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Therapeutic and prophylactic drugs to treat orthopoxvirus infections

Scott Parker, PhD,
Department of Molecular Microbiology & Immunology, Saint Louis University Health Sciences Center, St Louis, MO 63104, USA

Lauren Handley, PhD, and
Department of Molecular Microbiology & Immunology, Saint Louis University Health Sciences Center, St Louis, MO 63104, USA

R Mark Buller, PhD†
Department of Molecular Microbiology & Immunology, Saint Louis University Health Sciences Center, 1100 South Grand Blvd., St Louis, MO 63104, USA

Abstract

With the global eradication of smallpox in 1979, the causative agent, variola, no longer circulates in human populations. Other human poxvirus infections, such as those caused by vaccinia, cowpox virus and molluscum, are usually relatively benign in immunocompetent individuals. Conversely, monkeypox virus infections cause high levels of mortality and morbidity in Africa and the virus appears to be increasing its host range, virulence and demographic environs. Furthermore, there are concerns that clandestine stocks of variola virus exist. The re-introduction of aerosolized variola (or perhaps monkeypox virus) into human populations would result in high levels of morbidity and mortality. The attractiveness of variola as a bioweapon and, to a certain extent, monkeypox virus is its inherent ability to spread from person-to-person. The threat posed by the intentional release of variola or monkeypox virus, or a monkeypox virus epizoonosis, will require the capacity to rapidly diagnose the disease and to intervene with antivirals, as intervention is likely to take place during the initial diagnosis, approximately 10-15 days postinfection. Preimmunization of 'at-risk populations' with vaccines will likely not be practical, and the therapeutic use of vaccines has been shown to be ineffective after 4 days of infection with variola. However, a combination of vaccine and antivirals for those infected may be an option. Here we describe historical, current and future therapies to treat orthopoxvirus diseases.

Keywords

acyclic nucleoside phosphonates; animal model; antiviral; CMX001; monkeypox; orthopoxvirus; smallpox; ST-246; vaccinia

Human poxvirus diseases

A total of 14 poxviruses have been documented to infect humans, seven of which belong to the *Orthopoxvirus* genus (cowpox [CPXV], monkeypox [MPXV], buffalopox, cantagalo, aracatuba, vaccinia [VACV] and variola [VARV]), one to the *Molluscipox* genus (molluscum

†Author for correspondence: Department of Molecular Microbiology & Immunology, Saint Louis University Health Sciences Center, 1100 South Grand Blvd., St Louis, MO 63104, USA, Tel.: +1 314 997 8870, Fax: +1 314 997 8717, bullerrm@slu.edu

contagiosum), and one to the *Yatapoxvirus* genus (tanapox). The remainder belong to the *Parapoxvirus* genus (orf, paravaccinia, bovine popular stormatitis, deerpox and sealpox) [1]. All poxvirus diseases are zoonoses except for molluscum contagiosum and smallpox [2]. With rare exception, most human poxvirus infections, which usually occur through minor abrasions in the skin, fail to establish a human chain of transmission. MPXV, orf virus and molluscipox virus cause the most frequent human poxvirus infections worldwide. CPXV infections are sometimes acquired from cows, sheep and rodents, but the domestic cat is responsible for the majority of human CPXV infections. To address both natural and bioterror-related human orthopoxvirus disease, orthopoxviruses have received increased impetus for the development of prophylactic and therapeutic treatments.

Smallpox

History

Smallpox was named so to differentiate it from great-pox, now known as syphilis [3]. Smallpox is estimated to have infected approximately 400 million people in the 20th century alone. Historically, smallpox has had a close association with humans but the origin of the virus remains unknown. By the end of the 19th century, a milder and less lethal form of smallpox became apparent. This virus was named variola minor, to distinguish it from variola major (classic smallpox), and was first documented as Amass in South Africa in 1904 (Alastrim in South America). It is believed to have originated in several places throughout the world as the virus adapted to humans [3].

Clinical disease & disease transmission

The case-fatality rates for variola major (classic or ordinary smallpox) and variola minor are 16-30 and 1%, respectively. Clinically, smallpox in an unvaccinated person has a 7-19-day incubation period from the time infection is established within the respiratory tract until the first symptoms of fever, malaise, headache and backache occur, culminating in the start of the characteristic rash [3,4]. The rash begins with papules that sequentially transform into vesicles and then pustules. The rash is typically centrifugal (head and limbs), but centripetal (trunk) rashes have been reported. Lesions range from 0.5-1 cm in diameter and can spread over the entire body. Once pustules have dried, scabs will form, which eventually desquamate during the following 2-3-week period. The resultant feature of these cutaneous lesions is the formation of the classic pock scars which are apparent on the skin of surviving patients.

Two clinical variations of classic smallpox have been identified. Flat-type smallpox is a rare form of the disease (~6% in unvaccinated people) and is characterized by lesions that remain level with the skin. It was more frequently observed in children and usually resulted in death. Another variant of the disease is hemorrhagic smallpox (<2% in unvaccinated people), which occurred mainly in adults. Although a rare form of the disease, it also had a high mortality rate and is characterized by hemorrhages in the skin and/or mucous membranes early in the course of illness. Subconjuctival hemorrhages were most commonly observed, as well as bleeding from the gums and other parts of the body.

