Review

The Current State and Progress of Mpox Vaccine Research

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ABSTRACT

On July 23, 2022, the World Health Organization (WHO) declared the monkeypox (mpox) outbreak a "Public Health Emergency of International Concern." Since 2022, outbreaks of mpox in many countries around the world have primarily resulted in fatalities among immunocompromised individuals, such as untreated HIV/AIDS patients. Since the eradication of smallpox was declared by the WHO in 1980, the global vaccination against smallpox has been gradually discontinued. China also stopped routine smallpox vaccination in 1981. The protective effect of the smallpox vaccine has decreased over time due to aging and declining immunity in those who were vaccinated. For individuals, timely vaccination against smallpox is an effective means of protection against mpox. However, due to safety concerns with the smallpox vaccine and the limitations of current mpox vaccines, there is no vaccine that is safe, effective, and has low side effects applied in clinical settings. This article provides a comprehensive review of the development of mpox virus (MPXV) vaccines, their application in special populations, and the current state of vaccine research, considering the etiology, transmission, and prevention of the MPXV. Vaccination, as an effective method of epidemic prevention, can provide long-term immune protection and effectively reduce the severity of infection. However, as there is no licensed specific MPXV vaccine available globally, the vaccines currently used for mpox prevention are mostly smallpox vaccines. These smallpox vaccines can offer some degree of protection against mpox by activating cross-protection in the body.

INTRODUCTION

Overview of Mpox Virus (MPXV)

MPXV belongs to the Orthopoxvirus genus of the Poxviridae family, which includes other members like smallpox virus, vaccinia virus, cowpox virus, and rabbitpox virus, among its 14 members (*1*). MPXV is an enveloped double-stranded DNA virus with a size of about 200–250 nm, surrounded by a lipoprotein outer membrane, appearing oval or brick-shaped. Studies indicate that secondary transmission of MPXV among humans mainly occurs through prolonged contact with infected individuals, respiratory droplet transmission, direct or indirect contact with bodily fluids, and contaminated sources (*2*). Additionally, vertical transmission of the MPXV has been confirmed. In pregnant women infected with mpox, the virus's DNA can be detected in fetal tissue, the umbilical cord, and the placenta (*3*). Recent cases of mpox in multiple countries have shown that transmission through intimate contact, especially sexual transmission, is increasing. A report published in August 2022 identified MPXV in semen (*4*) and recent reports from various countries have linked mpox infections to close contact among males, primarily through sexual activity. The characteristic of close-contact transmission of the MPXV suggests that the infected population will continue to expand, with cases among women already reported internationally (*5*) and the first female case reported in the mainland of China on September 8, 2023.

The Development History of the Smallpox Vaccine

Due to the high genetic sequence similarity among Orthopoxviruses, they share many immunological epitopes and markers. The earliest evidence from animal studies in the 1960s showed that antibodies induced by the smallpox vaccine could bind and recognize various Orthopoxvirus proteins, providing cross-protection against mpox (*6*). Most people who were vaccinated with the Tian Tan strain (smallpox vaccine) before 1981 still maintain a certain level of MPXV-specific antibodies. Most of the Chinese population maintains vaccinia virus-specific IgG antibodies for 42 years or longer after vaccination, offering some degree of protection against mpox (*7*). This enduring immunity aligns with other studies indicating that smallpox vaccines, including more recent versions, offer effective cross-immunity against

mpox. This applies both as pre- and post-exposure prophylaxis (*8*). An England study demonstrated a 78% effectiveness of the Modified Vaccinia Ankara - Bavarian Nordic (MVA-BN) vaccine in preventing symptomatic Mpox 14 days after initial pre-exposure vaccination (*9*). Additionally, an observational study from the 2022 outbreak showed that post-exposure vaccination with a third-generation smallpox vaccine had an adjusted effectiveness of 88.8% [95% confidence interval (*CI*): 76.0–94.7] (*10*).

