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CRISPR, Recombinant DNA, and LAMP-Based Platforms for Monkeypox: Emerging Tools for Diagnosis and Therapeutic Development

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Abstract

Monkeypox, a zoonotic viral infection caused by the Monkeypox virus of the Orthopoxvirus genus, has recurred as a worldwide public health concern after recent outbreaks outside of its classical endemic areas in Central and West Africa. The resurgence has demanded a thorough reassessment of its epidemiology, mode of transmission, and clinical presentation. Considering these challenges, recombinant DNA technology (rDNA) has emerged as a candidate for developing vaccines, diagnostics, and therapeutic agents, as shown in the successful control of other viral diseases like hepatitis B and HPV. The combination of rDNA and CRISPR-based methods presents a revolutionary strategy for monkeypox surveillance and control. CRISPR-Cas systems – particularly Cas12a and Cas13a – have been shown to be effective for sensing MPXV with high sensitivity and specificity through fluorescence, lateral flow, and smartphone-based readouts. Challenges still exist in crRNA design, assay optimization, and standardization. Furthermore, Loop-mediated Isothermal Amplification (LAMP) directed against A27L and F3L genes of MPXV has shown encouraging sensitivity and specificity and was superior to traditional PCR approaches in some contexts. These diagnostic gains are matched by continued clinical trials for both conventional and emerging vaccine platforms – including MVA-BN, JYNNEOS, and RNA-based constructs such as BNT166a – and antiviral drugs like Tecovirimat. All the same, rapid diagnosis and availability of therapeutics remain significant challenges to monkeypox management. This review brings out the important contribution of new biotechnological tools, specifically rDNA, CRISPR, and LAMP, in revolutionizing existing diagnostic and treatment methods, hence leading to efficient disease management and future outbreak readiness.

Keywords: Assay optimization, CRISPR, Loop-mediated isothermal amplification (LAMP), monkeypox virus, Recombinant DNA technology, tecovirimat

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INTRODUCTION

Monkeypox is an emerging zoonotic viral infection caused by the virus of the genus Ortopoxvirus. It was recognized for the first time in laboratory monkeys in 1958, it has emerged more recently, in various ways, and now as a major public health concern, mainly. It causes of the following recent outbreaks [1]. It is traditionally endemic to Central and West Africa and the worldwide spread of monkeypox calls for going deeper into its epidemiology, understanding its transmission dynamics, and further developing clinical implications [2].

Numerous vaccines and diagnostic tools for the monkeypox virus can be created using recombinant

DNA (rDNA) technology. Recombinant DNA Technology is one of the major breakthroughs in biotechnology and medicine. It allows for the manipulation of genetic material to its most important benefits like vaccine production, gene therapy, and diagnostic industries [3]. Historical review shows that such technology has been very valuable to prevent infectious diseases through the development of effective vaccines against viruses like hepatitis B and human papillomavirus. It follows that the implementation of RDT in the context of monkeypox is both timely and crucial, since RDT holds such promise of revolutionizing treatment and prevention strategy [4].

The challenges include speedy diagnoses that are usually challenging and lack of therapeutic options in current management of monkeypox. The current resurgence of monkeypox calls for novelty in conducting surveillance, treatment, and prevention efforts. Time has come to revolutionize traditional approaches from the past to overcome the threats posed by novel emerging infectious diseases, like the current monkeypox outbreak, that requires advanced biotechnology solutions [5].

In this review, the renewed emergence and worldwide dissemination of monkeypox highlight the imperative to develop novel and effective approaches to counter this zoonotic infection. Recombinant DNA technology is a formidable weapon in this endeavor, holding the promise to accelerate vaccine development, refine diagnostic precision, and introduce new therapeutic agents. Since conventional methods are lacking in tackling the challenges of today, incorporating innovative biotechnological technologies, such as rDNA technology, is crucial in enhancing public health measures and stemming the effects of new infectious diseases like monkeypox.

MONKEYPOX VIRUS

Monkeypox is a well-known disease caused by the Monkeypox virus (MPXV). It is the most virulent ortopoxvirus that has challenged human health since the eradication of the Variola virus. MPXV, which is closely related to the Variola virus, can cause severe natural PO disease in human beings. Two distinct MPXV Klades are identified by total genome sequencing (WGS). Clads in Central Africa have 10.6% mortality and those in West Africa have 3.6% mortality. It was thought that MPXV might fill an ecological niche [6].

Human Monkeypox, especially in the Congo Basin, typically has symptoms identical to the modest, normal, natural po. Following a symptom-free incubation period of about 2 weeks, infected individuals get fever, followed by a general rash. Monkeypox and natural po are contagious and deadly, but Monkeypox is less lethal (10%) than Variola majors, and less effective in human infection [7] Democratic Republic of the Congo (DRC). The US outbreak also proved the capacity of the virus to infect new hosts and travel across the globe [8].

History of Monkeypox

Monkeypox was first observed in 1958 by Virologist Preben Christian Alexander Von Magnus in Denmark during investigations of smallpox-like outbreaks in laboratory monkeys. Phylogenetic analysis linked the virus to the West African clade, though some researchers believe it may have evolved earlier [9]. The initial human case was documented in 1970 in the Democratic Republic of the Congo (DRC). with subsequent cases appearing in Liberia, Sierra Leone, and Nigeria. Numerous outbreaks have since been reported, particularly in the DRC and Nigeria, with Two primary groups identified: the central African (Congo Basin) and western African clades [10]. The Timeline of MPV from origin is given in Figure 1.

The first monkeypox outbreak outside of Africa happened in the United States in 2003, and it was connected to prairie dogs that were infected. with no human-to-human transmission reported [11]. Though endemic to African rainforests, monkeypox has spread to other regions over time, partly due to reduced herd immunity after the cessation of the smallpox vaccination program in 1980. The virus offers important insights into host-pathogen interactions and the evolution of ancient viruses [12, 13].

