Review

Targeting Mpox: From Pharmacological Mechanisms to Next-Generation Therapies

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Abstract

Mpox, a zoonotic Orthopoxvirus, is a growing global health concern with clinical symptoms like smallpox, including fever, lymphadenopathy, and distinctive rashes. The increasing number of cases emphasizes how urgently antivirals and vaccinations are needed. Antiviral options like tecovirimat, cidofovir, and brincidofovir target DNA polymerase and viral assembly but show varying efficacy. Vaccines such as JYNNEOS and ACAM2000 provide cross-protection, while advancements in mRNA vaccines and recombinant viral vectors offer future promise. Mpox replicates its genome via DNA-dependent RNA polymerase, using envelope proteins for cell entry and evading immune responses through interferon signalling disruption and NF-κB activation. Challenges remain in improving animal models, addressing drug resistance, and ensuring equitable vaccine access. Bioinformatics-driven vaccine design and computational drug repurposing offer rapid solutions for Mpox. PoxApp, a mobile app for symptom tracking and AI analysis, and MPXV-CNN, a model for detecting Mpox image lesions, will accelerate diagnosis and monitoring. Strong pharmacovigilance, innovative treatments, and global collaboration are key to combating Mpox and improving public health resilience.

Keywords: Mpox; MPXV; antivirals; vaccines; emerging therapeutics; JYNNEOS; ACAM2000; LC16m8; Cidofovir; Brincidofovir; Tecovirimat; NIOCH-14

1. Introduction

Mpox is a zoonotic disease caused by infection with the Mpox virus (MPXV), a double-stranded DNA virus and a member of the Orthopoxvirus (OPXV) genus within the Poxviridae family, which also contains viruses that cause cowpox, horsepox, camelpox, and smallpox, a zoonotic viral disease [1]. Although the Mpox name implies that monkeys and other primates are the primary hosts [2], it causes a severe rash in humans, much like smallpox, caused by the OPXV genus's variola virus. Thus, the symptoms of Mpox and smallpox are identical [3]. The first recorded case of Mpox in Africa was in 1970, that of a 9-month-old baby [4,5], and the disease subsequently spread across the continent. In 2003, an outbreak emerged after it spread to Singapore, Israel, and the UK, and, by January 2022, all six WHO regions, 127 member States had reported Mpox cases. As of 31 May 2025, WHO had reported 149,005 cases (since 2022), resulting in 359 deaths, which usually occurred among immunosuppressed hosts [6]. In US (35,002), Democratic Republic of the Congo (25,481), Brazil (14,327), Spain (8,783), Uganda (6,636), France (4,487), Mexico (4,454), Germany (4,378), Colombia (4,285), and UK (4,264) are the top 10 nations that have been impacted globally since 1 January 2022 (https://worldhealthorg.shinyapps.io/mpx_global/#sec-global). These nations account for 77.8% of global cases [7]. In the past month, Angola reported its first case [8]. More recently, the Democratic Republic of Congo reported 17,104 cases and 88 deaths between 25 November 2024 and 5 January 2025. The FDA has approved two vaccines against smallpox (ACAM2000) and smallpox/Mpox (JYNNEOS; non-replicating) [8-10]. Human-to-wildlife contact and expanded urbanization have increased the risks of Mpox transmission to humans [11]. Mpox comprises two genetically distinct clades: Clade II, which was formerly known as the West African clade, and Clade I, which was once known as the Central African, Congo Basin clade [12]. Clade IIa and Clade IIb are subcategories of Clade II; with a 1% fatality rate, Clade II is a milder variant of the virus, and signs include fever, chills, migraines, muscle soreness, and fatigue [13,14]. The hands, chest, face, and genital areas of individuals infected with clade IIb are covered in rashes, nodules, and vesicles. During incubation, patients are asymptomatic for 3-17 days and last for 2-14 weeks [15], and those who are immune-compromised, pregnant, nursing, or younger than one year old are more likely to suffer from severe and sometimes fatal consequences [16]. Clade I exhibits increased clinical severity, enhanced interhuman transmissibility, and elevated fatality rates. Mpox is common in Africa, and is especially prevalent in primates, non-human primates, and rodents (squirrels, Gambian signed rats, and

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dormice). Hunting, trapping, and handling of diseased animals or their bodily fluids is a standard mode of transmission (Figure 1). Non-human primates can be infected with the virus and exhibit symptoms similar to those of humans, while small mammals may carry the virus without showing symptoms [11]. There are no drugs specifically for Mpox [17], however, for some Mpox patients, several medications used to treat smallpox may be recommended, as they are suitable for prevention and treatment [18].

1.1. Importance of Understanding Pharmacological Approaches

Understanding the pharmacology of drugs aids in improving drug therapy and developing new and safer drugs. Pharmacological treatments against MPXV infection have mainly been studied in animal models; human trials have only recently started. Cidofovir, for example, is an antiviral medication used against cytomegalovirus, herpesvirus, poxvirus, polyomavirus, and papillomavirus [19]. It can also be used against Mpox [20]. With the advancement of biotechnology, methodologies such as microRNAs and silencing RNAs are being developed to identify suitable targets against Mpox [21]. Research into biomarker-based therapeutics advances, enabling targeted drug design for various disorders. These methods support the screening, diagnosis, and treatment of viral diseases via vaccination and adaptive therapies [22]. Combining biomarkers with in silico analysis, bioinformatics, and molecular simulations could improve drug pharmacokinetics, reduce resistance, and minimize side effects (13, 14). Another strategy involves hybridizing nanoparticles with existing drugs to enhance efficacy, targeting, and distribution (15). These approaches are currently being explored for Mpox management, which are discussed. Herein, we summarize the most recent data concerning the principal antiviral treatments for Mpox, such as brincidofovir, cidofovir, TPOXX, vaccinia immune globulin, clinical studies, case reports, randomized trials, as well as a historical perspective on pharmacology, medications, and vaccinations [23].

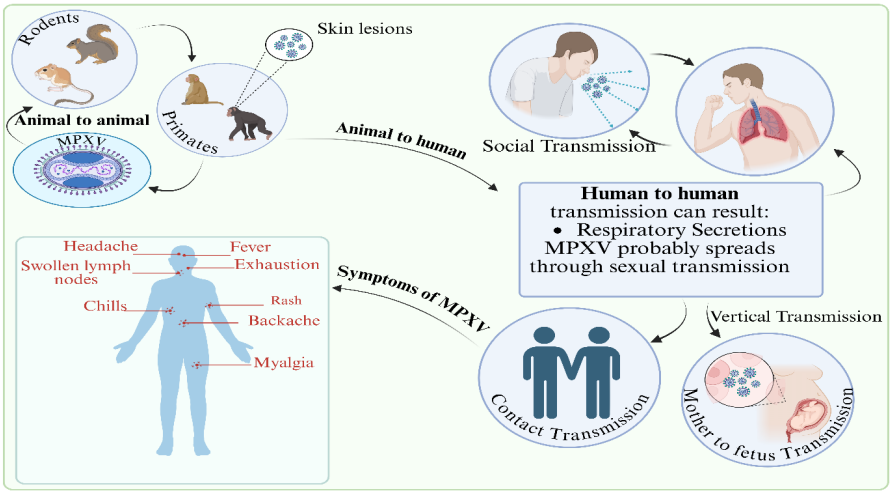

Figure 1. Mpox transmission, symptoms. Mpox is a zoonotic virus that primarily infects humans through skin lesions and contact with infected rats and primates. Direct interaction with an infected individual, respiratory secretions, and vertical transfer from mother to foetus are the three modes by which the disease can pass from one individual to another (social transmission). A typical rash, headache, chills, fatigue, enlarged lymph nodes, muscle and back pain, and fever are all signs of an Mpox.

