




Perspective

Mpox (Monkeypox) Virus and Its Co-Infection with HIV, Sexually Transmitted Infections, or Bacterial Superinfections: Double Whammy or a New Prime Culprit?

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Abstract: Epidemiologic studies have established that mpox (formerly known as monkeypox) outbreaks worldwide in 2022–2023, due to Clade IIb mpox virus (MPXV), disproportionately affected gay, bisexual, and other men who have sex with men. More than 35% and 40% of the mpox cases suffer from co-infection with HIV and sexually transmitted infections (STIs) (e.g., *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, and herpes simplex virus), respectively. Bacterial superinfection can also occur. Co-infection of MPXV and other infectious agents may enhance disease severity, deteriorate outcomes, elongate the recovery process, and potentially contribute to the morbidity and mortality of the ensuing diseases. However, the interplays between MPXV and HIV, bacteria, other STI pathogens and host cells are poorly studied. There are many open questions regarding the impact of co-infections with HIV, STIs, or bacterial superinfections on the diagnosis and treatment of MPXV infections, including clinical and laboratory-confirmed mpox diagnosis, suboptimal treatment effectiveness, and induction of antiviral drug resistance. In this review article, we will discuss the progress and knowledge gaps in MPXV biology, antiviral therapy, pathogenesis of human MPXV and its co-infection with HIV, STIs, or bacterial superinfections, and the impact of the co-infections on the diagnosis and treatment of mpox disease. This review not only sheds light on the MPXV infection and co-infection of other etiologies but also calls for more research on MPXV life cycles and the molecular mechanisms of pathogenesis of co-infection of MPXV and other infectious agents, as well as research and development of a novel multiplex molecular testing panel for the detection of MPXV and other STI co-infections.

Keywords: mpox; monkeypox virus; orthopoxvirus; co-infection; HIV; sexually transmitted infection; bacterial superinfection; trained immunity; diagnosis; treatment



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1. Introduction

Mpox (formerly known as monkeypox) virus (MPXV) belongs to the genus *Orthopoxvirus* (OPXV) and the family *Poxviridae*, which includes variola virus (causing smallpox), cowpox virus, vaccinia virus (VACV), and over 80 other species [1–3]. MPXV has long been recognized as a zoonosis that had been largely limited to regions of Africa, with

few human-to-human transmissions prior to 2022 [1,4]. Since May 2022, rapidly spreading MPXV outbreaks due to human transmissions have been reported outside of Africa and evolved into a global health emergency, with more than 94,200 confirmed global cases in over 100 countries, including over 32,000 cases and 58 deaths in the United States [5–10].

Epidemiologic studies have established that more than 90% of the confirmed MPXV cases in the 2022–2023 outbreaks were gay, bisexual, and other men who have sex with men (MSM), who represent a high-risk population for both MPXV and HIV infection [5–12]. Co-infection with HIV and sexually transmitted infections (STIs) was observed among 38% and 41% of 1969 mpox cases in 8 U.S. jurisdictions, respectively, in a study by Curran et al. [7]. MPXV-infected cases are reported in children and are more likely to be severe [13]. We have recently diagnosed and managed three young adult mpox cases at the Children’s National Hospital in Washington DC, which led the nation in MPXV cases per capita, with >564 confirmed cases and 24 hospitalizations [14]. These three cases presented atypical manifestations, fewer rashes, and an absence of prodromal symptoms (unpublished data). Although there are no FDA-approved treatments specifically for MPXV infection, tecovirimat and brincidofovir approved for antiviral therapy of smallpox disease are recommended for antiviral treatment of mpox disease [15,16]. Tecovirimat was approved by the European Medicines Agency for the treatment of mpox disease in adults and children (body weight ≥ 13 kg) in January 2022 [15,17]. Although smallpox vaccines JYNNEOS and ACAM2000 can provide protection against MPXV infections, limited vaccine supply and the associated side effects and contraindications underscore the importance of developing novel preventive and therapeutic strategies to manage MPXV and/or HIV infections for high-risk populations [18,19].

In this review article, we will update the progress and knowledge gaps in MPXV biology, antiviral therapy, pathogenesis of human MPXV and its co-infection with HIV, STIs or bacterial superinfections, as well as the impact of the co-infections on the diagnosis and treatment of mpox disease. This review calls for research on outstanding questions in the MPXV life cycle and the molecular mechanisms of pathogenesis of co-infection of MPXV and other pathogens, MPXV drug resistance surveillance in MPXV- and HIV-co-infected cases for public health purposes, as well as research and development of a novel multiplex molecular testing panel for the detection of MPXV and the co-infections.

2. MPXV Biology

There are two clades (I and II) of MPXV that have been recognized, with Clade II further classified into two subclades: IIa and IIb. Clade I present in the Congo Basin was first recognized and is responsible for up to 10% of human mortality [1]. It is mainly transmitted from rodents to humans with limited human-to-human spread. Clade IIa was subsequently recognized in West Africa with an approximately 1% mortality, though it has a 95% nucleotide sequence identity to clade I [20]. Further, clade IIb, the current outbreak strain of MPXV, has sequence similarity to clade IIa and a much lower mortality in immunocompetent individuals [21]. Consistent with the severity of clinical disease in humans, Americo et al. demonstrated that there was a significant difference in MPXV virulence in CAST/EiJ mice in the order clade I > clade IIa > clade IIb [1].

Like other OPXVs, MPXV replicates entirely in the cytoplasm and has similar life cycle steps (Figure 1) [2,22–26], among which viral DNA replication and intracellular virion wrapping steps are targeted by brincidofovir and tecovirimat, respectively [15] (to be discussed in the next section). By employing electron microscopy, Paniz-Mondolfi et al. [27] recently observed typical enveloped virions with brick-shaped morphologies containing surface protrusions in MPXV and abundant melanosomes near viral assembly sites in skin lesions isolated from patients affected by the 2022–2023 outbreak. Of note, there are many outstanding questions that remain unanswered in the MPXV life cycle (Figure 1), for example, the cellular receptor of MPXV and the molecular details of virion morphogenesis and egress. Generally speaking, in response to viral invasion, the innate immune system presents a front line of host defenses armed with multi-layered mechanisms [2,28–37]. Of

these, pattern recognition receptors, such as toll-like receptors (TLRs) and cyclic GMP-AMP synthase, sense OPXV nucleic acids, a major class of the viral pathogen-associated molecular pattern, triggering intracellular signaling pathways that activate the IFN production to restrict OPXV spread [2,36,37].

