccinia virus can be ACAM2000 (preferably as exemplified herein), a modified NYVAC (preferably as exemplified herein), JYNNEOS, or the modified vaccinia Ankara (MVA). Attenuated version of other vaccinia viruses are also contemplated herein.

[0021] The modified NYVAC virus, a preferred vaccine component of this disclosure, is a modified version of the vaccinia virus known in the art as NYVAC (or vP866), which was derived from the Copenhagen vaccine strain of vaccinia virus by deleting six nonessential regions of the genome encoding known or potential virulence factors (see, for example, U.S. Pat. Nos. 5,364,773 and 5,494,807; Tartaglia, et al. Virology, 188: 217-232 (1992)). The deletion loci were also engineered as recipient loci for the insertion of foreign genes. The deleted regions of the parental NYVAC vector are the thymidine kinase gene (TK; J2R); hemorrhagic region (u; B13R+B14R); A type inclusion body region (ATI; A26L); hemagglutinin gene (HA; A56R); host range gene region (C7L-K1L) (see below); and, large subunit, ribonucleotide reductase (I4L). NYVAC (vP866), vP994, vCP205, vCP1433, placZH6H4Lreverse, pMPC6H6K3E3 and pC3H6FHVB were also deposited with the ATCC under the terms of the Budapest Treaty, accession numbers VR-2559, VR-2558, VR-2557, VR-2556, ATCC-97913, ATCC-97912, and ATCC-97914, respectively. These modified vaccinia vectors were shown to exhibit altered host range and to be useful for expressing immunogens within a wide range of species. Such NYVAC vectors have been shown to be useful for expressing immunogens (antigens) (see, for example, U.S. Pat. No. 6,265,189 and U.S. Pat. No. 9,670,506) and used as recombinant vaccines against numerous pathogens and tumours in animal models and humans (Myagkikh *et al.*, 1996; Benson *et al.*, 1998; Siemens *et al.*, 2003; Franchini *et al.*, 2004). Clinical trials using NYVAC-based vectors showed an acceptable safety profile, with induction of high levels of immunity against heterologous antigens (Kanesa-thasan *et al.*, 2000; Gómez, C.E el al. 2007; Harari, A et al, 2008). Such vectors may be further modified by insertion or deletion of additional polynucleotides using the techniques described herein. Suitable polynucleotides may include, for example, those involved in host range, apoptosis, signaling, cytokine and/or chemokine expression or activity, cytokine and / or chemokine pathways, and / or the like, resulting in novel biological characteristics of the

vectors. The nomenclature of these sequences is related to the Copenhagen strain of vaccinia virus (GenBank Accession No. M35027; Goebel, et al. The complete DNA sequence of vaccinia virus. Virology 179 (1), 247-266 (1990); Goebel, et al. Appendix to 'The complete DNA sequence of Vaccinia virus'. Virology 179, 517-563 (1990)). Any of such polynucleotides may be modified (e.g, incorporated into a recombinant vector or as part of a composition containing multiple recombinant vectors) in combination with any other of such polynucleotides. Within vaccinia, the host range gene region (C7L-K1L) includes C1L (SEQ ID NO. 9), C2L (SEQ ID NO. 11), C3L (SEQ ID NO. 13), C4L (SEQ ID NO. 15), C5L (SEQ ID NO. 17), C6L (SEQ ID NO. 19), C7L (SEQ ID NO. 21), N1L (SEQ ID NO. 23), N2L (SEQ ID NO. 25), M1L (SEQ ID NO. 27), M2L (SEQ ID NO. 29) and K1L (SEQ ID NO. 31). These polypeptides have been shown to be involved in defining the "host range" or replication competence of the virus. Polynucleotides encoding such host range polypeptides are illustrated in SEQ ID NOS. 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32. In certain embodiments, one or more polynucleotides representing one or more of these host range genes can be introduced into the genome of a viral vector to affect the replication competence of the vector. In NYVAC, for example, one or more polynucleotides representing one or more of such host range genes may be re-incorporated into the NYVAC genome to modify its replication competence. In certain embodiments, as shown in the Examples, a preferred modified NYVAC is NYVAC-KC (which includes within its genome polynucleotides encoding C7L (e.g., SEQ ID NOS. 21, 22) and K1L (e.g., SEQ ID NOS. 31, 32) (e.g., as in NYVAC-KC-E3LΔ37N or NYVAC-KC-E3LΔ83N vector described and used in the Examples herein) which have been shown to effect replication competence as compared to the parental NYVAC. In certain embodiments, the modified NYVAC vector expresses at least one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of C1L (e.g., SEQ ID NOS. 9, 10), C2L (e.g., SEQ ID NOS. 11, 12), C3L (e.g., SEQ ID NOS. 13, 14), C4L (SEQ ID NOS. 15, 16), C5L (e.g., SEQ ID NOS. 17, 18), C6L (e.g., SEQ ID NOS. 19, 20), C7L (e.g., SEQ ID NOS. 21, 22), N1L (SEQ ID NOS. 23, 24), N2L (e.g., SEQ ID NOS. 25, 26), M1L (e.g., SEQ ID NOS. 27, 28), M2L (e.g., SEQ ID NOS. 29, 30), and K1L (e.g., SEQ ID NOS. 31, 32). Various combinations of such polynucleotides and / or polypeptides, as would be apparent to one of skill in the art, may be utilized in vectors. Certain of these polynucleotides (preferably C7L (e.g., SEQ ID NOS. 21, 22) and K1L (e.g., SEQ ID NOS. 31, 32)) and / or polypeptides may also be incorporated into modified NYVAC