2. Antivirals Against Mpox: Repurposed Drugs

2.1. Tecovirimat (TPOXX)

The antiviral tecovirimat (TPOXX or ST-246) was licensed to treat smallpox in 2018. Following studies in monkeys and rabbits, the FDA approved TPOXX under the 'animal rule', with only animal efficacy data available, making it adequate for approval without human trials due to the infeasibility

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of human trials. TPOXX is used to treat Mpox [24,25], by blocking the F13 protein (VP37/p37), essential for producing new enveloped viruses. This prevents viruses from escaping from infected cells, reducing viral transmission. The Mpox replicates in the cytoplasm, forming Guarnieri inclusion bodies and maturing to produce extracellular viruses, infecting other cells [26], exacerbating the disease. The F13 protein is conserved among OPXVs, making it a relevant target for several OPXV infections [27,28]. In organotypic raft cultures and cell monolayers, TPOXX inhibits vaccinia, camelpox, and cowpox [29]. In humans, TPOXX, when administered within the first week of Mpox symptoms, significantly improves symptoms, reduces the severity of the disease, and reduces hospitalizations and complications associated with MPXV infection [30]. TPOXX is associated with mild side effects, including headache, nausea, fatigue, elevated liver enzymes, and mental health issues, especially in those with pre-existing conditions. A clinical safety study noted no adverse effects after administering 600 mg of TPOXX orally twice daily for 14 days. Given its safety and efficacy profile, TPOXX is suitable as a first-line treatment for Mpox [31]. The 200 mg active pharmaceutical ingredient is typically administered in capsule form. The intravenous formulation is administered via gradual infusion for those weighing < 13 kg. Individuals weighing 40-120 kg are required to take 600 mg twice daily for 14 days to treat Mpox. For those weighing 25-40 kg, the dosage is 400 mg every 12 hours; for those weighing 13-25 kg, it is 200 mg twice daily. For 14 days, those who weigh >120 kg are administered 600 mg three times a day. If the patient cannot eat, 200 mg of TPOXX is given intravenously over 6-12 hours for 14 days. A clinical trial of TPOXX in the DRC showed that it was not effective for patients with clade I Mpox [32], suggesting the emergence of TPOXX-resistant Mpox variants and the need for further research and new antiviral drug development. Combination treatments that have demonstrated potent dose-dependent antiviral efficacy against Mpox and vaccinia virus, such as TPOXX and mycophenolate mofetil or TPOXX and N-myristoyltransferase inhibitor IMP-1088, are now being assessed [33].

2.2. Cidofovir and Brincidofovir

Cidofovir (CDV; brand name Vistide) is a small-molecule nucleoside analog DNA polymerase inhibitor, approved in 1996 for the treatment of AIDS-related cytomegalovirus retinitis. It has shown anti-viral activity in vitro against several other DNA viruses, including adenovirus, herpesvirus, papillomavirus, and poxviruses (including smallpox virus) [34] against bovine alphaherpesvirus-2 in sheep [35] and treats immunocompromised patients with refractory molluscum contagiosum [36]. CDV is metabolised intracellularly to its active form, CDV-diphosphate, within the infected cell, which competes with viral nuclear DNA polymerase, preventing DNA synthesis [37]. While it also inhibits human DNA polymerases, it is much less potent against them, being 8-600 times less active compared to the viral enzyme. The antiviral effects of CDV are prolonged within infected cells due to its long intracellular half-life of 65 hours and short plasma half-life of 2.4–3.2 hours. CDV is excreted via the kidneys, which is an essential consideration to those with renal dysfunction, as it can lead to significant drug-drug interactions, particularly with agents like probenecid, which inhibits CDV excretion, leading to increased serum levels and thus increased toxicity [38,39]. Further, a case report of two patients with HIV with cytomegalovirus retinitis developed iritis following CDV administration [40]. CDV is administered intravenously and usually together with probenecid and intravenous hydration, and common side effects include rash, anaemia, headache, fever, proteinuria, elevated serum creatinine, and in severe cases, can lead to nephrotoxicity and neutropenia [41]. In animal studies of monkeys and mice, CDV reduced viral load and protected cowpox virus, vaccinia virus, ectromelia virus, and variola virus when administered via different routes, including intraperitoneal, intranasal, subcutaneous, or aerosol [42]. In Mpox monkey models, CDV reduced Mpox lesions, mortality, and protected against laboratory signs of disease [42].

A phosphonate group on CDV is conjugated to a lipid to form a prodrug known as brincidofovir (BCV, also known as CMX001; brand name Tembexa), and is 50 times more active in vitro than CDV. Phosphorylation of BCV produces "CDV diphosphate". It is effective against DNA viruses by inhibiting smallpox virus DNA polymerase-mediated DNA synthesis [43-45]. The FDA authorized

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BCV to treat smallpox in June 2021 [46] under the Animal Rule, and research is being conducted to determine its effectiveness against the Ebola virus, cytomegalovirus, adenovirus, and poxviruses. BCV, however, has shown adverse reactions and no benefit in patients, leading to the discontinuation of several studies. For example, a randomized, double-blind, placebo-controlled phase III trial to prevent cytomegalovirus infection in stem cell transplant patients was prematurely stopped as it showed no benefit and serious adverse events such as gastrointestinal toxicity [47]. In an observational study in the UK, which included seven patients infected with Mpox, who received either BCV (200 mg/week) or TPOXX (600 mg twice a day for 2 weeks), those who received BCV had to stop treatment due to increased liver enzymes [48]. In a systematic review of 18 studies including 71 patients with Mpox, only 3 received BCV but had to be discontinued due to adverse events and increased liver enzymes; TPOXX was well tolerated in 61 of these subjects, with 83.1% showing complete resolution [49]. Further clinical studies are required to determine the efficacy of BCV in humans, given that several animal studies have shown efficacy against cytomegalovirus, poxviruses, and herpes simplex virus [50]. In mice, both CDV and BCV reduced clade IIb replication in vitro and in vivo, with BCV being superior as it also showed protection against the highly virulent clade IIa [51].

2.3. NIOCH-14

NIOCH-14 is an experimental antiviral drug, a prodrug of TPOXX, allowing it to interfere with viral replication and spread. It was developed as an anti-smallpox drug, and its efficacy against Mpox in animal models has shown therapeutic potential. Intragastric administration of NIOCH-14 at 5 g/kg was found to be safe with no signs of toxic effects in mice, and doses of 50 mg/kg in mice and 150 mg/kg in rats did not alter haematological parameters or microstructure of internal organs [52]. In mice and marmots, it showed similar antiviral efficacy compared to TPOXX against Mpox, variola virus, and ectromelia virus by reducing viral load and improving survival rates (60-100%) [53]. Similarly, oral administration of NIOCH-14 at 50 mg/kg in mice decreased Mpox viral titres in lungs [54]. In a phase I human clinical trial (NCT05976100), published recently, showed that 90 healthy volunteers who received NIOCH-14 (hard capsules) either as a single dose or repeated oral doses of 200, 600, and 1200 mg, was well tolerated and no changes noted in complete blood examination, urine and clinical parameters; one patient experienced mild, transient epigastric pain at 1200 mg dose [55]. This phase I study suggests that NIOCH-14 holds promise for further testing as a treatment for smallpox and Mpox.

2.4. Vaccinia Immune Globulin

Vaccina Immune Globulin (VIG) was approved for the treatment of adverse reactions as a result of smallpox vaccination [56]. It is used for conditions such as eczema vaccinatum, severe generalized vaccinia, progressive vaccina, widespread vaccinia, and for individuals with pre-existing conditions [57]. VIG has also been considered for use in severe cases of Mpox, in particular in immunocompromised individuals [58]. A dose of 6000 U/kg is given; however, it is increased to 9000 U/kg if the patient does not respond. VIG, a sterile, solvent-treated solution of the purified gamma globulin (IgG) component of human plasma, contains anti-vaccinia virus neutralizing antibodies [59]. It was previously shown that VIG also cross-reacted with cowpox and Mpox in murine models [60]. In another study, two humanized monoclonal antibodies (hMB621, hMB668) against the B6 protein of MPXV also bound to vaccinia virus, variola virus, cowpox virus, neutralized vaccinia virus in mice, and showed protection in a mouse model [61]. Further, monoclonal antibodies isolated from non-human primates that were previously immunized with vaccinia virus were shown to neutralize both vaccinia virus and Mpox, offering a therapeutic solution for OPXV infections and vaccine-related adverse reactions [62]. In a case report of a 29-year-old male with severe Mpox with HIV, improvements were seen as quickly as 2 weeks of 8 weeks of treatment with combination therapy of tecovirimat and VIG, which showed significant improvement with healing of lesions and resolution of new lesions [62]. Likewise, in a 49-year-old male with severe refractory Mpox who also had HIV,

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failed to respond to TPOXX, a combination of cidofovir and VIG led to significant improvement [63]. VIG is not considered a first-line treatment for MPXV infections and is used off-label, particularly showing potential as a treatment for severe Mpox cases in individuals with HIV.