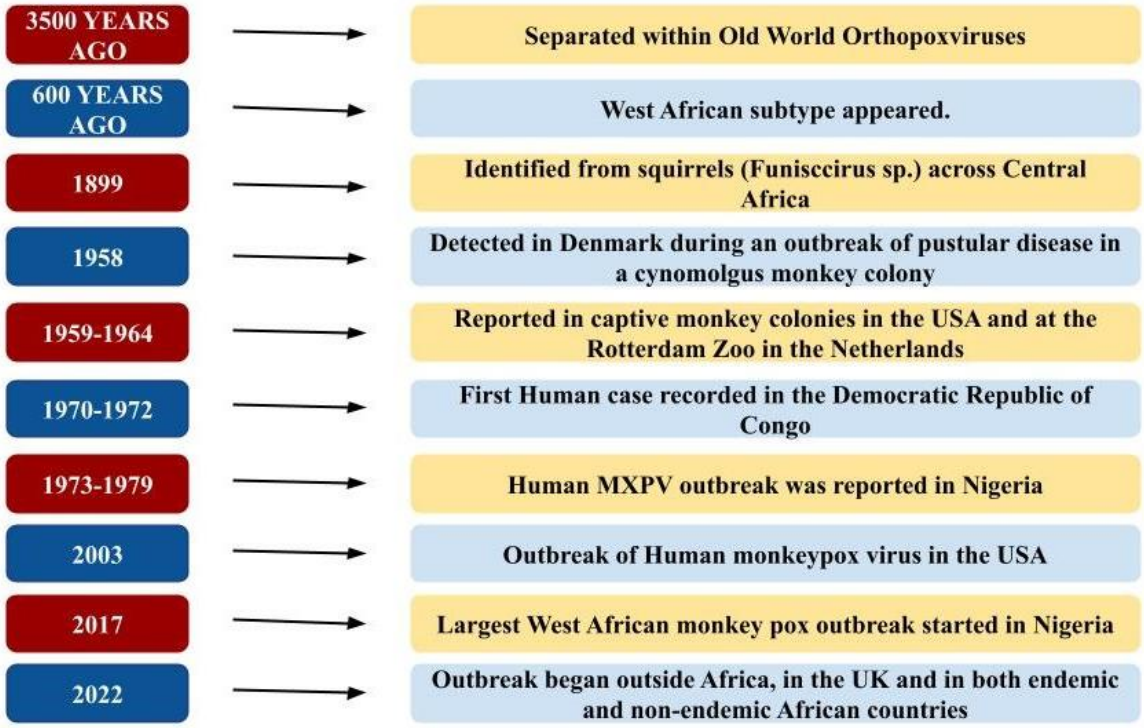

Figure 1. Historical timeline of the monkeypox virus.

Types of Monkeypox Virus

The monkeypox virus (MPXV) consists of two primary clades, labeled clades I and II, as well as two subdivisions within clade II, known as subclades IIa and IIb as shown in Figure 2. Originally, Clades I and II were categorized as the central African and west African clades, reflecting genetic, clinical, and geographic distinctions [14]. To avoid geographic labels, a new classification approach was recommended, which has since been endorsed by the WHO and the International Committee on Taxonomy of Viruses. Clade II now includes two subclades: IIa, previously the West African clade, and IIb, a newly recognized subgroup. Clade IIb has shown increased human-to-human transmission compared to Clades I and IIa and has potentially been circulating in various global regions for some time. While the clustering of clade IIb sequences aligns with samples from 2017 to 2019, limitations in diagnostic and surveillance capacities could affect these findings. Between 1970 and 2005, only ten monkeypox cases were reported in West Africa, yet the 2003 U.S. outbreak surpassed this with 47 probable or confirmed cases, all linked to zoonotic transmission. The monkeypox outbreak in 2022 has not been linked to any known animal source [15].

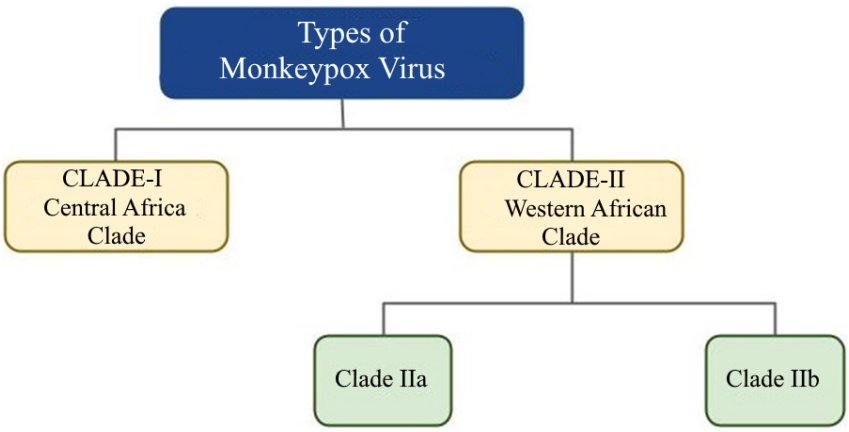

Figure 2. Distinct clades of MPXV.

Epidemiology of Monkeypox

Causative Agent and Virology

Monkeypox is caused by the monkeypox virus (MPXV), a large double-stranded DNA virus classified under the Poxviridae family and the Orthopoxvirus genus. This family also includes other viruses that affect humans, such as the cowpox virus, the vaccinia virus (used in smallpox vaccines), and the variola virus, which causes smallpox [16]. Although the two viruses are genetically related, the impact on human health of the monkeypox virus is greater than that of the variola virus, which causes smallpox.

The monkeypox virus is known to belong to two genetic clades: the Congo Basin (Central Africa) Clade, which has a documented history of greater virulence, and an increased alteration associated with greater disease severity and the West African clade which is associated with milder disease and lower mortality [17].

History and Emergence

The first documented case of monkeypox in humans was reported in 1970 in the Democratic Republic of Congo, at a time when efforts to eradicate smallpox were nearing completion [13]. The disease's name is derived from laboratory monkeys, which were the first to contract the disease in 1958. now referred to as Monkeypox, but a disease of rodents [18]. The disease has its endemic areas in Central and West Africa; however, it still covers its impacts. However, after the 1980s, when Smallpox vaccination was regularly practiced, helping to protect against Orth poxviruses, the incidence of monkeypox increased in public concern, particularly in those regions with limited coverage with a vaccination program [19].

Transmission Dynamics

Monkeypox has been identified as a zoonotic infection, where transmission mainly happens from animal reservoirs like rodents and possibly primates to humans through direct contact with blood, body fluids, skin lesions, or mucosal surfaces. Close physical contact, respiratory secretions, and contaminated materials aid human-to-human transmission, where the most common mode of transmission is direct contact. While airborne transmission through big respiratory droplets has been documented, it does not seem to be as effective. Recent outbreaks among Men who have Sex with Men (MSM) indicate the potential for sexual transmission, and this points to the ability of the virus to adapt to new socio-cultural practices. There are two modes of transmission for monkeypox. These are:

Zoonotic Transmission (Animal to Human)

Due to the transmission of the monkeypox virus by animals, it is referred to as a zoonotic disease [20] whose animal reservoirs are rodents such as squirrels, rats, and possibly simian species. Currently, the specific animal that acts as the natural reservoir is unknown, but rodents are likely the most important. Monkeypox can spread through direct contact with the blood, bodily fluids, skin lesions, or mucous membranes of infected animals [21].

Hunting and handling of bushmeat in areas with widespread occurrence of HIV, such as central and western Africa, are also important in zoonotic transmission [22].

Human-to-Human Transmission

Direct contact with body fluids from lesions, respiratory secretions, or mucosal surfaces of infected individuals is a primary risk factor for human-to-human transmission of monkeypox. This may include skin contact with the infected skin rashes or lesions, biting, and contact with fomites such as bedding or clothes. Figure 3 shows the transmission of Monkeypox from one organism to another [23]. Airborne droplet infection: contact of the infected person with a healthy person within less than 1 meter can lead to the transmission of the infection through large droplets in the aerosols. This mode of transmission is thought to be less effective than contact-based modes of human-to-human transmission [24].

It seems that sexual contact is one of the causes of transmission of the disease; in particular, the infection caused in 2022 and more recently in non-endemic populations among Men having Sex with Men (MSM) appears to be the pattern of intimate contact that includes sexual activity. This new mode of transmission is still subject to investigation but also confirms the adaptability of the virus to new socio-cultural habits [25].

