HHS Public Access

Author manuscript

Expert Opin Ther Targets. Author manuscript; available in PMC 2024 July 04.

Published in final edited form as:

Expert Opin Ther Targets. 2023 ; 27(6): 419–431. doi:10.1080/14728222.2023.2230361.

Potential therapeutic targets for Mpox: the evidence to date

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Abstract

Introduction: The global Mpox (MPX) disease outbreak caused by the Mpox virus (MPXV) in 2022 alarmed the World Health Organization (WHO) and health regulation agencies of individual countries leading to the declaration of MPX as a Public Health Emergency. Owing to the genetic similarities between smallpox-causing poxvirus and MPXV, vaccine JYNNEOS, and anti-smallpox drugs brincidofovir and tecovirimat were granted emergency use authorization by the United States Food and Drug Administration. The WHO also included cidofovir, NIOCH-14, and other vaccines as treatment options.

Areas covered: This article covers the historical development of EUA-granted antivirals, resistance to these antivirals, and the projected impact of signature mutations on the potency of antivirals against currently circulating MPXV. Since a high prevalence of MPXV infections in individuals coinfected with HIV and MPXV, the treatment results among these individuals have been included.

Expert opinion: All EUA-granted drugs have been approved for smallpox treatment. These antivirals show good potency against Mpox. However, conserved resistance mutation positions in MPXV and related poxviruses, and the signature mutations in the 2022 MPXV can potentially compromise the efficacy of the EUA-granted treatments. Therefore, MPXV-specific medications are required not only for the current but also for possible future outbreaks.

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KS (Kamal Singh) conceptualized the study; SNB, KS (Kamal Singh), and KS (Kalicharan Sharma), wrote the first draft. KS (Kamal Singh), SS, ASR, AA, SS, SNB, and CLL edited the final manuscript. KS (Kamal Singh) and SS conducted the literature search. KS (Kamal Singh) conducted the genetic analyses. KS (Kamal Singh) and KS (Kalicharan Sharma) conducted structural analyses. KS (Kamal Singh), SS, SNB, AA, KMK, and CLL edited the final manuscript and contributed to understanding the pathogenicity of MPXV. All authors approved the final manuscript.

Declaration of competing interest

CLL is co-founder and Chief Scientific Officer of Shift Pharmaceuticals, Overland Park, KS, USA. KS is a consultant for Sanctum Therapeutics Corporation, Sunnyvale, CA, USA.

Keywords

Mpox; Cidofovir; Brincidofovir; Tecovirimat; OPG71; OPG57; F8L; C19L

1. Introduction

The 2022 Mpox (MPX) outbreak has spread to more than 110 countries with approximately 90,000 documented cases. Due to the volume of the outbreak, and lessons learned from the Coronavirus Disease 19 COVID-19 pandemic, the WHO quickly acted by declaring MPX a Public Health Emergency (PHE) of international concern on July 23, 2022. Subsequently, the United States Human Health Services Administration (US-HHS) declared MPX as a PHE on August 5, 2022. Similar actions were taken by health regulatory agencies of individual countries worldwide. Although the PHE status of MPX was lifted on Jan. 31, 2023, by the US-HHS, new cases of MPXV will likely be identified. Additionally, the emergence of a virus closely related to MPXV in future outbreaks cannot be ruled out.

MPX is a zoonotic disease. It is caused by infection with Mpox (formerly known as monkeypox) virus (MPXV). MPXV is transmitted via infected skin, body fluids, and respiratory droplets. Symptoms include Flu-like conditions and rashes. MPXV is a linear double-stranded DNA virus with a genome length of ~200 kb, which encodes ~200 proteins. It belongs to the order *Chitovirales, the* family *Poxviridae*, and the genus *Orthopoxvirus*. The other examples of the *Orthopoxvirus* genus are the cowpox virus (CPXV), vaccinia viruses (VACV), and the variola virus (VARV). VACV has been extensively studied since all smallpox vaccines have been derived from VACV.

MPXV was first discovered in 1958 when two outbreaks of pox-like non-fatal disease were identified in cynomolgus monkeys during the summer of 1958[1]. These outbreaks occurred 51 and 62 days after the transport of monkeys from Singapore to Denmark, with 6% and 10% of the animals developing a pox-like disease, respectively. Additional MPX outbreaks were reported in 1968 from various countries (Panama, India, France, the USA, the Netherlands, Trinidad, Brazil, and Indonesia) in non-human primates[2, 3]. Concurrently, a pox-like disease was reported in humans, although the possibility of MPXV infections was ruled out at the time.[4] The first documented human MPXV infection was reported in 1970 in a 9-month old boy from the Democratic Republic of Congo[5].

Many sporadic outbreaks of MPXV have since been reported in different countries. Most of these were travel-related cases and restricted to travelers without any secondary transmission[6–11]. The first exported cases of MPXV infections were identified in the Midwestern USA in 2003, with 37 confirmed and 10 suspected infections[8, 12]. This outbreak was associated with exotic pocket pets (Prairie dogs) that were imported from Ghana. It is believed that the MPXV reservoir was the Gambian pouched rat, which transmitted the virus to the Prairie dogs. Therefore, while initially seemingly appropriate, the long-used name monkeypox is a misnomer as MPXV infects many animals[13, 14], and a definite MPXV reservoir has yet to be conclusively identified[7, 15].

2. MPXV genome replication cycle and therapeutic targets

In principle, all viral and host cell proteins that participate in viral replication can be antiviral therapeutic targets. However, as discussed in the following sections, only a few viral and/or host proteins routinely turn out to be viable therapeutic targets.