3. Mechanisms of Action

3.1. Viral Pathogenesis and Host Interactions

DNA viruses with genomes ranging from 150,000 to 300,000 base pairs make up the poxvirus family. Over 200 proteins involved in transcription, virion assembly, cellular entry, and genome replication are encoded by the 197 kb linear, double-stranded MPXV genome. The Mpox is a virus with an envelope 200-250 nm in size [64]. Mpox spreads via close contact between humans or animals. The respiratory or oropharyngeal mucosa is impacted by smallpox and Mpox. The virus enters through the injection site and uses ruptured tissue or mucosal membranes (those protecting the mouth, nose, eyes, urinary system, and rectum) to replicate in the surrounding tissues. It is an autonomous DNA virus that translates its viral proteins using the host's ribosomes. However, in the cytoplasm, it can also produce its individual replicating, transcription, and mRNA-making machinery [65]. Primary viremia spreads to regional lymph nodes. Secondary viremia enters lymph nodes and organs via blood. The first symptoms of MPXV infection are usually fatigue, fever, pain, and lymphadenopathy, which can occasionally be accompanied by severe inguinal lymphadenopathy. Research into virus-host cell interactions has consistently shown striking genetic variation between Clade I and Clade II genes [66]. Viral factories are perinuclear locations where early gene transcription activities occur once the virus infects the host cells. Mpox viruses are thought to attach to parts of the extracellular matrix, glycosaminoglycans within the target cell's surface, and proteins on the outside of the virus. The target cells' cytoplasm receives the python's viral envelope via low-pH enzymatic entry or plasma membrane contact at neutral pH. Poxviruses have host range factors (Hrfs) that alter the host's virus response. As an example, the Hrf protein BR-203 inhibits the antiviral host response cell-specifically by blocking the binding of IL-1 and IL-1b receptors [67]. For mature internal and enveloped exterior virions to attach to the cell, twelve distinct kinds of viral membrane proteins must be present [68]. Once inside, the virus copies itself using a special enzyme made of multiple parts called DNA-dependent RNA polymerase. Early, intermediate, and late protein translation on the host ribosomes comes next [69]. The latent Mpox can last for two weeks. During this time, Mpox patients are usually asymptomatic and lesion-free. Chills, fever, headache, muscle soreness, and lymphadenopathy are post-latent Mpox symptoms. Men who have sex with men are experiencing a rare clinical symptom of the current Mpox outbreak: anal or vaginal rashes that spread throughout the body [70,71]. The likelihood of these severe manifestations is higher in children, older adults, HIV patients, and anyone taking immunosuppressive medications. The virus then becomes tropic to other organs and travels through the bloodstream, which is how Mpox can lead to secondary viremia [66].

The Mpox entry, replication, and release in host cells are depicted in the diagram. Upon nucleation, the virus releases itself into the host cell through endocytosis. Its DNA is released into the cytoplasm. Proteins produced by viruses are the product of transcription and DNA replication. Newly formed virions undergo exocytosis after assembly and maturation in the Golgi apparatus. To prevent Mpox from spreading, various antiviral medications target distinct steps in the viral replication cycle. These include CDV, BCV, NIOCH-14, and TPOXX (Figure 2; site of action shown in red).

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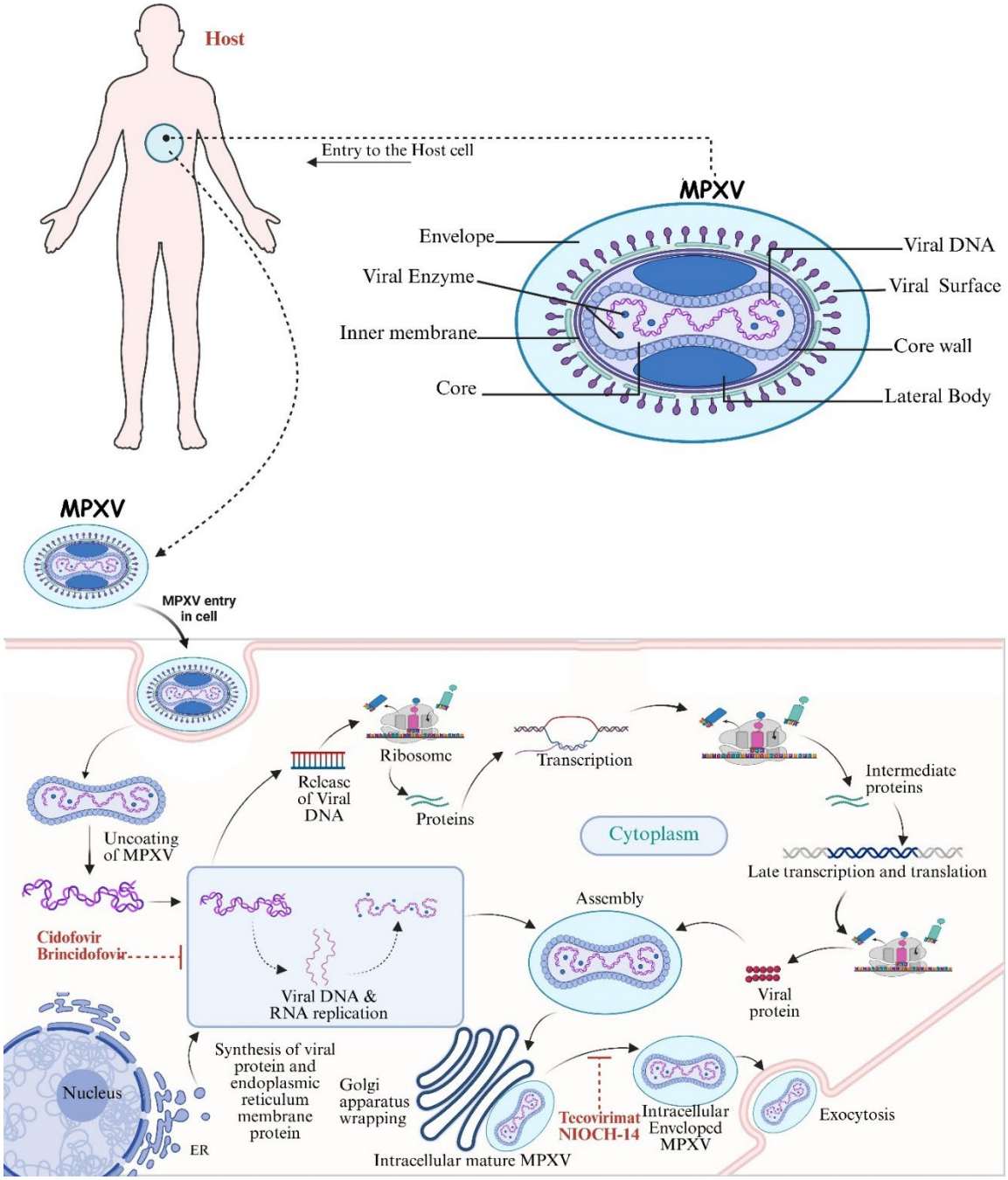

Figure 2. MPXV infection in the human host showing (red font) action point of cidofovir, brincidofovir, NIOCH-14, and TPOXX.

3.2. Targeting Viral Replication

Targeting viral replication in MPXV requires an understanding of the virus's life cycle, identifying essential components required for its replication, and developing strategies to interfere with these processes. As Mpox is a double-stranded DNA virus, viral enzymes are used for transcription and DNA replication, which occurs in the host cells' cytoplasm. MPXV infection and reproduction are in three stages (Figure 2): (i) invasion of the virus; (ii) synthesis of viral proteins and replication; and (iii) viral assembly, maturation, and release [72]. Several envelope proteins that enable the virus to penetrate host cells may be attractive targets for antivirals against MPXV, as the absence of a receptor has been identified on the host cell membrane. The interference of viral genome DNA or RNA synthesis aids in the development of MPXV antivirals [73]. CDV is a powerful antiviral