The relative infectiousness of variola major and variola minor, as measured by secondary attack rates, was similar and determined to be 58 and 61%, respectively [3]. Although smallpox is typically spread by respiratory droplets over a short distance, some examples of long distance transmission of classic smallpox exist: one such case occurred in a laboratory in 1978 in Birmingham, UK [3], one in a hospital at Meschede, Germany, in 1970 [5] and one on a trawler 15 km south of a Soviet bioweapons testing facility on Vozrozhdeniye island [6].

Human monkeypox

History

One could speculate that human MPXV infections have been occurring in Africa for centuries and were masked under the guise of smallpox [7]. MPXV was first isolated in 1958 from the vesiculopustular lesions found on infected cynomolgus macaques imported to the State Serum Institute of Copenhagen, Denmark [8]. During the next few years, similar outbreaks were reported in monkey colonies in the USA and in a zoo in Rotterdam, The Netherlands (reviewed in [9]).

Between 1970 and 1971, six cases of human MPXV infections were reported in Liberia, Sierra Leone and Nigeria, countries which had previously been free of smallpox for at least a year; the primary human monkeypox case discovered during this same period was in a 9-month old child in Zaire (now the Democratic Republic of the Congo [DRC]). Between 1970 and 1980, four of 47 (9%) cases were suspected to result from human-to-human transmission, with the remaining 43 (91%) human cases acquired from contact with animals [10]. The majority of infections were acquired in regions conterminous with the tropical rainforest.

Clinical disease, disease transmission & incidence

The most severe human MPXV infections have been reported in the Congo Basin, whereas attenuated human infections have generally occurred in West African countries. The difference is attributed to inherent differences in the virulence of circulating strains [11,12]. In the Congo Basin, human infections generally resulted from handling MPXV-infected animal tissues [13]. MPXV-infected humans develop a skin rash and follow a similar disease course to smallpox except for the occurrence of severely swollen lymph nodes (lymphadenopathy of the neck, inguinal and axillary regions). Human monkeypox is less severe than smallpox as approximately 58% of smallpox patients and 11% of human monkeypox (Congo Basin strain) cases presented with more than 100 pocks [7]. The case-fatality rate for human monkeypox is approximately 10%, compared with 10-30% for variola major [13-15] and is dependent on the MPXV strain. For example, no fatalities occurred in an outbreak in the USA; however, this is likely because the MPXV strain was a low-virulence West African isolate (reviewed in [7,9]).

Person-to-person transmission of MPXV appears to be on the rise, although accurate measurements are difficult to calculate because disease surveillance has improved, as has access to the Congo Basin for foreign scientists. Of the 338 cases documented in the intensive surveillance area within the DRC between 1981 and 1986, 93 of 245 (28%) were secondary cases, 69 were first generation, 19 were second generation and five were third or fourth generation. No transmission chains greater than five were observed. In July 1996, 42 cases, including three (7%) deaths, occurred in a village of 346 inhabitants. A male, identified epidemiologically as the primary case, is believed to have directly or indirectly passed the infection through eight generations [16]. These findings were interpreted as enhanced human-to-human transmission in the Congo Basin as compared with previous studies [8]. Moreover, in 2003, a hospital in the DRC reported six generations of human-to-human MPXV transmission, suggesting that the transmission efficiency may also be increasing [17]. A likely explanation for enhanced transmission may be due to the increasingly susceptible population that are often unhealthy and lack vaccination-induced immunity to smallpox. Cases of human monkeypox are increasing in the Congo Basin, the traditional home of human MPXV, and outbreaks have been observed in Sudan and the USA [10,16,18,19] (for a detailed review of increasing incidence and geographical range of human monkeypox see [7]).

Orthopoxviruses as biological weapons

The reality of the threat of state-sponsored bioweapons was revealed with the defections of two Biopreparat (Zagorsk, Russia) scientists from the former Soviet Union. With the break-up of the Soviet Union, terrorist groups are the greatest potential users of bioweapons. Indeed, in 1984, followers of the Indian guru Bhagwan Shree Rajneesh contaminated salad bars at ten restaurants in Dalles Oregon, USA, with salmonella and sickened approximately 750 people [20]. Furthermore, in 1993, Aum Shinrikyo released aerosolized *Bacillus anthracis* from a high-rise building in Tokyo, Japan (to no effect) prior to a sarin gas-attack on the Tokyo subway in 1995 that resulted in the deaths of 12 commuters and serious illness for 54 others [21]. The appeal of bioweapons lies in their low cost, technological simplicity and ease of concealment. There are a large number of biological agents which could be used as bioweapons. Orthopoxviruses, such as VARV and MPXV, would be ideal candidates because of their high transmissibility, high levels of mortality and morbidity and because their long incubation period would result in detection at 7-14 days after the perpetrator would have escaped. Furthermore, their propensity for secondary spread would likely result in widespread terror and anxiety.

In addition, there is concern that orthopoxviruses may be genetically engineered to make them more virulent and/or capable of breaking through the smallpox vaccine-induced immunity as has been demonstrated with a recombinant ectromelia virus (ECTV) expressing IL-4 [22].

With the global eradication of smallpox in 1979, the causative agent, VARV, no longer circulates in human populations; however, there is concern that clandestine stocks of VARV exist, and VARV could be re-introduced through bioterrorism and/or biowarfare. The release of aerosolized VARV (or perhaps MPXV) into human populations would result in high levels of mortality for several reasons:

- An efficacious antiviral therapy is not available for the treatment of exposed individuals;
- The routine immunization of the world's population with VACV had all but ceased in the late 1970s, resulting in the population under the age of 30 lacking cross-protective immunity to VARV;
- The strength of vaccine immunity in older individuals has decreased with the passage of time leaving these individuals with unknown protection against smallpox;
- A growing segment of the world's population is immunocompromised as a result of infection with HIV, and the use of immunosupressive drugs for treatment of cancer and the prevention of organ transplantation rejection;
- Individuals with skin conditions including atopic dermatitis are on the rise. As opposed to healthy persons, individuals with atopic dermatitis present with a range of clinically severe poxvirus infections [23,24].