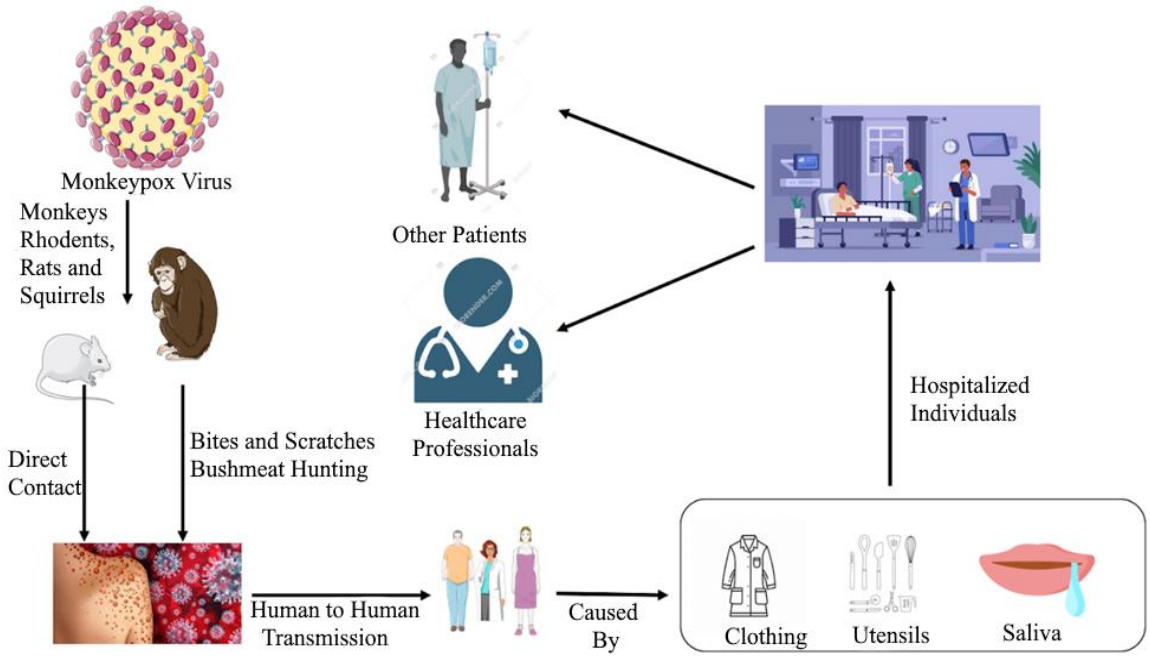

Figure 3. Routes of monkeypox virus transmission between hosts.

RECOMBINANT DNA TECHNOLOGY

Recombinant DNA (rDNA) technology is a genetic engineering method that involves introducing a specific gene into a carrier, such as a plasmid or bacteriophage, to form a new DNA molecule. This enables the mass production of the gene or its corresponding protein [26]. By using a genetically modified vector, the technology alters the phenotype of the host organism by incorporating the new DNA into its genome. Essentially, this method introduces foreign DNA into the genome where the target gene resides [27]. Figure 4 represents the process of rDNA technology. In recombinant DNA technology, the desired DNA fragment is inserted into a vector, a separate DNA molecule. This recombinant vector is then introduced into a host cell, where it replicates and produces multiple copies of the gene. The process includes several essential steps: isolating the gene of interest and the vector, cutting the DNA at specific sites, joining DNA fragments, transferring the recombinant DNA into the host cell, cloning, identifying cells containing the recombinant DNA, and analyzing gene expression [28].

The primary tools involved in rDNA technology are Restriction enzymes, DNA Ligase, Vectors, and hosts. Figure 5 gives the graphical representation of rDNA technology. The first step in recombinant DNA technology involves generating gene clones using a class of enzymes called restriction enzymes [29]. These enzymes specifically recognize and cut selected target regions on the DNA molecule. The restriction enzyme used in this process is known as Restriction Endonucleases (RE), which cut DNA at specific internal sites, known as recognition sequences or recognition sites. Once the DNA is cut, the resulting fragments have either blunt ends or sticky ends, which are joined by another enzyme called DNA ligase [30]. This enzyme ligates the double-stranded breaks in the DNA, requiring ATP for the process. To replicate or clone the target gene, it is inserted into a vector, which can autonomously replicate in the host cells. The vector must contain an Origin of Replication (ORI) to function. Common vectors include plasmids, phages, or viruses, which are small DNA molecules [31]. Various hosts can be used in recombinant DNA technology, including bacteria, yeasts, plant cells, mammalian cells, and

fish cell lines. Due to their availability, ease of cultivation, and well-characterized genomics, *E. coli* is the most used bacterial host, while *Saccharomyces cerevisiae* is frequently used as a yeast host due to its well-known genomics and accessibility [32].