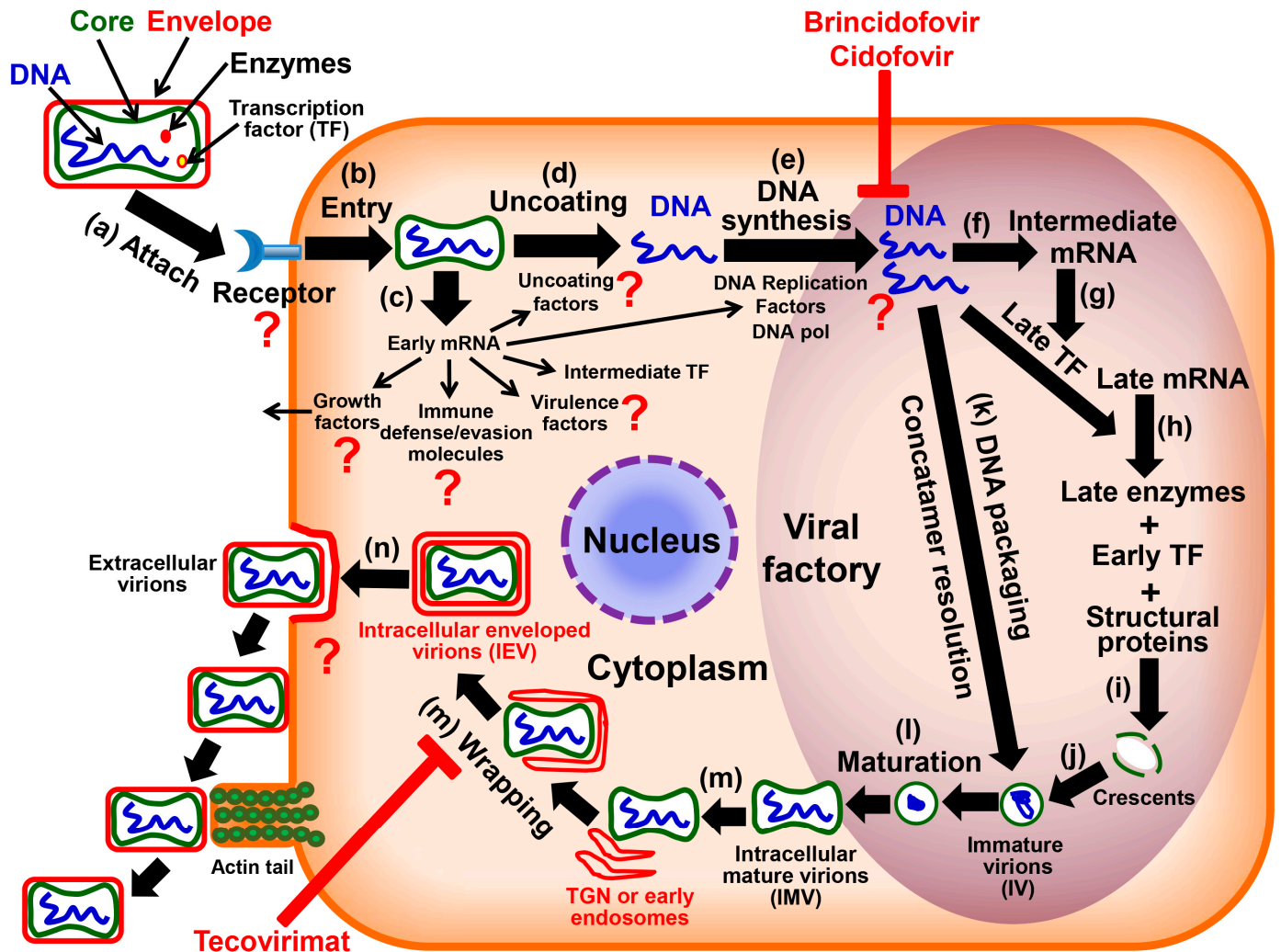


Figure 1. Life cycle of OPXVs including MPXV and molecular mechanisms of currently available antiviral strategies [2,15,17,22–24,38]. (Upper, left) MPXV is a DNA virus that has a membrane envelope surrounding an inner core containing viral DNA genome, transcription factors (TFs), and virion enzymes, e.g., DNA-dependent RNA polymerase and RNA-processing enzymes. MPXV replicates entirely in the cytoplasm. (a–c) After attachment and entry, viral early mRNAs are synthesized in the core and translated to generate non-structural proteins, including intermediate TFs and proteins necessary for immune defense, virulence (virulence factors), pathogenesis (pathogenic factors), and DNA replication. (d) The core wall opens and a nucleoprotein complex containing the genome is released from the core. (e) Replication of viral DNA genome is initiated, followed by the formation of viral factories. DNA synthesis is inhibited by cidofovir and brincidofovir. In the viral factory, the following steps take place, including (f–h) intermediate and late transcription, (i,j) crescent formation, (k) DNA packaging and concatamer resolution, as well as (l) virion maturation. Subsequently, (m) intracellular mature virions (IMV) become wrapped by a double membrane cisterna of the *trans*-Golgi network to form the intracellular enveloped virus (IEV), which is inhibited by tecovirimat. (n) IEV is capable of polymerizing actin tails, which facilitates the release (exocytosis) of the extracellular

enveloped virus into the extracellular space. Of note, there are many outstanding questions that remain to be answered (question marks) in terms of molecular details during different steps of the MPXV life cycle.

3. Antivirals against MPXV

Despite no MPXV-specific antivirals being approved, tecovirimat and brincidofovir (a prodrug of cidofovir), two FDA-approved, orally-bioavailable antivirals against smallpox in adults and children, are recommended for antiviral treatment of mpox disease, based on OPXV genome conservation, human safety profiles of these drugs, and their antiviral effectiveness in animal models [15,16,39]. Though lacking oral bioavailability, cidofovir is recommended by the Centers for Disease Control and Prevention (CDC) as one of the medical countermeasures available for the treatment of mpox due to its potent antiviral activities against OPXVs in *in vitro* and animal models and data on the effectiveness of brincidofovir [15,39]. As far as the mechanism of action is concerned, brincidofovir and cidofovir (a nucleoside analog) act as a DNA chain terminator to block DNA polymerase, thereby inhibiting OPXV DNA replication [15,39–42]. In contrast, tecovirimat suppresses the envelopment of intracellular mature virions and the formation of extracellular virus particles by inhibiting VP37 phospholipase (encoded by the *F13L* gene in VACV), thereby preventing the spread of the virus from an infected cell to other neighboring cells (Figure 1) [39–42].

Like other DNA viruses targeted by antivirals, e.g., hepatitis B virus, which accumulates antiviral drug resistance mutations [43,44], a potential issue that MPXV antiviral therapy may give rise to is the emergence of drug resistance, which has been a significant concern for the current antivirals [19,39]. Indeed, tecovirimat resistance has been identified in a small portion of patients with advanced HIV after treatment for periods of weeks to months [45]. Resistance to tecovirimat can develop in some patients who have subtherapeutic levels of the antiviral due to medication noncompliance or do not take the oral formulation within 30 min after eating full, fatty meals as indicated in the prescribing labeling [39]. Contrary to tecovirimat, MPXV seems less likely to develop resistance to brincidofovir or cidofovir partially due to the conservation of MPXV DNA polymerase [40,41]. However, mutations A314T and A684V in the VACV DNA polymerase (*E9L*) gene, which confer with cidofovir resistance, have been identified using *in vitro* selection model, which resides within the gene regions encoding the 3′-5′ exonuclease (A314T) and polymerase catalytic (A684V) domains [41].

The evolution of brincidofovir/cidofovir-resistant MPXV variants in patients is not fully understood. We aimed to examine the frequency and location of mutations in the MPXV DNA polymerase *F8L* gene (sharing ~98% homology with VACV *E9L*) and infer their impact on brincidofovir/cidofovir antiviral therapy. A total of 230 MPXV genome sequences were retrieved from the GenBank nucleotide sequence database, among which 110 and 120 strains were isolated before and during the 2022 mpox outbreak, respectively. Mutations in *F8L* gene sequences were identified and their potential impact on cidofovir efficacy was evaluated using structures of related viral and eukaryotic proteins and the structure prediction method AlphaFold. Phylogenetic analyses suggested that the included MPXV *F8L* gene sequences isolated before and during the 2022 outbreak did not cluster separately. Classic cidofovir resistance mutations A314T or A684V, as selected out in VACV [41], were not found among all MPXV sequences. However, 5 types of novel mutations were identified from 9 (7.5%, 9/120) sequences isolated during the 2022 mpox outbreak, including R25Q (1 strain), E45K (1 strain), A102T (2 strains), F108L (2 strains), and R366K (3 strains) (Figure 2). However, these mutations were not found in the included isolates before 2022. Given that topologically *F8L*-encoded DNA polymerase is responsible for ensuring DNA binding affinity of the replication complex, the newly identified mutations in the *F8L* gene may change the fidelity and processivity of MPXV DNA polymerase, thereby compromising its sensitivity to nucleoside inhibitors. These findings suggested that the circulating strains in the 2022 mpox outbreaks may accumulate novel, potential brincidofovir/cidofovir-resistant mutations in the MPXV DNA polymerase, whose clinical

significance needs further investigation. Genomic surveillance will be warranted to monitor the evolution of resistant MPXV variants.