Historically, the smallpox vaccine has undergone several iterations and upgrades. Based on the vaccine's preparation methods and protective principles, the smallpox vaccines used in clinical settings and those currently approved are divided into three generations (Table 1).

First-Generation Live Virus Vaccines

The first-generation smallpox vaccines were prepared from live, non-attenuated vaccinia viruses (VACV). Strains commonly used for producing the first-generation smallpox vaccines include the NYCBH strain (used in West Africa and North America), Lister/Elstree strain (UK), Tian Tan strain (China), and EM-63 strain (Russia and India) (*11*). With the rapid development of "vaccine farms" in the United States and Europe (*12*), extracting the virus from animals and preparing smallpox vaccines became a widely adopted and relatively safe method at the time, contributing significantly to the eventual eradication of smallpox. However, due to the use of live, non-attenuated vaccinia viruses sourced from live animals, the first-generation vaccines had notable safety and reliability concerns. Tens of deaths per million vaccinations were reported with the NYCBH strain, and up to 200 deaths per million occurred during the vaccination process with the Lister strain (*13*). The Dryvax vaccine, prepared from the NYCBH strain, could lead to side effects such as acute vaccinia syndrome, vaccine-related myocarditis, or myopericarditis (*14*). Due to these safety concerns and side effects, the use of first-generation smallpox vaccines has been discontinued.

Second-Generation Live Virus Vaccines

To reduce microbial contamination seen in the production of first-generation vaccines and to improve the side effects associated with them, second-generation vaccines utilized tissue culture or cell line cultures of live vaccinia virus, replacing the original vaccine production method. Main products of the second generation include ACAM1000, ACAM2000, CJ-50300, and APSV.

ACAM1000 and ACAM2000, derived from monoclonal virus isolates of the first-generation Dryvax vaccine, have shown immunogenicity in tests that are comparable to the first-generation live virus vaccines (*15*). They exhibit a reduced level of severe side effects

TABLE 1. The development of smallpox vaccines.

Generation	Vaccine name	Strain name	Preparation method	Advantages	Disadvantages
First-generation live virus vaccine	Dryvax	NYCBH strain	Unattenuated live vaccinia virus	Made significant contributions to the global eradication of smallpox campaign.	Live virus safety and reliability are lower, can produce serious side effects.
	Lister	Lister strain			
	Tiantan	Tiantan strain			
Second-generation live virus vaccine	ACAM2000	NYCBH strain	Unattenuated live vaccinia virus	Improved and simplified the production process of the first-generation vaccine, enhancing safety.	There is a certain probability of exhibiting serious adverse reactions, performing poorly in patients with compromised immune function.
	Elstree-BN	Lister strain			
	CJ-50300	NYCBH strain			
Third-generation attenuated vaccine	MVA	Ankara strain	Attenuated live vaccinia virus	Significantly improved safety, the strain's replication capability is reduced, suitable for patients with compromised immune function.	Situations with relatively low levels of neutralizing antibodies in vaccinated individuals exist, clinical reliability needs to be verified.
	LC16m8	Lister strain		Compared to the first and second-generation smallpox vaccines, enhanced safety, reduced theovvurrence of adverse reactions.	Clinical reliability needs to be verified.
	NYVAC	Copenhagen strain		Enhanced safety.	
	dVV-L	NYCBH strain			

compared to the first-generation vaccines but can still cause serious side effects, including encephalitis, encephalomyelitis, encephalopathy, and erythema multiforme. These vaccines are contraindicated in individuals with compromised immune function [such as those with leukemia, lymphoma, human immunodeficiency virus (HIV), infections, and acquired immune deficiency syndrome (AIDS)], potential heart disease, and in pregnant women. ACAM2000 carries a risk of causing myocarditis and/or pericarditis, with an average of 5.7 cases per 1,000 primary vaccine doses (*16*). In 2007, ACAM2000 was licensed by the U.S. Food and Drug Administration, replacing Dryvax as the only available smallpox vaccine in the United States (*17*).