In an effort to protect the increasingly vulnerable population, the National Institute of Allergy and Infectious Diseases (NIAID) support research to provide safer vaccines and to develop at least three antiviral compounds against human orthopoxvirus infection. This goal was supported independently by reports generated by the Institute of Medicine and the National Academy of Sciences, USA [25,26] (a more through review on the development of therapeutics and prophylactics against smallpox and monkeypox can be found in [27]).

Vaccines

Traditional vaccines: live, animal passaged & virulent

First-generation vaccines evolved from locally produced products that gained regional and/or national prominence through efficacy testing. These vaccines were neither clonal nor highly purified and were serially propagated on domesticated animals, most often calves or sheep (at least in the early years), meaning that microorganism contamination was frequent. Four major vaccines were used during the smallpox eradication program: Dryvax™ (USA), Lister (UK, Europe, Africa, Asia and Oceania), Temple of Heaven (China) and EM-63 (USSR). During the intensified smallpox eradication program, these vaccines were prepared locally to a uniform potency of 1×10^8 PFU/ml, which gave a presented dose of approximately 2.5×10^5 PFU per vaccination site when used with a bifurcated needle [28]. Although vaccines, regardless of source, gave similar levels of protection from severe smallpox, they varied in the severity of postvaccination complications.

Type & frequency of complications

Fenner and colleagues identified two major groups of VACV complications: abnormal skin eruptions (accidental infection, generalized vaccinia, eczema vaccinatum, erythema multiforme and progressive vaccinia [vaccinia necrosum]) and disorders affecting the CNS (encephalopathy and encephalitis) [3]. In the USA, the frequency of vaccinia virus-associated complications was thoroughly examined in the 1968 national and ten-state surveys [29]. The majority of vaccine-associated complications reportedly occurred after primary immunization and less frequently with re-vaccination, except in the case of progressive vaccinia. Using the ten-state survey, 1253.8 cases per one million primary immunizations were observed for all ages. More specifically, for every million vaccinations, 935.5 cases were serious, but not life-threatening, 52.3 were life-threatening and 1.5 resulted in death. For a thorough description of these complications see the review by Fulginiti *et al.* [30].

Contraindications to vaccination

Five conditions were traditionally accepted as contraindications for immunization with traditional VACV and its derivatives: immune disorders, young age (less than 2 years old), eczema, pregnancy and disorders of the CNS [3,30]. Although cardiac complications associated with the vaccination were not considered significant during the 1960s, several cardiac complications reported in early 2003 prompted the CDC to revise their recommendations for contraindications of vaccination to include heart disease. Women who are breastfeeding, persons less than 18 years of age and individuals with allergies to vaccine components have also been included as contraindicated to vaccination [31]. The current number of people afflicted with the contraindicated conditions have significantly increased since the eradication program. Thus, in the event of a bioterrorist attack, or the emergence of MPXV in the human population, it is inevitable that the number of adverse events associated with a mass vaccination program would be considerably more than during the eradication program. For this reason, the design and evaluation of safer vaccines have become a major research thrust.

New vaccines

With the realization of the threat of VARV as a bioweapon in the 1990s, additional stocks of vaccine were needed since there was a worldwide shortage of vaccine due, in part, to the failure to replace aging vaccine stocks and the destruction of others as a cost-cutting measure. As a means of increasing the number of available doses, diluting the available stock of Dryvax was evaluated. Indeed, a 1:10 dilution of Dryvax had been shown to maintain immunogenicity; however, even this, for example, would leave the US national stockpile millions of doses short

of vaccinating its total population. Dryvax was not a candidate to make up the shortfall in vaccine because its traditional method of manufacture, by passage through calf lymph, is not feasible for large-scale vaccine production, and it is deemed unacceptable owing to potential microbial contamination. Additionally, an unacceptable frequency of adverse reactions was associated with Dryvax, as previously discussed. For this reason, next generation vaccines were created with the aim of producing a sufficient stock of vaccine with equivalent efficacy to Dryvax and an improved safety profile. In this section we will discuss two of the leading vaccines that address these aims: ACAM2000, a vaccine derived from Dryvax, which was developed for use with the majority of the US population, and modified vaccinia virus ankara (MVA; see below), a more highly attenuated vaccine with an improved safety record, which was evaluated for use with an immunocompromised portion of the US population.

ACAM2000 vaccine—In partnership with the CDC, the NIAID supported the development and testing of a new generation of live vaccines based on the Dryvax vaccine, under the condition of them being produced using newer manufacturing approaches. Because Dryvax was produced by sequential passages in calf skin, it contains quasispecies, a population of viruses with distinct biological properties. From these quasispecies, six viral clones were isolated from a pool of ten vials of Dryvax. Clone 2 (named ACAM1000) was selected as the seed for the second generation smallpox vaccine based on comparable behavior to Dryvax when tested in mice, rabbits and monkeys for virulence and immunogenicity [32,33]. In partnership with Baxter BioScience (IL, USA), the ACAM1000 master seed virus was used to infect Vero cells under serum-free conditions to produce a second larger master seed virus stock named ACAM2000. ACAM2000 was evaluated in three Phase I clinical trials and produced major cutaneous reactions, evoked neutralizing antibody and cell-mediated responses, and had a reactogenicity profile similar to Dryvax [34]. Similarly, Phase II randomized, double-blinded, controlled trials found ACAM2000 to be equivalent to Dryvax in terms of cutaneous response rate, antibody responses and safety [35].

