Review

Advancements in Mpox Vaccine Development: A Comprehensive Review of Global Progress and Recent Data

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Abstract: Since May 2022, a severe global Mpox epidemic has underscored the urgent need for a preventative vaccine. On September 16, 2022, the mainland of China reported its first case of imported Mpox, which was subsequently followed by a significant rise in domestic infections commencing from June 2023. This alarming trend has escalated the likelihood of localized outbreaks and covert transmission, posing a heightened risk to public health. Notably, the United States, many European countries, and Japan have approved the use of smallpox vaccines for Mpox prevention and emergency vaccination post-exposure, based on their cross-protection efficacy. In recent years, virology research has broadened its scope to include investigations into various novel vaccine approaches, such as nucleic acid-based vaccines, protein subunit vaccines, and epitope peptide vaccines, and other related methodologies. This review offers a thorough examination of the current global landscape of Mpox prevalence, delves into the advancements in Mpox vaccine development, and highlights the progress achieved in Mpox vaccine research, serving as a valuable resource and providing technical insights essential for the effective prevention and control of Mpox.

Key words: Mpox vaccine; Vaccinia virus; Mpox virus; Smallpox vaccine

INTRODUCTION

Since May 2022, a severe Mpox epidemic has occurred globally. Mpox is a zoonotic disease caused by the Mpox virus (MPXV), which belongs to the genus *Orthopoxvirus* of the family Poxviridae along with the smallpox virus, cowpox virus, and vaccinia virus. These viruses exhibit cross-immune protection, meaning that immunity developed against one can offer protection against the others. The increase in Mpox infections has been attributed not only to mutations in the MPXV genome but also to expedited international travel and partly to men who have sex with men (MSM)[1], facilitating its widespread dissemination. Furthermore, the discontinuation of global smallpox vaccination efforts following its eradication has led to a growing population lacking immunity to *orthopoxviruses*, potentially contributing to the prevalence of Mpox[2].

After the declaration of smallpox eradication by the World Health Organization (WHO) in 1980, China ceased smallpox vaccination, resulting in a lack of immunity in subsequent generations because of the cross immune response among *orthopoxviruses*. Even individuals previously vaccinated against smallpox exhibited considerably reduced immunity[3]. To effectively combat the Mpox epidemic, in addition to prompt detection, timely reporting, immediate isolation, early treatment, and efficient containment of Mpox transmission, ensuring the effective protection of high-risk populations through pre- and post-exposure vaccination is imperative. The present article provides a comprehensive review of current progress in Mpox vaccine development, offering valuable insights for MPXV prevention and control.

MPOX AND MPXV

Before 2021, the majority of Mpox were reported in tropical rainforest regions in Africa due to transmission of MPXV to humans by animals carrying the virus. However, since 2022, new cases have predominantly resulted from human-to-human transmission. Infections can occur through contact with patient fluids, damaged skin, mucous membranes (such as the mouth or throat), respiratory droplets, contaminated items, or injuries caused by animals carrying the MPXV[4].

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The incubation period for Mpox is 5–21 d, typically ranging from 6 to 13 d. In most cases, Mpox is a self-limiting disease characterized by skin and mucous membrane rashes. Rashes progress through various stages, including macules, papules, vesicles, pustules, scabs, and scab peeling. Patients with Mpox commonly experience symptoms such as fever, headache, back pain, muscle aches, and swollen lymph nodes[3].

MPXV has a double-stranded DNA genome of approximately 197 kb in length. Based on sequence characteristics, the virus is categorized into two evolutionary clades: Clade I, previously named the Congo Basin (CB) or Central African clade; and Clade II, previously named the West African (WA) clade, which is further subdivided into lineages IIa and IIb. The diseases caused by Clade I are more severe and associated with higher mortality rates than those caused by Clade II[5].

Following the declaration of smallpox eradication in 1980, Mpox emerged as the most serious threat caused by orthopoxviruses. The genetic sequences of orthopoxviruses, especially those encoding immunorelevant proteins, exhibit a remarkable degree of similarity, with viral particles harboring numerous epitopes that are either identical or closely related. The 24 membrane and structural proteins found in orthopoxviruses have the capacity to elicit antibody responses, with several of these proteins containing epitopes that trigger T-cell responses, offering cross-protection against other orthopoxviruses. Of note, pre-exposure smallpox vaccinations have been shown to exhibit an impressive 85% clinical efficacy against Mpox, further highlighting the cross-protective nature of these viruses[2,6].

