Front. Med. 2022, 16(4): 507−517
https://doi.org/10.1007/s11684-022-0952-z

REVIEW

Unusual global outbreak of monkeypox: what should we do?

Miaojin Zhu[1,*], Jia Ji[1,*], Danrong Shi[1,*], Xiangyun Lu[1], Baohong Wang[1,2], Nanping Wu[1,2], Jie Wu (✉)[1,2], Hangping Yao (✉)[1,2], Lanjuan Li (✉)[1,2]

[1]*State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China;* [2]*Jinan Microecological Biomedicine Shandong Laboratory, Jinan 250117, China*

Abstract **Recently, monkeypox has become a global concern amid the ongoing COVID-19 pandemic. Monkeypox is an acute rash zoonosis caused by the monkeypox virus, which was previously concentrated in Africa. The re-emergence of this pathogen seems unusual on account of outbreaks in multiple nonendemic countries and the incline to spread from person to person. We need to revisit this virus to prevent the epidemic from getting worse. In this review, we comprehensively summarize studies on monkeypox, including its epidemiology, biological characteristics, pathogenesis, and clinical characteristics, as well as therapeutics and vaccines, highlighting its unusual outbreak attributed to the transformation of transmission. We also analyze the present situation and put forward countermeasures from both clinical and scientific research to address it.**

Keywords monkeypox; poxviruses; vaccine; infectious diseases

Introduction

Recently, cases of human-to-human transmission of monkeypox virus (MPXV), which was previously thought to be extremely rare, have been reported in several countries. According to the US Centers for Disease Control and Prevention (CDC), on July 8, 2022, over 50 countries/territories reported more than 8000 confirmed cases in total [1]. Monkeypox spread from Africa to the world in just one month, and most cases have come from Europe. The actual number of cases may be underestimated, in part because of the lack of early clinical recognition of infections previously familiar only in a few countries and limited mechanisms to strengthen surveillance in many countries for diseases previously unfamiliar to most health systems.

Among the family of orthopoxviruses, only four species, smallpox or variola virus (VARV), cowpox virus (CPXV), vaccinia virus (VACV), and MPXV, can cause human infection [2]. MPXV and VARV are close relatives and are the two most important pathogens with potential bioterrorism threats [3]. VARV was once a common severe infectious disease pathogen that has been circulating in humans for at least 3000 years. It is highly pathogenic (mortality rate ~30%) and transmissible [4,5], taking many people's lives and causing crushing blows to humans. In 1980, the WHO declared that the world had eradicated smallpox owing to the smallpox vaccine [6]. Soon after, the world gradually stopped the smallpox virus vaccination.

Monkeypox is considered to be the most important orthopoxvirus infection in humans since the eradication of smallpox. Monkeypox was first discovered in humans in 1970 [7] and has previously been rare outside of Central and West Africa. Two phylogenetically distinct branches of monkeypox virus have been identified by genome sequencing: the Central African (Congo Basin) branch and the West African branch. Typically, Central African monkeypox viruses are associated with more severe disease, higher mortality, and more frequent human-to-human transmission. Monkeypox virus, in the current outbreak outside of Africa, has been confirmed to belong to a West African strain. Monkeypox viruses have not previously been highly contagious, with a basic reproduction number (R0) lower than 1 in the 1980s [8], higher than 1 in a study previous to this outbreak [9], compared with a high average R0 of 9.5 for the SARS-CoV-2 Omicron strain (from November 1, 2021 to

Received: July 3, 2022; accepted: July 11, 2022

Correspondence: Lanjuan Li, ljli@zju.edu.cn;
Hangping Yao, yaohangping@zju.edu.cn;
Jie Wu, zjwujie@zju.edu.cn

*Miaojin Zhu, Jia Ji, and Danrong Shi contributed equally to this study.

February 9, 2022) [10]. This unusual outbreak requires us to reassess the virus.

Here, we reviewed the warning pathogen, including its epidemiology, biological characteristics, pathogenesis, and clinical characteristics, as well as therapeutics and vaccines, highlighting its unusual outbreak attributed to the transformation of transmission. We also analyzed the present situation and put forward countermeasures from both clinical and scientific research to address it.

Epidemiology

MPXV was first detected in 1958 and in an outbreak of vesicular disease in captive monkeys. This is why it is called "monkeypox" [11]. However, the largest animal reservoirs of this virus are not monkeys but rodents, including squirrels and kangaroos. Rodents are the largest group of mammals, with more than 1500 species. To date, MPXV has been detected in a variety of animal species, including squirrels (ropes and trees), rats, striped mice, dormice, and monkeys [12–14]. Like monkeys, humans are considered to be the host of the virus, but the mode of transmission of MPXV from animals to humans is still unclear. Aerosol transmission has been demonstrated in animals [15,16] and may explain a nosocomial outbreak in the Central African Republic [17]. Monkeypox virus is transmitted from one person to another by close contact with lesions, body fluids, respiratory droplets, and contaminated materials, such as bedding. In August 1970, in the equatorial village of Bukenda in Zaire (now the Democratic Republic of the Congo), a 9-year-old child was found to have vesicular skin disease similar to smallpox, which was the first reported human case of monkeypox [18]. The incubation period of monkeypox is usually 6 to 13 days, but it can also be between 5 and 21 days [19,20].

Since monkeypox was discovered 60 years ago, it has not received much attention. In recent years, the frequency and geographical distribution of monkeypox in humans have been increasing. In the past 20 years, the number of human cases of monkeypox has increased exponentially, exceeding the number of cases accumulated in the first 45 years after the first detection of monkeypox [19,20]. Poverty and persistent civil strife force people to hunt down small mammals (bush meat) to obtain protein-rich food, thereby increasing the chance of contact with wild rodents that may carry monkeypox [21]. Since its discovery, the disease has been endemic in Central Africa and West Africa, and intermittent and sporadic cases of monkeypox transmission in humans by local wild animals have been reported. In addition, the number of human monkeypox cases in Central Africa and West Africa is increasing, which is considered to be the result of the weakening of cross-protective immunity in the population after the smallpox vaccination was stopped in the early 1980s [20]. In addition, many human cases have been reported in nonendemic areas, including the United States, the UK, Israel, and Singapore [22]. These sporadic outbreaks in nonendemic areas are related to imported rodents or people with a history of travel to endemic areas.

In May 2022, multiple cases of monkeypox were detected in several nonendemic countries. None of the cases reported a travel history to the endemic areas of monkeypox, and there was no epidemiological link between the cases reported in different countries. The early epidemiology of the initial cases reported to the WHO by various countries showed that the cases were mainly reported in men who have sex with men [23]. Currently, research is underway to further understand the epidemiology, source of infection, and mode of transmission. The sudden appearance of monkeypox in several nonendemic countries indicates that there may be a period of undetected transmission, as well as recent expansion events.

Biological characteristics

Monkeypox virus belongs to the family Poxviridae, genus Orthopoxvirus. Poxviruses are the largest and most complex group of DNA viruses among all animal viruses [24]. Monkeypox virus is 200–300 nm in size, round brick-shaped or ovoid, and surrounded by a 30-nm outer membrane composed of irregularly shaped tubules. There exist two different forms of infectious virus particles: intracellular mature virions (MVs) and extracellular enveloped virions (EVs). EVs have an extra envelope than MVs. The interior of the viral particle consists of a dumbbell-shaped nucleus and a pair of lateral bodies between the concavities and the outer membrane (Fig. 1). Recently, researchers have predicted the structure of the whole proteome of monkeypox variants as well as the small-molecule binding pockets [25]. Monkeypox virus contains soluble antigens, nucleoprotein antigens, and erythrocyte agglutinins and is cross-immunogenic with three other orthopoxviruses that infect humans. Monkeypox virus shares structural and soluble antigens with other orthopoxviruses. It is difficult to distinguish between smallpox virus and poxvirus in complement binding tests and agar diffusion tests.