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against OPXV. Unlike CDV, BCV has superior bioavailability and lower nephrotoxicity since its metabolism does not depend on the renal ion pathway. Both drugs work by inhibiting DNA polymerase, thereby preventing the virus from spreading. On the other hand, TPOXX works by targeting the viral envelope, a crucial component that allows the virus to infect host cells and replicate. By inhibiting the formation of this envelope, TPOXX effectively blocks the virus from maturing and spreading, thus preventing its ability to replicate within the body [74]. NIOCH-14, an antiviral currently undergoing clinical testing, acts as a nucleoside analogue that integrates into the viral genome during replication. By incorporating itself into the viral RNA or DNA, it disrupts the replication process, preventing the virus from multiplying and spreading within the host [75]. The cytoplasmic replication of DNA viruses is dependent on DNA-dependent RNA polymerase (DdRp). DdRp's biological relevance makes it a potential Mpox therapeutic target. Using computational screening, modelling, docking, and molecular dynamics, further potential drug inhibitors can be identified [76]. In addition, RNA interference (RNAi) was shown to reduce Mpox replication in a sequence-specific and efficient way. As such, 48 siRNA constructs targeting 12 Mpox genes in 12 pools on viral replication were evaluated. The siRNAs' effects on Mpox replication varied in strength. Intense antiviral action was demonstrated by two siRNA pools, which decreased virus replication below 10% of that observed in untreated control cells. At an IC_{50} of less than 10 nM, a single siRNA construct targeting the A6R gene prevented the spread of viruses almost entirely [77,78]. SiA6-a similarly suppressed all virus replication at 20 nM and CDV at 100 nM [79]. SiRNA molecules effectively target the A6R and E8L genes (involved in immune evasion and replication) of Mpox, and SiA6-a and SiE8-d, respectively, considerably reduced Mpox replication [79]. In addition, compounds from the ReFRAME and NPC libraries that inhibit other RNA virus families were also noted to inhibit vaccinia virus and Mpox, suggesting they could be effective against multiple viral infections [80-84]. As with most antivirals that target viral protein function, the researchers explored compounds that target host components to prevent viral escape mutations. These results also point to the use of host (ReFRAME and NPC drugs) and viral (TPOXX) combinations to treat OPXV infections [82,85].

3.3. Modulation of Host Immune Responses

The severity and outcomes of MPXV infection are greatly affected by the host immune response, with MPXV proteins manipulating the host immune pathways and responses such as complement activation, inflammatory signalling, cytokine production, NF-κB regulation, and suppressing natural killer cells. Mpox encodes several BCL-2-like proteins, including A47, B13, P1, C6, and D11, which play a key role in immune evasion and persistence within the host [86,87]. The E3 protein, which is also produced by vaccinia virus, binds to double-stranded RNA and suppresses antiviral responses by blocking the protein kinase R pathway. The homologous protein F3, which Mpox expresses, has its N-terminus truncated [88], implying that additional Mpox proteins would make up for the partial loss, but have not yet been identified. Mpox uses a variety of strategies to inhibit innate immune responses actively. For example, the MPXV B19 protein directly inhibits the transcription factor IRF3, which is essential for the production of IFN-α/β. As such, the B19R gene reduces viral infection. MPXV also encodes several ankyrin-like proteins (B17R, D9L, J1R, N4R, J3L, D1L, D7L, O1L, C1L, and B5R) that inhibit NF-κB activation which modify host immune responses [89,90]. Understanding how MPXV modulates host immune responses is essential for developing effective antivirals to overcome its immune evasion strategies, ultimately reducing the impact of MPXV infections.

3.4. Challenges in Understanding Mechanistic Pathways

As Mpox is an emerging global health threat that has spread internationally, sensitive diagnostic methods are limited to a few national reference laboratories. Understanding the molecular pathways of Mpox is challenging due to a lack of effective animal models that replicate human transmission, pathogenic mechanisms, and outcomes such as morbidity and mortality. While mice, black Prairie dogs, African rope squirrels (*Funisciurus anerythrus*), and Gambian pouched rats (*Cricetomys gambianus*) are used, the virus does not infect them the same way as they do humans. In addition, the

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mechanism of how monocytes, macrophages, neutrophils, and dendritic cells contribute to viral transmission requires further work. Although the smallpox vaccine provides partial cross-protection against Mpox, its effectiveness against emerging Mpox mutations is still being studied [4,91-93]. Further, understanding the molecular basis of Mpox, such as immune evasion, replication, and spread, as well as viral mutations, will aid in the development of effective treatments and vaccines, as well as understanding host-host transmission [94,95]. As infection with Mpox shows similar symptoms initially (fever, lymphadenopathy, headache, muscle ache) to other viruses, such as smallpox, chickenpox, or hand-foot-mouth disease, diagnosis could be challenging and requires laboratory testing. Mpox, however, does show distinct features of skin lesions and could help identify Mpox at later stages. Identification and isolation of infected people are essential, as is maintaining adequate hand hygiene and sanitation, to reduce the risk of MPXV infection and transmission. Further, it is essential to understand the mechanistic pathways of Mpox as its interaction with the host immune system is quite complex. All these factors complicate the development of antivirals against Mpox.

4. Challenges in Drug Development

4.1. Barriers to Effective Therapeutics Development

Developing treatments for MPXV infection is complicated because of its many biological components. These include a limited understanding of virus-host dynamics, medication development challenges, vaccination distribution inequities, and global health initiatives for emerging diseases. Through the development of Mpox drugs, insights into drug repurposing, small-molecule drugs, AI-driven drug target discovery, antibody treatments, and preclinical drug development have been gained, advancing strategies for more effective therapies and enhancing the overall understanding of Mpox treatment options [71,96]. Regarding treatments, CDV has potent antiviral properties; however, it is not easily absorbed by the body, and in patients on dialysis or with renal failure, the metabolites can accumulate in the proximal tubular cells, leading to kidney damage [97,98]. In this regard, BCV was developed to eliminate these side effects. The FDA allowed BCV as a treatment for smallpox in 2021 after it was shown to work better at being absorbed and converted in the body due to lipid conjugation technology. However, as noted in the earlier section above, BCV causes increased liver enzymes and is not as well tolerated compared to CDV, and many clinical studies were prematurely stopped. As such, it is essential to consider the following: (i) Drug delivery and specificity should be enhanced to target Mpox at the infection site effectively; (ii) To prevent drug-resistant strains and maintain therapeutic efficacy, several anti-MPXV drugs should be developed, with each drug targeting different phases of MPXV infections; (iii) Several variations should be developed targeting different regions of the virus; (iv) Considerations for sequential and combination medication regimens; and (v) pharmacological adjustments to decrease toxicity and adverse reactions [99,100].

4.2. Regulatory Considerations and Clinical Trial Design

Mpox classification as a public health concern and the need for effective therapies and diagnostics influence the regulatory framework surrounding the disease. International regulators, including EMA and ICMRA, published a report outlining the outcomes of the discussions on the development, testing, and availability of MPXV antivirals and vaccines. Additionally, the FDA, JPMDA, and WHO reviewed the general characteristics, regulatory status, and available evidence for various Mpox vaccines and therapeutics [101,102]. Antivirals are the first focus in the DRC, where mortality is highest, particularly among children. To prevent infection from smallpox and Mpox, the MVA-BN (modified vaccinia Ankara-Bavarian Nordic, brand name JYNNEOS, also referred to as Imvamune, Imvanex) vaccine has been used safely in the United States [103] in children since the FDA gave it emergency use clearance (EUA). Regulatory approval routes for novel vaccines should be evaluated, along with alternatives to placebo-controlled trials, such as animal models and clinical

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immune-bridging, where placebo studies are not feasible. According to the study, 302 participants received a placebo and 295 people received TPOXX. The median duration from randomization to lesion resolution was 7 days with tecovirimat and 8 days with placebo. The competing-risks hazard ratio for lesion resolution was 1.13 (95% CI, 0.97 to 1.31; P=0.14). Results were similar for those who started the study regimen within 7 days of symptom onset. Tecovirimat did not reduce lesion resolution days in clade I MPXV individuals with Mpox. Researchers found no safety issues. NIAID and others funded PALM007 ClinicalTrials.gov number, NCT05559099 [104]. Currently, studies are also determining the effects of TPOXX and JYNNEOS vaccine combination in a multicentre, randomized, placebo-controlled, drug-vaccine interaction phase 2 study given 4 weeks apart, and the potential supporting the combined use of these drugs for post-exposure prophylaxis [105]. BCV, along with TPOXX, is the only antiviral eligible for clinical trials, requiring careful monitoring of patient safety. The STOMP (Study of TPOXX for Human Mpox) clinical trial is still ongoing, and only interim findings have been made publicly available to evaluate the safety and effectiveness of TPOXX for treating clade II Mpox. The prevalence of TPOXX-resistant Mpox forms in clinical Mpox instances, particularly in immune-compromised individuals receiving long-term therapy, suggests that the drug's insufficiency or resistance may cause the lack of efficacy. The new emergence of clade Ib Mpox in the DRC and Sweden, along with findings from the international clinical study STOMP (NCT05597735), noted that there is a need for new antivirals due to TPOXX not being effective against both clades [106]. Further, in an attempt to improve the JYNNEOS vaccine and allow more individuals to be vaccinated, a much lower dose, as much as 80% has been tested, via the same subcutaneous route, did not lower the effectiveness of the vaccine [107,108]. The vaccine options evaluated were ACAM2000 (second-generation vaccine against smallpox; Sanofi Pasteur) and LC16m8 (live attenuated smallpox vaccine derived from vaccinia virus; Sumitomo Dainippon Pharma Co., Ltd). In addition, single-dose vaccines, especially in endemic areas, are an option, and given that mRNA vaccines are being evaluated against Mpox (mRNA-1769; Moderna), this could be a possibility [108]. As such, the findings support the vaccine's potential and support clinical testing for orthopoxvirus protection, including Mpox, and for future pandemic prevention.