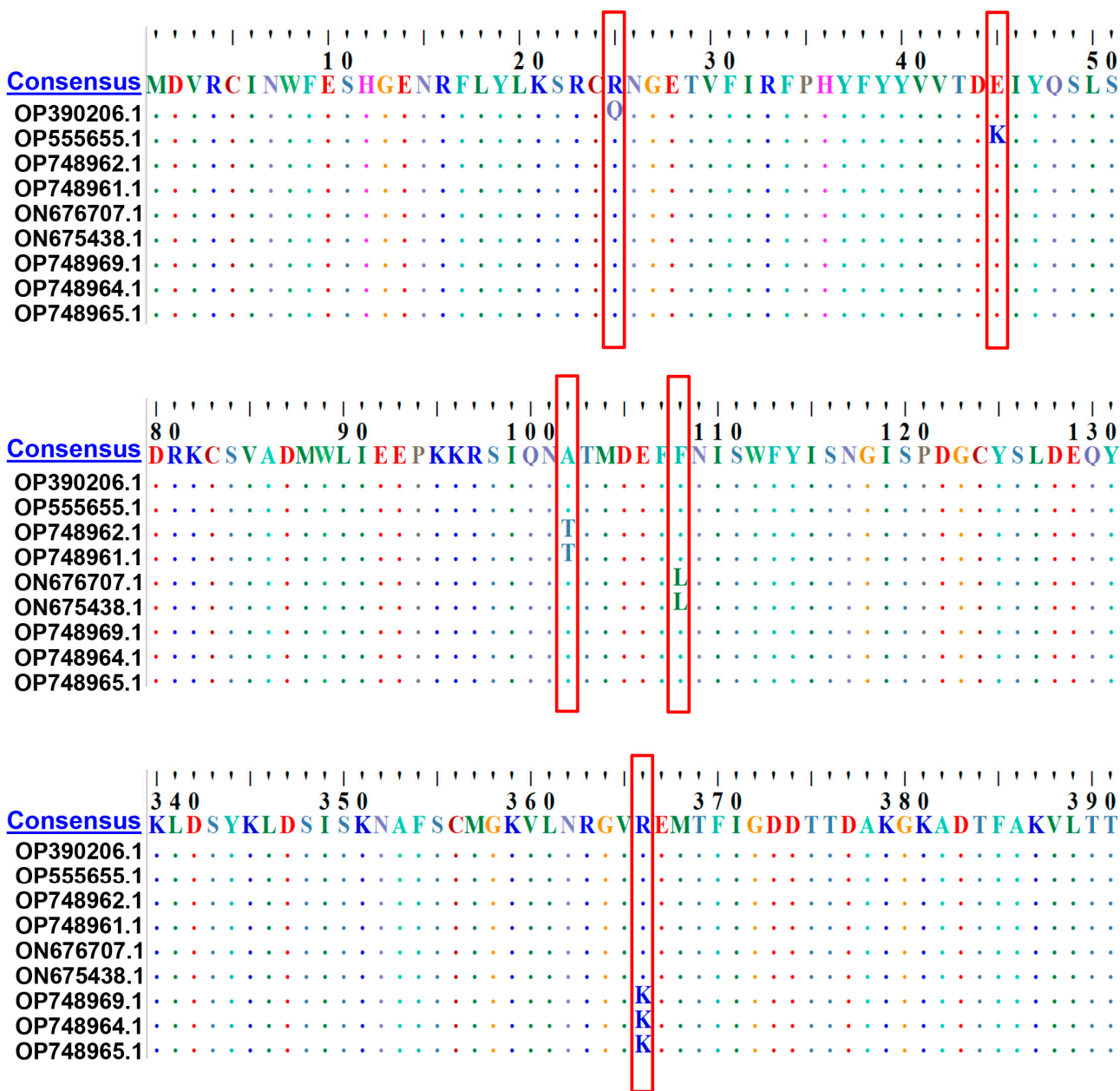


Figure 2. Identification of novel, potential brincidofovir/cidofovir antiviral resistance mutations in MPXV DNA polymerase (*F8L*) gene in the strains responsible for the 2022 mpx outbreak. A total of 230 MPXV *F8L* gene sequences were retrieved from whole genome sequences and aligned by ClustalW multiple alignment using BioEdit software, as reported elsewhere [46–49]. Among them, 110 and 120 sequences were isolated before and during the 2022 mpx outbreak, respectively. The consensus amino acid sequence of the *F8L* gene is shown at the top of the alignment. Although classic cidofovir resistance mutations [41] were not found among all MPXV sequences, 5 types of novel point mutations (R25Q (1/230), E45K (1/230), A102T (2/230), F108L (2/230), and R366K (3/230)) were identified among the 2022 mpx isolates (highlighted in red box).

4. Pathogenesis of Human MPXV and Its Co-Infection with HIV, STIs, or Bacterial Superinfections

Severe symptoms due to primary MPXV infection in humans implicate multiple organs and systems, which are aligned with the broad tropism of this virus (Figure 3, left) [2,50]. Humans get infected with MPXV via mucosa or skin, thereby leading to primary infection in the eye (e.g., conjunctivitis, blepharitis, and keratitis), respiratory system, skin, and genital sites (e.g., anal pain, proctitis, and genital lesions) [38,39,50–53]. MPXV infection can also cause neurologic symptoms, e.g., headache and, rarely, encephalitis. MPXV can disseminate via the lymphatic system and blood vessels, leading to lymphadenopathy and spreading to large organs, e.g., lungs, liver, and kidneys [39,50–53].

Co-infection of MPXV and HIV-1, STIs, or bacterial superinfections may enhance the severity, deteriorate outcomes of the symptoms, elongate the recovery process, and potentially contribute to morbidity and mortality of the ensuing disease [51–53]. First, a retrospective review of mpox (clade IIa) cases affected by a 2017–2018 outbreak in Nigeria suggested that HIV-1- and MPXV-coinfected patients suffered from more prolonged clinical course, extended lesions, and higher incidence of bacterial superinfections and genital ulcers, when compared with MPXV-positive but HIV-negative cases [54]. Second, MPXV and co-infection with STIs are reported in persons with HIV (PWH), e.g., *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, and herpes simplex virus (HSV) [7,53,55]. Among them, HSV-2 co-infection can exacerbate genital lesions, in which both MPXV and HSV-2 invade the skin and infect different types of cells, e.g., Langerhans, macrophages, and dendritic cells (Figure 3, bottom right). Furthermore, MPXV and bacterial superinfection, e.g., group A *Streptococcus* (GAS, i.e., *Streptococcus pyogenes*), can be found in the upper respiratory tract of both HIV-negative and HIV-positive cases [51] (Figure 3, upper right). GAS adheres and invades airway epithelial cells such as ciliated cells and induces cell death. MPXV also attaches and infects the same cell types and starts replication in the primary infection sites. GAS can damage the blood vessels, facilitating entry of GAS and MPXV into the bloodstream. Immune cells from peripheral blood infiltrate the infected tissue. Macrophages initiate phagocytosis to engulf GAS. Dendritic cells and macrophages can be infected with MPXV and enter blood vessels.

Notably, the co-infection of MPXV, STI pathogens, or bacteria superinfections in PWH could be attributed to behavioral factors, host immune status and responses, as well as virus–host interactions [1,5–12,56,57]. First, MSM-specific sexual exposures constitute a major risk factor for MPXV/HIV co-infection [5–12,56]. A recent systematic review [56] of 6345 cases affected by the 2022 mpox outbreaks (40.3% MPXV/HIV co-infection rate (2558/6345)) showed that 91.4% (5802/6345) of these cases were male patients, 56.2% (3259/5802) of whom had MSM sexual exposure, which may enhance the risk of infection of MPXV, HIV, STI pathogens, or bacteria superinfections. Second, weakened immune systems of PWH make them more vulnerable to infection with MPXV and other co-infections, e.g., STIs. Immunosuppressed/immunocompromised status, a low CD4+ T-cell count, and decreased immune response in PWH could give rise to the clinical severity of mpox disease, uncontrolled superinfection with other pathogens, and even mortality. For instance, two reported fatal cases with mpox and HIV co-infection had a CD4+ T-cells count < 200/ μ L [45,56]. In contrast, virus-specific T-cell responses can be detected among PWH participants after recovery from MPXV infection, indicating the involvement of T-cell responses in recovery from MPXV infection [57]. Third, generally speaking, innate immune responses, e.g., host restriction factors and cytokines/chemokines, and adaptive immune responses, e.g., T cell responses, play critical roles in sensing and restricting viral infections, including OPXVs [2,58–60]. However, MPXV has the ability to suppress cognate T cell activation and evade antiviral CD4+ and CD8+ T cell responses, thereby blocking inflammatory cytokine production and contributing to viral dissemination [61].