The genome of monkeypox virus is approximately 197 kb of bipartite covalently closed linear double-stranded DNA and contains an identical but oppositely oriented 6379 bp inverted terminal repeat (ITR) sequence at the end of the genome [26]. The virus contains 190 open reading frames, four of which are located in the ITR sequence [27] (Fig. 1). In the central region of the genome are mostly genes encoding important replication

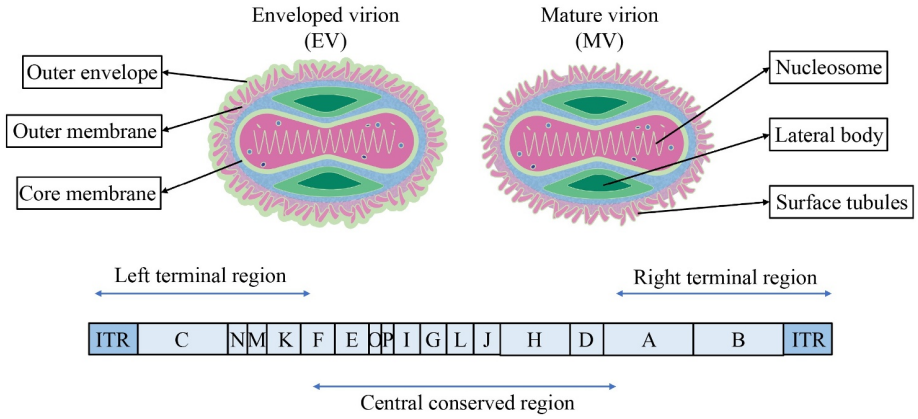

Fig. 1 Structure and genome of monkeypox virus. The top left and right parts of the figure show the structures of enveloped virions (EVs) and mature virions (MVs) respectively. EVs have an extra envelope than MVs. The surface of the outer membrane consists of irregularly shaped tubules. The interior of the viral particle consists of a dumbbell-shaped nucleus and a pair of the lateral bodies between the concavities and the outer membrane. The bottom part of the figure shows the genome of the monkeypox virus (data from ViralZone, SIB Swiss Institute of Bioinformatics). It is approximately 197 kb of double-stranded DNA and contains an inverted terminal repeat (ITR) sequence at the end of the genome. In the central region of the genome are mostly genes encoding necessary replication enzymes and structural proteins of the virus, which are highly homologous compared to the smallpox virus.

enzymes and structural proteins of the virus, which are highly homologous compared to smallpox virus, while genes encoding virulence and host tropism are mostly located at both ends of the genome, which are highly heterogeneous to smallpox virus. At present, the structure and function of many kinds of monkeypox proteins have been predicted and analyzed, and a number of studies on gene variation of monkeypox virus have been released, but the function and expression status of some genes still need further study.

The current outbreak was caused by the West African branch of the virus, which is generally less dangerous than the Congo Basin branch, and genetic differences between the viral genomes of these two branches may explain the differences in viral clearance and pathogenesis [28]. Studies have revealed that the virus strain associated with the monkeypox outbreak identified in May 2022 is a well-characterized divergent clade of monkeypox virus outbreaks in endemic countries in 2018–2019 or represents a recent evolutionary change in the virus [29]. Of course, differences in disease severity may also be influenced by the route of transmission, host susceptibility, and the amount of infected virus, as we will elaborate below.

Monkeypox virus is more stable in dry conditions and can remain viable for a long time in a 4 °C or −70 °C environment, but it does not tolerate high temperatures and can be rendered noninfectious in 20 min at 56 °C. It is resistant to ether but is easily inactivated by organic reagents, such as chloroform, methanol, formaldehyde, ethanol, sodium dodecyl acetate, and formalin.

Monkeypox virus can grow in cells originating from monkeys, rabbits, cattle, guinea-pigs and mice, as well as humans, and produce distinctive cavernous or plaque-like cytopathic effects. Most of the cells infected with monkeypox virus contain many small round or oval eosinophilic inclusion bodies.

Mechanisms of monkeypox virus transmission and pathogenesis

Monkeypox is a zoonotic disease that spreads relatively inefficiently from person to person [30,31], and the incidence of the disease is increasing year by year [22,30,32]. Transmission is believed to occur via saliva/respiratory excretions or contact with lesion exudate or crust material, which can infect the mucous membranes of the eyes, nose, throat and wounded skin. Studies have shown that it takes prolonged face-to-face contact to spread, such as lasting more than three hours within a 2-m radius without personal protective equipment [33,34]. Monkeypox virus can be transmitted from pregnant women to fetuses through the placenta, or from infected parents to children through skin contact during or after the birth of children [35]. The disease can also be caught by being bitten or scratched by infected animals and eating meat from infected animals that are not properly cooked [36,37], so attention should be given to the quarantine of imported animal monkeypox virus and the investigation of patients' travel history. In recent years, sexual contact has also been considered a potential route of human spread, especially male-to-male contact [38–40].

Between infection and the occurrence of rashes, a complicated series of events occur: the virus enters the human body through the mucous membrane or damaged skin and is disseminated through the host via the lymph and blood, primarily by infected leukocytes [41]. Then,

the virus replicates and multiplies in the regional lymph node and reaches skin epithelial cells and other tissues through the blood circulation [42]. Virus deposit in the skin multiplies for several days before any macroscopic lesions appear. Viral replication and invasion of skin epithelial cells cause superficial inflammation of the dermis, leading to vasodilation, hyperemia, and inflammatory cell infiltration, resulting in a rash (cutaneous vesicles) [43] (Fig. 2).

During infection, monkeypox virus replicates and multiplies in cells, and the host immune system recognizes the virus or its surface epitopes, causing an innate or adaptive immune response. Host pattern recognition receptors (PRRs) sense pathogen-associated molecular patterns (PAMPs), which then initiate innate immune responses and activate inflammatory signaling pathways, trigger local inflammation; and strongly secrete proinflammatory cytokines (including IL-6 and TNF-α), chemokines (including CXCL1 and IL-8) and complement proteins [44,45]. The secretion of cytokines and chemokines attracts more immune cells to the infected site and causes clinical symptoms, such as rash and skin lesions.

To counteract the host's immune defenses, monkeypox viruses have developed an assortment of immunomodulators that work in various ways to undermine or circumvent host antiviral responses triggered by PRRs. Monkeypox in Africa inhibits the activation of T cells through their receptors, preventing the release of inflammatory cytokines in human cells derived from patients who have already been infected with monkeypox [46]. In addition, compared with West African strains, Central African monkeypox strains selectively downregulated host responses, especially host cell apoptosis [47], and secreted immunomodulatory factors to inhibit complement enzymes, thereby increasing the virulence of Central African strains [48]. Clinical recovery and the disappearance of the virus are closely correlated with the appearance of circulating antibodies.