vectors modified not to express B8R (SEQ ID NOS. 5, 6) and / or B19R (SEQ ID NOS. 7,8), and/or not to contain functional B8R (SEQ ID NO. 5) and / or B19R (SEQ ID NO. 7) (the NYVAC-KC-ΔB8R-ΔB19R vector described and used in the Examples herein). Thus, the modified NYVAC vectors of this disclosure, in preferred embodiments, express neither B8R or B19R (i.e., NYVAC-KC-ΔB8R-ΔB19R; preferably this attenuated virus can also express ATV eIF2αH (SEQ ID NO: 33, preferably encoded by SEQ ID NO: 34)). In addition, a most preferred NYVAC-KC-ΔB8R-ΔB19R derivative of this disclosure has been further modified to encode a modified E3L having a N-terminal deletion of either 37 amino acids or 83 amino acids (e.g., SEQ ID NO: 1 or SEQ ID NO: 3, or a derivative thereof; these modified viruses being referred to as NYVAC-KC-ΔB8R-ΔB19R-E3LΔ37N or NYVAC-KC-ΔB8R-ΔB19R-E3LΔ83N, respectively). Suitable recombinant vectors for introduction or re-introduction of such host range genes include those from which such sequences have been previously deleted or those that otherwise do not contain such genes within the vector genome.

[0022] Pharmaceutical compositions may take any of several forms and may be administered by any of several routes. Preferred embodiments of administratable compositions include, for example, at least one pharmaceutically acceptable excipient (e.g., saline) and one or more nucleic acids and/or viral particles in a liquid preparation such as a suspension, syrup, or elixir. The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of a nucleic acids and/or viral particles as a pharmaceutical composition. A "pharmaceutical composition" is a composition comprising a therapeutically effective amount of a nucleic acid or polypeptide. The terms "effective amount" and "therapeutically effective amount" each refer to the amount of a nucleic acid or viral particles used to observe the desired therapeutic effect (e.g., induce or enhance and immune response against MPXV). Preferred injectable preparations include, for example, nucleic acids and/or viral particles suitable for parental, subcutaneous, intradermal, intramuscular or intravenous administration such as sterile suspensions or emulsions. Injectable preparations, such as sterile injectable aqueous or oleaginous suspensions, may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Suitable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride

solution, among others. For instance, a viral vector such as a poxvirus may be prepared in 0.4% NaCl or a Tris-HCl buffer, with or without a suitable stabilizer such as lactoglutamate, and with or without freeze drying medium. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0023] In general, the attenuated vaccinia viruses may be administered in pharmaceutical compositions in an amount of about 10^4 to about 10^9 pfu per inoculation; often about 10^4 pfu to about 10^6 pfu, or, 10^3 to 10^7 pfu. Higher dosages such as about 10^4 pfu to about 10^{10} pfu, e.g., about 10^5 pfu to about 10^9 pfu, or about 10^6 pfu to about 10^8 pfu, or about 10^7 pfu can also be employed. Ordinarily, suitable quantities of plasmid or naked DNA (e.g., encoding an attenuated vaccinia virus of this disclosure) are about 1 μg to about 100 mg, about 1 mg, about 2 mg, but lower levels such as 0.1 to 1 mg or 1-10 μg may be employed. Actual dosages of such compositions can be readily determined by one of ordinary skill in the field of vaccine technology.