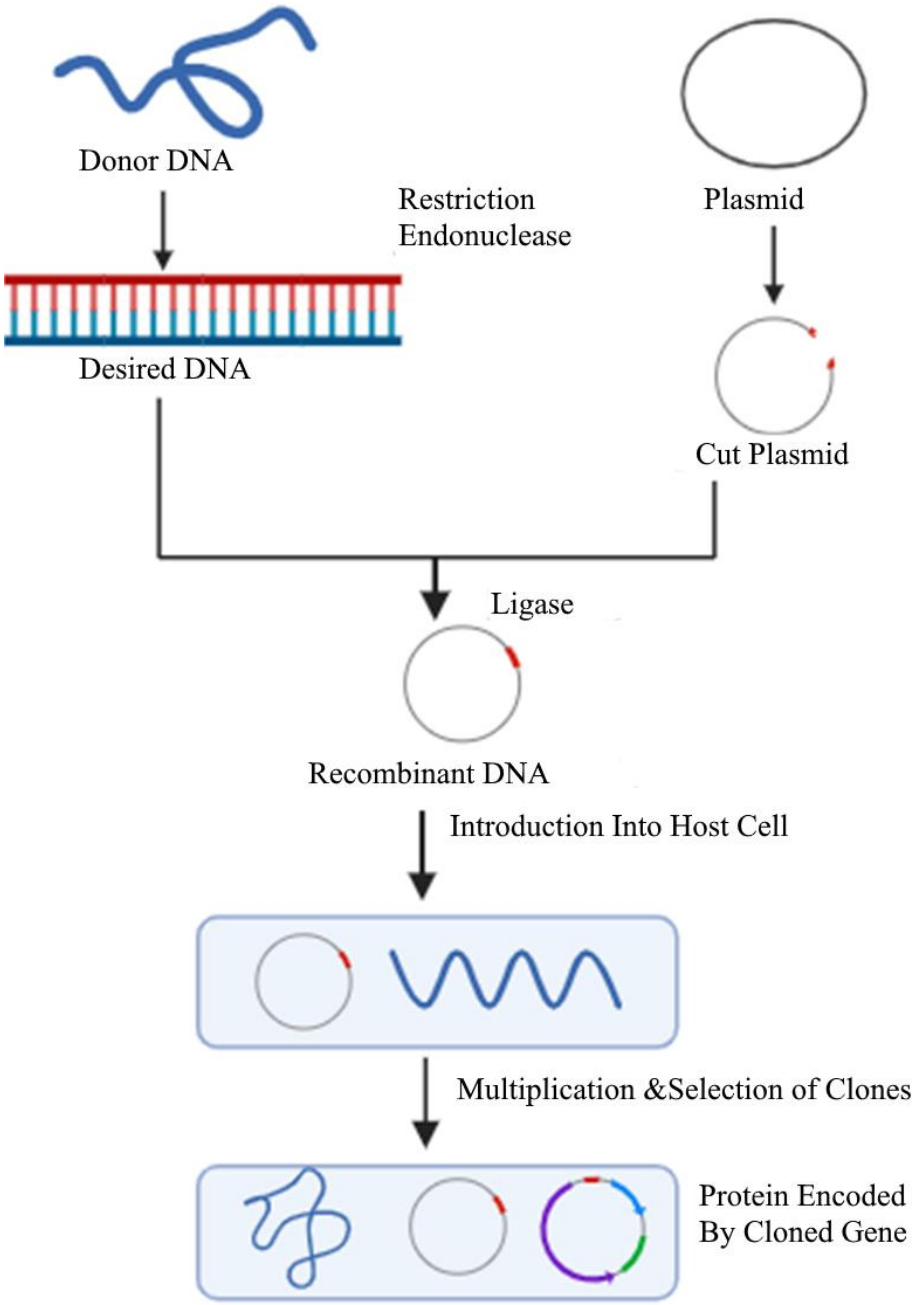

Figure 4. Schematic representation of rDNA technology.

There are three methods of DNA Recombination. Transformation, the first one, is the process by which bacteria take up external DNA fragments as single strands and incorporate them into their chromosome through homologous recombination [33]. Conjugation is a mechanism of genetic transfer where a donor cell directly transfers plasmid DNA or other mobile genetic elements, including segments of chromosomal DNA, to a recipient cell. This process typically occurs through physical contact facilitated by a specialized structure known as the conjugation or sex pilus [34]. In contrast, transduction occurs when donor bacterial DNA is introduced into a recipient bacterium via a bacteriophage, a virus that infects bacterial cells [33].

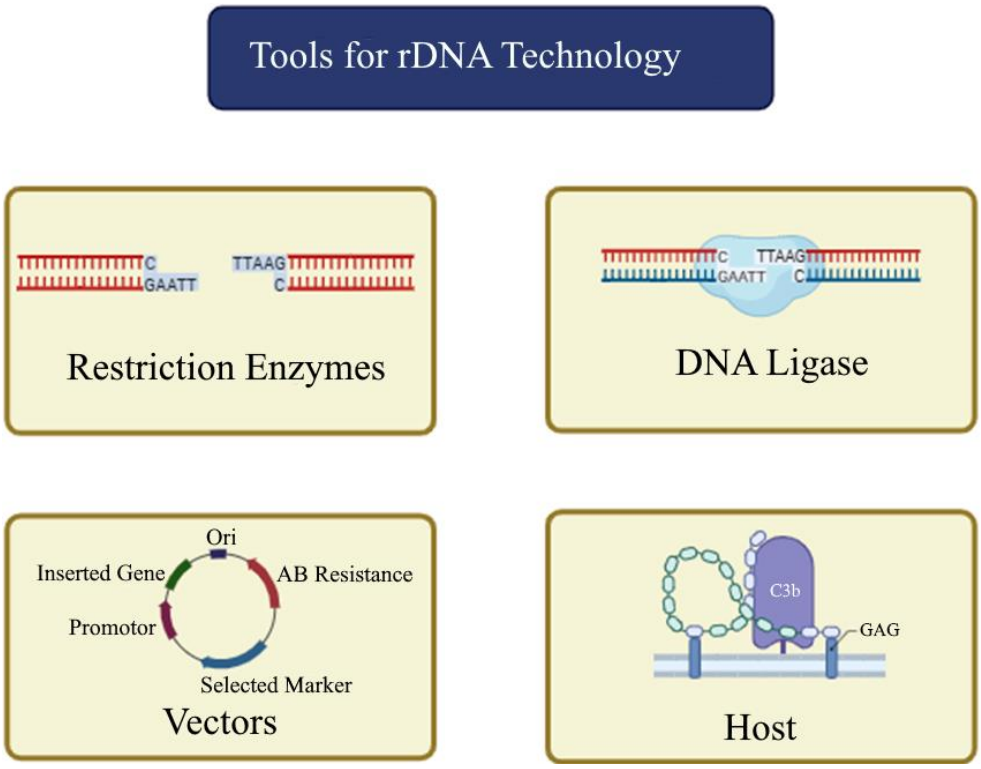

Figure 5. key tools utilized in recombinant DNA technology.

CRISPR Technique for Monkeypox Virus Detection

Originally introduced as a revolutionary gene-editing system, the CRISPR/Cas mechanism – consisting of clustered regularly interspaced short palindromic repeats and associated proteins – has since been adapted for various applications, including molecular diagnostics and biosensing [35]. The CRISPR/Cas12a platform merges with biorecognition, where Cas12a crRNA targets specific sequences, with signal transduction, allowing the activated Cas12a to indiscriminately cleave nearby single-stranded DNA reporters [36]. This method has proven effective in detecting various viruses, such as SARS-CoV-2, African swine fever virus (ASFV), and human immunodeficiency virus (HIV), and is valued for its mild reaction conditions, ease of use, high sensitivity, and robust signal amplification. While CRISPR-based assays for the monkeypox virus (MPXV) have been developed, limitations in systematic primer and crRNA design and screening have constrained their sensitivity, indicating the need for further investigation into CRISPR's potential in MPXV detection and prevention [37].

Unlike conventional nucleic acid recognition techniques that rely on sequence complementarity between the target and a complementary probe, the CRISPR/Cas system follows a two-step recognition process involving a Cas effector protein and guide RNA (gRNA). The gRNA directs the Cas nuclease to the target sequence, forming a complex that activates trans-cleavage activity [38]. In the Cas12a system, a single crRNA serves as the guide, binding to the Cas nuclease and leading it to the target gene adjacent to a protospacer adjacent motif (PAM), a short sequence of 2–5 base pairs essential for CRISPR activity. Upon PAM recognition, Cas12a is activated by double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA), resulting in the trans-cleavage of nearby nonspecific ssDNA [39].

The key properties of three Cas enzymes (Cas12a, Cas12b, and Cas13a) are used in MPXV detection. The properties include spacer length, requirement for tracrRNA, PAM/PFS recognition, substrate specificity, cleavage pattern, and trans-cleavage substrate. Each enzyme has distinct characteristics that make it suitable for specific applications in MPXV detection [40]. Cas12, which can target both single-stranded and double-stranded DNA, is the most commonly used enzyme in MPXV detection platforms.(Cas12b has been chosen over Cas12a in some instances due to its less restrictive PAM sequence (TTN),

compared to the more specific Cas12a PAM sequence (TTTN) [41]. Some researchers argue that CRISPR/Cas12a detection systems are more accessible due to their requirement for a shorter crRNA sequence (around 41 nucleotides), whereas Cas12b requires a longer gRNA, which consists of both crRNA and trans-activating CRISPR RNA (tracrRNA), totaling about 111 nucleotides. Beyond the Cas12 family, the Cas13a enzyme has been utilized for MPXV detection by attaching a label to the T7 promoter sequence at the 5' end of the upstream primer. This enables the recombinase-aided amplification (RAA) product to undergo transcription into RNA with the help of T7 transcriptase [42].