Clinical characteristics

Human monkeypox virus is a zoonotic orthopoxvirus with a presentation similar to that of smallpox virus. The incubation period (interval from infection to onset of symptoms) of monkeypox varies from 5 to 21 days [49]. Infected persons usually start with "flu-like" symptoms [50,51], including fever, headaches, fatigue, muscle soreness, and enlarged lymph nodes. Swollen lymph

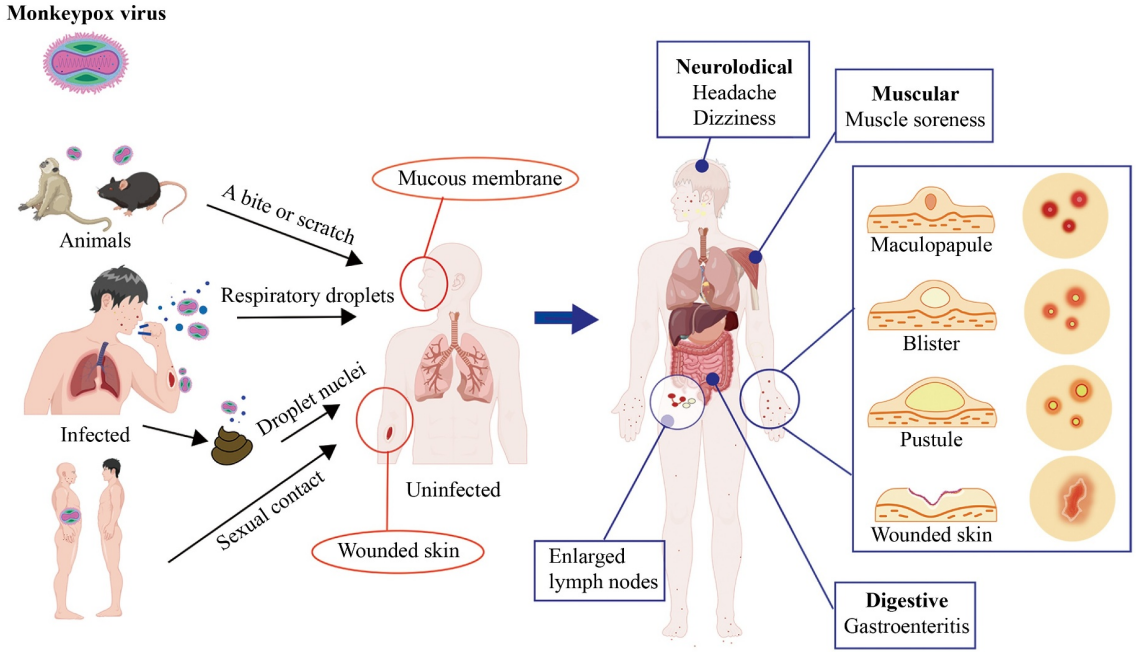

Fig. 2 Transmission routes and clinical symptoms of human monkeypox. The section on the left describes how the monkeypox virus is transmitted. Animals such as monkeys, rodents, and patients infected with monkeypox virus can be the source of infection. There are many modes of transmission: animal bites, respiratory droplets, sexual contact, and contact with body fluids of infected animals or patients. The section on the right describes the clinical signs of human monkeypox. Monkeypox virus infection can involve multiple body systems and is characterized by a centrifugal rash. The rash changes from macular papules to small blisters and pustules. Scabs formed after approximately 10 days and disappeared after approximately 3 weeks. Some patients with monkeypox have enlarged lymph nodes in the neck (submandibular and cervical), axillae, or groin during the rash phase, which is the clinical feature that distinguishes monkeypox from smallpox. This figure was drawn using Figdraw, an open access online scientific research drawing platform.

nodes (1–4 cm in diameter) are firm and tender, usually in the submandibular, cervical, or inguinal regions (Fig. 2).

A rash usually appears 1 to 3 days after the onset of fever and lymphadenopathy, beginning with macules (lesions with a flat base) and papules (raised firm painful lesions) [52]. Then, the lesions develop quickly into herpes, forming fluid-filled blisters and pus-filled pustules with local lymphadenopathy, followed by scabs or crusts, and each symptom may last 1–2 days [53,54]. Monkeypox lesions are firm, deep-seated, and well circumscribed with a central point of umbilication [55], and the exudate of the lesion is highly infectious. Often, the rash first appears on the face and quickly spreads to the extremities, which mainly affects the face, palms, and soles of the feet. It can also appear on the genitals, mouth, conjunctiva, and cornea [42,56]. In severe cases, the lesions can even cover the whole body. The rash always lasts up to 4 weeks until the lesion desquamates [57].

Monkeypox is often misdiagnosed as chicken pox and smallpox (up to 50% of cases in the Democratic Republic of the Congo were misdiagnosed) [54]. The major difference distinguishing human monkeypox (MPX) from smallpox is the lymph node enlargement caused by MPXV that often occurs at the onset of fever, and varicella zoster virus lesions are usually more superficial in appearance with irregular borders, can be present at multiple stages on any one site on the body, and rapidly evolve from macules to crusts within 24 h [58].

For most people, monkeypox is a self-limiting disease with mild symptoms that usually lasts two to four weeks and recovers completely. However, the severity of symptoms is also closely related to the transmission route, the degree of virus exposure and the patient's own basic diseases. Epidemiologic studies of human monkeypox infections have shown that younger children, pregnant women, and persons not vaccinated against smallpox may suffer from severe disease and complications, including secondary bacterial infections, respiratory distress, pneumonitis, sepsis, gastrointestinal involvement, encephalitis, and corneal infection with ensuing loss of vision [19,52,59]. The proportion of patients who die has varied between 0 and 11% in documented cases and has been higher among young children. The outbreak of monkeypox virus originated from the West African branch, and the mortality of this branch was 3.6%, while that of the evolutionary branch in Central Africa (Congo basin) was 10.6% [41].

Laboratory assays are important components for the diagnosis and identification of monkeypox infection. Medical professionals collected clinical specimens (including vesicular fluid, scabs or lesion swabs, throat swabs, whole blood, and serum) from confirmed or suspected monkeypox patients for further etiological detection [50]. Viral isolation and identification, electron microscopy and real-time PCR are the gold standard detection methods for confirming monkeypox infections, but require sophisticated laboratories with high containment facilities. Specimens of rash, blister fluid, crusts, oropharyngeal or nasopharyngeal secretions are collected and infected with Vero cells in virus growth medium for virus amplification until CPE advances throughout for virus isolation and identification. Intracytoplasmic, round-to-oval inclusions may be observed under an electron microscope measuring 200 to 300 μm with sausage-shaped structures centrally. Other molecular and antigen detection tests such as immunohistochemistry and immunofluorescence, can be used to assist the clinical diagnosis of monkeypox, and serological assays are mainly used for epidemiological investigation. Serologic testing requires paired acute and convalescent sera for MPXV-specific IgM detection within 5 days of presentation, or IgG detection after 8 days [52]. It is worth noting that monkeypox needs to be differentiated from other diseases such as smallpox, chicken pox, herpes zoster, and herpes simplex.

Therapeutics and vaccines

At present, there are no specific treatments available for monkeypox infection. As a self-limited disease, most people infected with monkeypox do not require special treatment. The main treatment methods are symptomatic support treatment; prevention and treatment of complications; rest; replenishment of water and nutrition; strengthening nursing; keeping eyes, nose, the oral cavity and skin clean; and antibiotics can be used to prevent secondary infection. There are antiviral drugs for smallpox that could also be used to treat monkeypox under certain circumstances. Tecovirimat, smallpox vaccine, cidofovir, brincidofovir, and vaccinia immune globulin (VIG) can be used to control a monkeypox outbreak [60] (Table 1). However, there are no data on the effectiveness of cidofovir, brincidofovir, and VIG in the treatment of monkeypox complications. It is unknown whether or not a person with severe monkeypox infection will benefit from treatment with either antiviral; however, its use may be considered to deal with emergencies in such instances.