[0024] The compositions are administered via a parenteral route (e.g., intradermal, intramuscular, subcutaneous, skin scarification) to induce an immune response in the host. For example, one or more attenuated poxviruses may be administered separately or together in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The composition may also be provided in lyophilized form for reconstituting, for instance, in isotonic aqueous, saline buffer. In addition, the compositions can be co-administered or sequentially administered with one another, other antiviral compounds and/or compounds that reduce or alleviate ill effects of such agents.

[0025] Administration of a composition of the present invention to a host may be accomplished using any of a variety of techniques known to those of skill in the art. The composition(s) may be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals (i.e., a "pharmaceutical composition"). The pharmaceutical composition is preferably made in the form of a dosage unit containing a given amount of DNA and/or viral particles, for example. A suitable daily dose for a human or other mammal may vary widely depending on the condition of the patient and other

factors, but, once again, can be determined using routine methods. The compositions are administered to a patient in a form and amount sufficient to elicit a therapeutic effect (e.g., vaccination against MPXV infection). The amounts that are effective for this use will depend on various factors, including for example, the particular composition of the vaccine regimen administered, the manner of administration, the stage and severity of the disease, the general state of health of the patient, and the judgment of the prescribing physician. The dosage regimen for immunizing a host or otherwise treating a disorder or a disease with a composition of this invention is based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular composition employed. The dosage regimen can include a single dose or multiple doses of a particular recombinant vector of this disclosure in a prime-boost protocol. For instance, a recombinant vector of this disclosure (preferably ACAM2000-E3LΔ37N or ACAM2000-E3LΔ83N), modified NYVAC, modified NYVAC-KC-ΔB8R-ΔB19R (preferably NYVAC-KCΔB8RΔB19R-E3LΔ37N or NYVAC-KCΔB8RΔB19R-E3LΔ83N)) can be administered as a first dose (the prime or priming dose) and followed by one or more subsequent doses (the boost or boosting dose(s)). In some preferred embodiments, the prime and boost doses include the same amount of recombinant vector in each dose. In some preferred embodiments, the prime and boost doses include different amounts of recombinant vector in each dose. Thus, the dosage regimen may vary widely, but can be determined using standard and routine methods in the art.

[0026] The pharmaceutical composition may be administered orally, parentally, by inhalation spray, rectally, intranodally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles.

[0027] While the compositions described herein may be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other compositions or agents (i.e., other immunogens, co-stimulatory molecules, adjuvants). When administered as a combination, the individual components can be formulated as separate compositions administered at the same time or different times, or the components can be combined as a single composition. In one embodiment, a method of administering to a host a first form of an immunogen and subsequently administering a second form of the immunogen, wherein the first and second forms are different, and wherein administration of the first form prior to administration of the second

form enhances the immune response resulting from administration of the second form relative to administration of the second form alone, is provided. Also provided are compositions for administration to the host. For example, a two-part immunological composition where the first part of the composition comprises a first form of an immunogen (e.g., a first type of attentuated vaccinia virus) and the second part comprises a second form of the immunogen (e.g., a second type of attentuated vaccinia virus), wherein the first and second parts are administered separately from one another such that administration of the first form enhances the immune response against the second form relative to administration of the second form alone, is provided. As mentioned above, approved mpox immunological compositions comprising MVA, JYNNEOS, IMVAMUNE, and/or ACAM2000TM (see, e.g., Peterson, et al. Antiviral Res., 162: 171-177 (2019); Preyamvada, et al. Vaccine, 40: 7321-7327 (2022); Nalca, et al. Drug Des. Devel. Ther. 4: 71-79 (2010); and, Kandeel, et al. Int. Immunopharmacol. 119: 110206 (2023)) are available and can be combined with the recombinant vectors and compositions of this disclosure (preferably ACAM2000-E3LΔ37N or ACAM2000-E3LΔ83N), modified NYVAC (NYVAC-KC-E3LΔ37N or NYVAC-KC-E3LΔ83N), or modified NYVAC-KC-ΔB8R-ΔB19R (preferably NYVAC-KCΔB8RΔB19R-E3LΔ37N or NYVAC-KCΔB8RΔB19R-E3LΔ83N)). Such a combination can be within the same or different formulations (e.g., a recombinant vector of this disclosure with any one or more of MVA, JYNNEOS, IMVAMUNE, and/or ACAM2000TM (or other similar vector or composition) (either as separate single formulations or combination formulations)). In some embodiments, one or more recombinant vectors of this disclosure can serve as a priming or boosting dose and another type of vector or composition can serve as the corresponding prime or boost dose (e.g., any one or more of MVA, JYNNEOS, IMVAMUNE, and/or ACAM2000TM). In some embodiments, one or the other type of mpox vector can be utilized multiple times in an extended prime-boost administration protocol. Other arrangements are also contemplated by this disclosure as would be understood by those of ordinary skill in the art.