4.3. Need for Comprehensive Pharmacovigilance

The Mpox outbreaks show how important pharmacovigilance is for ensuring that antivirals and vaccines are safe and effective. Due to the disease's global prevalence (Figure 3a) and ability to transmit zoonotically, robust monitoring methods are necessary to identify adverse reactions to antivirals and vaccines, assess the effectiveness of immunizations, and provide real-time data for public health measures. Additional clinical research and pharmacovigilance monitoring in high-risk groups are necessary to achieve herd immunity and effectively counter Mpox. Healthcare facilities must improve social and safety containment, monitoring, diagnostics, and infection prevention and control. Global efforts to provide vaccines, context-based risk communication, public awareness, and public engagement must prioritize the most vulnerable groups, such as the poor and wealthy living on the outbreak's edges and those in the most affected areas. A complete immunization plan is encouraged to prevent Mpox cases from increasing exponentially, which could pose a pandemic risk. Thus far, ACAM2000 and JYNNEOS smallpox vaccines are most suitable for laboratory workers, healthcare professionals, and frontline staff who may be exposed to MPXV in their workplace [109,110]. Further clinical trials and pharmacovigilance monitoring for at-risk groups are required to assess the safety and effectiveness of currently available vaccinations to acquire herd immunity against Mpox. These include implementing social and safety containment measures, improving hospital infection prevention and control, and enhancing surveillance diagnostics. In the current Mpox outbreak, coordinated efforts should be made to contain the spread, such as equitable risk sharing, public engagement, effective vaccine distribution, and tailored risk communication strategies for disadvantaged and vulnerable groups [110].

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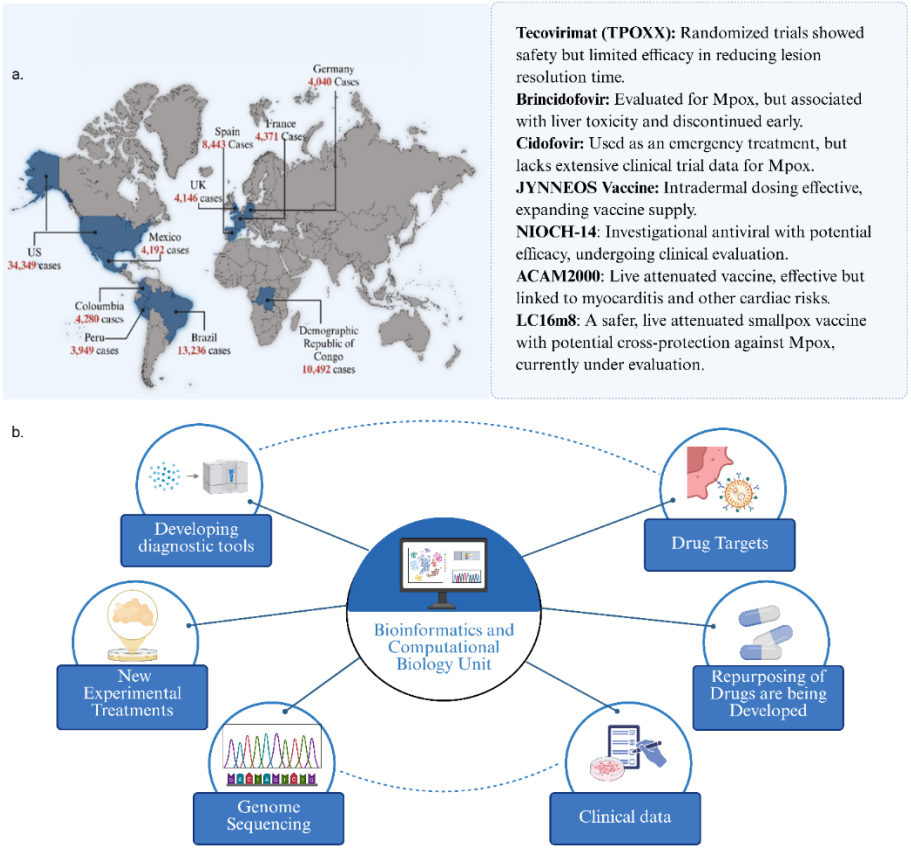

Figure 3. (a) Global distribution of Mpox cases, highlighting affected countries and reported case numbers [7]. **(b)** Role of bioinformatics and computational biology in Mpox research, including diagnostic tool development, genome sequencing, drug target identification, and clinical data analysis [124].

5. Next-Generation Therapeutic Approaches

5.1. Computational and Bioinformatics Approaches

Current trends in drug discovery include the repurposing of existing therapeutic molecules and the discovery of fresh lead compounds and possible molecular targets from natural sources. By combining computational techniques with biological research, bioinformatics has quickly developed into a crucial field (Figure 3b). When computational methods for protein sequence analysis emerged in the early 1960s, bioinformatics was established. Simultaneous developments in molecular biology methods made DNA analysis easier and allowed for more effective genetic data analysis, sequencing, and interpretation. Innovations in bioinformatics were a result of advances in computer science, which also produced more advanced software and more potent computer systems that improved the capacity to evaluate and comprehend intricate biological data [111,112]. Recent research has shown that using computational methods to repurpose FDA-approved drugs is an effective strategy for identifying drug targets for several diseases, including Mpox [113,114]. Statistical biology, data analysis, machine learning, and AI work together to identify lead drug candidates with optimal drug probability, pharmacokinetic properties, and toxicity profiles for development [115]. Computational models not only reduce costs and time, they also provide information regarding how to validate the results in experiments. As a result, the development of molecular docking and molecular dynamics techniques has increased the significance of computational research in the development of antivirals [116,117]. Common docking tools include Dock, Autodock, Gold, Gide, Zdock, Rdock, FlexX, AutoDock-4, and Autodock-Vina [118]. Virtual screening tool PyRx is used in computational drug discovery to match chemical groups to therapeutic targets. GOLD-Chemscore rates macromolecule-small molecular docking program. Run simulations with Molegro Virtual Docker, another docking simulation application [119]. Several FDA-approved drugs with the most active compounds against

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Mpox proteins, as well as model validation for MPXV cysteine proteinase, DNA polymerase, early transcription factor and DNA topoisomerase are described in Table 1. One negative issue of using computational techniques to screen for antivirals for MPXV is that these methods do not capture the complexity of biological in vivo physiology. While computational models can predict drug interactions and efficacy, they are not able to predict potential side effects in humans. Furthermore, the quality of the data that is currently available determines how accurate the projections are, which could restrict their dependability, particularly for recently discovered illnesses like Mpox.

Identifying and combining CD4 and CD8 T cell epitopes as well as B cell epitopes through a multi-epitope approach is necessary for developing effective vaccines. Strong multi-epitope vaccines stimulate humoral and cellular responses using antigenic peptide sequences that overlap B- and T-cell epitopes [120,121]. To improve the immunogenicity of the vaccines, bioinformatics approach combined with machine learning algorithms, can be used to identify enhancers by analysing large datasets from experimental studies; such enhancers can be branched peptides, adjuvants, immune modulating agents [122]. This approach significantly reduces time, money, and risk of failure in vaccine development. In vitro and in vivo assays are expensive, labour-intensive, potentially toxic, can induce allergic reactions, and often result in high rates of clinical trial failure. Employing bioinformatics technologies to identify non-toxic and non-allergenic antigens for a disease can conserve time and resources [123]. On the contrary using bioinformatics and prediction algorithms to develop vaccines does not fully account for the complexity of the immune system, complexities of pre-existing immunity and potential adverse effects. As a result, vaccines developed primarily through bioinformatics and prediction algorithms requires extensive clinical testing to confirm their safety and efficacy.