2.1 MPXV genome replication cycle

Details of the MPXV replication cycle steps have not been established. Therefore, other well-studied and closely related poxviruses, such as VACV, must be used as a surrogate for our understanding of the MPXV replication cycle. Two distinct forms of infectious poxvirus virions can infect a host cell: (i) a mature virion (MV), and (ii) an extracellular enveloped virion (EV) (Fig. 1). MV has a single membrane, whereas the EV has an additional outer membrane[16]. The additional EV outer membrane is disrupted prior to fusion, rendering EV similar to MV at the point of entry into the host cell[16]. A multitude (20–30) of VACV proteins constitute the MV membrane, while the EV has ~6 additional proteins within the outer membrane. Entry and fusion of the MVs and EVs involve multiple viral and cell-surface proteins[17–19]. In addition, the attachment of MV and EV differs significantly[16]. For example, proteinase treatment disrupts the binding of MV but not EV[16, 20]. Thus, poxvirus entry and fusion are multiplayer and complex processes, making it challenging to select feasible antiviral targets from many viral proteins.

A distinct structure of MV/EV particles is the core[21, 22], which contains a linear dsDNA genome, virus-encoded enzymes, and factors required to transcribe early viral genes. Following entry and fusion, the uncoating of the viral core releases the genome into the cytosol, where the synthesis of early mRNA and genes begins, which is succeeded by DNA genome replication. The replicated DNA serves as the template for the synthesis of intermediate and late mRNAs. After the late genes' translation, the assembly of new viral particles initiates, resulting in the formation of infectious MVs (Fig. 1). Some MVs are released from the cell by lysis, whereas a population of MVs acquires trans-Golgi and/or endosomal membranes to become triple membrane wrapped particles called wrapped virions (WVs)[16]. These virions are transported to the cell surface, where the outer membrane fuses with the plasma membrane, and the virion is released as an EV.

2.2 MPXV replication-associated proteins as therapeutic targets

The most sought-after antiviral targets for all viruses are the components of the nucleic acid replication machinery. To date, ~100 antivirals are available in the US. The majority of these drugs target Human Immunodeficiency Virus (HIV) (42), hepatitis C (18), hepatitis B (10), and herpesviruses (10). Fifteen out of 42 currently approved antiretrovirals target HIV-1 reverse transcriptase (HIV-1 RT), underlining that nucleic acid polymerase is the most important therapeutic target. In poxviruses, there are at least two nucleic acid replicating complexes: (i) a multi-subunit DNA-dependent RNA polymerase (vRNAP) complex and (ii) a multi-subunit DNA replication holoenzyme. The cryo-EM structure of the VACV vRNAP transcription complex provided the details of subunit arrangements and the mechanism of poxvirus transcription[23]. No antiviral drugs targeting vRNAP components have been approved for any poxvirus RNA transcription component.

VACV DNA genome replication is conducted by a holoenzyme consisting of multiple proteins[22]. An essential component of this holoenzyme is E9, the DNA-dependent DNA polymerase belonging to B family DNA polymerases. The MPXV genome encodes F8L (OPG71)[24], also a B family DNA-dependent DNA polymerase[25], which shares ~98% identity with VACV E9. The first B family DNA polymerase (RB69) structure showed an overall architecture of this class of enzymes[26]. This structure showed a canonical polymerase domain consisting of the Thumb, Palm, and Fingers subdomains, as seen in the structure of the Klenow Fragment (KF) of *E. coli* DNA polymerase I[27]. A notable difference between these polymerases is the relative position of the 3' – 5' exonuclease domain, which was ~180° opposite to that in KF relative to the polymerase active site. Subsequent crystal structures of the RB69 polymerase showed that residues of a β-hairpin positioned in the major groove of the template-primer played a role in the partitioning of primer to the 3' – 5' exonuclease site upon mismatch nucleotide incorporation[26, 28–30]. Indeed, a resistance mutation on topologically similar β-hairpin in poxviruses' DNA polymerase showed the relevance of the resistance mechanism of nucleotide analogs mediated by 3' – 5' exonuclease function (discussed below).

Numerous structures of B family DNA polymerases have since been reported[28, 31–39]. The most notable viral DNA polymerase structures include the structure of unliganded herpes simplex virus 1 (HSV-1) DNA polymerase[35], HSV1 DNA polymerase in complex with template-primer and 4-oxo-dihydroquinoline[39], the crystal structure of VACV DNA polymerase E9[38], and a recently reported cryo-EM structure of the MPVX DNA polymerase holoenzyme[40]. Notably, the VACV E9 structure was the first to provide details of poxvirus-specific inserts[38]. Before the cryo-EM structure of MPXV DNA polymerase holoenzyme, a homology built MPXV F8L based on this structure, AlphaFold[41], and ColabFold[42], predicted structures of processivity factor A22R (OPG148), PCNA (Proliferation Cell Nuclear Antigen) ortholog G9R (OPG93), homology-derived MPXV uracil DNA glycosylase (OPG116) were used to assemble an MPXV replication complex[43]. This MPXV replication complex was guided by a low-resolution structure of the vaccinia virus DNA replication machinery[44] and the structure of eukaryotic DNA polymerase δ bound to PCNA[45]. Mutations presented in the 2022 MPXV outbreak were mapped onto the assembled complex to predict the impact of these mutations on the replication of MPXV genome replication[43].