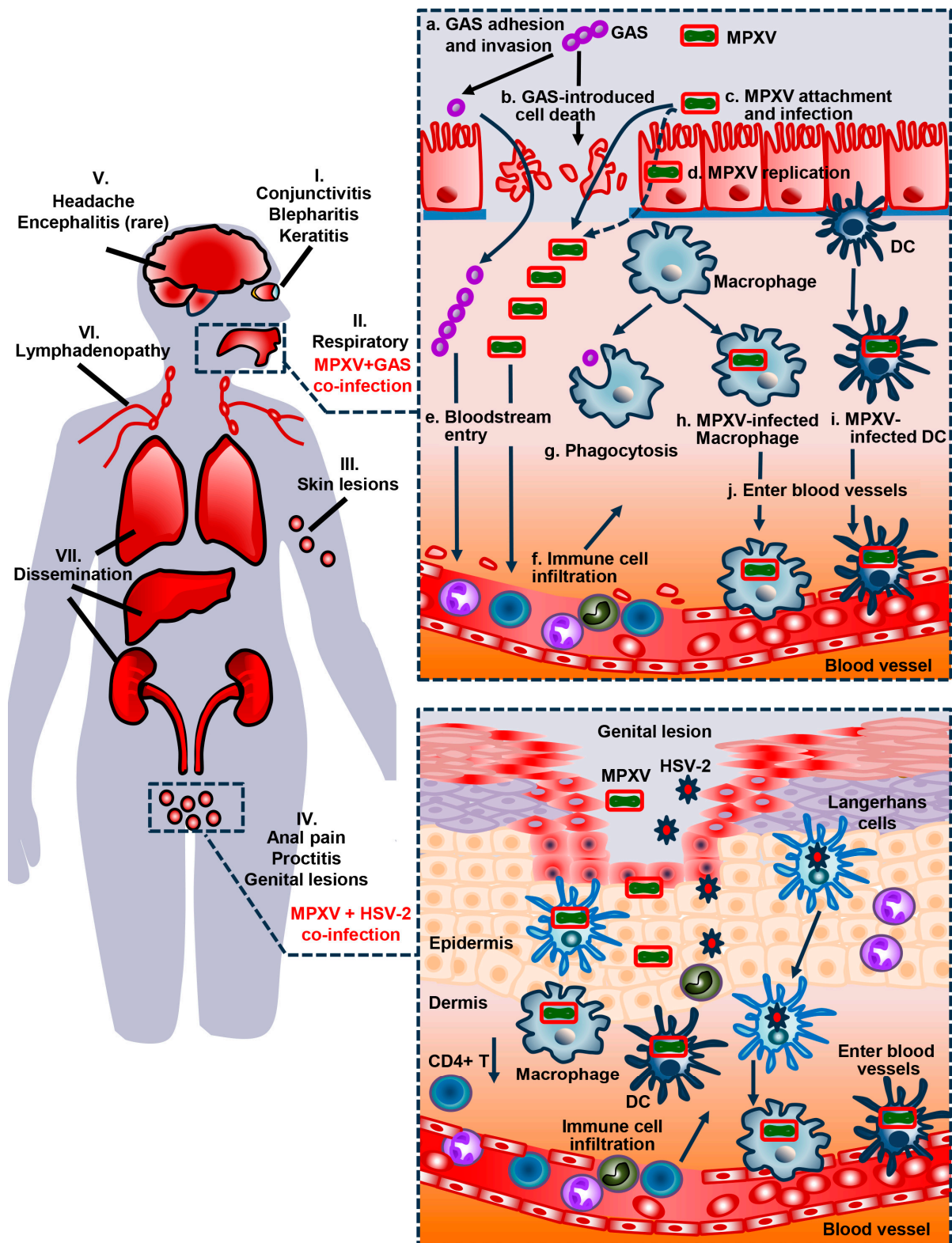


Figure 3. The pathogenesis of human MPXV and its co-infection with group A *Streptococcus* (GAS) and herpes simplex virus (HSV)-2. (Left) humans get primary MPXV infection via mucosa or skin, thereby leading to primary infection in (I) eye (e.g., conjunctivitis, blepharitis, and keratitis), (II) respiratory system, (III) skin, and (IV) genital sites (e.g., anal pain, proctitis, and genital lesions). (V) MPXV infection can cause neurologic symptoms, e.g., headache and, rarely, encephalitis. MPXV can disseminate via the lymph system and blood vessels, leading to (VI) lymphadenopathy and

spreading to (VII) large organs, e.g., lungs, liver, and kidneys. (**Upper, right**) MPXV and GAS co-infection can be found in the upper respiratory tract. In this scenario, (a) GAS adheres and invades airway epithelial cells such as ciliated cells, and (b) induces cell death. (c) MPXV also attaches and infects ciliated cells, and (d) replicates in the primary infection sites. (e) GAS damages the blood vessel, facilitating entry of GAS and MPXV into the bloodstream. (f) Immune cells in peripheral blood start to infiltrate. (g) Macrophages initiate phagocytosis to engulf GAS. (h,i) Dendritic cells and macrophages can be infected with MPXV, and (j) enter blood vessels. (**Bottom, right**) MPXV and HSV-2 co-infection can occur in genital lesions, in which both MPXV and HSV-2 invade the genital skin and infect different types of cells, e.g., Langerhans, macrophages, and dendritic cells. The infection can lead to decreased CD4+ T cell count, infiltration of other immune cells, and viral dissemination via blood vessels.

Before the 2022 MPXV outbreak, most of the PWH populations had not received the smallpox vaccine, which not only provides protection against MPXV but may also induce “trained immunity” to fend off other infections. The concept of trained immunity was introduced by Netea et al. [62–64] to describe the ability of innate immune cells (e.g., NK, dendritic cells, and monocytes/macrophages) to acquire an immunological memory to a “priming” pathogen (e.g., viruses, bacteria or fungi), a stimulus (e.g., TLR agonists, oxidized low-density lipoprotein, and aldosterone) or a vaccine (e.g., VACV-based smallpox vaccine), allowing them to trigger enhanced response to subsequent infection/stimulation by the same or unrelated agents/stimuli [65]. For example, innate immune responses associated with “trained immunity” persist after certain infections or vaccinations, e.g., with the smallpox vaccine. Weinstein et al. suggested that immunization with replicating smallpox vaccine, to some extent, might lead to a protective effect against subsequent HIV-1 infection [66], which could be explained by the concept of trained immunity. Compared with VACV-naive subjects, Weinstein et al. found that there was an up to five-fold reduction in the replication of CCR5-tropic, but not CXCR4-tropic, HIV-1 in the peripheral blood mononuclear cells (PBMCs) isolated from subjects vaccinated with replicating VACV smallpox vaccine Dryvax (Wyeth) 3–6 months before [66]. R5 HIV-1 replication in the PBMCs from VACV naive subjects, but not vaccinated subjects, was potentiated after adding autologous serum to the cell cultures, which is likely attributed to some activating effects in the serum of VACV naive subjects on the cultured PBMCs [66]. To establish trained immunity, metabolic pathways of innate immune cells are activated by the first stimulus, which lead to epigenetic changes and maintain the cell in a “trained” state. Innate immune memory or trained immunity can give rise either to stronger (enhancement) or weaker (suppression or tolerance) responses to subsequent infection or stimulation, which is dependent upon the strength and length of the first stimulation of the immune cells [65]. Similar to other functions of innate immunity, trained immunity has a broad range of responses and is quite different from the high specificity of classical memory T and B cells.

Similar to the concept of “trained immunity”, “heterologous immunity” is an immune response that is induced against a prior vaccination or exposure/infection with one pathogen but also can boost or weaken protective immunity against another unrelated pathogen, as suggested in studies with humans and mouse models [67–69]. Thanks to the cross-reactivity of T cell receptors, heterologous immunity to VACV infection has been experimentally shown in a mouse model that had been earlier exposed to infections with lymphocytic choriomeningitis virus, murine cytomegalovirus, Pichinde virus, or influenza A virus [67–69]. More research is needed to study the role of heterologous immunity and trained immunity in MPXV infection and co-infection with other etiologies.