Table 1. FDA approved drugs repurposed to target Mpox identified using computational studies.

Drug Name	Drug Bank ID	Protein Interaction	Mode of Action	Refs
Eluxadoline	DB09011	Mu-opioid receptor	Serves as both an agonist and an antagonist of mu-opioid receptors	[125]
Dihydroergotamine	DB00630	5-HT1B and 5-HT1D serotonin receptors	Vasoconstrictor, primarily used for migraine treatment	[126]
Tobramycin	DB00583	30S ribosomal subunit	Inhibits bacterial protein synthesis	[125]
Nebivolol	DB01298	Beta-adrenergic receptors	Selective beta-1 blocker with vasodilating properties	[125]
Pimozide	DB00501	Dopamine D2 receptor	Antipsychotic that blocks dopamine receptors	[126,127]
Triptorelin	DB00112	Gonadotropin-releasing hormone receptor	GnRH analog that inhibits gonadotropin release	[126]
Carfilzomib	DB08892	Proteasome	Proteasome inhibitor used in cancer therapy	[126]

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Tolvaptan	DB06155	Vasopressin V2 receptor	Vasopressin receptor antagonist used for treating hyponatremia	[126,127]
Cobicistat	DB09019	Cytochrome P450 3A4	CYP3A inhibitor that increases the effectiveness of certain HIV medications	[126]
Conivaptan	DB04874	Vasopressin V1A and V2 receptors	Dual vasopressin receptor antagonist used for hyponatremia treatment	[126,127]
Tenapanor	DB11607	Sodium/hydrogen exchanger NHE3	Inhibits NHE3 to reduce sodium absorption in the intestine	[126]
Fludarabine	DB00336	DNA polymerase	Antimetabolite that interferes with DNA synthesis in cancer cells	[28, 127, 128]
Tigecycline	DB02280	Bacterial ribosomal subunit	Tigecycline, a glycylcycline, binds to the 30S ribosomal subunit and prevents amino acyl tRNA molecules from entering the ribosome's A site, therefore inhibiting protein translation in bacteria	[127]
Eravacycline	DB12329	DNA-dependent RNA polymerase	Broad-spectrum antibiotic; inhibits bacterial protein synthesis.	[127]

5.2. Pipeline Pharmaceuticals and Vaccines Against Mpox

The development of pipeline for the treatment for Mpox has advanced significantly within the last few years. Currently Mpox vaccines include, JYNNEOS, LC16m8, Imvanex, ACAM2000, Orthopox vaccine (Figure 4, Table 2), which were mainly developed against vaccinia virus but is cross-protective against Mpox pre-clinical / animal studies (Table 3), (human clinical trials) (Table 4) Testing in primates, prairie dogs, mice, the Mpox pipeline (Table 2) show safety and immunogenicity. Evaluated for antiviral efficacy, including combination therapy with antivirals, are also being evaluated [129].

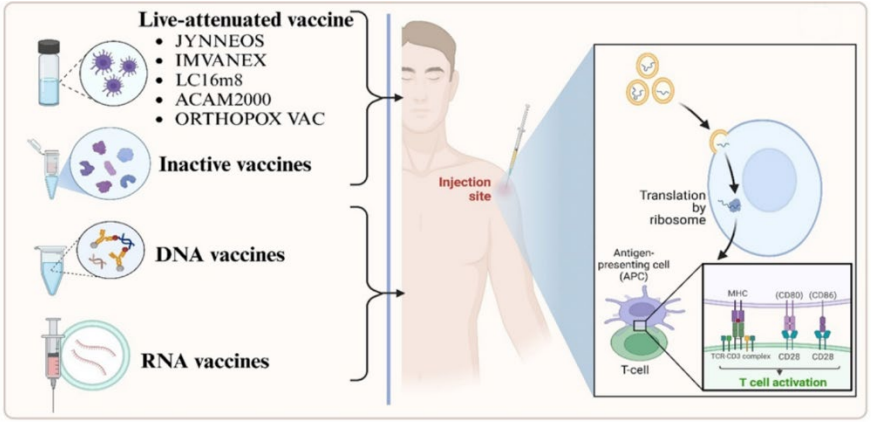

Figure 4. Methods of Mpox vaccination. Several vaccines (JYNNEOS, LC16m8, ACAM2000, ORTHOPOX VAC), inactivated, DNA, and RNA vaccines, are being evaluated. .

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Table 2. Vaccines against Mpox.

Vaccine Name	Description	Dosing Regimen	Approved Countries	Reported Side Effects	Refs
ACAM2000	Live, replicating vaccinia virus (NYCBH strain)	Single percutaneous dose (2.5 µL)	USA	Myocarditis, pericarditis, encephalitis, progressive vaccinia; contraindicated in immunocompromised individuals and those with certain skin conditions	[130,131]
JYNNEOS (also known as Imvanex® or Imvamune®)	Modified Vaccinia Ankara (MVA), non-replicating strain	Two subcutaneous doses (0.5 mL each), 4 weeks apart	USA, Canada, European Union	Mild to moderate local reactions (pain, redness, swelling); fewer systemic side effects; considered safe for immunocompromised individuals	[130,132]
LC16m8 (also known as LC16-KMB®)	Live attenuated, minimally replicating vaccinia virus	Single percutaneous dose (2 µL)	Japan	Generally well-tolerated; specific side effects not extensively reported; ongoing studies to assess safety profile	[131]
Orthopox Vaccine	Recombinant MVA-based vaccine (live vaccinia VACdelta6-based culture vaccine)	Dosing regimen not specified	Russian Federation (approved in November 2022)	Limited available data on side effects; further studies are required to establish safety and efficacy	[131]

Attenuated viral vaccines, like NYCBHAE3L [133], showed high survival rates and fewer lesions following the Mpox challenge in cynomolgus monkey tests as compared to non-vaccinated controls. As in cynomolgus monkeys and prairie dogs, Dryvax, when used in combination with TPOXX, induces sustained immune responses [134] and protects mice from viral challenge. Depending on when administered, these vaccines' post-exposure efficacy varied, but they both decreased viremia and generated neutralizing antibodies [135]. The development of non-replicating Mpox vaccines like JYNNEOS and ACAM2000 has advanced. The ACAM2000 works well. However, it can have serious side effects, like heart issues. In animal experiments, JYNNEOS exhibited robust immune responses in non-human primates, significantly diminishing illness severity and viral load. ACAM2000 offered protection but exhibited potential cardiac problems, such as myocarditis. In humans, JYNNEOS was, associated with minor cardiovascular adverse events such as tachycardia, palpitation, electrocardiogram changes such as T wave inversion, and ST elevation. Nonetheless, ACAM2000, has

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been reported to have major cardiovascular adverse events such as myocarditis, dilated cardiomyopathy, and heart failure [136,137].

The Mpox research program includes different antiviral and vaccine studies focused on tackling Mpox. Importantly, TPOXX has been assessed for lesion recovery and viral shedding in research carried out in Brazil, Europe, and the Democratic Republic of the Congo (DRC). The PALM007 study (NCT05559099) targeted adults and children affected by Clade I Mpox in the DRC, whereas the STOMP trial (NCT05534984) included participants with Clade II Mpox in the U.S. and additional nations [138]. No safety concerns were identified in PALM007 [104]. Furthermore, Phase 4 trials for Bavarian Nordic's smallpox vaccine have taken place in Nigeria and Uganda. Safety and immunogenicity assessments have also been conducted for NIOCH-14 and VACΔ6 in Russia, alongside LC16m8 in Colombia. Additionally, preclinical findings from the UK regarding BNT166a mRNA vaccination studies (NCT05988203) indicate robust protection. This Phase I/II study intends to assess the safety, tolerability, reactogenicity, and immunogenicity of BNT166a for active immunization against Mpox in adults between 18 and 65 years, conducted at sites in the US and UK, projected to conclude in May 2025 [139].

Table 3. Preclinical Mpox vaccines and animal models.