[0028] A kit comprising a composition of the present invention is also provided. The kit can include a separate container containing a suitable carrier, diluent or excipient. The kit may also include additional components for simultaneous or sequential-administration. In one embodiment, such a kit may include a first form of an immunogen and a second form of the immunogen. Additionally, the kit can include instructions for mixing or combining ingredients

and/or administration. A kit may provide reagents for performing screening assays, such as one or more PCR primers, hybridization probes, and / or biochips, for example.

[0029] This disclosure provides multiple embodiments and/or aspects as will be understood by those of ordinary skill in the art. In preferred embodiments, this disclosure provides the following aspects:

1. An attenuated vaccinia virus having a genome comprising a modified E3L coding sequence including at least one deletion therein, and/or polynucleotide comprising the same, the deletion preferably being an a N-terminal deletion of 37 ("Δ37") or 83 ("Δ 83") amino acids (preferably as shown in SEQ ID NO: 1 or SEQ ID NO: 3, or a derivative thereof), especially for use as a vaccine against monkeypox.

2. The attenuated vaccinia virus of aspect 1, wherein the vaccinia virus is selected from a modified ACAM2000 (preferably ACAM2000-E3LΔ37N or ACAM2000-E3LΔ83N), modified NYVAC (preferably NYVAC-KC-E3LΔ37N or NYVAC-KC-E3LΔ83N), modified NYVAC-KC-ΔB8R-ΔB19R (preferably NYVAC-KCΔB8RΔB19R-E3LΔ37N or NYVAC-KCΔB8RΔB19R-E3LΔ83N), a modified MVA (modified vaccinia Ankara), and modified JYNNEOS.

3. A composition comprising a for inducing an immune response against a monkeypox virus in a human being, the composition comprising an attenuated vaccinia of aspect 1 and/or 2.

4. The composition of aspect 3 comprising an attenuated vaccinia virus of aspects 1 and/or 2 and a pharmaceutically acceptable carrier.

5. The attenuated vaccinia virus or composition of any of aspects 1-4 wherein the E3L coding sequence encodes SEQ ID NO: 1 or SEQ ID NO: 3, or a polypeptide having at least 85% identity with SEQ ID NO: 1 or SEQ ID NO: 3.

6. The attenuated vaccinia virus or composition of any of aspects 1-5 wherein SEQ ID NO: 1 is encoded by SEQ ID NO: 2; SEQ ID NO: 3 is encoded by SEQ ID NO: 4; or a polynucleotide having at least 85% identity with SEQ ID NO: 2 or SEQ ID NO: 4.

7. A method of for producing an immune response against monkeypox, the method comprising administering to the host an attenuated vaccinia virus or composition of any of aspects 1-6 to the host.

8. The method of claim 7 which comprises immunizing a host against infection by monkeypox and/or preventing and/or reducing the symptoms of infection by monkeypox.

9. The method of aspect 7 and/or 8 wherein a single dose or at least two doses are administered to the host.

10. The method of aspect 9 wherein at least two doses are administered to the host wherein the second dose (the boosting dose) is administered three or four weeks after the first dose (the priming dose).

11. The method of any of aspects 7-10 wherein administration of the composition affects cells of the host immune system as determined by detecting a change in at least one immune cells characteristic related to monkeypox infection.

12. The method of aspect 11 wherein the immune cells comprise one or more cell types selected from the group consisting of dendritic cells, lymphocytes, monocytes, macrophages, natural killer cells, and granulocytes.

13. The method of aspect 12 wherein the lymphocytes are cytotoxic T cells and/or B cells.

14. The method of any one of aspects 7-13 wherein the method induces a protective immune response against monkeypox.

Other embodiments and/or aspects are also disclosed herein as will be apparent to those of ordinary skill in the art.

[0030] The terms "about", "approximately", and the like, when preceding a list of numerical values or range, refer to each individual value in the list or range independently as if each individual value in the list or range was immediately preceded by that term. The terms mean that the values to which the same refer are exactly, close to, or similar thereto. Optional or optionally means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. Ranges may be expressed herein as from about one particular value, and/or to

about another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent about or approximately, it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. Ranges (e.g., 90-100%) are meant to include the range *per se* as well as each independent value within the range as if each value was individually listed.

[0031] All references cited within this disclosure are hereby incorporated by reference in their entirety. Certain embodiments are further described in the following examples. These embodiments are provided as examples only and are not intended to limit the scope of the claims in any way.