At least 8 proteins participate in VACV DNA replication[22] (Table 1) and are conserved between VACV and MPXV. The DNA polymerase, helicase/primase, UDG, processivity factor (A20R/A22R), and SSB are essential for viral replication[16, 22]. Host protein kinase VRK1 can complement the replication defect due to the B1R VACV kinase mutant[46]. Similarly, host DNA ligase I has been shown to substitute for VACV A50R[47]. The absence of the FEN1 family endonuclease results in the reduced mean size of the replicated DNA and packaged virion with little or no viral DNA[48]. VACV A20R and MPXV A22R are processivity factors, and SSB is an ssDNA coating protein; neither protein exhibits any enzymatic activity. In addition to these, H5R (a multifunctional phosphoprotein), A22R (A23R in MPXV, Holliday junction resolvase), and H6R (G7R in MPXV, a Topoisomerase) participate in the completion of poxvirus (VACV) genome replication[21]. Thus, DNA

polymerase, helicase-primase, and topoisomerase remain attractive antiviral targets of MPXV infection.

2.2.1 DNA polymerase structure and the mechanism of CDV/BCV inhibition

—As the poxvirus DNA polymerase (*e.g.,* VACV E9 or MPXV F8L) is essential for viral replication[21, 49], it is the most sought-after antiviral therapeutic target against MPX. The FDA-approved drug brincidofovir (BCV) has been granted EUA, while the WHO has included the parent compound cidofovir (CDV) (Fig. 2) for the treatment of MPX. CDV is an acyclic nucleoside phosphonate (ANP), which was developed by Antonin Holy as an antiviral compound[50, 51]. ANPs have been extremely successful antivirals as tenofovir disoproxil (TDF) and tenofovir alafenamide (TAF), the prodrugs of tenofovir are the integral part of antiretroviral therapy against HIV. BCV is a CDV prodrug converted into CDV-diphosphate (CDVpp) by cellular kinases, becoming a ready-to-use substrate for the viral DNA polymerase. CDV is a broad-spectrum inhibitor of dsDNA viruses' DNA polymerases[52]. It inhibits viral DNA replication by multiple mechanisms: as a dCTP competitor, a nonobligate chain-terminator, resistant to 3'−5' exonuclease (when present at the penultimate 3'OH position), an inhibitor of template-directed trans-lesion synthesis, and as a mutagen[53–56] when present in the template strand. Since BCV is eventually metabolized to CDV-diphosphate (CDVpp), the inhibition mechanism of BCV is identical to CVD as far as the mutations in poxvirus DNA polymerases are concerned.

2.2.2 Resistance to CDV/BCV—

CDV resistance mutations in multiple orthopoxviruses have been identified[52, 57–62]. These mutations are ΔK174 (deletion of K174), A314T, S338F, A613T[61], A684V/T, T688A, T808M[61], T831I, and S851Y (Fig. 3a). The wild-type residues are conserved in all poxvirus DNA polymerases studied to date. Therefore, the CDV resistance mechanism appears to be shared among orthopoxviruses[61]. The crystal structure of VACV E9 was the first solved structure that provided the topological positions of the CDV resistance mutations relative to the two active sites (polymerase and 3'−5' exonuclease)[38]. However, a recently reported structure of MPXV DNA replication holoenzyme[41] showed the proximity of CDV resistance mutations to the template-primer and dNTP substrate (Fig. 3a). While this cryo-EM structure is a breakthrough in poxvirus structural biology, it is not a complete DNA replication holoenzyme, as the helicase-primase, an integral part of the holoenzyme, is missing[44].

Three CDV resistance mutation positions (174, 314, and 338) are in the 3'−5' exonuclease domain, whereas six (613, 684, 688, 808, 831, and 851), are in the polymerase domain (Fig. 3a). A CDV resistance mutation A314V/T has been identified in multiple poxviruses[52]. Mutation A314V in VACV DNA polymerase enhances the excision of CDV from the primer terminus[52]. A314 is part of a β-hairpin located within the major groove at the active site (Fig. 3b). Mutation A314V/T may change the interactions of this hairpin structure with the template-primer, resulting in the enhanced partitioning of the primer to the exonuclease site.

Two resistance mutation positions (684 and 688) are proximal to the CDVpp binding site (Fig. 3c). While these residues do not interact directly with the CDVpp (or dCTP), they do interact with critical dNTP binding pocket residues (Y554 and Y668) (Fig. 3c). Y554 is at the 5th position downstream of the first Motif A catalytic residue D549. A bulky

residue is essential at this position for the exclusion of ribonucleotides (NTPs) to bind at the DNA polymerase site[63–66]. Y668 is at a topologically equivalent position to F762 of *E. coli* DNA polymerase I. Mutation F762Y enables *E. coli* DNA polymerase I to accept dideoxy nucleotide triphosphate substrates[67]. Therefore, mutations at residues 684 and 688 can alter the dNTP pocket by indirectly changing the interactions of Y554 and Y668 such that the polymerase discriminates against CDVpp. Another CDV resistance mutation at position 831 is close to the primer strand. All other CDV resistance mutation positions are not within the interacting distance to either template-primer or the dNTP substrate. However, these resistance mutations can indirectly interfere with the binding of CDVpp or the primer 3'OH position.