Among CRISPR-based diagnostic technologies, Cas12a and Cas13a proteins are particularly notable for their high sensitivity, with Cas12a often paired with Recombinase Polymerase Amplification (RPA) to detect viral targets like F3L and B6R [43]. The detection methods are diverse, encompassing fluorescence readouts, lateral flow strips, UV and blue light, and advanced tools such as smartphone-assisted imaging and chemiluminescence analyzers. Sensitivity ranges widely, from single copies per microliter to higher levels, influenced by factors like assay complexity, light sources, and the use of RPA amplification [44].

For instance, when Cas12a is combined with RPA, it enables detection through both fluorescence and lateral flow, achieving sensitivities from 1–10 copies/μL in two-step processes or 100 copies/μL in single-step setups. Similarly, Cas13a with RAA, paired with UV or fluorescence readout, can detect as few as 4 copies. Emerging platforms like CRISPR-CUBE, CRISPR-SPR-FT biosensors, and smartphone-based imaging further enhance detection limits, reaching femtomolar (fM) and attomolar (aM) levels in simulated clinical and plasmid samples [45]. The flexibility and specificity of these CRISPR-based diagnostics make them particularly valuable for use in field and clinical environments, especially in settings where rapid and accurate detection is essential [46]. Result readout is a critical element in CRISPR-based diagnostics, as it enables direct visualization of detection outcomes through signal output. Widely adopted methods, such as fluorescence readouts, UV or blue light, and lateral flow strips, provide convenient options but can fall short in sensitivity [47]. To improve detection of low-concentration targets and enhance signal accuracy, several advanced strategies have been investigated [48].

Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a simple and efficient technique for detecting nucleic acids. It relies on Bst DNA polymerase to enable continuous strand displacement, allowing for rapid amplification. Originally introduced by Notomi et al., LAMP-based methods have since been adapted for diagnosing a wide range of pathogens, including viruses, bacteria, parasites, and fungi [49].

In the study, researchers focused on specific regions of the A27L and F3L genes in the monkeypox virus (MPXV) because these areas are identical within the MPXV genome but differ significantly from those in other related viruses. Using DNA from a pseudo virus that included these target sequences, they tested and optimized conditions for a loop-mediated isothermal amplification (LAMP) assay – a method used to quickly detect the presence of specific DNA sequences [50]. To achieve this, they designed five sets of primers for each gene and evaluated their effectiveness at detecting the A27L and F3L genes. Out of these sets, two primers, A27L-1 and F3L-1, showed the fastest response at 63°C, making them the best candidates for further testing. The researchers then fine-tuned the temperature settings, experimenting within a narrow range from 60°C to 65°C, to determine the ideal conditions for the LAMP assay. They found that 63°C worked best for detecting the A27L and F3L genes, so this temperature was used for all subsequent tests [51].

LAMP Assay Sensitivity

DNA from the MPXV pseudo virus, which contained target sequences for the A27L and F3L genes, was extracted using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) and serially diluted in 10-fold steps to achieve concentrations ranging from 10^7 copies/μL to 1 copy/μL. This DNA was used as a template to assess the sensitivity of the LAMP assay. With primer set A27L-1, detection times varied

from 16 minutes at a concentration of 10^7 copies/μL to 31 minutes at 10^1 copies/μL. For primer set F3L-1, detection was achieved between 18 minutes at 10^7 copies/μL and 55 minutes at 10^1 copies/μL. Results obtained from both turbidimetric, and color-based visual detection methods were consistent. Both primer sets, A27L-1 and F3L-1, reached a detection threshold of 20 copies per reaction mixture, whereas conventional PCR exhibited sensitivities of 2×10^3 and 2×10^4 copies per reaction for the A27L and F3L genes, respectively [52].

LAMP Assay Specificity

To evaluate the specificity of the LAMP assay, recombinant plasmids (pUC57-A27L and pUC57-F3L) and MPXV pseudo virus samples at a concentration of 1×10^7 copies/μL were used as positive templates, while sterile water served as the negative control. Additionally, the assay was tested against 21 other human pathogens at the same concentration to ensure specificity. Both turbidity monitoring and visual inspection revealed that only the positive control samples (MPXV pseudovirus and plasmids with A27L or F3L genes) resulted in a positive reaction. All other pathogen samples and the blank control produced negative results, demonstrating that the LAMP assay was highly specific for MPXV pseudoviruses and plasmids containing the A27L or F3L genes [52].

Clinical Presentation

The clinical presentation of monkeypox resembles that of smallpox, but it is typically milder. After an incubation period of 5 to 21 days, symptoms manifest in two distinct phases.

- *Prodromal Phase:* This phase is marked by fever, malaise, headache, lymphadenopathy (swollen lymph nodes), and myalgia (muscle aches). The presence of lymphadenopathy distinguishes monkeypox from smallpox [53].
- *Rash Phase:* A characteristic rash begins on the face and later spreads to other parts of the body. The rash progresses through a cycle, starting as macules, then progressing to papules, vesicles, and pustules before forming a crust and peeling off within 2 to 4 weeks [54].

Risk Factors

- *Geographic and Ecological Factors:* Vectors are typically found in forests where reservoirs are common; most. However, within Central and West Africa, handling of bushmeat and rural residences increases contact, thus reducing access to health care [55].
- *Immunization Gaps:* Stopping vaccination programs against smallpox causes the level of herd immunity to orthopoxviruses to decrease. Individuals born after the global effort to eradicate smallpox are more susceptible to monkeypox which is causing an increase in cases in endemic areas [56].
- *Human Behavior:* International travel, globalization, and social behavior, for example, close physical contact in the common environment or during sexual activity have all contributed to recent outbreaks outside Africa [57].
- *Public Health Interventions:* Monitoring of monkeypox shall require surveillance, vaccination, and other public health measures.
- *Surveillance:* Intensified monitoring and case reporting in both endemic and non-endemic areas is imperative for early outbreak detection and containment.
- *Vaccine:* The smallpox vaccine confers cross-protection against monkeypox. A newer, licensed, non-replicating JYNNEOS vaccine, also known as Imvamune or Imvanex, is licensed for the prevention of both smallpox and monkeypox and was used in recent outbreaks [58].
- *Isolation and Quarantine:* The infected should be kept in isolation to prevent transmission. Contact tracing would also be done to identify and monitor people who may have been exposed [59].

Pathway of Disease

MPXV pathogenesis starts with viral transmission, thus taking two main routes: transmission from animals to humans or zoonotic transmission and human to human [60]. Zoonotic transmission is especially started through contact with contaminated animals, among them rodents and non-human primates. Other facilitating factors in the transmission to humans include direct contact through bites,

scratches, or touching fluids or objects contaminated by the animal itself (bedding, carcasses) [61]. The second route by which this virus may be transmitted between humans includes human-to-human, mainly during outbreaks within crowded or close-contact settings [62]. Human-to-human transmission occurs through several modes: Direct contact with infectious body fluids, skin lesions, or respiratory droplets from an infected person [63]. Importantly, monkeypox is usually spread through large droplets that are formed during acute illness; hence, face-to-face exposure is maintained for some time for respiratory transmission to occur. There is also fomite transmission, where the material of contact, such as clothing or bedding, is contaminated with the virus. In recent outbreaks, close physical or intimate contact, including sexual activity, has been identified as a notable transmission route, particularly among specific populations [64].