EXAMPLES

[0032] As shown in these examples, the exemplifed attenuated vaccinia viruses of these examples (E3LΔ83 and E3LΔ37) were found to provide protection equal to that of the wild type vaccine strain and prevent MPXV from escaping the cellular programmed death process of necroptosis, reducing the ability of MPXV to spread and cause disease while yet remaining replication competent, and therefore, introduce the immune system to a full complement of virus proteins. Other advantages of the reagents and methods of using the same are also provided herein, as would be understood by those of ordinary skill in the art.

[0033] CAST/EiJ mice were mock vaccinated, vaccinated with wild-type ACAM2000 ($8x10^6$ pfu in 20 µl), or vaccinated with attenuated ACAM2000 by deleting the N-terminal 83 amino acids thereof (ACAM-E3Δ83N ($1.4x10^7$ pfu in 20 µl). An illustration of the construction of this vector is shown in **Fig. 1**. Mice were boosted on Day 28 and challenged on Day 42 with monkeypox virus (strain 7-61, WRAIR, $3x10^7$ pfu in 20 µl). Vaccination and challenge was by scarification on tails. Clinical scores were assigned according to the pock formation measured on the indicated days post challenge. Scoring was based on erythema, swelling, scabs, ulceration, and spread. As shown in **Figs. 2A**, **2B** and **2C**, respectively, show that the clinical score after challenge with MPXV was much improved as compared to mock infected mice following administration of wild-type ACAM2000 or attenuated ACAM-E3Δ83N. As shown in **Figs. 3** and **4**, respectively, show that the clinical score following administration or NYVAC-KC-ΔB8R-

ΔB19R-E3LΔ37N, ACAM2000-E3LΔ83N, or wild-type (WT) ACAM2000 after challenge with MPOXV (MPX7-61 (Clade II). **Fig. 3** illustrates the results following a first priming dose, followed three weeks later with a boosting dose, and then the challenge with MPX7-61 (Clade II) two weeks after administration of the boost. **Fig. 4** illustrates the results following a first priming dose, followed four weeks later (as compared to three weeks as presented in **Fig. 3**) with a boosting dose (prime-boost or "P/B") of NYVAC-KC-E3LΔ37N, and then the challenge with MPX7-61 (Clade II) two weeks after administration of the boost. **Fig. 4** also illustrates the results following a first priming dose ("Prime") and then the challenge with MPX7-61 (Clade II) two weeks after administration of the priming dose (i.e., no boosting dose / only a single administration). The data shows much improved immune response against the challenge in prime-boosted (P-B) and prime only ("Prime") mice as compared to mock (control) vaccinated mice.

[0034] Thus, this data shows the attenuated E3LΔ83 and E3LΔ37 attenuated vaccinia viruses improve clinical scores in animals challenged with MPXV (MPX7-61 (Clade II)).

[0035] While certain embodiments have been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the following claims.

CLAIMS

What is claimed is:

1. An attenuated vaccinia virus for use as a vaccine against monkeypox, the attenuated vaccinia virus having a genome comprising a modified E3L coding sequence that renders E3L non-functional, optionally wherein the deletion comprises an a N-terminal deletion of 37 amino acids (SEQ ID NO: 1) or 83 amino acids (SEQ ID NO: 3).

2. The attenuated vaccinia virus of claim 1, wherein the vaccinia virus is selected from a modified ACAM2000, ACAM2000-E3LΔ37N, ACAM2000-E3LΔ83N, modified NYVAC, NYVAC-KC-E3LΔ37N, NYVAC-KC-E3LΔ83N modified NYVAC-KC-ΔB8R-ΔB19R, NYVAC-KCΔB8RΔB19R-E3LΔ37N, NYVAC-KCΔB8RΔB19R-E3LΔ83N, modified JYNNEOS, and a modified vaccinia Ankara (MVA).

3. A composition for inducing an immune response against a monkeypox virus in a human being, the composition comprising an attenuated vaccinia of claim 1.

4. A composition inducing an immune response against a monkeypox virus in a human being, the composition comprising an attenuated vaccinia of claim 2.

5. The composition of claim 3 comprising an attenuated vaccinia virus and a pharmaceutically acceptable carrier.

6. The composition of claim 4 comprising an attenuated vaccinia virus and a pharmaceutically acceptable carrier.

7. The attenuated vaccinia virus or composition of claim 1 wherein the E3L coding sequence encodes SEQ ID NO: 1 or SEQ ID NO: 3, or a polypeptide having at least 85% identity with SEQ ID NO: 1 or SEQ ID NO: 3.