5. The Clinical Impact of Co-Infections with HIV, STIs, or Bacterial Superinfections on the Diagnosis of MPXV Infections

With the advent of molecular microbiology, molecular testing has become the mainstay for infectious disease diagnostics with rapid turnaround time and high sensitivity, specificity, and accuracy [70–72]. Real-time PCR has played an important role in diagnosis

of mpox infection in indicated populations in the 2022–2023 outbreaks. Per CDC guidelines [73], testing for MPXV infections is only recommended when there is a rash that is consistent with mpox. However, during the 2022–2023 mpox outbreaks, co-infections with different bacteria (e.g., GAS), STIs (e.g., syphilis), and other viruses (e.g., HSV-1, HSV-2, and varicella zoster virus) may complicate the differential diagnosis in adults as the co-infected organisms may present similar maculopapular rash, vesicles and pustule manifestations as MPXV does [51–53] (Figure 3), which may cause missing identification (false negativity) of MPXV if sampling the rashes or genital lesions due to co-infected pathogens (e.g., HSV-2) other than MPXV. For example, the symptoms of MPXV infection, e.g., lesions and rash, may overlap with other infections, such as GAS pharyngitis reported by Kaiser et al. [51]. Thus, the diagnosis of MPXV infection may be delayed. As another example in the pediatric world, enteroviruses (EV), coxsackievirus and human parechovirus with worldwide distribution may cause some symptoms that mimic the presentations of MPXV infection, e.g., hand-foot-and-mouth disease (HFMD) [71,74,75]. Similar to the findings by Fathi and Schmiedel [74], we tested skin swabs of atypical rashes from five pediatric cases with suspicion of mpox with Cepheid Xpert EV assay (off-label use) [72] between July and December 2022 at Children’s National Hospital in Washington, DC, which ended up being positive for EVs but negative for OPXV, suggesting atypical HFMD as an important differential diagnosis of mpox among pediatric populations.

Of note, a drawback of the currently available diagnostic real-time PCR assays employed in some reference CLIA labs (e.g., Mayo Clinic and ARUP Laboratories) in the U.S. during the 2022–2023 mpox outbreaks is that they are pan-OPXV real-time PCR, which fails to differentiate MPXV from other OPXVs, e.g., smallpox vaccine strains and molluscum contagiosum virus (MOCV) [76,77]. For example, co-infection with MOCV, an OPXV commonly responsible for self-limited infectious dermatosis among pediatric populations, may cause cross-reactions in the pan-OPXV PCR assays, when testing patients with suspicion of MPXV [77].

6. The Clinical Impact of Co-Infections with HIV, STIs, or Bacterial Superinfections on the Antiviral Treatment of MPXV Infections

Co-infections with different bacteria, STIs, and other viruses in mpox cases, especially those mpox and HIV co-infected cases, may complicate the management of the diseases, especially antiviral treatment [51–53]. First, co-infection with multiple organisms in mpox cases can be especially dangerous as the symptoms of the comorbidities can be exacerbated, making them more difficult to treat [56]. Second, co-infections with other organisms, e.g., STI pathogens, may affect the effectiveness of antiviral treatment for mpox and virus clearance. Opardija et al. recently reported delayed clearance of MPXV in a patient with co infection with secondary syphilis, despite a 14-day course of tecovirimat therapy [52]. However, in another cohort of patients with severe mpox who were treated with tecovirimat, treatment outcomes were not affected by HIV status [78]. Given the limited research in this area, it is important to acknowledge the caveat of limited data while highlighting potential outcomes on both fronts. Moreover, the delayed viral clearance due to co-infections of other pathogens may induce antiviral drug resistance and viral dissemination. Alarcón et al. reported a fatal mpox (clade IIb) and HIV co-infected cases (CD4+ T-cell count < 35 per cubic millimeter) with recently treated syphilis, who developed tecovirimat resistance developed after 2 courses of oral tecovirimat [45]. Last but not least, antivirals against MPXV may have undesirable interactions with drugs used to treat co-infections [39]. For example, tecovirimat may lead to decreased levels of rilpivirine, a non-nucleoside reverse transcriptase inhibitor, whereas brincidofovir concentration may be elevated by protease inhibitors, cobicistat, and fostemsavir [39]. While cidofovir has minimal drug interactions, probenecid, which is co-administrated with cidofovir to minimize nephrotoxicity, has multiple drug interactions, e.g., zidovudine, beta-lactam antibiotics, diuretics, non-steroidal anti-inflammatory drugs, and angiotensin-converting enzyme inhibitors [39].

7. Conclusions

As an OPXV, clade IIb MPXV is responsible for the global 2022–2023 mpox outbreaks, with mild symptoms presented in most cases, though severe manifestations were observed in disseminated or immunocompromised cases. While there are no approved drugs specifically for MPXV, tecovirimat, brincidofovir, and cidofovir are recommended as medical countermeasures by the CDC.

More than 90% of the recently confirmed mpox cases were gay, bisexual, and other MSMs. Co-infection with HIV and STIs (e.g., *C. trachomatis*, *N. gonorrhoeae*, *T. pallidum*, and HSV) account for more than 35% and 40% of the mpox cases, respectively. Bacterial superinfection (e.g., GAS) can also occur. MSM-specific sexual behaviors constitute a major risk factor for MPXV/HIV co-infection. The co-infection of MPXV and other pathogens in PWH could be attributed to behavioral factors (e.g., MSM sexual exposure to MPXV, HIV, STI, and other pathogens), host immune status and responses (e.g., low CD4+ T cells, low trained or heterologous immunity), and virus-host interactions (e.g., virulent MPXV strains evading T cell responses).

Co-infections with HIV, STIs, or bacterial superinfections have a dramatic clinical impact on the pathogenesis, diagnosis, and treatment of MPXV infections. First, the co-infections of other pathogens may exacerbate symptoms and prolong illness. Second, co-infection of other pathogens mimicking mpox clinical presentations may lead to missed identification (e.g., false negative diagnostic MPXV PCR due to sampling HSV-2 genital lesions) or misidentification (e.g., false positive MPXV PCR due to cross-reactions with MOCV by pan-OPXV PCR) of MPXV, respectively. Third, the delayed viral clearance due to co-infections in mpox cases may induce antiviral drug resistance and viral dissemination.

8. Future Directions

This review calls for research on outstanding questions in the MPXV life cycle and the molecular mechanisms of pathogenesis of co-infection of MPXV and HIV, STIs, or bacterial superinfections. Research on the interplay between MPXV and other infectious agents and host cells in MPXV- and HIV-co-infected cases is warranted and will shed light on the mechanisms under which multiple organisms contribute to the pathogenesis in the context of mpox and HIV infections.

Drug resistance to tecovirimat, brincidofovir, and cidofovir should be monitored in MPXV- and HIV-co-infected cases for public health surveillance purposes. The current definition of drug resistance to these antivirals against MPXV is “clinical resistance” observed in the clinical response or MPXV viral load change in response to antiviral treatment. More sensitive and faster molecular testing for antiviral drug resistance mutations will be a valuable tool for screening and detection of “genotypic resistance” to predict “clinical resistance”. Moreover, identification and research of MPXV mutations conferring primary antiviral drug resistance (“primary drug-resistant” mutations) or compensated fitness (“secondary compensatory” mutations) are warranted.

MPXV-specific diagnostic real-time PCR assays, rather than pan-OPXV PCRs, should be used for definitive diagnosis of MPXV infection, which will allow for differentiation of MPXV from smallpox vaccine strains and MOCV. Considering the incidence of co-infection of MPXV and STIs, multiplex molecular testing panel for the detection of MPXV and co-infected STI pathogens (e.g., HSV-1, HSV-2, *T. pallidum*, *N. gonorrhoeae*, and *C. trachomatis*) in genital and anorectal lesions/ulcers is worthy development and clinical validation.