2.2.3 Mutations in F8L 2022 outbreak—A temporal analysis showed that two mutations in F8L (L108F and W411L) emerged during the 2022 outbreak[43]. Residue position 411 belongs to poxvirus-specific 'insert 2'. Many phosophonoacetic acid (PAA) resistance mutations are in this insert[68]. Insert 2 is close to the Fingers subdomain which is involved in substrate binding. Therefore, W411 may affect the binding affinity of dNTP substrate resulting in the change in replication kinetics of the polymerase. Alternately, as seen in the reported MPXV replication holoenzyme[40], L411 is exposed to the surface (Fig. 4a). For a hydrophobic residue to be exposed at the surface is highly unusual. It is possible that 'insert 2' also interacts with another factor, and this residue is buried at the interface of two proteins[43]. F8L amino acid position 108 is close to the ssDNA overhang of the template strand. As previously proposed, mutation L108F should increase the binding affinity of template-primer with F8L, which can enhance the processivity and/or polymerase strand-displacement DNA synthesis[69]. The phenylalanine residues at a topologically equivalent position in HIV-1 reverse transcriptase[70, 71] and *E. coli* DNA polymerase I have been shown to interact with the template base in the ssDNA region and to contribute to strand-displacement DNA synthesis[69, 72, 73]. Therefore, it is possible that mutation L108F emerged to enhance the binding of template-primer with the polymerase and facilitate strand-displacement DNA synthesis.

3. ST-246 or tecovirimat

Tecovirimat (initially known as ST-246) (Fig. 2) was first reported in 2005 as an inhibitor of extracellular virus formation, and it protected mice from multiple orthopoxviruses including VACV, MPXV, camelpox, cowpox, mousepox, and VARV[74]. The G277C resistance mutation that emerged in the cowpox V061 gene suggested that ST-246 targeted the V061 gene[74], an ortholog of F13L (VACV) and C19L (MPXV). F13L and C19L have phospholipase activity[75], are palmitylated[76], and participate in enveloping the intracellular MV to generate extracellular EV particle[77, 78].

Tecovirimat is the most characterized among three poxvirus antivirals (CDV, BCV and ST-246). Its efficacy against MPXV infection has been tested in cell culture[79–81], and animal models[82–85] including non-human primates[86–89]. ST-246 showed strong inhibitory activity with good pharmacokinetics in all models (reviewed by Duraffour et al.[90], and by Smee[91]). *In vitro* potency of tecovirimat (IC_{50} = 12.7 nM) was recently reported in cell-based assays using MPXV/France/IRBA2211i/2022 isolate[92]. Tecovirimat showed a synergistic effect

when combined with BCV[82]. Co-administration of tecovirimat and vaccine ACAM2000 suggested that tecovirimat can be safely used after vaccination[93].

Tecovirimat resistance mutations have been identified in F13L and its orthologs in different orthopoxviruses[74, 94]. Seven resistance mutations (F25V, H194N, G277C, D283Y, A290V, L315M , and I372N) and an insertion of an SVK triplet at positions 303–305 have been reported[94]. Most of these resistance mutations emerge in combination with other mutations[94]. Two resistance mutations, A290V, and L315M, were identified by next-generation sequencing of a clinical isolate obtained from an immunosuppressed patient with progressive vaccinia who received ST-246 and vaccinia immune globulin intravenous and BCV[94, 95].

The structure of F13L or its orthologs has not been solved. However, a homology-derived model of MPXV C19L using the crystal structure of Phospholipase D from *Streptomyces sp*. as a template (PDB entry 1V0W)[96] shows that there is only one pocket in *Streptomyces sp*. Phospholipase D or in the C19L homology model, where tecovirimat can be docked with a minimal conformational change in the protein. The best docked pose obtained based on the ‘Glide’ score (Schrödinger LLC, NY) is shown in Fig. 5. Most of the resistance mutations are around the docked tecovirimat. Three mutations emerged in the 2022 outbreak: V5A, S250N, and E353K (our unpublished results). The selection pressure of these mutations remains unknown. One of these mutations, S250N is in the vicinity of docked tecovirimat (Fig. 5). It is possible that S250N may impart some resistance to tecovirimat. A prodrug of tecovirimat, NIOCH-14 has been reported to have comparable efficacy in animal model[97–99].

4. Efficacy of CDV, BCV, and tecovirimat against currently circulating MPXV

The FDA has not approved CDV for treating MPXV infections. Instead, it has been approved for cytomegalovirus retinitis in HIV-infected patients. However, CDV has shown antiviral activity against molluscum contagiosum in HIV-infected patient[100]. BCV has been approved for smallpox treatment as an oral drug. The efficacy studies of these drugs against currently circulating MPXV viruses are limited. In a recent report, the efficacy of CDV, BCV and tecovirimat was evaluated using 12 patient isolates in relevant cell models (human foreskin fibroblasts and human foreskin keratinocytes)[101]. The IC_{50} of tecovirimat, cidofovir, and brincidofovir was 4000 nmol, 80 μmol, and 600 nmol, respectively[101], and were reportedly within the range of therapeutic concentrations in plasma[101]. In another study, the plaque formation assay was used to evaluate the potency of tecovirimat, and CDV using a patient isolate (MPXV/France/IRBA2211i/2022). The results showed that tecovirimat IC_{50} was 12.7 nM whereas CDV IC_{50} was 30.4 μM, suggesting that tecovirimat was ~2400-fold more potent than CDV. This difference in Mpox inhibition[92] does not corroborate with the results of Bojkova et al.[101], where CDV is only 80-fold less potent than tecovirimat.