Third-Generation Attenuated Vaccines

Compared to the unattenuated live viruses used in the first two generations of vaccines, the third generation selected vaccinia viruses that had been passaged multiple times, resulting in reduced virulence and replication capabilities. There are mainly four strains used for the third-generation attenuated vaccines: Ankara strain, Lister strain, Copenhagen strain, and NYCBH strain. Among these, the Modified Vaccinia Ankara (MVA) vaccine and the LC16m8 vaccine are particularly representative (*18*).

The Ankara strain vaccine for the modified vaccinia virus. Replication of MVA is weakened in primary chicken embryo fibroblast (CEF) cells after more than 570 continuous passages, making it suitable for the preparation of third-generation attenuated smallpox vaccines. The reduced replication ability of MVA in mammals (*19*) makes it an ideal choice for immunocompromised patients (*20*). Clinical efficacy data show that volunteers who received two doses of MVA vaccine achieved similar overall peak neutralizing antibody titers to those observed after a single dose of ACAM2000 vaccine (*21*). Although no safety issues related to MVA vaccine have been reported so far (*22*), the administration of this vaccine can still cause certain side effects, including injection site reactions, headache, myalgia, fatigue, nausea, fever, and lymphadenopathy. There are also reports indicating that the levels of neutralizing antibodies against MPXV generated by the administration of two doses of JYNNEOS vaccine in healthy individuals are relatively low with poor neutralizing capacity (*21*).

The LC16m8 vaccine. In the 1970s, Japan developed a highly attenuated live vaccine, LC16m8, at the Chiba Serum Institute, aiming to replace first-generation vaccines such as Lister and Dryvax. The virus in LC16m8 is attenuated due to the lack of the B5R envelope protein gene, and its replication ability in vaccine recipients is limited (*23*). LC16m8 showed no severe adverse reactions in 100,000 infants and was proven to have the same immunogenicity as its parent strain. Although LC16m8 is the only smallpox vaccine approved for use in children, its effectiveness against MPXV in humans has not yet been reported. In response to mpox outbreaks, LC16m8 has been approved in Japan as a smallpox vaccine for children and other non-immunocompromised individuals (*24*). Side effects of the LC1618 vaccine include lymph node enlargement, fatigue, fever, rash, erythema, and swelling at the injection site, with side effects being more common in first-time vaccine recipients than in those receiving revaccination.

The development of smallpox vaccines has undergone significant advancements in three generations. The initial generation employed live, non-attenuated vaccinia viruses, specifically the NYCBH and Lister strains. Although effective, these vaccines raised safety concerns. The second generation, exemplified by ACAM2000, improved safety by utilizing tissue culture methods but still posed risks for certain populations. The third generation vaccines, such as MVA and LC16m8, offered enhanced safety with reduced virulence, making them suitable for a broader range of individuals, including those with compromised immune systems.

THE APPLICATION OF SMALLPOX VACCINE

The Application of Smallpox Vaccine in Susceptible Populations

To address the increasingly severe mpox outbreak, medical institutions worldwide have implemented widespread measures. Containment of mpox requires a comprehensive approach, which includes pre- and post-exposure vaccinations for at-risk groups, early detection and screening, isolation or minimizing close contact, and dissemination of accurate information to potentially exposed individuals. The development and application of vaccines are particularly important. The MVA-BN-based JYNNEOS vaccine was approved by the U.S. Food and Drug Administration (FDA) on September 24, 2019, for the prevention of smallpox and mpox diseases (*25*). On June 23, 2023, JYNNEOS (also known as Imvamune or Imvanex)

became the only FDA-approved non-replicative smallpox and mpox vaccine for both military and non-military purposes. Currently, there are three options for mpox vaccination: ACAM2000, MVA-BN, and LC-16. All three vaccines have been approved for use against mpox in various jurisdictions. However, it is important to note that their availability varies across different geographical regions (*26*). The similarities and differences among these three vaccines are summarized in Table 2.