The MPXV was first discovered in 1985 in a Mangabey monkey, while the first human Mpox case was reported in Africa in 1970. In 2003, Mpox spread from Africa to the United States. Before 2021, sporadic cases were reported in several European and Asian countries. However, a global Mpox epidemic erupted in May 2022[7]. As of October 19, 2023, 115 countries and regions had reported 91123 confirmed cases. In September 2022, the mainland of China confirmed the first imported case of Mpox[8]. From June to October 2023, China (excluding Hong Kong, Macau, and Taiwan) reported 1,530 cases of Mpox, indicating increased risks of local outbreak and covert transmission[9].

MPOX VACCINES

Approved Mpox Vaccines

Following this epidemic, the Food and Drug Administration of the United States and several European countries urgently approved the smallpox vaccines ACAM2000 and JYNNEOS[10], whereas Japan and Russia approved LC16m8 and OrthopoxVac (VACΔ6), respectively, for Mpox prevention[11,12] (Table 1).

ACAM2000 ACAM2000 is a second-generation smallpox vaccine, derived from the low neurovirulent first-generation smallpox vaccine strain Dryvax and produced using mammalian cell culture[13,14]. However, the vaccine is associated with various adverse reactions such as fever, headache, muscle pain, myocarditis, pericarditis, and post-vaccination encephalitis, posing a significant limitation to its widespread application[15]. Nonetheless, it may be used for emergency vaccination in individuals at high exposure risk.

JYNNEOS The vaccinia virus Ankara strain (modified vaccinia Ankara, MVA)-derived smallpox vaccine, also known as JYNNEOS in the United States, IMVANEX in the European Union, and

Table 1. Smallpox vaccines used as Mpox vaccines[6]

Mpox vaccine	Licensed	Replication	Pre-exposure indications	Post-exposure indications
ACAM2000	USA	Replication competent	High-risk occupational groups; not recommended for the general population	Close contact with someone or something that has Mpox
JYNNEOS	USA	Replication- deficient modified	High-risk occupational groups; preferred for those with contraindication	Same as above; high-risk occupational groups; preferred for those with contraindication; pregnant women
LC16m8	Japan	Attenuated, minimally replication competent	High-risk occupational groups; preferred for those with contraindication; not recommended for the general population	Same as above; high-risk occupational groups; preferred for those with contraindication; pregnant women not inoculated with JYNNEOS; licensed in Japan for use in children
OrthopoxVac	Russia	Attenuated	High-risk occupational groups	Close contact with someone or something that has Mpox

IMAMUNE in Canada, is obtained through continuous passage into cells, resulting in the loss of approximately 10% of its genome[13]. Compared with the parental strain, MVA exhibits six major genomic deletion regions, including deletions, truncations, or mutations in virulence genes such as C12L, K1L, N1L, M1L, and A53R[14]. Administering two doses of JYNNEOS demonstrated immunogenicity similar to that induced by ACAM2000[16], but with fewer side effects. The MVA vaccine exhibits favorable safety and immunogenicity profiles, with low incidences of both severe adverse events and adverse events overall[17]. Therefore, it is also used in HIV-positive individuals and those prone to eczema[18]. Research indicated that MVA was well-tolerated in children and induced the production of poxvirus IgG antibodies, as well as antibodies against whole modified vaccinia virus Ankara and a select pool of conserved pan-Poxviridae peptides, for up to 15 weeks post-vaccination[19]. Additionally, MVA vaccination has demonstrated some protective efficacy in high-risk populations[20,21]. However, a study indicated that administering two doses of MVA resulted in lower levels of Mpox neutralizing antibodies[22], which may lead to breakthrough Mpox infections[23].