5. Efficacy of CDV, BCV, and tecovirimat MPXV in the context of HIV co-infection

Clinical studies have shown a significant prevalence (20% - 74%) of MPXV infection among HIV-infected patients[102–109]. Additionally, most (95% – 98%) MPXV infections among these clinical studies involved men with sex men (MSM) and bisexuals, suggesting that MSM and bisexual individuals may be highly susceptible to MPXV infection. Therefore, an MPXV treatment strategy for this group of individuals must be in place. CDV has broad-spectrum antiviral activities[110, 111]. However, only a few studies have been reported where orthopoxvirus infections have been treated with CDV, BCV and tecovirimat as described below. Early studies demonstrated that lipid esters of CDV (including BCV) were effective as prophylaxis in mice infected with cowpox or vaccinia virus[112]. CDV treatment cleared recalcitrant molluscum contagiosum, a poxvirus, in an AIDS patient[100]. Recently, a patient coinfected with HIV, MPXV, and primary syphilis was successfully treated with CDV[113]. There are a few examples of successful MPXV treatment by tecovirimat among patients coinfected with MPXV and HIV[114, 115]. One report showed that one of the two patients (Patient A) coinfected with HIV and ocular MPXV infection suffered profound visual impairment despite treatment with tecovirimat[115]. In contrast, the other patient (Patient B) recovered from the ocular MPXV infection after treatment with tecovirimat[115]. It is unclear why CDV or BCV was not prescribed to Patient A. In a 28-year-old patient infected with HIV and MPXV, the treatment with tecovirimat for 14 days resulted in decreased skin lesions and decreased MPXV viral load without any adverse events[114].

6. Summary

Here we presented our opinion on the feasibility of EUA-granted antivirals for the treatment of MPX. We discussed the impact of resistance mutations to these antivirals, the probable effect of signature mutations in the 2022 outbreak on the antiviral targets at the atomic level, the potency of these antivirals on currently circulating MPXV, and the latest results on the outcomes of EUA-granted MPXV treatments in the cases of MPXV and HIV co-infections. The limited number of clinical trials show that all three drugs: CDV, BCV, and tecovirimat appear to have good efficacy against currently circulating MPXV viruses. Future clinical results will provide a clearer picture of the antiviral activities of these compounds. It is almost certain that resistance mutations will emerge as the treatment becomes widely available or the population that has received treatment becomes significant. Since the resistance mutations of the three drugs have been evaluated in MPXV-related poxviruses, analogous mutations in MPXV can also be reasonably deduced. However, new resistance mutations may emerge as the current MPXV circulates among various conditions, including HIV infected/immunocompromised patients. An additional challenge is that only three currently available anti-MPXV drugs (as access to NIOCH-14 is limited). Therefore, more drugs targeting F8L and C19L are needed for the current outbreak or for possible future outbreaks with variants of MPXV. Thus, broad-spectrum inhibitors such as cytosine arabinoside (ara-C, also known as cytarabine) and 9-β-D-arabinofuranosyladenine (ara-A) that have shown antiviral activities should also be considered[116]. Due to the reported high

prevalence of MPX among MSM and bisexual individuals, individuals from this community must be advised to adopt safe practices and treatment strategies to control the spread of MPXV[118].

Acknowledgments

K. Singh acknowledges the computation facilities of the Molecular Interactions Core at the University of Missouri, Columbia, MO 65212. We thank the laboratories that have generously deposited sequences into the NCBI database. We also thank numerous laboratories that have enormously contributed to poxvirus research, but we could not cite their work.

Funding

K. Singh was partially funded by the Bond Life Sciences Center (Early Concept grant), a subcontract from Emory University (5R37AI076119), and the University of Missouri startup support.

Data Sharing

Genomic sequences used in this study were obtained from NCBI GenBank.

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Article highlights

1. The 2022 Mpox outbreak and the volume of infections showed that poxviruses remain a constant threat to global health. Signature mutations in currently circulating Mpox viruses (MPXV) may be contributing in unknown ways to the outbreak. Therefore, the role of these mutations in viral replication needs to be established.
2. There are no Mpox-specific treatments available. Limited studies indicate that the treatments that have been granted emergency use authorization (EUA) have good efficacy against MPXV. However, additional, and more robust studies are needed to establish the potency of these drugs against MPXV.
3. Nucleic acid polymerases are the most sought-after antiviral targets. Considering their essential role in virus replication, research to discover poxvirus DNA polymerase inhibitors is needed. Additionally, other components of viral DNA replication holoenzyme, such as DNA helicase, should be extensively characterized so that new antivirals can be developed against such targets.
4. The current MPXV was most prevalent among a specific group of individuals (men who have sex with men, and bisexuals). Many of these individuals are coinfected with HIV and MPXV. Therefore, drugs targeting MPXV and HIV coinfection need to be developed.