Application of smallpox vaccine in immunocompromised populations. Individuals with impaired immune function include those with active cancer, organ transplant recipients, those with immunodeficiencies, people undergoing immunosuppressive therapy, and HIV-infected individuals. Considering the principles of vaccine preparation, contraindications, and clinical trial results of various vaccines, not all smallpox vaccines are suitable for immunocompromised populations. For instance, ACAM2000 is not recommended for individuals with severe immunodeficiency, as it may cause severe localized or systemic vaccinia skin ulcer infections in those with weakened immune systems; LC16 is also not suitable for individuals with severe immunodeficiency or those undergoing immunosuppressive treatment (*23*), among others.

In a study involving 24 hematopoietic stem cell transplant recipients, researchers randomly divided the subjects into two groups, with one group receiving the MVA-BN vaccine, and then compared neutralizing antibody titers between the groups. The results showed that no vaccine-related severe adverse reactions occurred in the MVA-BN group, and the antibody titers indicated good immunogenicity of MVA-BN in this population (*27*). Another Phase II trial involving HIV-infected individuals (previously with AIDS) assessed the safety, tolerability, and immunogenicity of three dosing regimens of MVA-BN, concluding that MVA-BN demonstrated good tolerability and immunogenicity in the study population (*28*). These findings suggest that for patients with immunosuppression, high risk of infection, or exposure to vaccinia skin ulcer cases, priority should be given to vaccination with the MVA-BN vaccine.

Application of smallpox vaccine to pregnant women and newborns. The MPXV has been proven to be transmissible vertically, making pregnant women a focus of concern during mpox outbreaks. There is an urgent need to explore effective means to protect pregnant women and newborns. A case of household transmission following smallpox vaccination was reported in 2004 (*29*), where a male member of the household was vaccinated with the second-generation live virus vaccine ACAM2000. A week later, his wife developed vesicles on her areola. Approximately two weeks later, their breastfed daughter developed papules, and the PCR test for the smallpox virus was positive. This was the first global case of mother-to-child transmission via breastfeeding following vaccination. The CDC explicitly prohibits the vaccination of pregnant women, breastfeeding women, and infants under one year of age with the ACAM 2000 vaccine (*30*).

Compared to the ACAM 2000 vaccine, the JYNNEOS vaccine is a non-replicating, attenuated live vaccinia virus vaccine. JYNNEOS was tested in developmental toxicity studies in rats and rabbits. None of these studies reported vaccine-related fetal malformations, developmental delays before weaning, or impacts on maternal fertility (*31*). Currently, there is no clear evidence indicating a definitive relationship

TABLE 2. Similarities and differences of ACAM2000, JYNNEOS, and LC16m8 vaccines.

Feature	ACAM2000	JYNNEOS	LC16m8
Vaccine Type	Live vaccine, based on Vaccinia virus	Live, non-replicating vaccine, based on Modified Vaccinia Ankara	Live, attenuated vaccine, based on Vaccinia virus
Composition	Vaccinia virus	Modified Vaccinia Ankara	Vaccinia virus from Lister strain
Effectiveness	High efficacy, similar to Dryvax	Verified in clinical studies, similar immune response to ACAM2000	Good safety and immunogenicity shown in Japanese clinical trials
Side Effects	Muscle pain, fever, myocarditis and/or pericarditis, etc.	Redness, pain, swelling, and itching at the injection site, fatigue, headache, and muscle pain	Limited detailed information, but generally considered to have fewer side effects
Applicable Population	Not suitable for people with immune deficiencies, HIV, and potential heart disease, and in pregnant women, etc.	Vaccination may be postponed for certain groups(e.g., pregnant, breastfeeding women)	Widely used in children and adults(non-immunocompromised individuals)
Method of Administration	Bifurcated needle	Needle and syringe(subcutaneous administration)	Bifurcated needle
Storage Conditions	Generally refrigerated	Generally refrigerated	Generally refrigerated

between the use of JYNNEOS vaccine in pregnant women and pregnancy outcomes.