Tecovirimat (TPOXX), an antiviral drug used to treat smallpox, was also approved by the Food and Drug Administration (FDA) for monkeypox in January 2022. Tecovirimat targets and inhibits the activity of the highly conserved orthopoxvirus VP37 protein and blocks the interaction with cellular Rab9 GTPase and TIP47, to prevent the formation of egress competent enveloped virions necessary for cell-to-cell and long-range dissemination of the virus, inhibiting long-range dissemination of the virus [81]. Studies on the therapeutic efficacies of the antiviral drug Tecovirimat in a variety of animal species have shown that Tecovirimat is effective

Table 1 Prevention and treatment of monkeypox

Name	Manufacturer	Mechanism of action	Administration and dosage	Clinical trial information	Indications and usage	References
Antiviral therapeutics						
Tecovirimat	SIGA Technologies	Inhibits the activity of the orthopoxvirus VP37 envelope wrapping protein and prevents virus replication and intracellular viruses release	PO, IV Adults: 600 mg twice daily for 14 days Pediatrics (13 kg or more): dosage reduction	The completed phase 3 clinical trials to evaluate the safety, tolerability, and pharmacokinetics of tecovirimat showed the drug was safe with minor side effects	Licensed by FDA for the treatment of human smallpox disease since July 2018 and urgently licensed by FDA for treatment of monkeypox in 2022	[61,62]
Brincidofovir	Chimerix	Phosphorylated cidofovir diphosphate acts as a competitive substrate inhibitor of DNA polymerase to reduce DNA synthesis and terminating chain elongation	PO Most frequently administered at an oral dose of 200 mg twice weekly	The completed phase 1/2/3 clinical trials showed the drug had effective antiviral effects and the main dose-limiting toxic effects are gastrointestinal events	Licensed by FDA for treatment of human smallpox disease in adult and pediatric patients in the US since June 2021	[63,64]
Cidofovir	Gilead	Inhibits CMV replication by selective inhibition of viral DNA polymerase, thereby preventing viral replication and transcription	IV 5 mg/kg once weekly for the first 2 weeks, and thereafter once every other week	The completed phase 2/3 clinical trials to evaluate the effectiveness, safety of cidofovir in AIDS patients with CMV retinitis showed the drug was effective, but with the potential risk of nephrotoxicity	Developed for the treatment of cytomegalovirus retinitis in patients with AIDS, which has been used in the treatment of poxvirus infections	[65–67]
Blood products						
Vaccinia Immune Globulin	Cangene Corporation/ DynPort Vaccine	Antibodies isolated from blood of healthy adults vaccinated with vaccinia neutralize the virus to inhibit viral infection and provide passive immunity	IV Severe complications of vaccinia vaccination: 6000 U/kg with a maximum speed of 2 mL/min as soon as symptoms appear Persistent or severe symptoms: considered repeated administration	An open-label phase 4 study is under way to collect additional data to assess the safety and efficacy of VIGIV in healthy people	Licensed by FDA for the treatment of complications caused by vaccinia vaccination since February 2005, considered emergency treatment of orthopoxviruses in the case of a serious outbreak	[68,69]
Vaccines						
MVA-BN	Bavarian Nordic	Based on a live, highly attenuated vaccinia virus, unable to achieve complete replication in humans but can induce an effective immune response	IH A total of 2 doses (0.5 mL per dose) were injected subcutaneously with an interval of 4 weeks Subjects previously vaccinated against smallpox: a single 0.5 mL dose	The completed phase 2/3 clinical trials showed it had excellent safety and tolerability in both healthy and low immunity people, and its immunogenicity was not inferior to ACAM2000	Licensed by FDA to prevent monkeypox in September 2019, applicable to adults over 18 years old with high risk of monkeypox infection	[70–73]
ACAM2000	Acambis	A live, replication-competent vaccinia virus vaccine derived from a plaque-purified clone can induce an effective immune response	TDD Administered in a single 2.5 μL dose by the percutaneous route (scarification) using 15 jabs bifurcated needle to the deltoid muscle of the upper arm	The completed phase 1/2/3 clinical trials have shown it had excellent immunogenicity and safety, but with the concerns about the incidence of vaccination-related myopericarditis	Licensed by FDA for people at high risk of exposure to smallpox since August 2007	[70,74,75]
APSV	Aventis Pasteur	A liquid formulation of calf-lymph vaccine produced by vaccinia virus derived from the NYCBOH strain can induce an effective immune response	TDD Administered in a single 2.5 μL dose by the percutaneous route (scarification) using 15 jabs bifurcated needle to the deltoid muscle of the upper arm	The completed phase 1/2 clinical trials to evaluate the safety, dose and preliminary efficacy of APSV in vaccinia-naive adults showed it was still effective at diluted doses, but its reactogenicity did not decrease	Submitted a pre-EUA to FDA for the use of APSV diluted 1:5 to vaccinate persons during a declared public health emergency involving smallpox	[70,76]
Tiantan	Sinopharm	A live, highly attenuated vaccinia virus isolated from the blister scab of patients with smallpox in 1926 can induce an effective immune response	TDD Administered in a single 10 μL dose by the acupuncture or scarification route inoculation on the deltoid muscle of the upper arm	The completed phase 2 clinical trials of recombinant Tiantan vaccinia virus-vectored/HIV-1 booster in healthy people showed excellent performance in immunogenicity and safety	Licensed for use in China. Novel smallpox vaccines constructed by deleting some genes of Tiantan strain, have been used in the research of vaccines such as HIV, HBV, HCV and so on	[77–80]

in treating orthopoxvirus-induced disease, and the clinical signs of disease were elevated in Tecovirimat-treated animals, compared to placebo-treated animals [82–84]. Human clinical trials indicated the drug was safe and tolerable with only minor side effects [85,86]. However, the data on the effectiveness of Tecovirimat in treating human cases of monkeypox are not available.

Because monkeypox virus is closely related to the virus that causes smallpox, the smallpox vaccination also has cross-immunity to other orthopoxvirus infections, including monkeypox. Studies suggest that the smallpox vaccine is at least 85% effective in preventing monkeypox. However, since the World Health Organization declared that smallpox was eradicated in 1980 [6], countries gradually stopped the smallpox vaccination, so that people under the age of 40 or 50 years around the world no longer benefit from the protection provided by the previous smallpox vaccination program and are not immune to monkeypox. Even people who have been vaccinated against smallpox have limited protection against the monkeypox virus due to the decline in their immunity over time.