Owing to more stringent exclusion criteria than previous vaccine trials and because ACAM2000 is a clonal, cell culture-derived vaccine, it was thought that the incidence of vaccine-associated adverse events would decrease. Interestingly, the safety profile of ACAM2000 does not significantly differ from that of Dryvax, suggesting that vaccine-associated complications are due to the virus itself, and not the preparation of the vaccine. In Phase II clinical trials, 100% of Dryvax and ACAM2000 vaccine recipients experienced at least mild adverse reactions (including pain and erythema); however, 50% fewer volunteers receiving ACAM2000 developed a fever and the degree of erythema in these patients was less. In addition, there were no reports of progressive vaccinia or eczema vaccinatum in patients receiving ACAM2000; however, these trials were powered to detect reactogenicity, not serious adverse reactions. Of greater concern was the increased incidence of myocarditis/pericarditis detected in both ACAM2000 and Dryvax vaccine recipients [36]. Based on the ACAM2000 clinical trials, it is estimated that one in 175 primary ACAM2000 vaccine recipients experienced myocarditis and/or pericarditis, similar to that seen for Dryvax [37]. The incidence rate of vaccination-associated heart conditions was higher in these studies than in previously reported smallpox vaccine clinical trials; this is likely due to more stringent screening [35]. Nevertheless, Phase III clinical trials for ACAM2000 were put on hold. Upon review, it was determined by the US FDA that the safety profile of ACAM2000 was not significantly different from that of Dryvax, and ACAM2000 has since replaced Dryvax as the US national stockpile of smallpox vaccine [38].

MVA vaccine—To circumvent the adverse reactions associated with live viral preparations of smallpox vaccinia-based vaccines (e.g., Dryvax and ACAM2000), replication-deficient strains of vaccinia are under evaluation for efficacy and immunogenicity. Of these, MVA is the most promising candidate vaccine. MVA was developed by growing the Ankara strain of

VACV for greater than 500 passages on chicken embryo fibroblasts, which dramatically reduced its virulence by restricting its ability to replicate in human cells [39]. Since 1971, MVA has been safely used in more than 100,000 humans without documentation of any of the adverse reactions associated with other VACV vaccines [40]. That said, MVA vaccinated individuals have never been subjected to actual challenge by VARV and it has been shown that immunogenicity is dose-dependent [41]. Prophylactic MVA immunization of mice protects as efficiently as Dryvax against a lethal intranasal challenge with VACV strain WR [42,43] and MPXV [44], but fails to protect when delivered as a postexposure treatment [45]. Similarly, cynomolgus monkeys immunized with MVA survived a lethal intravenous or respiratory challenge with MPXV, although in the former study, two doses of MVA were required to block skin lesion formation, compared with one dose of Dryvax [42,44]. Importantly, there are limited data in mouse models to suggest that MVA can efficiently protect against a lethal VACV intranasal challenge under some immunosuppressive conditions (e.g., B-cell deficient and β_2-microglobulin-deficient mouse strains), but not others (e.g., RAG-$1^{-/-}$ mouse strain and mice with decreased CD4 or MHC class II expression and double-knockout mice deficient in MHC class I- and II-restricted T-cell activities) [45,46]. The safety, immunogenicity and efficacy of the MVA vaccine (strain TBC, Therion Biologics Corporation, MA, USA) has recently been demonstrated against a Dryvax challenge in vaccinia-naive and -immune volunteers [47]. IMVAMUNE®, an MVA vaccine (strain BN, Bavarian Nordic GmbH, Berlin, Germany), has been tested for safety and immunogenicity in Phase I and II clinical trials, which revealed that it produced comparable cellular and humoral immune responses to one dose of Dryvax. Furthermore, IMVAMUNE vaccination prior to Dryvax vaccination reduced viral replication at the Dryvax site and decreased the size of the vaccination lesion [41,48]. Because the MVA virus does not replicate efficiently in human cells, one to two vaccine doses containing approximately 100-times more MVA virus than Dryvax may be required to induce equivalent immune responses and protection, making this a potentially expensive vaccine in the absence of adjuvants.

Passive immunoprophylaxis

Vaccinia immune globulin

Intramuscular administration of vaccinia immune globulin (VIG), a product derived from the pooled plasma of vaccinated individuals, is indicated for treatment of generalized vaccinia, progressive vaccinia (vaccinia necrosum), eczema vaccinatum and certain auto-inoculations. Although efficacy has not been demonstrated through controlled clinical trials, VIG was reported to halt the formation of new lesions and cause rapid clinical improvement in cases of generalized vaccinia and eczema vaccinatum [49]. One large study suggested that postexposure treatment of contacts of patients with smallpox with vaccination and VIG appeared more efficacious than vaccination alone (Table 1). Smallpox developed in five of 326 contacts who received VIG compared with 21 of 379 controls, with a relative efficacy of 70% in preventing smallpox [50,51]. In 2005, the FDA approved the manufacture of new stocks of VIG by DynPort Vaccine Company LLC (MD, USA).