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References

1. Americo, J.L.; Earl, P.L.; Moss, B. Virulence differences of mpox (monkeypox) virus clades I, IIa, and IIb.1 in a small animal model. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2220415120. [CrossRef] [PubMed] [PubMed Central]
2. Moss, B. Poxviridae: The Viruses and Their Replication. In *Fields Virology*, 5th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2006.
3. McInnes, C.J.; Damon, I.K.; Smith, G.L.; McFadden, G.; Isaacs, S.N.; Roper, R.L.; Evans, D.H.; Damaso, C.R.; Carulei, O.; Wise, L.M.; et al. ICTV Virus Taxonomy Profile: Poxviridae 2023. *J. Gen. Virol.* **2023**, *104*, 001849. [CrossRef] [PubMed]
4. Di Giulio, D.B.; Eckburg, P.B. Human monkeypox: An emerging zoonosis. *Lancet Infect. Dis.* **2004**, *4*, 15–25, Erratum in *Lancet Infect. Dis.* **2004**, *4*, 251. [CrossRef] [PubMed] [PubMed Central]
5. Tarín-Vicente, E.J.; Alemany, A.; Agud-Dios, M.; Ubals, M.; Suárez, C.; Antón, A.; Arando, M.; Arroyo-Andrés, J.; Calderón-Lozano, L.; Casañ, C.; et al. Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: A prospective observational cohort study. *Lancet* **2022**, *400*, 661–669. [CrossRef]
6. Philpott, D.; Hughes, C.M.; Alroy, K.A.; Kerins, J.L.; Pavlick, J.; Asbel, L.; Crawley, A.; Newman, A.P.; Spencer, H.; Feldpausch, A.; et al. Epidemiologic and Clinical Characteristics of Monkeypox Cases—United States, May 17–July 22, 2022. *MMWR Morb. Mortal Wkly. Rep.* **2022**, *71*, 1018–1022. [CrossRef]
7. Curran, K.G.; Eberly, K.; Russell, O.O.; Snyder, R.E.; Phillips, E.K.; Tang, E.C.; Peters, P.J.; Sanchez, M.A.; Hsu, L.; Co-hen, S.E.; et al. HIV and Sexually Transmitted Infections among Persons with Monkeypox—Eight U.S. Jurisdictions, May 17–July 22, 2022. *MMWR Morb. Mortal Wkly. Rep.* **2022**, *71*, 1141–1147. [CrossRef] [PubMed]
8. Hoffmann, C.; Jessen, H.; Wyen, C.; Grunwald, S.; Noe, S.; Teichmann, J.; Krauss, A.; Kolarikal, H.; Scholten, S.; Schuler, C.; et al. Clinical characteristics of monkeypox virus infections among men with and without HIV: A large outbreak cohort in Germany. *HIV Med.* **2022**, *24*, 389–397. [CrossRef] [PubMed]
9. WHO Director-General Declares the Ongoing Monkeypox Outbreak a Public Health Emergency of International Concern. Available online: <https://www.who.int/europe/news/item/23-07-2022-who-director-general-declares-the-ongoing-monkeypox-outbreak-a-public-health-event-of-international-concern> (accessed on 10 May 2024).
10. Ongoing 2022 Global Outbreak Cases and Data. Available online: <https://www.cdc.gov/poxvirus/mpox/response/2022/index.html> (accessed on 10 May 2024).
11. Technical Report 2: Multi-National Mpox Outbreak, United States. 2022. Available online: <https://www.cdc.gov/poxvirus/monkeypox/cases-data/technical-report/report-2.html#global> (accessed on 10 May 2024).
12. Joint ECDC-WHO Regional Office for Europe Mpox Surveillance Bulletin. Available online: <https://monkeypoxreport.ecdc.europa.eu> (accessed on 10 May 2024).
13. World Health Organization. Multi-Country Monkeypox Outbreak in Non-Endemic Countries. 21 May 2022. Available online: <https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385> (accessed on 10 May 2024).
14. Available online: <https://dchealth.dc.gov/node/1611066> (accessed on 10 May 2024).
15. Wang, J.; Mahmud, S.A.; Chen, A.; Li, K.; Tan, H.; Joyce, R. An Overview of Antivirals against Monkeypox Virus and Other Orthopoxviruses. *J. Med. Chem.* **2023**, *66*, 4468–4490. [CrossRef] [PubMed]
16. Available online: <https://www.cdc.gov/poxvirus/mpox/clinicians/treatment.html> (accessed on 10 May 2024).
17. Hermanussen, L.; Brehm, T.T.; Wolf, T.; Boesecke, C.; Schlabe, S.; Borgans, F.; Monin, M.B.; Jensen, B.-E.O.; Windhaber, S.; Scholten, S.; et al. Tecovirimat for the treatment of severe Mpox in Germany. *Infection* **2023**, *51*, 1563–1568. [CrossRef] [PubMed] [PubMed Central]
18. Deputy, N.P.; Deckert, J.; Chard, A.N.; Sandberg, N.; Moulia, D.L.; Barkley, E.; Dalton, A.F.; Sweet, C.; Cohn, A.C.; Little, D.R.; et al. Vaccine Effectiveness of JYNNEOS against Mpox Disease in the United States. *N. Engl. J. Med.* **2023**, *388*, 2434–2443. [CrossRef] [PubMed]

19. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Division of Earth and Life Studies; Board on Health Sciences Policy; Board on Global Health; Board on Life Sciences; Committee on the Current State of Research, Development, and Stockpiling of Smallpox Medical Countermeasures; Gostin, L.O.; Brown, L.; Singaravelu, S.; et al. (Eds.) National Academies of Sciences, Engineering, and Medicine. In *Future State of Smallpox Medical Countermeasures*; The National Academies Press: Washington, DC, USA, 2024. [[CrossRef](#)]
20. Likos, A.M.; Sammons, S.A.; Olson, V.A.; Frace, A.M.; Li, Y.; Olsen-Rasmussen, M.; Davidson, W.; Galloway, R.; Khristova, M.L.; Reynolds, M.G.; et al. A tale of two clades: Monkeypox viruses. *J. Gen. Virol.* **2005**, *86*, 2661–2672. [[CrossRef](#)] [[PubMed](#)]
21. Gigante, C.M.; Korber, B.; Seabolt, M.H.; Wilkins, K.; Davidson, W.; Rao, A.K.; Zhao, H.; Smith, T.G.; Hughes, C.M.; Minhaj, F.; et al. Multiple lineages of monkeypox virus detected in the United States, 2021–2022. *Science* **2022**, *378*, 560–564. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
22. Liu, B.; Panda, D.; Mendez-Rios, J.D.; Ganesan, S.; Wyatt, L.S.; Moss, B. Identification of Poxvirus Genome Uncoating and DNA Replication Factors with Mutually Redundant Roles. *J. Virol.* **2018**, *92*, e02152-17. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
23. Yang, Z. Monkeypox: A potential global threat? *J. Med. Virol.* **2022**, *94*, 4034–4036. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
24. Rampogu, S.; Kim, Y.; Kim, S.-W.; Lee, K.W. An overview on monkeypox virus: Pathogenesis, transmission, host interaction and therapeutics. *Front. Cell. Infect. Microbiol.* **2023**, *13*, 1076251. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
25. Yang, Z.; Reynolds, S.E.; Martens, C.A.; Bruno, D.P.; Porcella, S.F.; Moss, B. Expression profiling of the intermediate and late stages of poxvirus replication. *J. Virol.* **2011**, *85*, 9899–9908. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
26. Yang, Z.; Bruno, D.P.; Martens, C.A.; Porcella, S.F.; Moss, B. Simultaneous high-resolution analysis of vaccinia virus and host cell transcriptomes by deep RNA sequencing. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 11513–11518. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
27. Paniz-Mondolfi, A.; Reidy, J.; Pagani, N.; Lednicky, J.A.; McGrail, J.P.; Kasminskaya, Y.; Patino, L.H.; Garcia-Sastre, A.; Palacios, G.; Gonzalez-Reiche, A.S.; et al. Genomic and ultrastructural analysis of monkeypox virus in skin lesions and in human/animal infected cells reveals further morphofunctional insights into viral pathogenicity. *J. Med. Virol.* **2023**, *95*, e28878. [[CrossRef](#)] [[PubMed](#)]
28. Liu, B.; Li, N.L.; Shen, Y.; Bao, X.; Fabrizio, T.; Elbahesh, H.; Webby, R.J.; Li, K. The C-Terminal Tail of TRIM56 Dictates Antiviral Restriction of Influenza A and B Viruses by Impeding Viral RNA Synthesis. *J. Virol.* **2016**, *90*, 4369–4382. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
29. Liu, B.; Li, N.L.; Wang, J.; Shi, P.-Y.; Wang, T.; Miller, M.A.; Li, K. Overlapping and distinct molecular determinants dictating the antiviral activities of TRIM56 against flaviviruses and coronavirus. *J. Virol.* **2014**, *88*, 13821–13835. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
30. Shen, Y.; Li, N.L.; Wang, J.; Liu, B.; Lester, S.; Li, K. TRIM56 Is an essential component of the TLR3 antiviral signaling pathway. *J. Biol. Chem.* **2012**, *287*, 36404–36413. [[CrossRef](#)]
31. Liu, S.; Qiu, C.; Miao, R.; Zhou, J.; Lee, A.; Liu, B.; Lester, S.N.; Fu, W.; Zhu, L.; Zhang, L.; et al. MCP1P1 restricts HIV infection and is rapidly degraded in activated CD4+ T cells. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 19083–19088. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
32. Liu, B.M.; Martins, T.B.; Peterson, L.K.; Hill, H.R. Clinical significance of measuring serum cytokine levels as inflammatory biomarkers in adult and pediatric COVID-19 cases: A review. *Cytokine* **2021**, *142*, 155478. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
33. Yang, D.; Li, N.L.; Wei, D.; Liu, B.; Guo, F.; Elbahesh, H.; Zhang, Y.; Zhou, Z.; Chen, G.-Y.; Li, K. The E3 ligase TRIM56 is a host restriction factor of Zika virus and depends on its RNA-binding activity but not miRNA regulation, for antiviral function. *PLoS Neglected Trop. Dis.* **2019**, *13*, e0007537. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
34. Wei, D.; Li, N.L.; Zeng, Y.; Liu, B.; Kumthip, K.; Wang, T.T.; Huo, D.; Ingels, J.F.; Lu, L.; Shang, J.; et al. The Molecular Chaperone GRP78 Contributes to Toll-like Receptor 3-mediated Innate Immune Response to Hepatitis C Virus in Hepatocytes. *J. Biol. Chem.* **2016**, *291*, 12294–12309. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
35. LeMessurier, K.S.; Rooney, R.; E Ghoneim, H.; Liu, B.; Li, K.; Smallwood, H.S.; E Samarasinghe, A. Influenza A virus directly modulates mouse eosinophil responses. *J. Leukoc. Biol.* **2020**, *108*, 151–168. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
36. El-Jesr, M.; Teir, M.; Maluquer de Motes, C. Vaccinia Virus Activation and Antagonism of Cytosolic DNA Sensing. *Front. Immunol.* **2020**, *11*, 568412. [[CrossRef](#)]
37. Li, K.; Lemon, S.M. Innate immune responses in hepatitis C virus infection. *Semin Immunopathol.* **2013**, *35*, 53–72. [[CrossRef](#)] [[PubMed](#)]
38. Lucena-Neto, F.D.; Falcão, L.F.M.; Vieira-Junior, A.S.; Moraes, E.C.S.; David, J.P.F.; Silva, C.C.; Sousa, J.R.; Duarte, M.I.S.; Vasconcelos, P.F.C.; Quaresma, J.A.S. Monkeypox Virus Immune Evasion and Eye Manifestation: Beyond Eyelid Implications. *Viruses* **2023**, *15*, 2301. [[CrossRef](#)]
39. Rao, A.K.; Schrodtt, C.A.; Minhaj, F.S.; Waltenburg, M.A.; Cash-Goldwasser, S.; Yu, Y.; Petersen, B.W.; Hutson, C.; Damon, I.K. Interim Clinical Treatment Considerations for Severe Manifestations of Mpox—United States, February 2023. *MMWR. Morb. Mortal. Wkly. Rep.* **2023**, *72*, 232–243. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
40. Duraffour, S.; Lorenzo, M.M.; Zöller, G.; Topalis, D.; Grosenbach, D.; Hruby, D.E.; Andrei, G.; Blasco, R.; Meyer, H.; Snoeck, R. ST-246 is a key antiviral to inhibit the viral F13L phospholipase, one of the essential proteins for orthopoxvirus wrapping. *J. Antimicrob. Chemother.* **2015**, *70*, 1367–1380. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]