International cross-sectional studies on the vaccination of newborns with live smallpox vaccines indicate that the risk of adverse reactions to the vaccine in infants under one year old is very high. However, there is still no global consensus on the most appropriate timing for vaccinating newborns against smallpox.

THE NEW GENERATION OF MPOX VACCINES

Preparation of The New Generation of Mpox Vaccines

Despite the important role of smallpox vaccines in combating the current MPXV outbreak, their efficacy is still questioned. Their side effects and limitations in application hinder their protective function in specific populations, and their effectiveness in controlling and preventing MPXV is not entirely satisfactory. Therefore, in response to the current mpox outbreak, there is an urgent need for a safer, more effective, and highly specific vaccine targeted specifically at the MPXV. However, the research progress on mpox vaccines faces several challenges, including slow progress in animal experiments, lack of clinical trial data, and issues related to the current variations and specificity of the MPXV.

Recent reports have identified several potential vaccine targets for the MPXV and highlighted effective immunogens (such as L1R, B5R, A27L, and A33R). These immunogens can be integrated into vaccines to enhance their protective effect against MPXV infection. At the same time, due to the mechanisms of MPXV transmission and infection, intracellular mature virions (IMV) and extracellular enveloped virions (EEV) are also expected to become important targets for the development of specific vaccines against mpox (*32*).

The 4pox DNA Vaccine

The 4pox DNA vaccine is developed targeting the immunogenic sites L1, A27, B5, and A33. Compared to mRNA vaccines, which have less stability and require drug delivery systems such as lipid nanoparticles (LNP) to deliver mRNA to target cells, the 4pox DNA vaccine does not require formulation and has sufficient immunogenicity as demonstrated by researchers (*33*). Additionally, it offers protection similar to traditional smallpox vaccines (ACAM2000, MVA) and demonstrates superior performance in preventing virus transmission, reducing the likelihood of viral shedding.

Studies have shown that the 4pox DNA vaccine, inducing an immune response with a small amount of viral antigen, can produce protective immunity against lethal orthopoxvirus attacks in mice and non-human primates. In the MPXV model of non-human primates, two doses of the 4pox DNA vaccine provided protection equivalent to MVA (*34*). Subsequent studies further proved the effectiveness of the 4pox DNA vaccine in preventing aerosolized poxvirus in highly susceptible animal models (*33*). However, this vaccine has not yet undergone clinical validation, and further investigation is needed to assess its safety and reliability for human use.

Multivalent MRNA Vaccines

The principle of DNA and mRNA vaccines involves injecting genetic material into the host to express target proteins, thereby eliciting cellular and humoral immunity. In recent years, numerous research institutions globally have focused on the development of mRNA vaccines and have made certain advancements.

Internationally, research institutions have designed a multivalent mRNA vaccine candidate, MPXVac-097, targeting five MPXV antigens: A29L, E8L, M1R, A35R, and B6R. This vaccine has demonstrated specific T-cell responses against MPXV and protection against vaccinia virus attacks in mouse models (*35*). Domestic research targeting the mature virion (MV) and enveloped virion (EV) particles involved in MPXV replication has produced four types of mRNA vaccines using combinations of antigens from EV (A35R and B6R), MV (A29L, E8L, H3L, and M1R), and surface proteins of EV and MV (*36*). These vaccines were evaluated in mice for their protective efficacy. Studies indicate that mRNA vaccines with various combinations of EV and MV surface antigens protect the mouse model from lethal doses of vaccinia virus, with the vaccine containing both EV and MV antigens providing the strongest protection, laying the groundwork for the further development of safe and effective mRNA vaccines.

Protein-Based Subunit Vaccines

Subunit vaccines, unlike attenuated or live virus vaccines, do not contain the complete pathogen but

typically only antigenic components, such as proteins or polysaccharides, theoretically eliminating the risk of causing the disease. However, subunit vaccines also have drawbacks: they often require adjuvants or booster shots to achieve the desired efficacy in recipients. Researchers have experimented with vaccines in mice using CpG-ODN and aluminum as adjuvants, containing envelope proteins from MV and EV. Mice vaccinated with these showed significantly lower viral titers compared to the unvaccinated control group (*37*). Currently, protein-based subunit vaccines are still at the laboratory stage and have not yet undergone related clinical trials.