Antibody therapy

The production of large quantities of VIG is inherently reliant on the continued vaccination of volunteers with VACV. This alone severely limits the wide-scale use of VIG and the problem is compounded because VIG lots may have different potencies and lots have the potential to transmit other pathogens because they are derived from human blood. This problem stimulated the development of a monoclonal antibody cocktail that could replace VIG. Lustig *et al.* showed that BALB/c mice could be protected from mortality with a combination of three to four intracellular mature virus and extracellular enveloped virus (EEV) antibodies, and that this protection was superior to that provided by VIG [52]. The efficacy of VIG has been shown to

be largely dependent on the neutralizing activity of anti-B5 antibodies directed against the EEV membrane (Figure 1 & Table 1) [53]. Furthermore, anti-B5 and anti-A33 chimpanzee Fab fragments have been isolated and converted into humanized monoclonal antibodies via the addition of the human heavy chain constant region [54,55]. These antibodies demonstrated higher efficacies than VIG against VACV and VARV *in vitro* and protected mice against a lethal intranasal VACV challenge when administered up to 2 days postinfection. They are therefore indicated as potential replacements for VIG, and several companies are evaluating the production of anti-orthopoxvirus monoclonal antibodies.

Orthopoxvirus antivirals

History

During the smallpox eradication program, a number of compounds were shown to have efficacy against orthopoxvirus infections in tissue culture, and some were actually tested in field conditions (Table 1). Thiosemicarbazone and metisazone were administered prophylactically in a series of trials in India and showed some protective effect; however, their administration was associated with severe nausea and vomiting. The drug is no longer available [3]. Cytosine arabinoside and adenine arabinoside were also used to treat variola major and minor, but failed to affect the case mortality rate or the clinical progression of disease. Rifampicin showed antiviral activity against VACV in a mouse model, but was never tested clinically against VARV. Its use as an anti-orthopoxvirus drug is probably limited by the requirement for high doses and its inherent toxic activity as demonstrated *in vitro* (Figure 1 & Table 1) [56].

Targets of host processes

Abl-family tyrosine kinase inhibitors—Many other drugs have proven to have anti-orthopoxvirus characteristics, such as Gleevec, an Ablfamily tyrosine kinase inhibitor licensed for the treatment of chronic myeloid leukemia. Gleevec has been shown to protect mice against lethal intranasal VACV infections by blocking EEV release. However, the *in vivo* effects are somewhat difficult to interpret because Gleevec was continually administered to the mice via a subcutaneous osmotic pump (Figure 1 & Table 1) [57]. The advantage of targeting a host process, rather than a viral one, is that the chances of the virus developing resistance are significantly reduced. Moreover, such targets have the potential to have activities against a broad-spectrum of virus families.

EGF signal transduction inhibitors—The EGF-like domains carried by orthopoxviruses target host ErbB-1, which ultimately induces viral replication. Chemical interference with the signal transduction pathways mediated by ErbB-1 can lead to the control of orthopoxvirus *in vivo*. CI-1033, an EGFR kinase inhibitor, has been shown to protect mice from intranasal VACV infections; however, its efficacy in delayed treatment has not been tested beyond 6 h postinfection (Table 1) [58].

Innate immunomodulators—Interferons are well characterized as playing key roles in the innate and adaptive immune responses to many different viruses. Poxviruses elegantly perturb interferon signaling by encoding their own interferon-binding proteins [59]. Nevertheless, loading mice with excess amounts of interferon has been shown to overwhelm the poxvirus-encoded binding proteins and provide some protection against disease [60-62]. Furthermore, synthetic peptide agonists and mimetics of IFN-γ have been developed and shown to protect mice from lethal intranasal VACV infections when delivered orally up to 2 days postinfection. In addition, these mimetics have powerful adjuvant activities that boost the humoral and cellular immune responses to VACV infection [63].

Targets of viral DNA synthesis

Thymidine analogs—Some thymidine analogs have activity against orthopoxvirus DNA synthesis. For example, *N*-methanocarbathymidine has been shown to be efficacious against VACV in mice even when treatment is delayed until 2-3 days postinfection [64]. This drug, although typically administered intraperitoneally in mice, also has some limited oral bioavailability and could potentially be developed further (Table 1). Although the poxvirus thymidine kinase is homologous to its cellular counterpart, certain compounds, such as 5-substituted deoxyuridine analogs, require phosphorylation by the viral thymidine kinase before they have antiviral activity [65,66]. Thus, selective phosphorylation by viral kinases is a potential avenue that could contribute to the development of new antiviral drugs [67].

Nucleoside phosphonates—Acyclic nucleosides and their analogs, such as acyclic nucleoside phosphonates (ANPs), have proven track records as efficacious antiviral compounds used to treat both DNA and RNA viral infections [68,69]. ANPs make up a large proportion of candidate antiviral drugs. Acyclic nucleosides and their derivatives have a detailed history dating back to the 1980s and have demonstrated good efficacy in the management of several different viral infections, including herpes simplex virus, HIV, HBV, adenoviruses and orthopoxviruses. Cidofovir (CDV) (Table 1) is currently the only drug licensed to treat orthopoxvirus infections but its clinical use is governed by its Investigational New Drug (IND) status. Adefovir dipivoxil, an oral prodrug derivative of adefovir, was licensed for the treatment of HBV in 2002, but it has been shown to have anti-orthopoxvirus properties that could be exploited. Not all ANP drugs have anti-orthopoxvirus qualities, for example, tenofovir and its derivatives.