41. Andrei, G.; Gammon, D.B.; Fiten, P.; De Clercq, E.; Opendakker, G.; Snoeck, R.; Evans, D.H. Cidofovir resistance in vaccinia virus is linked to diminished virulence in mice. *J. Virol.* **2006**, *80*, 9391–9401. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
42. Yang, G.; Pevear, D.C.; Davies, M.H.; Collett, M.S.; Bailey, T.; Rippen, S.; Barone, L.; Burns, C.; Rhodes, G.; Tohan, S.; et al. An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus challenge. *J. Virol.* **2005**, *79*, 13139–13149. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
43. Li, X.; Liu, B.; Xu, J.; Liu, X.; Ding, H.; Li, T. Discrepancy of potential antiviral resistance mutation profiles within the HBV reverse transcriptase between nucleos(t)ide analogue-untreated and -treated patients with chronic hepatitis B in a hospital in China. *J. Med. Virol.* **2012**, *84*, 207–216. [[CrossRef](#)] [[PubMed](#)]
44. Peng, Y.; Liu, B.; Hou, J.; Sun, J.; Hao, R.; Xiang, K.; Yan, L.; Zhang, J.; Zhuang, H.; Li, T. Naturally occurring deletions/insertions in HBV core promoter tend to decrease in HBeAg-positive chronic hepatitis B patients during antiviral therapy. *Antivir. Ther.* **2015**, *20*, 623–632. [[CrossRef](#)] [[PubMed](#)]
45. Alarcon, J.; Kim, M.; Terashita, D.; Davar, K.; Garrigues, J.M.; Guccione, J.P.; Evans, M.G.; Hemarajata, P.; Wald-Dickler, N.; Holtom, P.; et al. An Mpox-Related Death in the United States. *N. Engl. J. Med.* **2023**, *388*, 1246–1247. [[CrossRef](#)] [[PubMed](#)]
46. Liu, B.-M.; Li, T.; Xu, J.; Li, X.-G.; Dong, J.-P.; Yan, P.; Yang, J.-X.; Yan, L.; Gao, Z.-Y.; Li, W.-P.; et al. Characterization of potential antiviral resistance mutations in hepatitis B virus reverse transcriptase sequences in treatment-naïve Chinese patients. *Antivir. Res.* **2010**, *85*, 512–519. [[CrossRef](#)] [[PubMed](#)]
47. Yang, J.-X.; Liu, B.-M.; Li, X.-G.; Yan, C.-H.; Xu, J.; Sun, X.-W.; Wang, Y.-H.; Jiao, X.-J.; Yan, L.; Dong, J.-P.; et al. Profile of HBV antiviral resistance mutations with distinct evolutionary pathways against nucleoside/nucleotide analogue treatment among Chinese chronic hepatitis B patients. *Antivir. Ther.* **2010**, *15*, 1171–1178. [[CrossRef](#)] [[PubMed](#)]
48. Ding, H.; Liu, B.; Zhao, C.; Yang, J.; Yan, C.; Yan, L.; Zhuang, H.; Li, T. Amino acid similarities and divergences in the small surface proteins of genotype C hepatitis B viruses between nucleos(t)ide analogue-naïve and lamivudine-treated patients with chronic hepatitis B. *Antivir. Res.* **2014**, *102*, 29–34. [[CrossRef](#)] [[PubMed](#)]
49. Liu, B.; Yang, J.-X.; Yan, L.; Zhuang, H.; Li, T. Novel HBV recombinants between genotypes B and C in 3'-terminal reverse transcriptase (RT) sequences are associated with enhanced viral DNA load, higher RT point mutation rates and place of birth among Chinese patients. *Infect. Genet. Evol.* **2017**, *57*, 26–35. [[CrossRef](#)] [[PubMed](#)]
50. Lum, F.-M.; Torres-Ruesta, A.; Tay, M.Z.; Lin, R.T.P.; Lye, D.C.; Rénia, L.; Ng, L.F.P. Monkeypox: Disease epidemiology, host immunity and clinical interventions. *Nat. Rev. Immunol.* **2022**, *22*, 597–613. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
51. Kaiser, R.M.; Cash-Goldwasser, S.; Lehnertz, N.; Griffith, J.; Ruprecht, A.; Stanton, J.; Feldpausch, A.; Pavlick, J.; Bruen, C.A.; Perez-Molinar, D.; et al. Pharyngeal Co-Infections with Monkeypox Virus and Group A *Streptococcus*, United States, 2022. *Emerg. Infect. Dis.* **2023**, *29*, 1855–1858. [[CrossRef](#)] [[PubMed](#)]
52. Opardija, A.; Sivasubramanian, G. Delayed clearance of monkeypox virus in a patient with co infection with secondary syphilis. *IDCases* **2023**, *31*, e01707. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
53. Zlámál, M.; Bartovská, Z.; Burantová, A.; Zákoucká, H.; Jiřincová, H.M.; Chmel, M.M.; Holub, M. Monkeypox and Herpes Simplex Virus Type 2 Coinfection: Case Report of Perianal Lesions in HIV-Positive Patient. *Sex. Transm. Dis.* **2022**, *49*, 769–770. [[CrossRef](#)] [[PubMed](#)]
54. Ogoina, D.; Iroezindu, M.; James, H.I.; Oladokun, R.; Yinka-Ogunleye, A.; Wakama, P.; Otike-Odibi, B.; Usman, L.M.; Obazee, E.; Aruna, O.; et al. Clinical Course and Outcome of Human Monkeypox in Nigeria. *Clin. Infect. Dis.* **2020**, *71*, E210–E214. [[CrossRef](#)] [[PubMed](#)]
55. Vivancos-Gallego, M.J.; Sanchez-Conde, M.; Rodriguez-Dominguez, M.; Fernandez-Gonzalez, P.; Martinez-Garcia, L.; Garcia-Mouronte, E.; Martinez-Sanz, J.; Moreno-Zamora, A.M.; Casado, J.L.; Ron, R.; et al. Human Monkeypox in People With HIV: Transmission, Clinical Features, and Outcome. *Open Forum Infect. Dis.* **2022**, *9*, ofac557. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
56. Ortiz-Saavedra, B.; Montes-Madariaga, E.S.; Cabanillas-Ramirez, C.; Alva, N.; Ricardo-Martínez, A.; León-Figueroa, D.A.; Barboza, J.J.; Mohanty, A.; Padhi, B.K.; Sah, R. Epidemiologic Situation of HIV and Monkeypox Coinfection: A Systematic Review. *Vaccines* **2023**, *11*, 246. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
57. Traut, C.C.; Jones, J.L.; A Sanders, R.; Clark, L.R.; Hamill, M.M.; Stavrakis, G.; Sop, J.; Beckey, T.P.; Keller, S.C.; A Gilliams, E.; et al. Orthopoxvirus-Specific T-Cell Responses in Convalescent Mpox Patients. *J. Infect. Dis.* **2023**, *229*, 54–58. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
58. Wang, J.; Liu, B.; Wang, N.; Lee, Y.-M.; Liu, C.; Li, K. TRIM56 is a virus- and interferon-inducible E3 ubiquitin ligase that restricts pestivirus infection. *J. Virol.* **2011**, *85*, 3733–3745. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
59. Liu, B.M.; Hill, H.R. Role of Host Immune and Inflammatory Responses in COVID-19 Cases with Underlying Primary Immunodeficiency: A Review. *J. Interf. Cytokine Res.* **2020**, *40*, 549–554. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
60. Liu, B.M.; Li, N.L.; Wang, R.; Li, X.; Li, Z.A.; Marion, T.N.; Li, K. Key roles for phosphorylation and the Coiled-coil domain in TRIM56-mediated positive regulation of TLR3-TRIF-dependent innate immunity. *J. Biol. Chem.* **2024**, *300*, 107249. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
61. Hammarlund, E.; Dasgupta, A.; Pinilla, C.; Norori, P.; Früh, K.; Slifka, M.K. Monkeypox virus evades antiviral CD4⁺ and CD8⁺ T cell responses by suppressing cognate T cell activation. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14567–14572. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]