8. The attenuated vaccinia virus or composition of claim 7 wherein SEQ ID NO: 1 is encoded by SEQ ID NO: 2; SEQ ID NO: 3 is encoded by SEQ ID NO: 4; or a polynucleotide having at least 85% identity with SEQ ID NO: 2 or SEQ ID NO: 4.

9. A method for producing an immune response against monkeypox, the method comprising administering to the host a vaccinia virus of claim 1 to the host.

10. A method of claim 9 wherein the vaccinia virus is selected from a modified ACAM2000, ACAM2000-E3LΔ37N, ACAM2000-E3LΔ83N, modified NYVAC, modified NYVAC-KC-ΔB8R-ΔB19R, NYVAC-KCΔB8RΔB19R-E3LΔ37N, NYVAC-KCΔB8RΔB19R-E3LΔ83N, modified JYNNEOS, and a modified vaccinia Ankara (MVA).

11. The method of claim 9 which comprises immunizing a host against infection by monkeypox and/or preventing and/or reducing the symptoms of infection by monkeypox.

12. The method of claim 9 wherein a single dose or at least two doses are administered to the host.

13. The method of claim 12 wherein at least two doses are administered to the host wherein the second dose is administered three or four weeks after the first dose.

14. The method of claim 7 wherein the administration affects cells of the host immune system as determined by detecting a change in at least one immune cells characteristic related to monkeypox infection.

15. The method of claim 14 wherein the immune cells comprise one or more cell types selected from the group consisting of dendritic cells, lymphocytes, monocytes, macrophages, natural killer cells, and granulocytes.

16. The method of claim 15 wherein the lymphocytes are cytotoxic T cells and/or B cells.

17. The method of claim 9 wherein the method induces a protective immune response against monkeypox.

FIG. 1

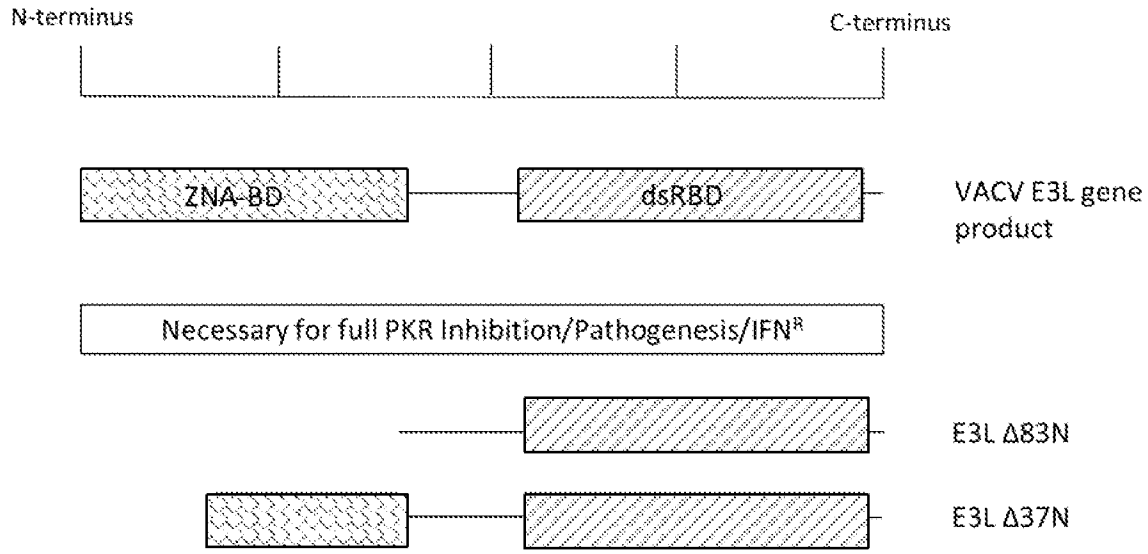

N-terminus
C-terminus
ZNA-BD
dsRBD
VACV E3L gene product
Necessary for full PKR Inhibition/Pathogenesis/IFN^R
E3L Δ83N
E3L Δ37N