THE CURRENT PROGRESS OF MPOX VACCINE IN CHINA

Recently, there has been a focus on the development of mRNA vaccines in Chinese research institutions. Studies have shown that multivalent mRNA vaccines, which combine various EV and MV antigens, offer better protection in mouse models compared to traditional vaccines (*36*). The China National Biotec Group (CNBG) research team has encoded the mpox proteins M1R and A35R, and their research has demonstrated promising immune responses against A35R and M1R. They have tested three poxvirus mRNA vaccines (VGPox 1-3), which have shown similar levels of A35R antibodies. The mRNA encoding a fusion form of A35R and M1R (VGPox 1 and VGPox 2) effectively induces high levels of A35R and M1R IgG and can neutralize live viruses at early stages (*38*). The research team at the Seventh Affiliated Hospital of Sun Yat-sen University has selected highly conserved targets, including A27, A33, B5, and L1 antigens, using a 2003 isolate from Clade II of the mpox evolutionary branch. Serum from all mice was collected 21 days after the first immunization to assess humoral immunity. The combination of the four types of mRNA-LNP resulted in average IgG titers of 3,000, 4,965, 22,518, and 14,388, targeting each of the four MPXV antigens respectively. This provides strong evidence for the efficacy and safety of the vaccine (*39*).

Currently, there is no available vaccine against mpox in the Chinese population. However, domestic development of mpox vaccines is progressing rapidly, with China's own Mpox mRNA vaccine soon to enter clinical trials. According to "mpox prevention guideline for the public (2023)" released by the National Institute for Infectious Diseases (Huashan Hospital, Fudan University) and the Chinese Preventive Medicine Association, mpox vaccination is currently not recommended for the general population (*40*). However, research shows that there is a low vaccine hesitancy rate among high-risk populations, particularly men who have sex with men (MSM) in China. It is essential to enhance health education and implement the mpox vaccine inoculation plan to address the current mpox situation (*41*).

CONCLUSION AND DISCUSSION

On May 12, 2023, WHO declared that the mpox outbreak no longer constituted a Public Health Emergency of International Concern. However, the number of confirmed mpox cases in China is still on the rise, with 491 cases reported in July, a significant increase from 106 cases in June. In response, the National Health Commission of China included mpox in the category B infectious diseases for management on September 20, 2023, adopting preventive and control measures for category B infectious diseases. These developments not only reflect the challenges in epidemic prevention and control and the continuous risk of imported cases in China but also provide a realistic basis for the application and development of mpox vaccines in the mainland of China.

In light of the current progress in vaccine research both domestically and internationally, protection against MPXV still relies on the application of smallpox vaccines. The first-generation smallpox vaccines have been largely replaced by second and third-generation vaccines. These newer generations of smallpox vaccines are produced through more advanced and modern cell culture methods and have shown good immune effects in treating mpox and other orthopoxvirus diseases. Clinical trials have confirmed that smallpox vaccines, represented by MVA-BN, have not caused severe adverse reactions in immunocompromised individuals, providing an important basis for expanding the eligible population for vaccination and reducing the mortality rate of mpox infections.

Currently, countries are intensifying efforts to develop next-generation (fourth-generation) vaccines. These efforts aim to address issues related to manufacturing processes and the ability to rapidly respond to new poxviruses, with the hope of further enhancing safety and protection, reducing the pathogenicity and transmissibility of the MPXV. The new generation of mpox-specific vaccines is expected to

be more suitable for vaccination in populations with immune deficiencies, skin diseases, or cardiovascular conditions. Although these vaccines are mostly in the experimental stage and have not yet entered clinical trials, requiring long-term testing and observation to verify their safety and reliability, the current results from related animal experiments strengthen our confidence in the new generation of vaccines for the prevention and treatment of mpox.

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