Once inside, the virus is spread into the human body through broken skin and mucous membranes that involve the eyes, mouth, and nose or via the respiratory tract [59]. Inside, the virus invades epithelial cells and initiates viral replication. Unlike most DNA viruses, which typically replicate in the nucleus of their host cell, the monkeypox virus replicates entirely within the cytoplasm of infected cells [17]. It relies completely on its viral machinery for replicating its double-stranded DNA as well as to produce viral proteins that must be assembled into new virions [65]. After their assembly in special areas in the cytoplasm known as viral factories, the newly formed virions are released for infecting neighboring cells [66].

Once the virus has been replicated at the entry site, it continues to spread to adjacent lymph nodes [67]. This lymphatic involvement is the characteristic feature of monkeypox, which leads to lymphadenopathy and is one of the important clinical signs that distinguish the disease from other members of poxviruses, including smallpox [68]. The virus remains multiplying in the lymphatic system before entering the bloodstream, hence leading to primary viremia. At this stage, the virus spreads systemically to different parts of the body and gives the infection its characteristic rash and lesions responsible for the disease monkeypox [69].

During this phase of secondary viremia, when the infection progresses, the virus spreads further in the body. This infection has an onset of nonspecific signs and symptoms, which usually occur 1–3 days before the onset of rash, that include fever, malaise, headaches, muscle aches, and fatigue [70]. The rash evolves from being initially flat macules to eventually papules, vesicles, pustules, and scabs in a dramatic clinical presentation evolving over several weeks. The rash first begins to appear on the face but soon spreads over most parts of the body and the palms of the hands and the soles of the feet [71]. Apart from this, another characteristic feature of the illness is a centrifugal pattern of distribution of rash, with more marked involvement on the extremities and face. Lesions have appeared in the genital and perianal regions in some patients, particularly in the recent outbreaks and often related to sexual transmission [72, 73].

Immune Response of the Host

In checking the spread and worsening of the infection, there is a critical role that must be given to the immune response of the body. The innate immune response is activated immediately as the host immunity recognizes the virus. It triggers cytokine and interferon production to bring about interference with viral replication [65]. Various mechanisms allow MPXV to avoid the immune system of the host. The virus produces a virus protein that inhibits interferon signaling; therefore, it avoids early recognition and lysis by the immune system, facilitating multiplication of the virus [74]. Upon progressing infection, the adaptive immune response is induced. T-cells and B-cells play a significant role in identifying and lysing infected cells. Antibodies of B-cells neutralize the virus; this results in further infection [75]. While the virus has immune evasion strategies, most people with competent immunity are able to clear the infection within 2–4 weeks [76].

Even though most cases are self-limiting, severe complications can arise, especially among immuno-compromised patients, children, and pregnant women [77]. These complications include secondary bacterial infections, pneumonia, sepsis, encephalitis, and corneal infections that can permanently impair

vision [78]. Besides this, it varies with the clade of monkeypox infections; the Central African, or Congo Basin clade has a higher virulence with a CFR of up to 10%, while that of West Africa has a lower CFR of about 1–3%. In essence, the pathogenesis of monkeypox encompasses an initial entry and replication in epithelial cells followed by systemic spread through lymphatics and bloodstream, which gives rise to the characteristic clinical features [79]. Ability of the virus to evade immune detection lets it replicate and spread before the body mounts a robust immune response [80]. While most patients recover without serious complications, this potential in serious outcomes underlines the importance of early diagnosis and treatment, particularly in vulnerable populations [54].

SYMPTOMS OF MONKEYPOX

MPXV has an incubation period of five days to three weeks, with symptoms lasting 2–5 weeks. Early signs include chills, headaches, fainting, backaches, and muscle pain, which are non-specific. The most common symptoms prior to rash development are fever, restlessness, and lymphadenopathy, the latter being a hallmark of monkeypox, as it appears in 90% of cases and is rare in smallpox. Swollen lymph nodes can be observed in the neck, groin, and submandibular areas [81].

Rashes develop within five days of fever onset, starting on the face and spreading to the trunk and limbs, including the palms and soles. These lesions typically measure 0.5 to 1cm in diameter and progress through various stages, eventually forming crusts that fall off during healing [82]. Co-infection of lesions is common and can lead to lasting skin changes. Although MPX symptoms are generally milder than smallpox, it remains a potentially fatal disease, with a mortality rate of up to 10%, especially in children, young adults, and immunocompromised individuals [83]. Complications may include co-infections, respiratory issues, encephalitis, keratitis leading to blindness, and gastrointestinal symptoms such as vomiting and diarrhea [84]. Due to the varied and non-specific symptoms of MPXV, differential diagnoses can include chickenpox, warts, measles, rickettsial disease, staphylococcal infections, anthrax, scabies, syphilis, and drug reactions [85]. Lymphadenopathy is a key distinguishing feature of monkeypox, highlighting its importance in the initial assessment of suspected cases [86].

Diagnosis of Monkeypox

A combination of clinical evaluation and laboratory testing diagnoses Monkeypox. First, the clinician evaluates the symptoms [72]. These include fever, headache, myalgias, lymphadenopathy, and an evolving rash with characteristic lesions. A history of exposure, either through contact with infected animals or human inhabitants, is equally important [85]. Laboratory diagnosis is done mainly through Polymerase Chain Reaction (PCR) testing, which detects the viral genetic material from lesions, blood, or other body fluids [87]. This has been the most frequently used and dependable test for monkeypox [88]. In addition, serological tests may be used to detect antibodies; however, those tests tend to be used less often during the acute phase of the infection [89]. In some cases, virus isolation from lesions or other samples could be done. Healthcare providers also carry out differential diagnosis to rule out other diseases such as chickenpox, smallpox, and other viral exanthems [90].

Genes Which Can Be Utilized in Technology to Treat Monkeypox

Several host and viral genes play crucial roles in monkeypox infection, immune response, and potential therapeutic targets. These genes are involved in viral recognition, immune modulation, and host-pathogen interactions. Table 1 lists key genes associated with monkeypox that may be useful in diagnostic and treatment strategies.

Clinical Trials for Treatment

The establishment of highly effective vaccines and therapeutic treatments against monkeypox (Mpox) has been an urgent field of research due to recent epidemics. Multiple clinical trials have been initiated internationally to evaluate candidate vaccines and antiviral medicines for safety, efficacy, and immunogenicity. These processes are necessary for informing evidence-driven Mpox response and enhancing public health readiness. A list of some of the current and concluded clinical

trials is provided in Table 2 with data obtained from the ClinicalTrials.gov database (https://clinicaltrials.gov/search?cond=Monkeypox).

Table 1. Representing the genes associated with monkeypox.