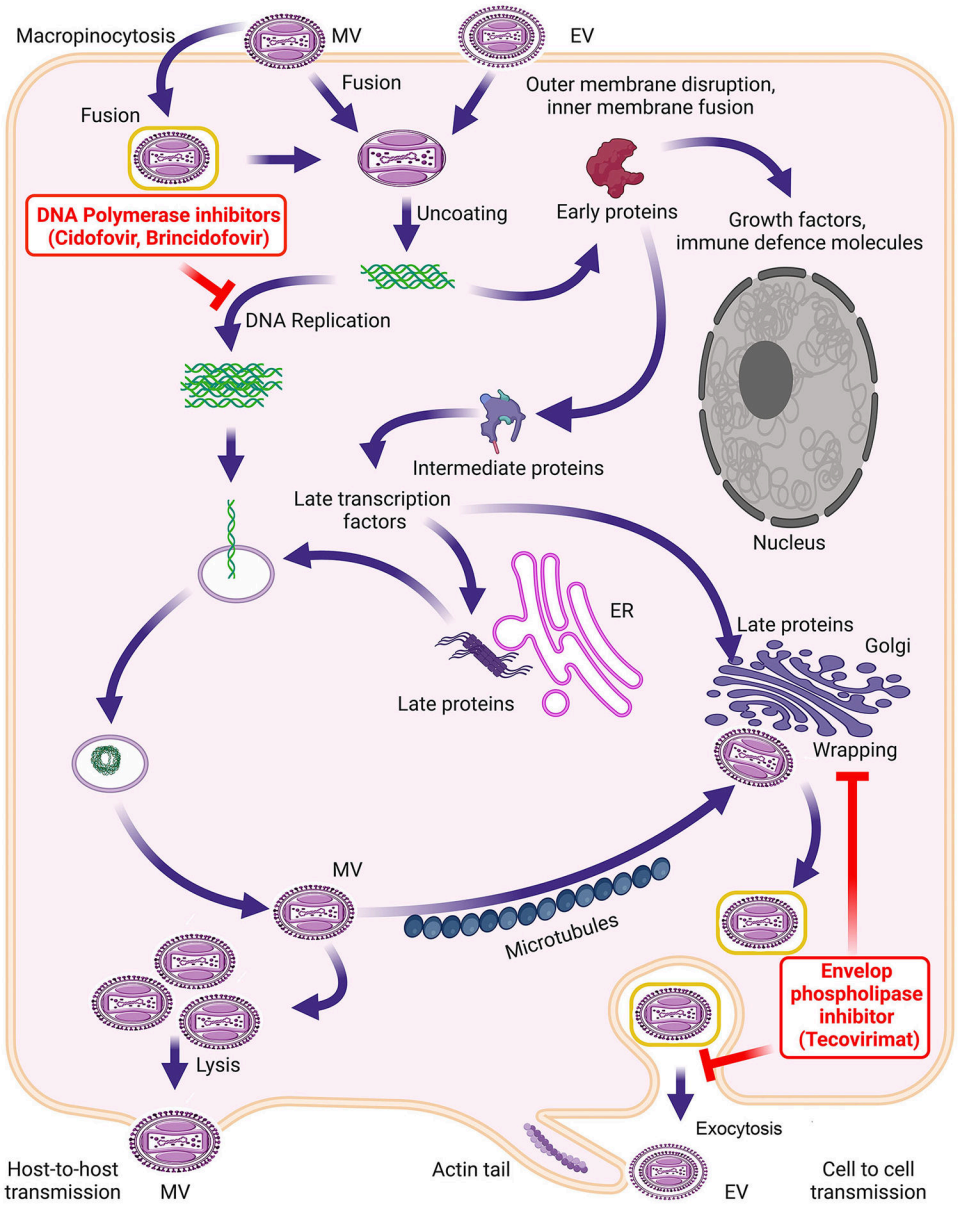
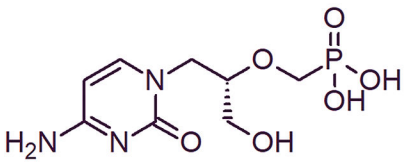
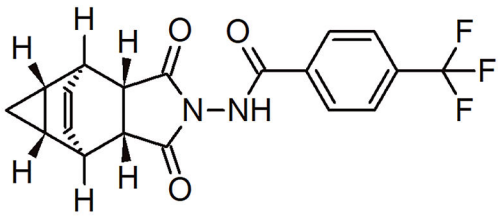


Figure 1. Replication cycle of MPXV and steps targeted by CDV, BCV and tecovirimat

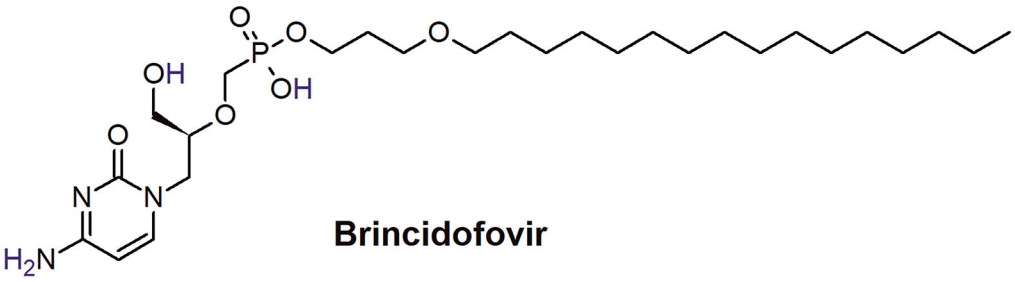
– The orthopoxviruses enter the cell either directly by binding to the cell surface receptors or through micropinocytosis. Following fusion, the core containing linear dsDNA genome and proteins required for early transcriptions are released into the cytosol. The replication of the dsDNA genome at this step serves as the template for intermediate and late gene transcription. CDV and BCV target the replication of the dsDNA genome. Late proteins and viral genomes assemble to form the MV. Some MVs acquire trans-Golgi and/or endosomal membranes to become triple-membrane-wrapped particles. The membrane biogenesis is mediated by the orthopoxvirus phospholipase protein (*e.g.,* MPXV C19L), which is targeted by tecovirimat. The wrapped virions are transported to the cell surface, where the outer membrane fuses with the plasma membrane, and the virion is released as an EV.



Cidofovir



Tecovirimat



Brincidofovir

Figure 2.
Chemical structures of CDV, BCV, and tecovirimat.