FIG. 2

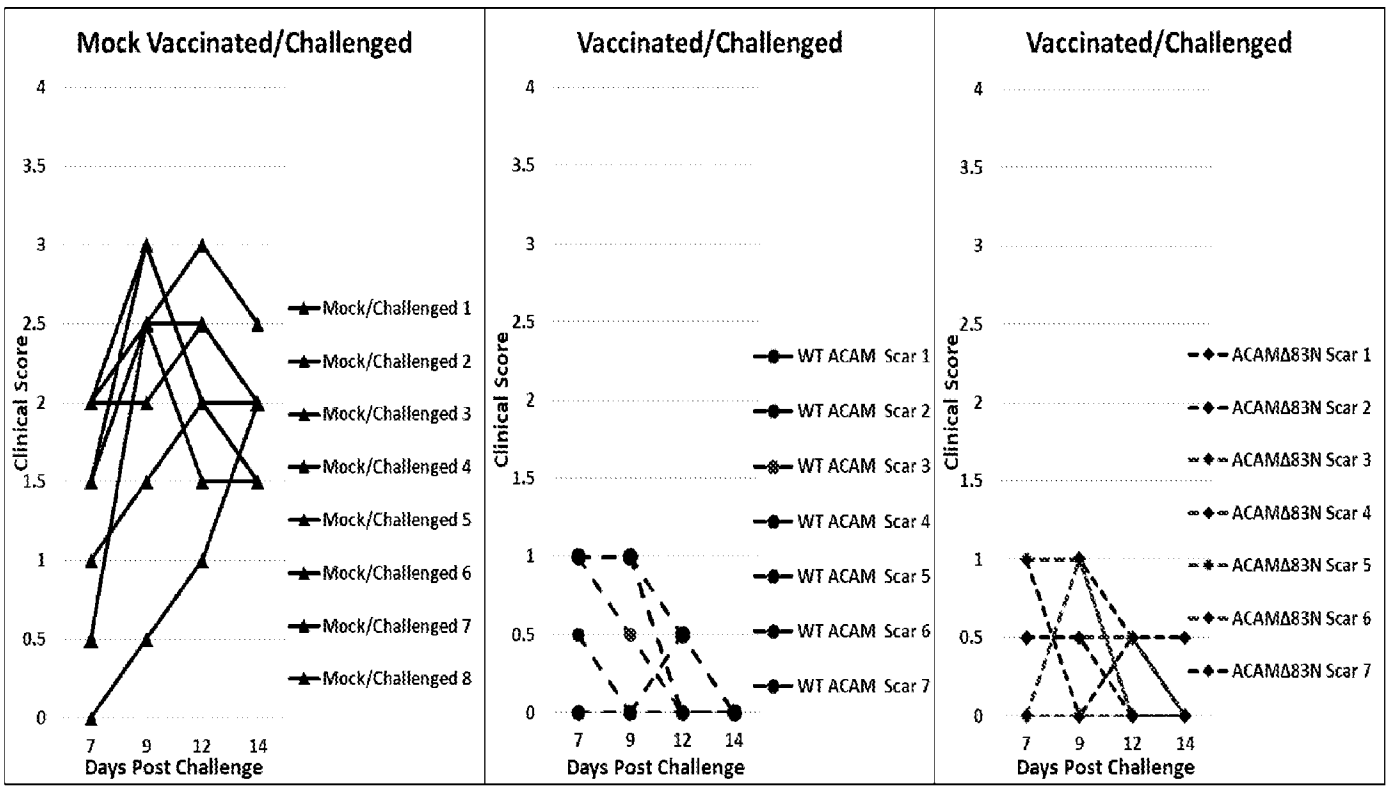

Mock Vaccinated/Challenged
Clinical Score
Days Post Challenge
Mock/Challenged 1
Mock/Challenged 2
Mock/Challenged 3
Mock/Challenged 4
Mock/Challenged 5
Mock/Challenged 6
Mock/Challenged 7
Mock/Challenged 8
Vaccinated/Challenged
WT ACAM Scar 1
WT ACAM Scar 2
WT ACAM Scar 3
WT ACAM Scar 4
WT ACAM Scar 5
WT ACAM Scar 6
WT ACAM Scar 7
Vaccinated/Challenged
ACAMΔ83N Scar 1
ACAMΔ83N Scar 2
ACAMΔ83N Scar 3
ACAMΔ83N Scar 4
ACAMΔ83N Scar 5
ACAMΔ83N Scar 6
ACAMΔ83N Scar 7