Acyclic nucleoside phosphonates—CDV is a broad spectrum antiviral with activity against herpes, polyoma, papilloma, and adeno- and poxviruses. It has been used topically to treat human molluscum infections and intravenously in humans and several animal models to treat other orthopoxvirus infections. CDV, although efficacious, accumulates in the renal proximal tubes and induces nephrotoxicity. Unfortunately, this inherent nephrotoxicity is common amongst the ANPs and requires the administration of probenecid to alleviate toxicity in the kidneys. In the case of CDV, its use is restricted to 5 mg/kg/week for 2 weeks followed by every-other-week dosings. The requirement for careful clinical attention to CDV patients, coupled with its requirement to be delivered intravenously, makes CDV an unsuitable option for the treatment of wide-scale poxvirus infections [70,71].

ANPs are converted to 5′-nucleotides by various kinases. Generally, the nucleoside analogs require triphosphorylation before they can function in an antiviral capacity. ANP drugs such as adefovir and CDV are already phosphorous modified, thus reducing the required cellular phosphorylation steps to two. Nonphosphorylated acyclic nucelosides, such as acyclovir, are generally less effective antivirals because they rely on an extra host-mediated step (three phosphorylation steps instead of two) for their activation. This first phosphorylation step is often catalyzed by viral kinases, which makes acyclovir very effective against herpes infections but not poxvirus infections. ANPs exert their antiviral effect by binding to viral DNA polymerases and reverse transcriptases with higher affinity than they do to host enzymes (Figure 2). In the case of CDV, two adjacent incorporations are required in the elongating DNA chain to inhibit the viral polymerase and induce termination (Figure 2) [72]. However, it has recently been show that incorporation into the template strand strongly inhibits trans-lesion DNA synthesis [73].

Orally bioavailable ANPs (CMX001)—Owing to the phosphorous moiety, ANPs typically exhibit poor bioavailability compared with nonphosphorylated acyclic nucleosides. Drugs such as adefovir dipivoxil and tenofovir disoproxil fumarate have overcome this defect by

conversion of the nucleoside to the prodrug form, which can be administered orally. These prodrugs undergo ester bond cleavage to release the respective ANP [70].

Similar prodrug delivery systems have been exploited for the delivery of CDV with encouraging results. CMX001 is a CDV prodrug synthesized by covalently coupling CDV to the hexadecyl propanediol alkoxyalkanol (HDP-CDV) [71]. The conjugate was designed to act as a natural lipid that mimics lysophosphatidylcholine and its natural pathway, which involves absorption through the small intestine [70,71]. The parental CDV compound lacks oral bioavailability and induces nephrotoxicity, but 88% of HDP-CDV is bioavailable and is distributed to tissues via plasma and/or lymph without significant concentration in the kidney, and is predicted to lack nephrotoxicity. Following uptake into the plasma membrane, the HDP-CDV molecule is cleaved by phospholipase enzymes, which liberate CDV into the cytosol. After cellular uptake, CDV is phosphorylated twice by host kinases and is available to competitively inhibit the virus-encoded host polymerase (Figure 2) [72,74]. This method of drug delivery is the same as that exploited by HDP-(*S*)-HPMPA (see earlier). Exhaustive preclinical antiviral efficacy studies have been carried out in mice with ECTV, VACV and CPXV and in rabbits with rabbitpox virus (RPXV). With regard to the former, dose-optimization studies and clinical biomarkers of drug efficacy have been evaluated, which have facilitated the development of a 4-day postinfection, single-dose regimen of CMX001 to protect mice from lethal intranasal ECTV challenges [75,76]. Chimerix (NC, USA) received an IND license for CMX001 in 2006 and Phase I/II clinical trials are ongoing.

CDV is not the only antiviral drug that has been coupled to HDP or other lipids. For example, (*S*)-HPMPA is active against several orthopoxviruses *in vitro* but has no *in vivo* activity. However, ether lipid esters, such as ODE-(*S*)-HPMPA and HDP-(*S*)-HPMPA, have increased efficacy *in vitro*, and good bioavailability and efficacy *in vivo* against CPXV and VACV infections in mice [77].

Inhibition of viral release (ST-246)

ST-246 (4-trifluoromethyl-*N*-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-etheno-cyclo-prop[f] isoindol-2(1H)-yl)-benzamide) is active against multiple species of orthopoxviruses, including two strains of VARV [78]. Resistance mapping studies indicate that ST-246 targets the VACV F13L ortholog family. The F13L open reading frame encodes a major envelope protein, p37, which is required for production of extracellular, but not intracellular, virus (Figure 1) [79]. Thus, ST-246 is unique in that it does not affect the actual production of infectious virus, only its efficient release from cells. Preclinical efficacy studies have shown ST-246 to be highly effective when administered shortly after CPXV, VACV and ECTV intranasal infections of mice. Importantly, ST-246 was highly effective at treating CPXV and ECTV infections as late as 72 h postinfection [80]. Considering that death of untreated controls occurs 7-10 days following infection, this is quite an impressive feat. In once instance, ST-246 was used to treat a severe case of eczema vaccinatum in a 2-year-old male infant. The patient had seemingly failed to respond to VIG and CDV treatment but recovered following FDA emergency approval for the administration of ST-246. However, it should be noted that this was not a controlled study and the true recovery-inducing agent remains unknown. SIGA (OR, USA) received IND status for ST-246 in 2005 and Phase II clinical trials are ongoing and indicate that ST-246 is well received in humans [81].