Modified Vaccinia Ankara (MVA) is an attenuated vaccinia virus that cannot achieve complete replication in mammalian cells. MVA-BN (also known as Imvamune, Imvanex, or Jynneos) is an alternative smallpox vaccine, which the FDA licensed to prevent monkeypox in 2019. At present, it is mainly used for work that has exposure risk and as a vaccination for an exposed population, and it is not currently available to the general public. The effectiveness of MVA against monkeypox was concluded from a clinical study on the immunogenicity of MVA and efficacy data from animal studies [87–89]. The immunogenicity of MVA-BN showed noninferiority compared with another FDA-licensed live smallpox vaccine, ACAM2000 [90,91]. However, the traditional replicative smallpox vaccine has safety problems in the general population, and the safety of MVA has been fully proven in a number of clinical trials. MVA-BN has excellent safety, tolerability, and immunogenicity. There were no major safety problems, especially regarding cardiac safety, in both healthy and immunocompromised people in trials [72,92]. In addition, in people who have been vaccinated, MVA-BN can quickly boost the long-term B cell memory response induced by the previous traditional smallpox vaccine [93]. As reserve resources, smallpox vaccines such as Tiantan strian developed and produced by China and Aventis Pasteur Smallpox Vaccine (APSV) of the United States have the potential to prevent monkeypox in case of emergency.

Future countermeasures

Today, the monkeypox virus has spread outside of Africa, and this outbreak is unusual in that there are so many confirmed cases of monkeypox of unknown origin in a short period of time in a nonendemic area that it is impossible not to be concerned. There are many possible reasons for this unusual outbreak of monkeypox, including a decrease in the proportion of people immunized against smallpox, increasingly frequent international travel, and mutations in the adaptation of monkeypox viruses to human hosts [23,29,94]. However, with years of human experience in fighting infectious diseases, we do not need to panic.

In essence, zoonotic diseases are infectious diseases. Therefore, the same principles of prevention and control of infectious diseases apply to zoonotic diseases, such as monkeypox. These principles can include the following: controlling the source of infection, cutting off the transmission route, and protecting vulnerable and susceptible populations.

To prevent underdiagnosis and misdiagnosis, clinicians, especially dermatologists and epidemiologists, should treat monkeypox infection as a clinical syndrome of varicella for differential diagnosis and should contact disease prevention and control or relevant agencies for sequencing identification [40]. Positive and suspected patients and animals should be isolated promptly, and reverse and positive contact tracing should be initiated.

Severe restrictions on the importation of wildlife, especially any African rodents, should also be imposed. Residents and travelers to endemic countries should avoid contact with sick animals (rodents, marsupials, primates), dead or alive, that may carry monkeypox virus and should avoid eating or handling wild animals. Any illness during travel or after return from an endemic area should be reported to a health professional. Health workers and other caregivers who care for patients with suspected or confirmed monkeypox should implement standard control precautions for contact and droplet infection. Samples collected from suspected monkeypox patients or animals suspected of being infected with monkeypox virus should be handled safely by trained personnel in an appropriately equipped laboratory.

Emphasis should always be placed on maintaining good hygiene habits. Other prevention measures include applying suds or alcohol-based disinfectants to maintain hand hygiene before eating and after using the toilet and after contact with suspected infected people or animals. Medical masks should also be worn in crowded places.

Since the global declaration of smallpox eradication in the 1980s, populations around the world no longer benefit from the protection provided by previous smallpox vaccination programs, and thus, populations born after this time generally lack the appropriate immunity and are in a susceptible group. In addition, even those who have been vaccinated against cowpox are at risk, as antibody

immunity greatly reduces with time. If a vaccine is available, the vaccination of high-risk close contacts should be considered after a risk-benefit assessment.

We should not relax our vigilance; instead, we should strengthen awareness of the prevention of the monkeypox virus, and scientists should strengthen the research of the virus itself to fully understand the virus and explore the cause of this unusual outbreak. In addition, we should develop better and more accurate diagnostic reagents for the timely detection of imported cases. At the same time, one should start stockpiling potent vaccines and antiviral drugs to avoid being in a passive situation when cases arise.

For thousands of years, the development of human society has always been intertwined with the emergence, prevalence, and control of different infectious diseases. Only by continuously gaining a deeper understanding of the epidemiological patterns of infectious diseases can we do our best to keep human beings free from infectious diseases and guard their health and dignity.

Acknowledgements

This work was supported by the National Key Research & Development Program of China (No. 2021YFC2301204), Zhejiang Provincial Key Research & Development Program of China (No. 2021C03043), and Research Project of Jinan Microecological Biomedicine Shandong Laboratory.

Compliance with ethics guidelines

Miaojin Zhu, Jia Ji, Danrong Shi, Xiangyun Lu, Baohong Wang, Nanping Wu, Jie Wu, Hangping Yao, and Lanjuan Li declare no competing financial interests. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. CDC. 2022 Monkeypox Outbreak Global Map. 2022. Available at the US CDC website (accessed July 8, 2022)
2. Frey SE, Belshe RB. Poxvirus zoonoses—putting pocks into context. N Engl J Med 2004; 350(4): 324–327
3. Henderson DA. The looming threat of bioterrorism. Science 1999; 283(5406): 1279–1282
4. Weiss MM, Weiss PD, Mathisen G, Guze P. Rethinking smallpox. Clin Infect Dis 2004; 39(11): 1668–1673
5. Bozzette SA, Boer R, Bhatnagar V, Brower JL, Keeler EB, Morton SC, Stoto MA. A model for a smallpox-vaccination policy. N Engl J Med 2003; 348(5): 416–425
6. World Health Assembly. Report of the global commission for the certification of smallpox eradication. World Health Organization: Geneva, 1980
7. Ladnyj ID, Ziegler P, Kima E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. Bull World Health Organ 1972; 46(5): 593–597
8. Fine PE, Jezek Z, Grab B, Dixon H. The transmission potential of monkeypox virus in human populations. Int J Epidemiol 1988; 17(3): 643–650
9. Grant R, Nguyen LL, Breban R. Modelling human-to-human transmission of monkeypox. Bull World Health Organ 2020; 98(9): 638–640
10. Liu Y, Rocklöv J. The effective reproductive number of the Omicron variant of SARS-CoV-2 is several times relative to Delta. J Travel Med 2022; 29(3): taac037
11. Magnus P, Andersen EK, Petersen KB, Birch-Andersen A. A pox-like disease in cynomolgus monkeys. Acta Pathol Microbiol Scand 1959; 46(2): 156–176
12. Sklenovská N, Van Ranst M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. Front Public Health 2018; 6: 241
13. Reynolds MG, Carroll DS, Karem KL. Factors affecting the likelihood of monkeypox's emergence and spread in the post-smallpox era. Curr Opin Virol 2012; 2(3): 335–343
14. Schneider KA, Eichner M. Does it matter who is spreading monkeypox? Lancet Infect Dis 2022; [Epub ahead of print] doi:10.1016/S1473-3099(22)00431-5
15. Wenner HA, Macasaet FD, Kamitsuka PS, Kidd P. Monkey pox. I. Clinical, virologic and immunologic studies. Am J Epidemiol 1968; 87(3): 551–566
16. Prier JE, Sauer RM. A pox disease of monkeys. Ann N Y Acad Sci 1960; 85(3): 951–959
17. Nakoune E, Lampaert E, Ndjapou SG, Janssens C, Zuniga I, Van Herp M, Fongbia JP, Koyazegbe TD, Selekon B, Komoyo GF, Garba-Ouangole SM, Manengu C, Manuguerra JC, Kazanji M, Gessain A, Berthet N. A nosocomial outbreak of human monkeypox in the Central African Republic. Open Forum Infect Dis 2017; 4(4): ofx168
18. Marennikova SS, Seluhina EM, Mal'ceva NN, Cimiskjan KL, Macevic GR. Isolation and properties of the causal agent of a new variola-like disease (monkeypox) in man. Bull World Health Organ 1972; 46(5): 599–611
19. Sklenovská N, Van Ranst M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. Front Public Health 2018; 6: 241
20. Durski KN, McCollum AM, Nakazawa Y, Petersen BW, Reynolds MG, Briand S, Djingarey MH, Olson V, Damon IK, Khalakdina A. Emergence of monkeypox—West and Central Africa, 1970–2017. MMWR Morb Mortal Wkly Rep 2018; 67(10): 306–310
21. Quiner CA, Moses C, Monroe BP, Nakazawa Y, Doty JB, Hughes CM, McCollum AM, Ibata S, Malekani J, Okitolonda E, Carroll DS, Reynolds MG. Presumptive risk factors for monkeypox in rural communities in the Democratic Republic of the Congo. PLoS One 2017; 12(2): e0168664
22. Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, Steffen R. The changing epidemiology of human monkeypox—a potential threat? A systematic review PLoS Negl Trop Dis 2022; 16(2): e0010141
23. Adegboye OA, Eugenia Castellanos M, Alele FO, Pak A, Ezechukwu HC, Hou K, Emeto TI. Travel-related monkeypox outbreaks in the era of COVID-19 pandemic: are we prepared? Viruses 2022; 14(6): 1283