Vaccine Description	Animal Model	Key Findings	Refs
Canine distemper virus (CDV) and Vaccinia immune globulin (VIG)	EiJ mice (aged 6–12 weeks)	Immunological responses and protective effectiveness	[140]
Dryvax vs non-vaccinated Control	Prairie dogs	Viral pathophysiology and vaccine-induced immunity	[141]
Sham-vaccinated and Elstree-RIVM vaccine	Cynomolgus macaques (*Macaca fascicularis*)	Evaluated the safety of vaccines and protective immunity; safe and immunogenic	[142]
Attenuated virus NYCBH-AESL (E3L Gene Deleted)	Cynomolgus monkeys	Examined the immune system's reaction to genetic changes	[143]
Modified vaccinia Ankara (MVA)	Cynomolgus monkeys	Demonstrated safety and immunogenicity of MVA-based vaccines	[144]
Recombinant MVA	Rhesus macaques (*Macaca mulatta*)	Explored enhanced immunogenicity through genetic modifications	[145]
MVA or Dryvax	Cynomolgus monkeys	Immune responses to conventional and modified vaccinations were compared	[146]
Dryvax and TPOXX	BALB/c mice	Evaluated the combined effect of vaccination and antiviral treatment which was shown to be effective	[147]
JYNNEOS or ACAM2000	Cynomolgus monkeys	Compared efficacy and safety profiles of both vaccines, with safety profiles	[148]

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LC16m8 and Lister (Elstree Strain)	Cynomolgus monkeys	Immunogenic and safe	[149]
Dryvax, ACAM2000, or JYNNEOS	Prairie dogs	Vaccine induced protection	[150]
JYNNEOS or ACAM2000	Prairie dogs	Vaccine efficacy against orthopoxvirus infections.	[151]
Live smallpox vaccine	BALB/c mice	Immune responses and durability of immunity	[152]
ACAM2000 and TPOXX	Cynomolgus monkeys	Synergistic effects of vaccination and antiviral therapy	([153]
Dryvax	Cynomolgus monkeys	Safety and long-term immunity of vaccines	[154,155]
Brincidofovir	Black-tailed prairie dogs	Examined the effectiveness of antiviral drug in treating poxvirus infections which showed good responses	[44]

Table 4. Human clinical trials against Mpox [156].

Intervention Trial No.	**Country**	**Year (Phase), Status**	**Number of Subjects**	**Primary Endpoints**	**Key Findings**
TPOXX NCT05597735	Brazil	2024 (3), recruiting	Not specified	Reduction of illness duration and contagiousness	The primary result is the duration required for the healing and re-epithelialization of all visible lesions (skin and mucosal).
TPOXX Oral Capsule NCT06156566	Belgium, France, Spain	2024 (4), ongoing	150	Anal pain assessments using the health-related symptom Index, on days 7, 14, and 90	The health-related Symptom Index was utilized to evaluate anal pain on days 7, 14, and 90. From the 28th day of randomization until the first day, every lesion

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					was fully healed and new skin grown
TPOXX Oral Capsule NCT05559099	The Democratic Republic of the Congo	2022 (Randomized, placebo-controlled, double-blind study) completed	597	The time to lesion resolution, measured in days until all lesions are scabbed or a new layer of skin is developed	Without posing any serious safety risks, the treatment greatly decreased viral shedding, accelerated lesion healing, and alleviated symptoms.
TPOXX (antiviral medication) NCT00728689	United States	2008 (1), completed	12	A single dose of ST-246 Form I versus Form V was tested for pharmacokinetics and safety in healthy volunteers	The study found no serious adverse events with ST-246 Form I and Form V. Pharmacokinetic parameters were similar for both forms
MVA-BN (JYNNEOS) NCT06549530	The Democratic Republic of the Congo	2024 (2), ongoing	460	Two weeks after the second MVA-BN injection, serum neutralizing antibodies against the vaccinia virus were measured using plaque reduction neutralization tests (PRNTs).	Test MVABN standard regimen neutralizing antibody response durability
JYNNEOS (vaccine for the prevention of smallpox and Mpox)	United States of America	2022 (2), completed	229	The PRNT assay was used to analyse venous blood on Study Day 43 to determine if the	At six weeks, the dose-sparing intradermal Mpox immunization regimen

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NCT05512949				intradermal regimen of $2x10^7$ TCID50 MVA-BN and $1x10^7$ TCID50 MVA-BN were non-inferior to the licensed subcutaneous regimen of $1x10^8$ TCID50 MVA-BN	induced antibody responses comparable to the conventional regimen. The vaccine was found to be safe
Bavarian Nordic smallpox vaccine NCT05745987	Nigeria, Uganda	2024 (4), ongoing	1560	Degree of symptoms and RT-PCR-confirmed Mpox	This research will ascertain whether the smallpox vaccine lessens the frequency of recurrent infections and the intensity of symptoms in those who have been exposed to Mpox.
MVA-BN vaccine NCT05734508	The Democratic Republic of the Congo	2023 (4), completed	500	New drug healing time and symptom reduction	The MVA-BN vaccine was safe, with no serious adverse events. Minor injection-site reactions were most common
NIOCH-14 (National Institute of Occupational Health and Safety) NCT05976100	Russian Federation	2020 (1), completed	90	Monitoring various blood parameters (like erythrocyte, leukocyte, and platelet levels), biochemical markers (such as glucose and creatinine levels), and the	NIOCH-14 was determined to be safe and well-tolerated in healthy volunteers; no significant adverse effects were noted

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				occurrence of adverse events over time	
VACΔ6 vaccine NCT05846243	Russian Federation-Novosibirsk Region	2021 (2 and 3), completed	334	The percentage of vaccinees with a titre of virus-neutralizing antibodies to vaccinia virus ≥ 1:40	VACΔ6 vaccine was safe and well-tolerated, with no adverse and only mild reaction at the site of injection
LC16m8 live attenuated vaccine of vaccinia virus NCT06223919	Colombia	2024 (3), active, not recruiting, Replicative live attenuated vaccinia virus vaccine: A randomized trial	8686	Based on each cohort's Poisson distribution, the laboratory-confirmed Mpox incidence rate will be computed with a 95% CI. To assess efficacy, immunogenicity and, safety	The vaccine was well-tolerated, with no serious adverse events observed.
BNT166a (mRNA vaccines by BioNTech NCT05988203	United Kingdom	2023 (2), Active, not recruiting, A Phase I/II randomized, partially observer-blind, dose-escalation trial of investigational RNA-based Mpox vaccine	96	The proportion of individuals who experienced at least one adverse event of special interest (AESI) between Dose 1 and day 201 after dose 1, inclusive. Overall, Safety and immunogenicity	No clinical results are published yet. Preclinical studies showed strong immune responses and full protection in animal models.

6. Pharmacological Landscape: A Comparative Analysis

The pharmacological landscape for Mpox evaluates both existing and new vaccines and antivirals to overcome disease. While Mpox symptoms are generally less severe as those of smallpox, Mpox can still cause significant disease. As described thus far, the pharmacological landscape to Mpox includes, supportive care, antivirals and vaccines. A comparative analysis of these treatment options are summarised as follows:

Supportive care and management

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- Whilst Mpox is self-limiting and usually resolve without treatment. Those with symptoms can be managed with anti-histamines for rashes, pain relief medications for headache and muscle aches, topical creams (such as antiseptics, hydrocortisone) for skin lesions, and regular hydration. In rare instances complications such as pneumonia, secondary bacterial infections, eye infections and encephalitis may occur, and medical help is advised. In such instances antivirals may be used based on the specific circumstances. Vaccines are used to prevent further spread during outbreaks.
- Individuals positive for Mpox are generally isolated to prevent spread and during outbreaks personal protective equipment such as masks, hygiene practices and social distancing are encouraged.

Antivirals

- TPOXX, approved by the FDA as a smallpox antiviral, has been shown to be effective against Mpox, reducing the mortality rate from 3.6% to 1.7% [157]; but was not effective against clade I Mpox lesions. It is currently used as a frontline treatment
- CDV an antiviral that inhibits the synthesis of viral DNA, is used to treat MPXV, in particular those with severe diseases or who have not responded to TPOXX.
- BCV a derivative of cidofovir has been investigated to treat Mpox. Its mechanism of action is similar to cidofovir with better safety profile to cidofovir. Both CDV and BCV have been shown to considerably reduce the viral replication of Mpox clade IIa and IIb [51].
- The development of drug-resistant Mpox strains emphasizes the requirement for continuous research and surveillance to develop new antivirals or use of combination treatments [158].
- New experimental therapies are currently being developed and tested, including vaccinia immune globulin, antibodies targeting the vaccinia virus that may also cross-react with Mpox, and repurposed drugs identified through computational and biological testing for their potential effectiveness in treating Mpox.