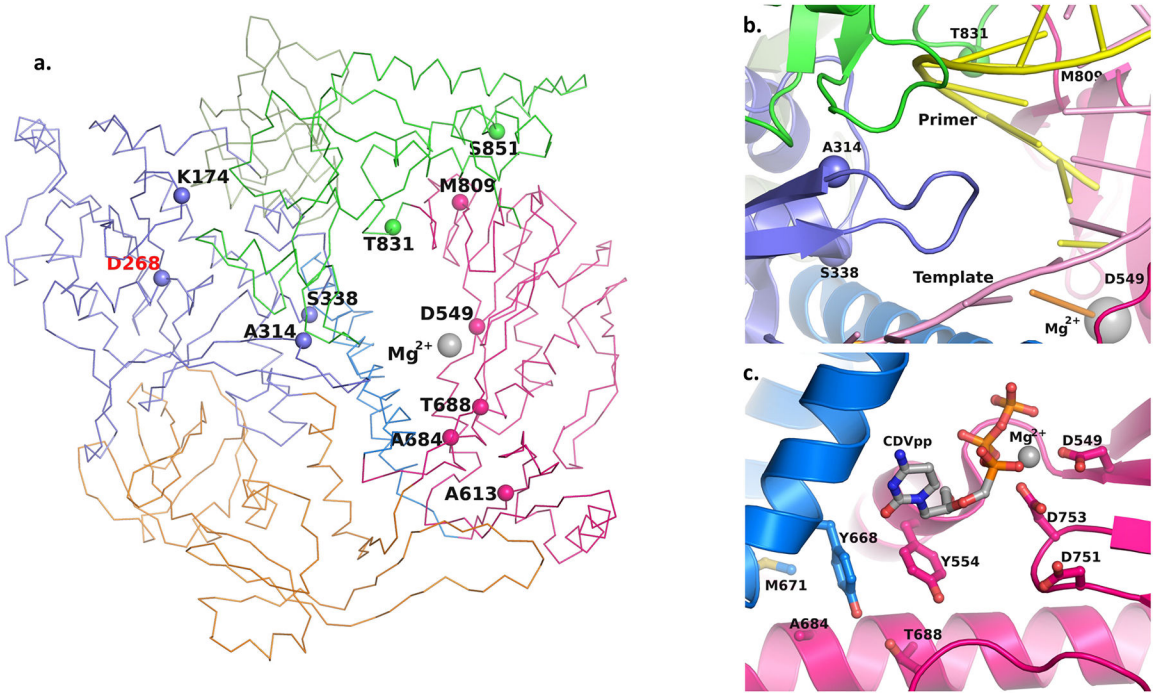


Figure 3. The topological position of CDV/BCV resistance mutations in MPXV F8L

– Panel a shows Cα trace of F8L structure and topological position of the CDV/BCV resistance mutation positions in 3' – 5' exonuclease domain (purple), and subdomains fingers (blue), thumb (green) and palm (dark pink). The N-terminal domain of F8L is colored orange. The Cα atom of the resistance mutation residues is shown as a solid sphere. This structure was generated by homology modeling using a recently reported cryo-EM structure of MPXV holoenzyme[40] (PDB entry 8HG1) as a template structure with Modeller[117]. Mg^{2+} and D549 represent the polymerase active site of F8L, whereas D268 represents 3' – 5' exonuclease active site. Panel b shows the resistance mutation position 314 on a β-hairpin located in the major groove of the template (light pink) and primer (yellow). Panel c shows the resistance mutation positions 684 and 688 and critical amino acids near these residues. The CDVpp was modeled using the dNTP substrate in PDB entry 8HG1. It is clear from this figure that the dNTP binding residues are in the proximity of resistance mutation positions 684 and 688. The α-helices and β-sheets are rendered as ribbons in panels b and c.

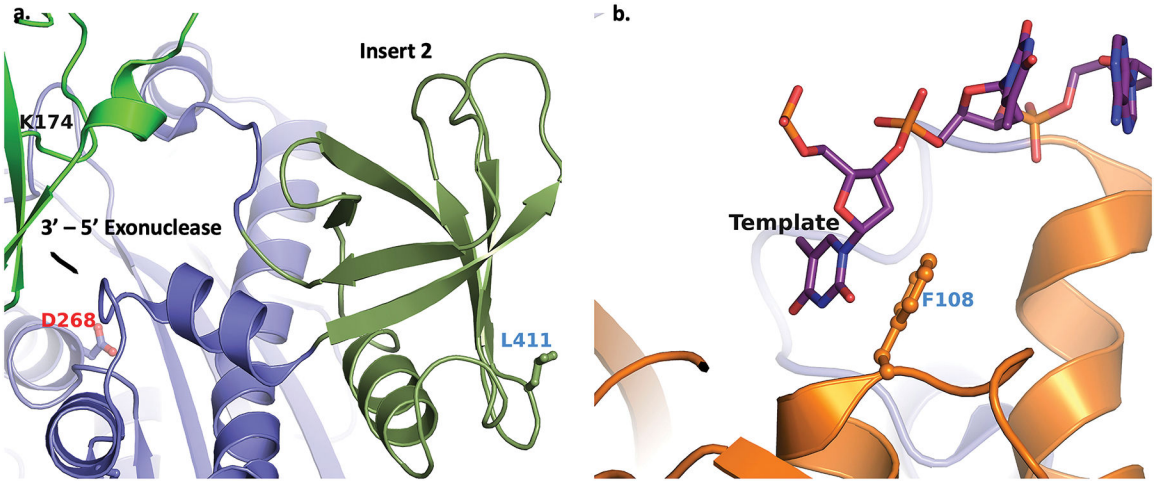


Figure 4. The topological position of signature mutations emerged in the 2022 outbreak in MPXV F8L

– Panel a depicts the signature mutation W411L in the poxvirus-specific 'insert 2' (rendered as forestgreen ribbons). The relative position of the 3'- 5' exonuclease site (D268) is also a reference. Panel b shows that the 2022 MPXV signature mutation L108F (ball-and-stick) within the N-terminal domain (represented as orange ribbons) stacks against the template base as seen in the cryo-EM structure of MPXV DNA replication holoenzyme (PDB entry 8HG1).