24. Hughes AL, Irausquin S, Friedman R. The evolutionary biology of poxviruses. Infect Genet Evol 2010; 10(1): 50–59
25. Zheng L, Meng J, Lin M, Lv R, Cheng H, Zou L, Sun J, Li L, Ren R, Wang S. Structure prediction of the entire proteome of monkeypox variants. Acta Materia Medica 2022; 1(2): 260–264
26. Vandenbogaert M, Kwasiborski A, Gonofio E, Descorps-Declère S, Selekon B, Nkili Meyong AA, Ouilibona RS, Gessain A, Manuguerra JC, Caro V, Nakoune E, Berthet N. Nanopore sequencing of a monkeypox virus strain isolated from a pustular lesion in the Central African Republic. Sci Rep 2022; 12(1): 10768
27. Shchelkunov SN, Totmenin AV, Safronov PF, Mikheev MV, Gutorov VV, Ryazankina OI, Petrov NA, Babkin IV, Uvarova EA, Sandakhchiev LS, Sisler JR, Esposito JJ, Damon IK, Jahrling PB, Moss B. Analysis of the monkeypox virus genome. Virology 2002; 297(2): 172–194
28. Babkin IV, Babkina IN, Tikunova NV. An update of orthopoxvirus molecular evolution. Viruses 2022; 14(2): 388
29. Isidro J, Borges V, Pinto M, Sobral D, Santos JD, Nunes A, Mixão V, Ferreira R, Santos D, Duarte S, Vieira L, Borrego MJ, Núncio S, de Carvalho IL, Pelerito A, Cordeiro R, Gomes JP. Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. Nat Med 2022; [Epub ahead of print] doi:10.1038/s41591-022-01907-y
30. Parker S, Nuara A, Buller RM, Schultz DA. Human monkeypox: an emerging zoonotic disease. Future Microbiol 2007; 2(1): 17–34
31. Nalca A, Rimoin AW, Bavari S, Whitehouse CA. Reemergence of monkeypox: prevalence, diagnostics, and countermeasures. Clin Infect Dis 2005; 41(12): 1765–1771
32. Rimoin AW, Mulembakani PM, Johnston SC, Lloyd Smith JO, Kisalu NK, Kinkela TL, Blumberg S, Thomassen HA, Pike BL, Fair JN, Wolfe ND, Shongo RL, Graham BS, Formenty P, Okitolonda E, Hensley LE, Meyer H, Wright LL, Muyembe JJ. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. Proc Natl Acad Sci USA 2010; 107(37): 16262–16267
33. Hutson CL, Olson VA, Carroll DS, Abel JA, Hughes CM, Braden ZH, Weiss S, Self J, Osorio JE, Hudson PN, Dillon M, Karem KL, Damon IK, Regnery RL. A prairie dog animal model of systemic orthopoxvirus disease using West African and Congo Basin strains of monkeypox virus. J Gen Virol 2009; 90(2): 323–333
34. Rao AK, Schulte J, Chen TH, Hughes CM, Davidson W, Neff JM, Markarian M, Delea KC, Wada S, Liddell A, Alexander S, Sunshine B, Huang P, Honza HT, Rey A, Monroe B, Doty J, Christensen B, Delaney L, Massey J, Waltenburg M, Schrodt CA, Kuhar D, Satheshkumar PS, Kondas A, Li Y, Wilkins K, Sage KM, Yu Y, Yu P, Feldpausch A, McQuiston J, Damon IK, McCollum AM; July 2021 Monkeypox Response Team. Monkeypox in a traveler returning from Nigeria—Dallas, Texas, July 2021. MMWR Morb Mortal Wkly Rep 2022; 71(14): 509–516
35. Kisalu NK, Mokili JL. Toward understanding the outcomes of monkeypox infection in human pregnancy. J Infect Dis 2017; 216(7): 795–797
36. Silva NIO, de Oliveira JS, Kroon EG, Trindade GS, Drumond BP. Here, there, and everywhere: the wide host range and geographic distribution of zoonotic orthopoxviruses. Viruses 2020; 13(1): 43
37. Bonilla-Aldana DK, Rodriguez-Morales AJ. Is monkeypox another reemerging viral zoonosis with many animal hosts yet to be defined? Vet Q 2022; 42(1): 148–150
38. Kupferschmidt K. Why monkeypox is mostly hitting men who have sex with men. Science 2022; 376(6600): 1364–1365
39. Heskin J, Belfield A, Milne C, Brown N, Walters Y, Scott C, Bracchi M, Moore LS, Mughal N, Rampling T, Winston A, Nelson M, Duncan S, Jones R, Price DA, Mora-Peris B. Transmission of monkeypox virus through sexual contact — a novel route of infection. J Infect 2022; [Epub ahead of print] doi:10.1016/j.jinf.2022.05.028
40. Bellinato F, Gisondi P, Girolomoni G. Monkeypox virus infection: what dermatologist needs to know? J Eur Acad Dermatol Venereol 2022; [Epub ahead of print] doi:10.1111/jdv.18299
41. Kabuga AI, El Zowalaty ME. A review of the monkeypox virus and a recent outbreak of skin rash disease in Nigeria. J Med Virol 2019; 91(4): 533–540
42. Girometti N, Byrne R, Bracchi M, Heskin J, McOwan A, Tittle V, Gedela K, Scott C, Patel S, Gohil J, Nugent D, Suchak T, Dickinson M, Feeney M, Mora-Peris B, Stegmann K, Plaha K, Davies G, Moore LSP, Mughal N, Asboe D, Boffito M, Jones R, Whitlock G. Demographic and clinical characteristics of confirmed human monkeypox virus cases in individuals attending a sexual health centre in London, UK: an observational analysis. Lancet Infect Dis 2022; [Epub ahead of print] doi:10.1016/S1473-3099(22)00411-X
43. Fenner F. The pathogenesis of the acute exanthems; an interpretation based on experimental investigations with mousepox; infectious ectromelia of mice. Lancet 1948; 252(6537): 915–920
44. Shepardson KM, Schwarz B, Larson K, Morton RV, Avera J, McCoy K, Caffrey A, Harmsen A, Douglas T, Rynda-Apple A. Induction of antiviral immune response through recognition of the repeating subunit pattern of viral capsids is Toll-like receptor 2 dependent. MBio 2017; 8(6): e01356–e01317
45. Yu H, Bruneau RC, Brennan G, Rothenburg S. Battle royale: innate recognition of poxviruses and viral immune evasion. Biomedicines 2021; 9(7): 765
46. Hammarlund E, Dasgupta A, Pinilla C, Norori P, Früh K, Slifka MK. Monkeypox virus evades antiviral $CD4^+$ and $CD8^+$ T cell responses by suppressing cognate T cell activation. Proc Natl Acad Sci USA 2008; 105(38): 14567–14572
47. Kindrachuk J, Arsenault R, Kusalik A, Kindrachuk KN, Trost B, Napper S, Jahrling PB, Blaney JE. Systems kinomics demonstrates Congo Basin monkeypox virus infection selectively modulates host cell signaling responses as compared to West African monkeypox virus. Mol Cell Proteomics 2012; 11(6): M111.015701
48. Estep RD, Messaoudi I, O'Connor MA, Li H, Sprague J, Barron A, Engelmann F, Yen B, Powers MF, Jones JM, Robinson BA, Orzechowska BU, Manoharan M, Legasse A, Planer S, Wilk J, Axthelm MK, Wong SW. Deletion of the monkeypox virus inhibitor of complement enzymes locus impacts the adaptive immune response to monkeypox virus in a nonhuman primate model of infection. J Virol 2011; 85(18): 9527–9542
49. Miura F, van Ewijk CE, Backer JA, Xiridou M, Franz E, Op de Coul E, Brandwagt D, van Cleef B, van Rijckevorsel G, Swaan C, van den Hof S, Wallinga J. Estimated incubation period for monkeypox cases confirmed in the Netherlands, May 2022. Euro Surveill 2022; 27(24): 2200448
50. Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye

A, Ihekweazu C, Zumla A. Human monkeypox: epidemiologic and clinical characteristics, diagnosis, and prevention. Infect Dis Clin North Am 2019; 33(4): 1027–1043

51. Adler H, Gould S, Hine P, Snell LB, Wong W, Houlihan CF, Osborne JC, Rampling T, Beadsworth MB, Duncan CJ, Dunning J, Fletcher TE, Hunter ER, Jacobs M, Khoo SH, Newsholme W, Porter D, Porter RJ, Ratcliffe L, Schmid ML, Semple MG, Tunbridge AJ, Wingfield T, Price NM; NHS England High Consequence Infectious Diseases (Airborne) Network. Clinical features and management of human monkeypox: a retrospective observational study in the UK. Lancet Infect Dis 2022; [Epub ahead of print] doi:10.1016/S1473-3099(22)00228-6
52. McCollum AM, Damon IK. Human monkeypox. Clin Infect Dis 2014; 58(2): 260–267
53. Erez N, Achdout H, Milrot E, Schwartz Y, Wiener-Well Y, Paran N, Politi B, Tamir H, Israely T, Weiss S, Beth-Din A, Shifman O, Israeli O, Yitzhaki S, Shapira SC, Melamed S, Schwartz E. Diagnosis of imported monkeypox, Israel, 2018. Emerg Infect Dis 2019; 25(5): 980–983
54. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, Mamadu I, Akinpelu A, Ahmad A, Burga J, Ndoreraho A, Nkunzimana E, Manneh L, Mohammed A, Adeoye O, Tom-Aba D, Silenou B, Ipadeola O, Saleh M, Adeyemo A, Nwadiutor I, Aworabhi N, Uke P, John D, Wakama P, Reynolds M, Mauldin MR, Doty J, Wilkins K, Musa J, Khalakdina A, Adedeji A, Mba N, Ojo O, Krause G, Ihekweazu C; CDC Monkeypox Outbreak Team. Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report. Lancet Infect Dis 2019; 19(8): 872–879
55. Macneil A, Reynolds MG, Braden Z, Carroll DS, Bostik V, Karem K, Smith SK, Davidson W, Li Y, Moundeli A, Mombouli JV, Jumaan AO, Schmid DS, Regnery RL, Damon IK. Transmission of atypical varicella-zoster virus infections involving palm and sole manifestations in an area with monkeypox endemicity. Clin Infect Dis 2009; 48(1): e6–e8
56. Patrocinio-Jesus R, Peruzzu F. Monkeypox genital lesions. N Engl J Med 2022; 387(1): 66
57. Walter K, Malani PN. What is monkeypox? JAMA 2022; [Epub ahead of print] doi:10.1001/jama.2022.10259
58. Hughes CM, Liu L, Davidson WB, Radford KW, Wilkins K, Monroe B, Metcalfe MG, Likafi T, Lushima RS, Kabamba J, Nguete B, Malekani J, Pukuta E, Karhemere S, Muyembe Tamfum JJ, Okitolonda Wemakoy E, Reynolds MG, Schmid DS, McCollum AM. A tale of two viruses: coinfections of monkeypox and varicella zoster virus in the Democratic Republic of Congo. Am J Trop Med Hyg 2021; 104(2): 604–611
59. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. Lancet Infect Dis 2004; 4(1): 15–25
60. Rizk JG, Lippi G, Henry BM, Forthal DN, Rizk Y. Prevention and treatment of monkeypox. Drugs 2022; [Epub ahead of print] doi:10.1007/s40265-022-01742-y
61. Hoy SM. Tecovirimat: first global approval. Drugs 2018; 78(13): 1377–1382
62. Grosenbach DW, Honeychurch K, Rose EA, Chinsangaram J, Frimm A, Maiti B, Lovejoy C, Meara I, Long P, Hruby DE. Oral Tecovirimat for the treatment of smallpox. N Engl J Med 2018; 379(1): 44–53
63. Alvarez-Cardona JJ, Whited LK, Chemaly RF. Brincidofovir: understanding its unique profile and potential role against adenovirus and other viral infections. Future Microbiol 2020; 15(6): 389–400
64. Chittick G, Morrison M, Brundage T, Nichols WG. Short-term clinical safety profile of brincidofovir: a favorable benefit-risk proposition in the treatment of smallpox. Antiviral Res 2017; 143: 269–277
65. De Clercq E. Cidofovir in the therapy and short-term prophylaxis of poxvirus infections. Trends Pharmacol Sci 2002; 23(10): 456–458
66. Kravcik S. Cidofovir for cytomegalovirus retinitis. Ann Intern Med 1997; 127(6): 490–491
67. Holland GN, Van Natta ML, Goldenberg DT, Ritts R Jr, Danis RP, Jabs DA; Studies of Ocular Complications of AIDS Research Group. Relationship between opacity of cytomegalovirus retinitis lesion borders and severity of immunodeficiency among people with AIDS. Invest Ophthalmol Vis Sci 2019; 60(6): 1853–1862
68. Wittek R. Vaccinia immune globulin: current policies, preparedness, and product safety and efficacy. Int J Infect Dis 2006; 10(3): 193–201
69. Hopkins RJ, Kramer WG, Blackwelder WC, Ashtekar M, Hague L, Winker-La Roche SD, Berezuk G, Smith D, Leese PT. Safety and pharmacokinetic evaluation of intravenous vaccinia immune globulin in healthy volunteers. Clin Infect Dis 2004; 39(6): 759–766
70. Petersen BW, Damon IK, Pertowski CA, Meaney-Delman D, Guarnizo JT, Beigi RH, Edwards KM, Fisher MC, Frey SE, Lynfield R, Willoughby RE. Clinical guidance for smallpox vaccine use in a postevent vaccination program. MMWR Recomm Rep 2015; 64(RR–02): 1–26
71. Gilbert SC. Clinical development of Modified Vaccinia virus Ankara vaccines. Vaccine 2013; 31(39): 4241–4246
72. Overton ET, Lawrence SJ, Stapleton JT, Weidenthaler H, Schmidt D, Koenen B, Silbernagl G, Nopora K, Chaplin P. A randomized phase II trial to compare safety and immunogenicity of the MVA-BN smallpox vaccine at various doses in adults with a history of AIDS. Vaccine 2020; 38(11): 2600–2607
73. Volz A, Sutter G. Modified Vaccinia virus Ankara: history, value in basic research, and current perspectives for vaccine development. Adv Virus Res 2017; 97: 187–243
74. Greenberg RN, Kennedy JS. ACAM2000: a newly licensed cell culture-based live vaccinia smallpox vaccine. Expert Opin Investig Drugs 2008; 17(4): 555–564
75. Russell PK. Vaccines in civilian defense against bioterrorism. Emerg Infect Dis 1999; 5(4): 531–533
76. Talbot TR, Stapleton JT, Brady RC, Winokur PL, Bernstein DI, Germanson T, Yoder SM, Rock MT, Crowe JE Jr, Edwards KM. Vaccination success rate and reaction profile with diluted and undiluted smallpox vaccine: a randomized controlled trial. JAMA 2004; 292(10): 1205–1212
77. Hou J, Wang S, Li D, Carpp LN, Zhang T, Liu Y, Jia M, Peng H, Liu C, Wu H, Huang Y, Shao Y. Early pro-inflammatory signal and T-cell activation associate with vaccine-induced anti-vaccinia protective neutralizing antibodies. Front Immunol 2021; 12: 737487
78. Wen B, Deng Y, Chen H, Guan J, Chuai X, Ruan L, Kong W, Tan W. The novel replication-defective vaccinia virus (Tiantan strain)-based hepatitis C virus vaccine induces robust immunity in