LC16m8 LC16m8 is another third-generation smallpox vaccine developed from the Lister strain through multiple passages in rabbit kidney cells, which resulted in changes in the viral genome and alterations in the functionality of membrane protein B5, thereby reducing its pathogenicity[6]. LC16m8 induces immune responses of similar intensity to those of the Lister strain[24,25], with only a few recipients experiencing mild adverse reactions and no severe adverse effects[26,27]. LC16m8 effectively shields nonhuman primates from Mpox viral infection[18], exhibiting a robust neutralizing antibody response against MPXV in healthy adults[28]. The LC16m8 vaccine approved by Japan has fewer side effects but induces a weaker immune response. Its protective effect is equivalent to that of the original strain. In Japan, LC16m8 can also be administered to children[11].

OrthopoxVac The State Research Center of Virology and Biotechnology (SRC VB) VECTOR of Russia (SRC VB VECTOR) has also carried out relevant research and successfully obtained the highly attenuated VACΔ6 derivative strain. The vaccine has completed comprehensive preclinical research and clinical trials, demonstrating a good effect in preventing smallpox and other orthopoxvirus infections. In November 2022, OrthopoxVac, a live vaccine prepared using VACΔ6, was licensed in Russia, providing a new option for the epidemic prevention and control efforts of the country. OrthopoxVac (VACΔ6)[12] is a recombinant attenuated derivative of VACΔ6, featuring targeted knockdown of six genes, A56R, B8R, J2R, C3L, N1L, and A35R. VACΔ6 is based on the LIVP VACV strain, which is utilized in the Russian Federation for human vaccination. The VACΔ6 strain is significantly less reactogenic and neurovirulent and more immunogenic than the parent LIVP strain. Double subcutaneous injection of the recombinant VACΔ6 variant induced significantly higher levels of virus-neutralizing antibodies in mice than did the parental LIVP strain and provided complete protection to mice against the highly pathogenic ectromelia virus (ECTV), as opposed to the effect of the LIVP strain in this model, which is approved as a smallpox vaccine[29].

Other Potential Vaccinia Virus Vaccines

In China, the vaccinia virus Tian Tan strain (VTT) has been demonstrated to be highly effective in the prevention of smallpox. Remarkably, specific IgG antibodies against VTT remain detectable even more than 40 years after vaccination with the strain[30]. As a precautionary measure, China has maintained a stockpile of VTT strains (strain number: 752-1) for emergency smallpox vaccinations. However, it is crucial to acknowledge the potential for adverse reactions following VTT vaccination, which may include localized reactions at the vaccination site, post-vaccination encephalitis, allergic purpura, necrotic pustules, and systemic effects. Specific VTT proteins, including A27, L1, D8, B5, and A33, have been found to elicit cross-immune responses against MPXV in the range of 60%–95%, thereby contributing significantly to the effective prevention of MPXV infection. Notably, individuals born before 1980 exhibit a seroprevalence of 58.8% (10/17) for neutralizing antibodies against MPXV[31,32].

Using molecular biology techniques, 26 gene segments, specifically including C19L–K3L, were deliberately removed from VTT, leading to the creation of a non-replicating Tian Tan vaccinia virus (NTV). NTV is capable of replicating within chicken embryo fibroblast cells, but is unable to do so in most human cells. Comparable to MVA, NTV exhibits a lower virulence than its parent strain. The advancement of NTV serves to mitigate the potential security risks that arise from relying on viral proliferation for replicating vectors, as documented in previous studies[33-38]. Therefore, NTV stands out as a promising contender for the safe and effective development of next generation smallpox vaccines[39]. Furthermore, NTV has demonstrated cross-neutralization of MPXV in both mouse and rhesus monkey models. In mice, intramuscular administration of NTV elicited comparable levels of neutralizing antibodies against VACV and cross-neutralization against MPXV, akin to those achieved through skin-scratch inoculation with VTT[40]. These results provide compelling evidence for the further development of NTV as a promising candidate for Mpox vaccines. On July 13, 2023, the Beijing Institute of Biological Products Co., Ltd. received approval from the Center for Drug Evaluation of the National Medical Products Administration of China (NMPA) for their application to use NTV as a candidate Mpox vaccine (Acceptance Number: CXSL2300478). This milestone represents a significant leap forward in utilizing NTV for Mpox prevention. In addition to NTV, China has conducted extensive research on various modifications of VTT to establish it as a safe vaccine vector. These modifications involve the deletion of VTT genes associated with virulence, including M1L-K2L, C8-K3L, C7L-K3L, and A35R. Through these genetic manipulations, the virulence of VTT has been reduced to varying degrees, laying a solid foundation for the development of a new generation of smallpox vaccines[41-43].