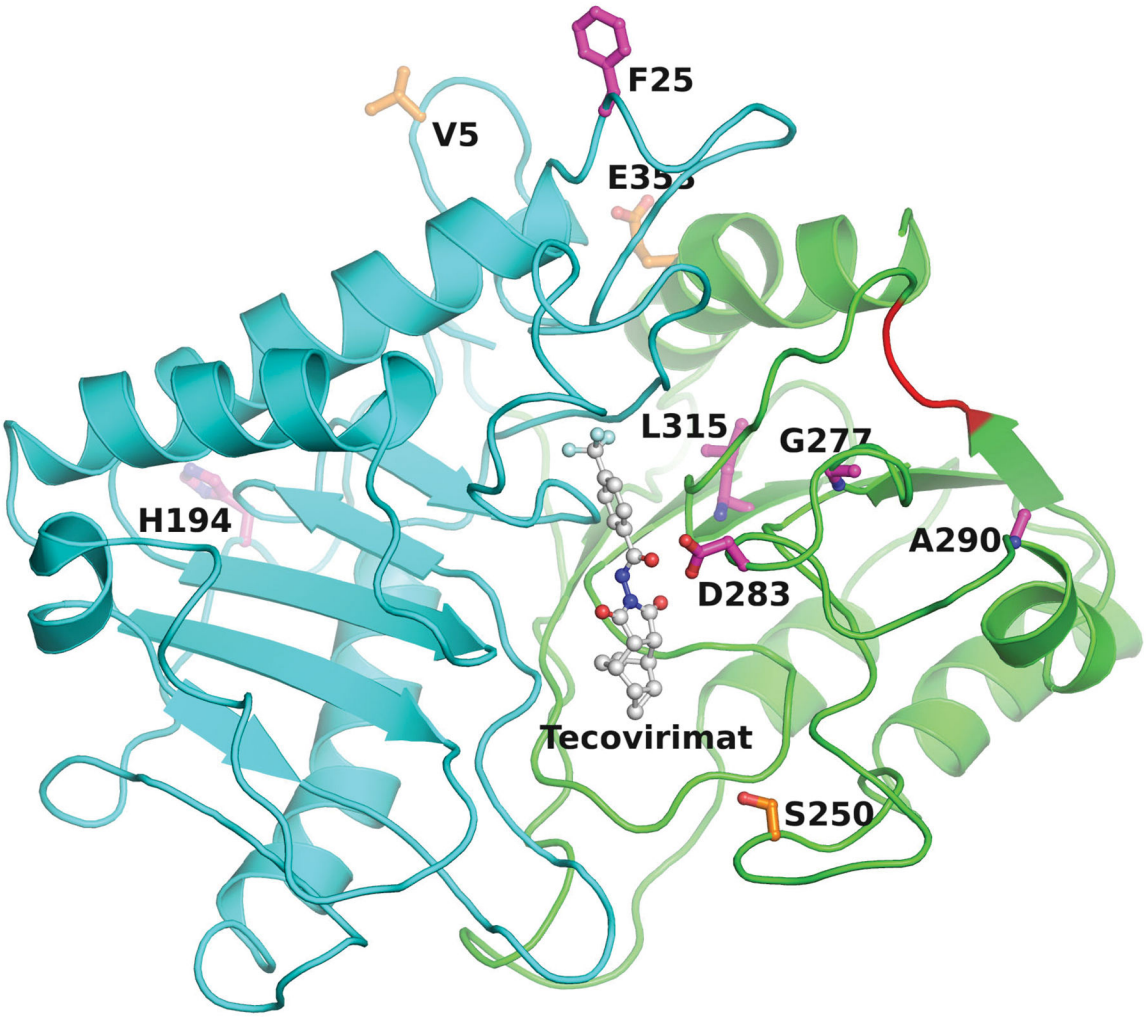


Figure 5. Docked tecovirimat in the homology-derived molecular model of C19L

– This figure shows the most favorable docked pose of tecovirimat in the modeled structure of MPXV C19L phospholipase rendered as ribbons in cyan (N-terminal domain) and green (C-terminal domain). This figure also shows tecovirimat resistance mutation positions (in magenta balls-and-sticks) and signature mutations that emerged in the 2022 outbreak (in orange balls-and-sticks). Most of the tecovirimat resistance mutations are close to tecovirimat. The red loop represents the SVK triplet insertion at positions 303–305.

Table 1.

Potential MPXV Therapeutic targets.

Protein[a]	VACV	MPXV	Essential
Replication			
DNA polymerase	E9L	F8L	Yes
Helicase–primase	D5R	E5R	Yes
UDG	D4R	E4R	Yes
Processivity factor	A20R	A22R	Yes
Protein kinase	B1R	B3R	Host-dependent
SSB	I3L	I3L	Yes
DNA ligase	A50R	A50R	Host-dependent
FEN1-like nuclease	G5R	G5R	Impaired

[a]SSB – single strand DNA binding protein

UDG – Uracil DNA glycosylase