Serial number	Gene	Function	Role in mpox infection (proposed/observed)	Reference
1	GPR182	Encodes a G-protein-coupled receptor involved in immune signaling.	May regulate chemokine clearance and affect immune cell trafficking during mpox.	[105]
2	CYB5R3	Plays a role in electron transport and oxidative stress response.	Oxidative stress modulation may impact viral replication and host defense.	[106]
3	CD4	Essential for immune system activation and T-cell response.	Targeted T-cell depletion observed in mpox infection stages	[107]
4	CHRM1	Involved in neurotransmission and immune system interactions.	Neuroimmune interaction may influence mpox symptomology.	[108]
5	CD8A	Important for cytotoxic T-cell function in antiviral immunity.	CD8+ T cells are key in clearing mpox infection.	[107]
6	TNXB	Associated with extracellular matrix integrity and immune regulation.	May influence inflammation and tissue damage during infection.	[109]
7	VPS54	A component of intracellular trafficking pathways affecting immune response.	May alter antigen presentation or viral egress pathways.	[110]
8	IFNG	Encodes interferon-gamma, crucial for antiviral immune defense.	Upregulated in response to orthopoxvirus infection.	[111]
9	VPS52	Functions in vesicular transport, potentially impacting viral replication.	May facilitate or inhibit mpox viral assembly.	[110]
10	ERV3-1	A human endogenous retrovirus gene with potential immunological roles.	Possibly modulates innate immunity or viral mimicry mechanisms.	[112]

Table 2. Ongoing and completed clinical trials for monkeypox virus.

S.N.	Therapy	Study phase	Sponsor/ collaborator	Treatment approach/intervention	Trial status	NCT number
1	Bavarian Nordic smallpox vaccine	IV	McMaster University	Parallel assignment determining if smallpox vaccine could reduce secondary cases and symptom severity in persons exposed to Mpox.	Recruiting	NCT05745987
2	MVA-BN Vaccine	IV	National Institute of Allergy and Infectious Diseases (NIAID)	Single group assignment estimating the incidence of serious adverse events (SAEs) in all individuals vaccinated with the MVA-BN vaccine.	Completed	NCT05734508
3	Tecovirimat	IV	European Clinical Research Alliance for Infectious Diseases (ECRAID), and others	Parallel assignment tests the drug capsules in Mpox patients.	Recruiting	NCT06156566
4	LC16m8 Mpox accine	III	Universidad Nacional de Colombia	Parallel assignment to have cross-efficacy and immunogenicity against MPXV.	Active, not recruiting	NCT06223919
5	Tecovirimat	III	University Hospital, Geneva	Parallel assignment evaluating whether tecovirimat is an efficient and safe antiviral in the treatment of monkeypox in adults and adolescents (14 years	Recruiting	NCT05597735

				old and older).		
6	JYNNEOS	III	Centers for Disease Control and Prevention	Sequential assignment with two doses of JYNNEOS subcutaneously injected in Mpox patients.	Active, not recruiting	NCT02977715
7	MVA-BN	III	Université de Kinshasa	Parallel assignment to compare the safety and immunogenicity of a full-dose regimen versus a half-dose regimen in this population.	Not yet recruiting	NCT06844487
8	MVA-BN	III	Université de Kinshasa	Parallel assignment evaluating safety and immunogenicity of the MVA-BN mpox vaccine in pregnancy.	Not yet recruiting	NCT06844500
9	VACΔ6 vaccine	II/III	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	Parallel assignment confirming the safety and tolerability of vaccines.	Completed	NCT05846243
10	Tecovirimat	II	National Institute of Allergy and Infectious Diseases (NIAID)	Parallel assignment evaluating if tecovirimat is a safe and effective drug to treat monkeypox (Mpox) in combination with standard of care (SOC).	Completed	NCT05559099
11	MVA-BN	II	National Institute of Allergy and Infectious Diseases (NIAID)	Parallel assignment evaluating to determine if peak humoral immune responses.	Completed	NCT05512949
12	MVA-BN	II	National Institute of Allergy and Infectious Diseases (NIAID)	Parallel assignment evaluating standard SC regimen in adolescents ages 12 through 17 years.	Completed	NCT05740982
13	MVA-BN	II	Institute of Tropical Medicine	Parallel assignment evaluating immunogenicity and safety trial of the MVA-BN vaccine for the prevention of Mpox in adults living with HIV with different level of CD4 counts.	Not yet recruiting	NCT06839989
14	MVA-BN	II	Bavarian Nordic	Single group assignment 2 vaccinations of the same dose of MVA-BN vaccine 4 weeks apart.	Recruiting	NCT06549530
15	BNT166a	I/II	BioNTech SE	Sequential assignment evaluating safety, tolerability, reactogenicity and immunogenicity for active immunization against Mpox.	Active, not recruiting	NCT05988203
16	ST-246	I	SIGA Technologies	Crossover assignment to 12 healthy, fed volunteers between the ages of 18 and 50 years.	Completed	NCT00728689
17	NIOCH-14	I	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	Parallel assignment evaluating safety, tolerability, pharmacokinetics of NIOCH-14 in volunteers Aged 18–50.	Completed	NCT05976100
18	VACΔ6	I	Federal Budgetary Research Institution State	Parallel assignment studying the safety and tolerability of the live cell-based vaccine against	Completed	NCT05762523

			Research Center of Virology and Biotechnology	smallpox and other orthopoxvirus infections (VACΔ6 vaccine) based on vaccinia virus, in intracutaneous administration.		
19	MVA Strain Mpo vaccines	I	Shanghai Institute of Biological Products	Parallel assignment evaluating the safety and immunogenicity of receiving two doses of MVA strain monkeypox.	Not yet recruiting	NCT06771479

The clinical trials for monkeypox (Mpox) vaccines and treatments represent a concerted global effort to mitigate the impact of this zoonotic orthopoxvirus. Among the vaccines under investigation, the Bavarian Nordic smallpox vaccine, currently in Phase IV trials, is being studied for its potential to minimize secondary cases and alleviate symptoms in individuals exposed to Mpox [91]. The MVA-BN vaccine, a modified vaccinia Ankara strain, has been extensively studied across multiple phases. Phase IV trials led by NIAID have assessed its safety, focusing on serious adverse events [92]. Antiviral treatments, like Tecovirimat, are also a focal point. This drug is being tested in Phase III trials to determine its safety and efficacy in treating Mpox in adults and adolescents [93]. Other antivirals, such as ST-246 and NIOCH-14, have completed early-phase studies assessing their safety and pharmacokinetics. Novel vaccine candidates, like VACΔ6 and BNT166a, are being developed to enhance prevention strategies. The VACΔ6 vaccine has undergone Phase II/III trials confirming its safety and tolerability, while BioNTech's RNA-based BNT166a is in Phase I/II trials evaluating its immunogenicity against Mpox. Furthermore, the JYNNEOS vaccine, approved for smallpox prevention, is being repurposed for Mpox with sequential dosing regimens tested in active Phase III trials [94].

Some of the Potential Upcoming Therapies Include

MVA-BN

MVA-BN, a weakened virus vaccine in development, is being studied for its effectiveness against monkeypox and related viruses. It lowers the risk of monkeypox by 62% to 85% during outbreaks and by 20% in those already exposed [95]. Additionally, it may help reduce disease severity. This non-replicating vaccine is safe for children. Common side effects include pain, redness, swelling, and muscle pain. Millions, including thousands with HIV, have received it. One of its latest ongoing Phase II studies (NCT06549530) is examining its use in children aged 2 to under 12 years compared to adults for preventing smallpox, monkeypox, and other orthopoxvirus infections, conducted by Bavarian Nordic

LC16m8

LC16m8 is a vaccine therapy that also shields animals from fatal doses of monkeypox and related viruses. However, data on its effectiveness during outbreaks is limited. Reported side effects include fever, fatigue, redness, swollen lymph nodes, and itching. It has been administered to over 90,000 people, including 50,000 children, with no major safety issues. However, it is not recommended for immunosuppressed individuals, those with certain skin conditions, or pregnant women. The latest ongoing potential trial in Phase III is going on in Colombia [96].

mRNA-1769

Moderna is running a Phase I/II trial of mRNA-176, a therapy for monkeypox (NCT05995275). When tested in combination with MVA, this vaccine offered strong protection against a lethal MPXV challenge and provided better defense against weight loss, number of lesions, lesion duration, and viral replication in both the throat and blood [97].