KVAC103 is a naturally attenuated strain obtained from the second-generation smallpox vaccine, Lancy-Vaxina (originating from the Lister strain), by serial passaging (103 passages) in Vero cells. KVAC103 harbors a 19.5 kb deletion at the left terminal region (including C9L-F2L) and a 2.5 kb deletion at the right terminal region of its genome. In vivo studies have demonstrated significantly reduced neurovirulence and dermal toxicity, along with effective induction of protective immune responses against the WR strain[44,45]. Hence, it holds potential value as a Mpox vaccine.

NYVAC is a recombinant vaccine derived from the Copenhagen strain of vaccinia virus (VACV-COP) following the precise deletion of 18 open reading frames (ORFs). The deleted region encompasses 12 ORFs from C7L to K1L, as well as J2R (TK), B13R, B14R, A26L, A56R (HA), and I4L, which include genes related to pathogenicity, virulence, and host range. This deletion results in a significant reduction in the replication capacity of the strain on various human and mammalian cell lines, incapacity to disseminate in immunodeficient mice, markedly attenuated virulence, and inability to generate progeny viruses. While NYVAC exhibits improved safety, its immunogenicity is lower than that of the Lister strain vaccine. Even after two doses of NYVAC vaccination, the antibody levels induced by NYVAC are lower than those induced by Lister. Consequently, NYVAC is unlikely to be a candidate for a novel vaccine. Although safe for patients with contraindications, it does not elicit a sufficiently robust response to be effective[46,47].

Subunit, Peptide and Nucleic Acid-Based Vaccines

With the rapid development of biotechnology, the research and development strategies for new vaccines based on the vaccinia virus are constantly innovating and optimizing. Among the approximately 200 genes encoded by the vaccinia virus, one-third to half are non-essential for replication, and the proteins encoded by these genes affect host range, virulence, or immune evasion. Strategies targeting the host range of vaccinia virus and inhibiting immune response genes have shown great potential in the development of new vaccines.

In addition to using the vaccinia virus to prevent Mpox, several subunit Mpox vaccines, including recombinant proteins, and nucleic acid vaccines, are being developed globally using genetic engineering techniques. These vaccines utilize components such as nucleic acids or proteins to induce effective immune responses, combining multiple antigenic sites to elicit strong immune-protective responses[18,48-50]. The creation of a new-generation Mpox vaccine predominantly entails harnessing specific proteins, namely A29 and M1, derived from the MPXV intracellular mature virus (IMV), as well as particular proteins, A35 and B6, originating from the extracellular enveloped virus (EEV) or their corresponding homologs in VACV. These proteins, along with designated protein nucleic acids or antigenic sites, are employed in the construction of protein subunits, mRNA, or antigenic site vaccines. Studies have shown that the combination of IMV- and EEV-specific immunogens confers superior protection compared with the use of either immunogen individually.

Protein Subunit Vaccines Protein subunit vaccines are prepared by purifying the expressed target protein, resulting in a vaccine that is safer than attenuated vaccines because of non-replication and induction immune protection. Immunization of non-human primates, specifically crab-eating macaques, with a combination of A33, B5, L1, and A27 proteins from VACV together with adjuvants, conferred protection against lethal intravenous challenge with MPXV[51]. Fogg et al.[52] utilized the A33, B5, and L1 proteins, while Davies et al.[53] utilized a recombinant H3 protein from VACV for immunizing mice. They observed a significant increase in neutralizing antibody titers, which protected mice against WR challenge. Yang et al.[54] immunized mice with the A29, M1, A35, and B6 proteins from MPXV. The antibody titers in mouse sera increased sharply after the initial boost, while the ability of immune cells to produce interferons increased earlier than that in mice immunized with VTT, enhancing Th1 cell-mediated cellular immune levels. The vaccine-induced neutralizing antibodies significantly inhibited MPXV replication in mice, thereby reducing organ pathology. The novel Mpox vaccine DAM, developed by Wang et al.[55], which was fused with M1 and A35 antigens derived from the Mpox virus, exhibited stronger A35-specific and M1-specific antibody responses and *in vivo* protective efficacy against vaccinia virus (VACV) than those achieved using co-immunization strategies while eliciting 28 times stronger response than that induced by live VACV vaccine. Furthermore, it protected mice from a lethal VACV challenge with a safety profile.