Combination therapy

To date, ST-246 and CMX001 are the most promising anti-orthopoxvirus compounds. Both drugs are in intermediate-advanced stages of the drug approval processes. Although both drugs have potent efficacies, it has been demonstrated that different single point mutations can confer resistance to both CDV and ST-246; however, fortuitously CDV-resistant viruses are

attenuated *in vivo* [58,74,82,83]. The advantage of combination therapy is that the chance of developing drug resistance would be reduced as could the drug dosage, which would potentially reduce any toxic side effects. Furthermore, combination therapy has been shown to increase the survival rate of mice infected with CPXV when the intervention time is reduced to at least 6 days postinfection [84].

Potential antiviral compounds

Several drugs, such as aurintricarboxylic acid, mitoxantrone, tetrapyroles and distamycin, have demonstrated antiviral activity against poxviruses *in vitro* (Table 1) [85-89]. Unfortunately few, if any, of these are being evaluated in clinical trials. However, some do show promise. Aurintricarboxylic acid, for example, has good efficacy and has demonstrated antiviral activity at the both the genetic and enzymatic level, thus making it a good candidate for future evaluation.

Other drugs that have been tested *in vivo* have had varying degrees of success. For example, *in vitro* inhibitors of the lipoxygenase pathway (ETYA and BW755c) have been shown to specifically block orthopoxvirus replication [90]; however, these inhibitors were never tested *in vivo*. Ribavirin is of limited use at protecting mice from high-dose challenges and has not been demonstrated to have any delayed efficacy [91-94]. That said, several analogs have increased efficacy and the drug should be considered as a potential prophylactic option in the event of an orthopoxvirus outbreak [95]. Prostaglandin demonstrated good antiviral efficacy, but it has not been demonstrated to protect following delayed treatment and has not been shown to be orally bioavailable [96,97]. Thus, prostaglandin is, like ribavirin, of limited value in therapeutically treating orthopoxvirus infections. However, human β-defensin, which is produced by keratinocytes following skin injury and induces prostaglandin release, has been shown to have potent efficacy against VACV *in vitro* [98]. Thus, β-defensin therapy could provide an alternative method of circumventing any disadvantages inherent to prostaglandin treatment. Novobiocin, a drug that could have further been evaluated as an anti-orthopoxvirus drug, has been removed from the market [99-101].

Efficacy testing

Animal efficacy rule

Naturally occurring smallpox was eradicated in the late 1970s by a global vaccination program sponsored by the WHO. Human monkeypox, although on the rise, is still sporadic and usually occurs in the road-less tropical rainforest of the Congo Basin. In recognition of this problem, the FDA promulgated the so-called ‘Animal Efficacy Rule’, which acknowledges that therapeutics and prophylactics against NIAID Category A biothreat agents, such as VARV and MPXV, can be licensed under an alternative regulatory path [102]. The Animal Efficacy Rule permits the use of well-controlled animal efficacy data to support a New Drug Application for licensure of drugs and biological products intended to treat, or prevent, serious or life-threatening conditions in humans resulting from exposure to biological, chemical, radiological or nuclear substances (a similar directive is in place in the EU). Product licensure requires that the Animal Efficacy Rule be utilized if human challenge or protection efficacy trials to test the product would be unethical owing to the risks associated with exposure, or when clinical field trials are unfeasible, for example, in the case of rare, naturally occurring human diseases caused by dangerous infectious agents. Although the selection of animal models is left up to the scientific judgment of the principal investigator, a typical choice would involve at least one rodent and nonhuman primate model.

Animal models

Mousepox—The Animal Efficacy Rule demands a greater understanding of the animal models used to generate efficacy data for product licensure. Mousepox, rabbitpox and monkeypox experimental models together recapitulate most of the important features of human orthopoxvirus infections, although each has deficiencies. Mousepox has at least four features similar to smallpox. First, a relatively small dose of virus is required to initiate disease in the upper and lower respiratory tract (the actual LD_{50} for VARV remains unknown, but most virologists agree that the infectious dose is low, as determined by anecdotal evidence). This is supported by the low doses of ECTV and RPXV required to initiate lethal infections in mice and rabbits, respectively. Second, following a low dose intranasal infection there is no obvious lung involvement during the course of early disease. Third, virus can be detected in respiratory gases during the pre-exanthem period [103]. Finally, both diseases present with a characteristic exanthematous rash, although in the case of mousepox, rash development is dependent on a number of parameters including mouse strain, virus strain, route of inoculation and virus dose [104]. Mousepox differs from smallpox in at least two features following respiratory tract infection. First, the disease course in mousepox is shorter as compared with smallpox. Death in fatal cases of mousepox usually occurs 7-14 days postinfection, whereas death in ordinary smallpox infection occurs approximately 18-22 days postinfection. Second, the major lesions in mousepox are observed in the liver and spleen, whereas these organs are relatively uninvolved in smallpox [3,105].

Rabbitpox—The resemblance of rabbitpox to smallpox is striking. Both diseases are initiated with a relatively small dose of virus (≤100 virions). Also, there is a late onset of virus transmissibility that, for both diseases, occurs at about the beginning of the exanthem. Additionally, the viremia of rabbitpox resembles that reported for smallpox in its occurrence at the onset of overt disease, in the direct relationship between virus titer and severity of disease and in its absence in some fatal cases [106]. Furthermore, the early deaths in rabbitpox, which differed from the late deaths by the presence of a blood coagulation defect and a progressively increasing viremia, bare an uncanny resemblance to severe purpuric or hemorrhagic forms of smallpox [107,108]. However, rabbitpox differs from smallpox in its shorter incubation period, greater severity and more dramatic involvement of the upper respiratory tract late in the disease.