Current Prescriptions Patterns/Treatments Landscape

Symptomatic Treatments Using Drugs

Several antiviral medicines initially tested against smallpox have also been explored to be used to control monkeypox infections. Symptomatic management drugs among these include their application as Tecovirimat (TPOXX), brincidofovir (TEMBEXA), and cidofovir (VISTIDE), respectively targeting

various aspects of viral life cycles or completion of maturation. While these medications have had regulatory approval for smallpox through different emergency or orphan drug designations, they are used investigatively for monkeypox, with access frequently limited to clinical trials or expanded access programs. Beyond antiviral treatments, two vaccines against smallpox, JYNNEOS and ACAM2000, have been repurposed to provide protective immunity for monkeypox infection. Current studies will further define the safety and efficacy of such therapies in monkeypox cases and create more focused therapeutic choices for future epidemics. Currently, no medications are specifically approved for the treatment of monkeypox (Mpox). However, symptomatic treatments are available, including drugs such as:

TPOXX (Tecovirimat)

The US Food and Drug Administration (FDA) approved TPOXX (developed by SIGA Technologies) as the first drug with an indication for the treatment of smallpox in July 2018. TPOXX's effectiveness against smallpox was proven through animal studies with viruses related to smallpox, showing higher survival rates in treated animals compared to those given a placebo. In safety trials involving 359 healthy human volunteers, common side effects included headache, nausea, and abdominal pain. The drug has received designations such as Fast Track Designation (FTD), Orphan Drug Designation (ODD) from the FDA. It received marketing authorization from the European Medicines Agency in January 2022. It is not currently approved for use in children, as there is no standardized dosage for patients under 13 kg. A liquid oral suspension for pediatric patients is in development. No dosage adjustments are required for patients with kidney or liver failure [98, 99].

Generic tecovirimat is a derivative of 4-trifluoromethyl phenol, a low-molecular-weight compound that prevents the virus from exiting the infected cell by targeting the VP37 protein. This inhibits the final stages of virus maturation [100]. According to the U.S. Centers for Disease Control and Prevention (CDC), animal studies suggest that TPOXX may help treat the virus causing monkeypox, but more research is needed to confirm its effectiveness in humans. Since there isn't enough data to prove its safety and efficacy for monkeypox, TPOXX remains an investigational drug. As such, it is only available through the STOMP clinical trial or CDC's expanded access (compassionate use) program. TPOXX can only be prescribed by healthcare providers to individuals with monkeypox who meet specific treatment criteria. Using TPOXX frequently for those with milder symptoms could increase the risk of the virus developing resistance, which may make the medication less effective against monkeypox [101].

TEMBEXA (Brincidofovir)

In April 2021, TEMBEXA, a product of Chimerix (sold to Emergent BioSolutions in September 2022), received FDA approval for the treatment of smallpox. The safety data for TEMBEXA approval came from clinical trials for non-smallpox conditions, mainly in patients who underwent hematopoietic stem cell transplants. Extended use of TEMBEXA (beyond the recommended two doses on days 1 and 8) showed an increased risk of death in cytomegalovirus disease [102]. Common side effects include diarrhea, nausea, vomiting, and abdominal pain. The drug has also received ODD and FTD.

Brincidofovir, also called CMX001 or hexadecyloxy propyl-cidofovir, is a lipid-conjugated cidofovir analogue that inhibits DNA polymerase by integrating into viral DNA. It has better cellular uptake and conversion to its active form compared to cidofovir. Brincidofovir is effective against herpes viruses (including acyclovir-resistant strains), adenovirus, polyomaviruses (JC and BK), poxviruses, and has been used to treat ganciclovir-resistant CMV infections (Florescu et al., 2021). Given smallpox's potential as a bioweapon, brincidofovir may be considered a crucial part of a broader defense strategy against orthopoxviruses, including monkeypox. However, a recent retrospective study observed no significant clinical benefit from brincidofovir and found it was associated with increased liver enzymes, a known side effect, in a small group of three patients with monkeypox.

VISTIDE (Cidofovir)

In June 1996, the US FDA approved VISTIDE (cidofovir), developed by Gilead Sciences, for treating cytomegalovirus retinitis in AIDS patients. Like other nucleotide analogues, cidofovir is an acyclic

cytidine nucleoside phosphonate that inhibits the replication of DNA viruses [103]. It is recommended to take brincidofovir along with cidofovir for the treatment of monkeypox.

It may be too early to anticipate a specific vaccine for monkeypox, as outbreaks have only recently been reported in various countries. Previously, monkeypox was confined to a few endemic regions in Africa. However, with the virus now spreading across different nations, researchers have accelerated their efforts to develop a targeted vaccine for monkeypox, along with effective antiviral treatments [104]. Currently, smallpox vaccines, which offer up to 85% protection, are being used as a starting point in combating monkeypox. Currently, only two smallpox vaccines have been approved for monkeypox which are:

JYNNEOS

JYNNEOS is approved for use in individuals aged 18 and older who are considered at high risk for smallpox or monkeypox infection. In August 2022, the FDA granted an emergency use authorization (EUA) for JYNNEOS, allowing healthcare providers to administer the vaccine intradermally (between the skin layers) to individuals 18 and older who are at high risk of monkeypox infection.

ACAM2000

ACAM2000 is approved for preventing smallpox and monkeypox in individuals deemed at high risk for these infections. It is a second-generation vaccine produced by Acambis and Baxter [105–112].

CONCLUSION

Monkeypox, once regarded as a rare zoonotic infection, has increasingly become a public health concern due to its capacity for human-to-human transmission and the occurrence of recent outbreaks. With the emergence of recombinant DNA technology, there are promising new paths for developing advanced diagnostics, vaccines, and treatment options. A deep understanding of the virus's history, epidemiology, genetic structure, transmission pathways, and modes of infection is essential for devising effective strategies to combat it.

Clinically, monkeypox shares many characteristics with smallpox, though distinct features, such as lymphadenopathy, set it apart. Common symptoms include fever, rash, and muscle aches, and risk factors, such as contact with infected individuals or animals and travel to endemic regions, underscore the need for strong preventive measures. Public health responses, including smallpox vaccinations, case isolation, and contact tracing, have played an important role in controlling outbreaks. Further research into viral mechanisms has identified specific genes that could be targeted for therapeutic intervention, and ongoing clinical trials are investigating antiviral treatments and immune-modulating therapies.

Ongoing trials continue to explore innovative approaches, including gene therapies, to address the shortcomings of existing treatments. Moving forward, the synergy of molecular research, public health initiatives, and advancements in treatment will be crucial for effectively managing and possibly eliminating monkeypox outbreaks in the future.

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