Epitope Peptide Vaccines Epitope peptide vaccines typically utilize reverse vaccinology to predict and select antigenic sites (epitopes), that are specifically recognized by immune cells, such as helper T lymphocytes, cytotoxic T lymphocytes, and B cell epitopes; this process harnesses the advantages of both bioinformatics and vaccinomics. Vaccines constructed from antigenic epitopes selected from three target proteins of the MPXV, A35, B6, and H3 from EEV and IMV exhibited strong stability in binding to Toll-like receptors and major histocompatibility molecules. Immunological simulation experiments indicated the effective induction of protective immunity[56]. Quach et al.[57] evaluated 116 immunogenic peptides within the NYCBH protein and selected the four most immunogenic peptides (encoded respectively by F11L, F1L, G5R, and J3R) to prepare a vaccine. Mice immunized with these peptide segments and challenged with a lethal dose of vaccinia virus exhibited 100% protection. Additionally, five months after the second immunization, the T-cell immune response induced by the short peptide vaccine was five times higher than that induced by the live virus vaccine.

Nucleic Acid-Based Vaccines Nucleic acid-based vaccines have been developed based on DNA or mRNA sequences of antigens that strongly stimulate cellular and humoral immune responses. During the Coronavirus disease 2019 pandemic, they have attracted more attention from pharmaceutical companies, experts, and researchers. DNA vaccines containing VACV immunogens (L1R, A27L, A33R, and B5R) have demonstrated the ability to prevent lethal MPXV challenge in non-human primates and reduce disease severity from aerosol challenge by preventing poxvirus infection[58,59]. mRNA vaccination with the A29L, E8L, M1R, A35R, and B6 immunogens elicited specific T-cell responses against MPXV[60]. Furthermore, vaccines, using A29L, M1R, A35R, B6R, H3L, and E8L among others, induced the production of MPXV-specific IgG antibodies and vaccinia virus-neutralizing antibodies in mice, eliciting a highly robust cell-mediated immune response, establishing long-lasting immune memory, and providing protection against lethal doses of the vaccinia virus[61-64].

CONCLUSION AND OUTLOOK

Currently, four vaccines based on the vaccinia virus have been approved for Mpox prevention. The vaccinia virus has played a pivotal role in the eradication of smallpox and has since reemerged as a focal point in vaccine research and development. Thanks to technological advancements, notable progress has been achieved in creating novel vaccinia virus vaccines. However, comprehensive clinical trials are still required to firmly establish their safety and efficacy.

Vaccine development endeavors primarily concentrate on two fronts: refining traditional vaccines and forging ahead with innovative vaccine designs. Although the smallpox vaccine is widely considered safe for the majority of individuals, rare cases of severe adverse reactions have been reported, emphasizing the importance of continual monitoring and rigorous safety assessments. Simultaneously, continued modification of the vaccinia virus is required for ensuring its suitability for a broader spectrum of populations.

Furthermore, the adequate supply and strategic stockpiling of Mpox vaccines are paramount in ensuring a swift and effective response during Mpox outbreaks. Nonetheless, this endeavor presents significant challenges due to the intricate nature of vaccine production and storage, coupled with the global declaration of smallpox eradication. Hence, accelerating the development and rigorous testing of next-generation vaccines, such as those based on proteins and nucleic acids, to assess their safety, efficacy, and feasibility for large-scale use, is imperative.

No licensed Mpox vaccine is currently available in China. As an emergency stockpile for the prevention of smallpox, China has developed a smallpox vaccine using the vaccinia virus Tian Tan strain and multiple novel technologies, establishing a robust foundation for the future development of Mpox vaccines. Nevertheless, a research gap is apparent when benchmarked against international standards, and like many other countries internationally, China also needs to optimize conventional vaccines and innovate novel vaccine types. Accelerating the progress in Mpox vaccine development by increasing investments in funding and manpower, coupled with robust technical support, is imperative for achieving this goal.

Competing Interests The authors declare no competing interests.

Ethics This article constitutes a comprehensive review of the existing literature and does not encompass any original research involving human or animal participants conducted by the authors themselves.

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