FIG. 3

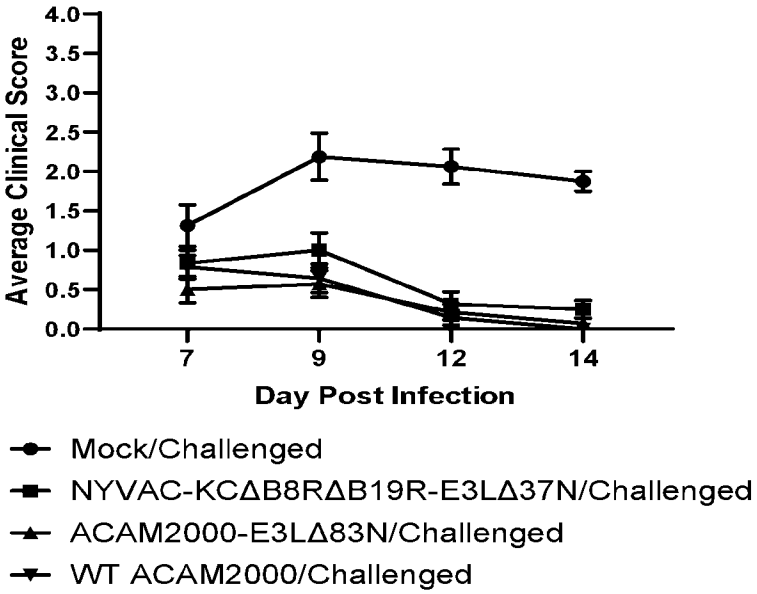
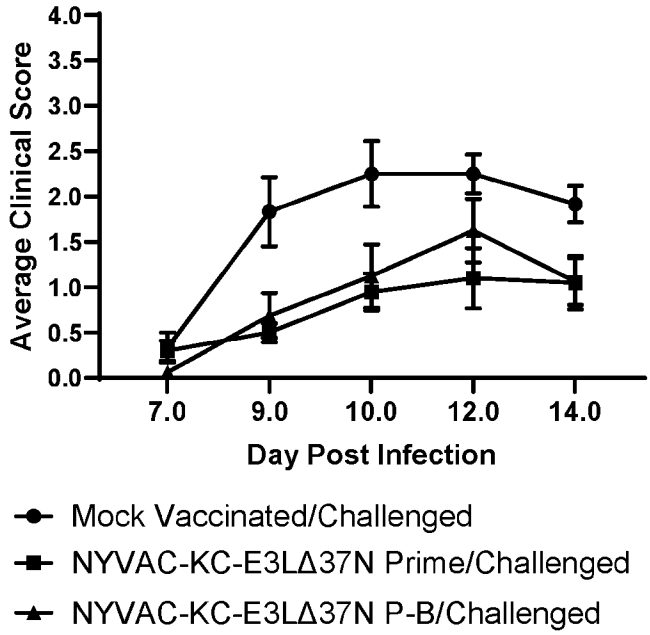

Average Clinical Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
7
9
12
14
Day Post Infection
Mock/Challenged
NYVAC-KCΔB8RΔB19R-E3LΔ37N/Challenged
ACAM2000-E3LΔ83N/Challenged
WT ACAM2000/Challenged

FIG. 4


Average Clinical Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
7.0
9.0
10.0
12.0
14.0
Day Post Infection
Mock Vaccinated/Challenged
NYVAC-KC-E3LΔ37N Prime/Challenged
NYVAC-KC-E3LΔ37N P-B/Challenged

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/044723

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12N 7/04*** (2024.01); ***A61K 39/285*** (2024.01); ***C07K 14/005*** (2024.01); ***C12N 15/39*** (2024.01)

CPC: ***C12N 7/04; C07K 14/005; A61K 39/285;*** *C12N 2710/24121*

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)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	US 2022/0056475 A1 (MEMORIAL SLOAN KETTERING CANCER CENTER) 24 February 2022 (24.02.2022)	
X	entire document	1-6
Y	entire document	9-17
	US 2003/0211964 A1 (JACOBS et al.) 13 November 2003 (13.11.2003)	
X	entire document	1, 7
	WO 2014/071963 A1 (BIONTECH AG et al.) 15 May 2014 (15.05.2014)	
A	entire document	1-17
	US 2013/0216572 A1 (HOCHREIN et al.) 22 August 2013 (22.08.2013)	
A	entire document	1-17
	US 2002/0110565 A1 (JACOBS et al.) 15 August 2002 (15.08.2002)	
A	entire document	1-17
	US 2006/0099224 A1 (KIRN) 11 May 2006 (11.05.2006)	
A	entire document	1-17

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"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)
"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
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2024 (14.10.2024)	**01 November 2024 (01.11.2024)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450 UNITED STATES OF AMERICA**	**TAINA MATOS**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/044723

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/044723

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PRIYAMVADA et al. "Serological responses to the MVA-based JYNNEOS monkeypox vaccine in a cohort of participants from the Democratic Republic of Congo," Vaccine, 04 November 2022 (04.11.2022), Vol. 40, Pgs. 7321-7327. entire document	9-17