macaques. Mol Ther 2013; 21(9): 1787–1795
79. Deng Y, Chuai X, Chen P, Chen H, Wang W, Ruan L, Li W, Tan W. Recombinant vaccinia vector-based vaccine (Tiantan) boosting a novel HBV subunit vaccine induced more robust and lasting immunity in rhesus macaques. Vaccine 2017; 35(25): 3347–3353
80. Liu Q, Li Y, Luo Z, Yang G, Liu Y, Liu Y, Sun M, Dai J, Li Q, Qin C, Shao Y. HIV-1 vaccines based on replication-competent Tiantan vaccinia protected Chinese rhesus macaques from simian HIV infection. AIDS 2015; 29(6): 649–658
81. Merchlinsky M, Albright A, Olson V, Schiltz H, Merkeley T, Hughes C, Petersen B, Challberg M. The development and approval of tecoviromat (TPOXX®), the first antiviral against smallpox. Antiviral Res 2019; 168: 168–174
82. Russo AT, Berhanu A, Bigger CB, Prigge J, Silvera PM, Grosenbach DW, Hruby D. Co-administration of tecovirimat and ACAM2000™ in non-human primates: effect of tecovirimat treatment on ACAM2000 immunogenicity and efficacy versus lethal monkeypox virus challenge. Vaccine 2020; 38(3): 644–654
83. Russo AT, Grosenbach DW, Brasel TL, Baker RO, Cawthon AG, Reynolds E, Bailey T, Kuehl PJ, Sugita V, Agans K, Hruby DE. Effects of treatment delay on efficacy of Tecovirimat following lethal aerosol monkeypox virus challenge in cynomolgus macaques. J Infect Dis 2018; 218(9): 1490–1499
84. Berhanu A, Prigge JT, Silvera PM, Honeychurch KM, Hruby DE, Grosenbach DW. Treatment with the smallpox antiviral tecovirimat (ST-246) alone or in combination with ACAM2000 vaccination is effective as a postsymptomatic therapy for monkeypox virus infection. Antimicrob Agents Chemother 2015; 59(7): 4296–4300
85. Whitehouse ER, Rao AK, Yu YC, Yu PA, Griffin M, Gorman S, Angel KA, McDonald EC, Manlutac AL, de Perio MA, McCollum AM, Davidson W, Wilkins K, Ortega E, Satheshkumar PS, Townsend MB, Isakari M, Petersen BW. Novel treatment of a vaccinia virus infection from an occupational needlestick—San Diego, California, 2019. MMWR Morb Mortal Wkly Rep 2019; 68(42): 943–946
86. Grosenbach DW, Honeychurch K, Rose EA, Chinsangaram J, Frimm A, Maiti B, Lovejoy C, Meara I, Long P, Hruby DE. Oral Tecovirimat for the treatment of smallpox. N Engl J Med 2018; 379(1): 44–53
87. Earl PL, Americo JL, Wyatt LS, Eller LA, Whitbeck JC, Cohen GH, Eisenberg RJ, Hartmann CJ, Jackson DL, Kulesh DA, Martinez MJ, Miller DM, Mucker EM, Shamblin JD, Zwiers SH, Huggins JW, Jahrling PB, Moss B. Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. Nature 2004; 428(6979): 182–185
88. Earl PL, Americo JL, Wyatt LS, Espenshade O, Bassler J, Gong K, Lin S, Peters E, Rhodes L Jr, Spano YE, Silvera PM, Moss B. Rapid protection in a monkeypox model by a single injection of a replication-deficient vaccinia virus. Proc Natl Acad Sci USA 2008; 105(31): 10889–10894
89. Stittelaar KJ, van Amerongen G, Kondova I, Kuiken T, van Lavieren RF, Pistoor FHM, Niesters HGM, van Doornum G, van der Zeijst BAM, Mateo L, Chaplin PJ, Osterhaus ADME. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. J Virol 2005; 79(12): 7845–7851
90. McCurdy LH, Larkin BD, Martin JE, Graham BS. Modified vaccinia Ankara: potential as an alternative smallpox vaccine. Clin Infect Dis 2004; 38(12): 1749–1753
91. Phelps AL, Gates AJ, Hillier M, Eastaugh L, Ulaeto DO. Comparative efficacy of modified vaccinia Ankara (MVA) as a potential replacement smallpox vaccine. Vaccine 2007; 25(1): 34–42
92. Overton ET, Lawrence SJ, Wagner E, Nopora K, Rösch S, Young P, Schmidt D, Kreusel C, De Carli S, Meyer TP, Weidenthaler H, Samy N, Chaplin P. Immunogenicity and safety of three consecutive production lots of the non replicating smallpox vaccine MVA: a randomised, double blind, placebo controlled phase III trial. PLoS One 2018; 13(4): e0195897
93. Greenberg RN, Hay CM, Stapleton JT, Marbury TC, Wagner E, Kreitmeir E, Röesch S, von Krempelhuber A, Young P, Nichols R, Meyer TP, Schmidt D, Weigl J, Virgin G, Arndtz-Wiedemann N, Chaplin P. A randomized, double-blind, placebo-controlled phase II trial investigating the safety and immunogenicity of Modified Vaccinia Ankara Smallpox Vaccine (MVA-BN®) in 56-80-year-old subjects. PLoS One 2016; 11(6): e0157335
94. Brown K, Leggat PA. Human monkeypox: current state of knowledge and implications for the future. Trop Med Infect Dis 2016; 1(1): 8