Vaccines

- JYNNEOS vaccine against smallpox and Mpox, a modified non-replicating vaccinia Ankara virus is safe, provides strong immune responses to both smallpox virus and Mpox, and is the preferred vaccine for use during Mpox outbreaks.
- ACAM2000 vaccine against smallpox, is a live vaccinia virus vaccine and is restricted in its use against Mpox, especially in those who are immunocompromised; it has shown some protection against MPXV infections
- JYNNEOS and ACAM2000 are regarded as safer alternatives to the older smallpox vaccines, like Dryvax, which were used during the smallpox eradication effort. While effective, the older smallpox vaccines use live, replication-competent vaccinia virus vaccine, and their use is not preferred due to the risks associated with live virus vaccines.
- LC16m8 has received emergency use authorization, LC16m8 shields animals from deadly dosages of viruses like Mpox. Little information is available on LC16m8 usage during Mpox outbreaks. The virus in LC16m8 is weakened. Itching, redness, swollen lymph nodes, fever, and exhaustion are some of the side symptoms. The vaccine virus has the potential to spread to other bodily parts. To date, LC16m8 has been donated to over 90,000 individuals. These dosages did not result in any notable safety signals, even in 50,000 youngsters. Pregnant women, those with specific skin conditions, and immunocompromised individuals should not get LC16m8 [159].

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7. Future Directions in Mpox Research

7.1. Integrating Mechanistic Insights into Drug Development

Mechanistic insights into Mpox replication provide important targets for antiviral intervention. For the development of antivirals, investigating immune evasion mechanisms and virus host interactions is still essential. The focus of research is on comprehending the entire lifecycle of the Mpox, from entry to replication and assembly. One idea is that the Mpox ability to change the way host cells work, for example, by turning the endoplasmic reticulum into replication factories, may make it more resistant to immune responses and apoptosis, opens up new treatment targets. High-throughput screening techniques improve our understanding of the host factors that viruses use, while research continues to understand the pathogenetic and structural mechanisms, replication processes, and interactions of the Mpox with the innate immune system [95,160]. Despite achievements, further research is required to develop treatments for Mpox to prevent drug-resistant strains and long-term outbreaks. Improving the specificity and accessibility of antiviral, preventing resistant strains, and evaluating combination therapy to treat various infection stages are required. Reducing side effects requires lowering drug toxicity. Pharmaceutical development must be rapid and robust to combat the global Mpox outbreak and prepare for future public health emergencies [161,162].

7.2. Collaborative Efforts in Global Health Contexts

Collaboration between governments, research institutions, and health organizations has increased significantly as a result of the global response to Mpox. These collaborations concentrate on developing antivirals, re-purpose drugs, vaccines, monitoring, diagnosis, and provide equal access to care. Global health agencies like the WHO and the Centers for Disease Control and Prevention (CDC) are imperative to global efforts to combat Mpox due to their monitoring of outbreaks, support research, and advise nations on containment strategies. The Clade IIb-driven global Mpox outbreak in 2022-2023 highlighted the necessity of concerted efforts as cases extended outside of Central and West Africa's endemic areas. Further, Clades Ia and Ib are also in circulation, with Clade Ib most recently being detected outside of Africa [163,164]. Vaccination campaigns continue to be a top priority, especially for high-risk groups. Global collaboration efforts are necessary to prevent future outbreaks, strengthening disease surveillance, increasing data sharing, and strengthening public health infrastructure. The success of prevention, control, and treatment strategies for Mpox and other emerging infectious diseases relies on a coordinated global response [165].

7.3. Potential for Personalized Medical Approaches

Personalized medicine customizes medical treatments based on unique characteristics, preferences, and genetic profiles, potentially improving the efficacy of interventions for diseases such as Mpox. Vaccines can be used in personalized medicine to direct vaccination strategies based on an individual's specific health needs, genetic makeup and whether they are immunocompromised, in order to reduce side effects and improve outcomes [166]. In addition, artificial intelligence (AI) driven proficiency can improve patient outcomes by making it easier to develop personalized treatment plans that are specific to each patient's needs [167,168]. AI uses patient data to recommend personalized drugs, increasing the effectiveness of treatment and reducing adverse effects [169]. Incorporating AI into healthcare processes for Mpox management is expected to improve productivity and reduce diagnostic errors [170]. AI can be used to tailor treatments by adjusting them based on patient information, such as underlying health conditions, previous vaccination history and immune status [171]. The **PoxApp (https://poxapp.stanford.edu/) was developed as** a mobile application to support a personalized medical approach to monitor and manage symptoms or diagnosis of **Mpox** cases (and other pox-related diseases). By providing real-time data on symptoms, exposures, and patient progress, it assists in personalized treatment decisions, offers diagnostic

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support, and monitors the effectiveness of interventions, leading to a more tailored and efficient response to outbreaks. Furthermore, a new personalized medical approach using an image-based deep convolutional neural network (MPXV-CNN) shows promise in detecting MPXV infections earlier, which could help prevent outbreaks by identifying characteristic skin lesions. MPXV-CNN has shown to have strong sensitivity and specificity across different skin tones and body regions. As such, MPXV-CNN is accessible through a web-based app for patient guidance, enhancing early detection and isolation efforts [172]. Correct diagnosis of MPXV infection necessitates laboratory validation, since clinical symptoms alone cannot be differentiated from other poxvirus infections. PCR testing of viral DNA from lesion samples (scabs, discharge, or vesicular lesions) continues to be the WHO-recommended gold standard diagnostic technique; however, specimens need to be preserved under sterile, cool, and dark conditions. Other diagnostic methods consist of immunohistochemistry, viral cultures obtained from nasopharyngeal or oropharyngeal swabs, evaluation using electron microscopy, and serological antibody analysis through ELISA. Samples obtained from vesiculopustular rashes or vesicular lesions usually provide the most precise outcomes [173]. As the recommended technique for identifying MPOX infections, PCR testing can improve infection control strategies by facilitating the early diagnosis of new cases [174]. Given its effectiveness, the Mpox vaccine ought to be made available to everybody who could be at risk of contracting the disease. To guarantee prompt diagnosis and suitable treatment, professionals need to be aware of the distinctive signs and symptoms of Mpox, a disease that is a constantly changing worldwide health concern.

8. Limitations

This review has several limitations. First, although it provides a comprehensive overview of current antiviral agents and vaccines for Mpox, much of the clinical evidence remains limited to observational studies, animal models, or early-phase trials, reducing the generalizability of findings to broader populations. Second, the effectiveness of treatments against Clade I and emerging variants of Mpox is not fully established, and data are especially lacking in vulnerable populations such as children, pregnant women, and immunocompromised individuals. Third, the computational drug repurposing strategies discussed require further experimental validation before clinical application. Finally, while global disparities in access to therapeutics and vaccines are acknowledged, this review does not address in-depth the socio-political and logistical barriers to equitable distribution, which remain critical for effective Mpox control worldwide.

9. Conclusions

A comprehensive approach to the prevention, treatment, and control of Mpox is essential as the virus poses an increasing threat to global health. Herein, we highlighted the challenges and progress in combating MPXV infections. While antiviral drugs such as TPOXX, BCV, and CDV show promise, further clinical trials are required to confirm their effectiveness and safety. TPOXX, which targets the F13L protein essential for viral envelope formation, plays a key role in modern treatment strategies. However, its limited efficacy against clade I variants highlights the need for new antivirals with broader effectiveness. Vaccination remains the most effective prevention method, with JYNNEOS and ACAM2000 leading the efforts. These vaccines, derived from the vaccinia virus, offer cross-protection against Mpox. Advancements in vaccine technologies, like mRNA and viral vectors, are transforming immunoprophylaxis, but equitable vaccine distribution, especially in endemic areas, remains a significant challenge. Research on Mpox replication and immune evasion has highlighted treatment targets, and host-directed therapies are gaining attention for improving antiviral efficacy. Despite progress, challenges such as limited animal models, viral mutations, and resource scarcity slow down treatment development. Computational biology and bioinformatics offer promising solutions for faster therapeutic identification and mutation prediction. Addressing Mpox requires global collaboration among researchers, policymakers, and agencies (CDC, WHO) to improve

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monitoring, diagnostics, and health measures, ensuring fair access to treatments and preparedness for future pandemics.

Abbreviations

MPXV	Monkeypox Virus
Mpox	Monkeypox
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
TPOXX	Tecovirimat
HIV	Human Immunodeficiency Virus
STIs	Sexually Transmitted Infections
FDA	Food and Drug Administration

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