62. Netea, M.G.; Domínguez-Andrés, J.; Barreiro, L.B.; Chavakis, T.; Divangahi, M.; Fuchs, E.; Joosten, L.A.B.; van der Meer, J.W.M.; Mhlanga, M.M.; Mulder, W.J.M.; et al. Defining Trained Immunity and Its Role in Health and Disease. *Nat. Rev. Immunol.* **2020**, *20*, 375–388. [[CrossRef](#)] [[PubMed](#)]
63. Netea, M.G.; Quintin, J.; van der Meer, J.W. Trained Immunity: A Memory for Innate Host Defense. *Cell Host Microbe* **2011**, *9*, 355–361. [[CrossRef](#)] [[PubMed](#)]
64. van der Heijden, C.D.C.C.; Keating, S.T.; Groh, L.; Joosten, L.A.B.; Netea, M.G.; Riksen, N.P. Aldosterone Induces Trained Immunity: The Role of Fatty Acid Synthesis. *Cardiovasc. Res.* **2019**, *116*, 317–328. [[CrossRef](#)] [[PubMed](#)]
65. Sviridov, D.; Miller, Y.I.; Bukrinsky, M.I. Trained Immunity and HIV Infection. *Front. Immunol.* **2022**, *13*, 903884. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
66. Weinstein, R.S.; Weinstein, M.M.; Alibek, K.; I Bukrinsky, M.; Brichacek, B. Significantly reduced CCR5-tropic HIV-1 replication in vitro in cells from subjects previously immunized with Vaccinia Virus. *BMC Immunol.* **2010**, *11*, 23. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
67. Agrawal, B. Heterologous Immunity: Role in Natural and Vaccine-Induced Resistance to Infections. *Front. Immunol.* **2019**, *10*, 2631. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
68. Welsh, R.M.; Selin, L.K. No one is naive: The significance of heterologous T-cell immunity. *Nat. Rev. Immunol.* **2002**, *2*, 417–426. [[CrossRef](#)] [[PubMed](#)]
69. Selin, L.K.; Brehm, M.A.; Naumov, Y.N.; Cornberg, M.; Kim, S.; Clute, S.C.; Welsh, R.M. Memory of mice and men: CD8⁺ T-cell cross-reactivity and heterologous immunity. *Immunol. Rev.* **2006**, *211*, 164–181. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
70. Liu, B.; Totten, M.; Nematollahi, S.; Datta, K.; Memon, W.; Marimuthu, S.; Wolf, L.A.; Carroll, K.C.; Zhang, S.X. Development and Evaluation of a Fully Automated Molecular Assay Targeting the Mitochondrial Small Subunit rRNA Gene for the Detection of *Pneumocystis jirovecii* in Bronchoalveolar Lavage Fluid Specimens. *J. Mol. Diagn.* **2020**, *22*, 1482–1493, Erratum in *J. Mol. Diagn.* **2021**, *23*, 506. [[CrossRef](#)] [[PubMed](#)]
71. Liu, B.; Forman, M.; Valsamakis, A. Optimization and evaluation of a novel real-time RT-PCR test for detection of parechovirus in cerebrospinal fluid. *J. Virol. Methods* **2019**, *272*, 113690. [[CrossRef](#)] [[PubMed](#)]
72. Liu, B.M.; Mulkey, S.B.; Campos, J.M.; DeBiasi, R.L. Laboratory diagnosis of CNS infections in children due to emerging and re-emerging neurotropic viruses. *Pediatr. Res.* **2024**, *95*, 543–550. [[CrossRef](#)] [[PubMed](#)]
73. Available online: <https://www.cdc.gov/poxvirus/mpox/testing/testing-basics.html> (accessed on 10 May 2024).
74. Fathi, A.; Schmiedel, S. Hand, Foot, and Mouth Disease as Differential Diagnosis of Monkeypox, Germany, August 2022. *Emerg. Infect. Dis.* **2022**, *28*, 2586. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
75. Liu, B. Universal PCR Primers Are Critical for Direct Sequencing-Based Enterovirus Genotyping. *J. Clin. Microbiol.* **2016**, *55*, 339–340. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
76. Mbala, P.K.; Huggins, J.W.; Riu-Rovira, T.; Ahuka, S.M.; Mulembakani, P.; Rimoin, A.W.; Martin, J.W.; Muyembe, J.T. Maternal and Fetal Outcomes among Pregnant Women with Human Monkeypox Infection in the Democratic Republic of Congo. *J. Infect. Dis.* **2017**, *216*, 824–828. [[CrossRef](#)] [[PubMed](#)]
77. Hussain, A.; Kaler, J.; Lau, G.; Maxwell, T. Clinical Conundrums: Differentiating Monkeypox from Similarly Presenting Infections. *Cureus* **2022**, *14*, e29929. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
78. McLean, J.; Stoeckle, K.; Huang, S.; Berardi, J.; Gray, B.; Glesby, M.J.; Zucker, J. Tecovirimat Treatment of People with HIV during the 2022 Mpox Outbreak: A Retrospective Cohort Study. *Ann. Intern. Med.* **2023**, *176*, 642–648. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]

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