Monkeypox & variola virus in nonhuman primates—The clinical features of natural or experimental monkeypox infection can vary from subclinical to fatal depending on the primate species and routes of inoculation. Monkeypox in monkeys is very similar to monkeypox and smallpox in humans, justifying its choice as a preclinical model for smallpox. Monkeypox virus can be transmitted by aerosol administration or intranasal instillation, as well as by parenteral inoculation by any route [108-111]. Aerosolized administration of high doses (10^6-10^7) of VARV, MPXV, RPXV strain Utrecht, VACV or CPXV to cynomolgus monkeys resulted in a febrile reaction with variable mortalities, ulcerative bronchiolitis, bronchitis and peribronchitis; however, as in the human disease, only the monkeys infected with VARV and MPXV developed the typical exanthema [109]. Currently, the efficacy of orthopoxvirus prophylaxis and therapeutics are evaluated in cynomolgus monkeys challenged intravenously with 5×10^7 PFU of MPXV.

VARV aerosol and intravenous infections of cynomolgus monkeys have also been examined as models for human smallpox [112]. No monkeys exposed to the highest achievable aerosol dose of $10^{8.5}$ PFU developed severe disease, although all exposed animals became infected. Intravenous infection of monkeys with either 10^9 PFU of the Harper or India 7124 strains of VARV produced a uniformly acute and lethal infection. A lower intravenous dose of 5×10^8 PFU resulted in less fulminant, systemic disease and 33% mortality. This lower challenge dose

has been used to evaluate antivirals, using death and day of death, virus load in blood and skin lesions as end points.

Future perspective

Currently, the likely response to a large scale orthopoxvirus outbreak would be ring-vaccination with Dryvax, ACAM2000 or MVA (if available) - this ring-vaccination technique proved to be successful during the smallpox eradication program. The efficiency of the response will be highly dependent on the population demographic, population immunocompetence and the availability of vaccines. Except for perhaps some postexposure vaccination, the initial or primary cases will likely be untreatable. Individuals with recent exposure histories of fewer than 4 days could be vaccinated; otherwise, the use of VIG remains the only postexposure therapeutic. The young, gravid and immunocompromised will likely incur the highest mortality and morbidity rates.

Executive summary

Human poxviruses

- A total of 14 poxviruses have been documented to infect humans.
- Seven are of the *Orthopoxvirus* genus (cowpox, monkeypox, buffalopox, cantagalo, Aracatuba, vaccinia and variola), one is of the *Molluscipox* genus (molluscum contagiosum), one is of the *Yatapoxvirus* genus (tanapox) and five are of the *Parapoxvirus* genus (orf, paravaccinia, bovine popular stormatitis, deerpox and sealpox).

Vaccines

- First-generation vaccines were propagated in domesticated animals. Contamination was frequent.
- Four different vaccinia virus vaccines were used in the smallpox eradication program.
- Complications to vaccination were frequent and often lethal.
- A large proportion of the population is contraindicated to vaccination.

New vaccines

- ACAM2000 has similar efficacy and contraindications to Dryvax™ - the vaccine previously used in the USA.
- ACAM2000 has the advantage of being clonal and not several quasispecies (unlike Dryvax).
- The MVA vaccine is a highly passaged vaccinia virus with limited capability to replicate in human cells. Fewer side effects are noted but the efficacy remains similar to that of ACAM2000.
- The MVA vaccine has been demonstrated to be safe in some immunocompromised individuals.
- A higher virus dose and up to two vaccinations may be required for MVA to demonstrate similar efficacy to ACAM2000 or Dryvax.

Current treatments

- Intramuscular administration of vaccinia immune globulin (VIG) is the only approved postexposure therapy.

- The efficacy of VIG remains controversial.
- Humanized antibodies will potentially replace VIG in the next decade.
- Cidofovir is efficacious against poxviruses but requires rigorous clinical management and induces nephrotoxicity.

Antivirals

- Other than cidofovir, no antivirals are approved for the treatment of poxviruses.
- Gleevec and CI-1033 have demonstrated limited efficacy at targeting host processes.
- Interferon mimetics appear promising in animal studies.
- Several nucleoside analogs have efficacy against poxviruses. Coupling the nucleoside to lipid molecules has been shown to improve bioavailability and efficacy, as demonstrated by CMX001.
- CMX001 and ST-246 are the only anti-poxvirus drugs currently in clinical trial. Both are performing exceptionally.
- Combination therapy to increase efficacy and to prevent the generation of viruses resistant to CMX001 and ST-246 is an option.

In the future it is hoped that a different scenario will be painted. Newer and safer vaccines, such as MVA, will likely reduce the number of people contraindicated to vaccination thus enabling the vaccination of large percentages of the population. This step, coupled with prophylactic antiviral therapy, will probably be the most crucial in containing orthopoxvirus outbreaks. Initial or primary contacts that are not vaccinated will be rapidly diagnosed using a plethora of techniques not available in the smallpox epoch. Orally bioavailable antivirals, such as CMX001 and ST-246, will provide further protection to vaccinated as well as unvaccinated individuals. The use of antivirals in combination has not yet been evaluated in humans but has demonstrated efficacy in animal models [84]. Combination therapy, coupled with ring-vaccination, would be expected to provide the best method to impede virus dissemination. Furthermore, antibodies, mimetic interferons or a combination thereof appear to be alternative treatment regimens that could have clinical utility. These therapies could feasibly be used independently or in combination with CMX001 and ST-246 to treat postexposure cases.

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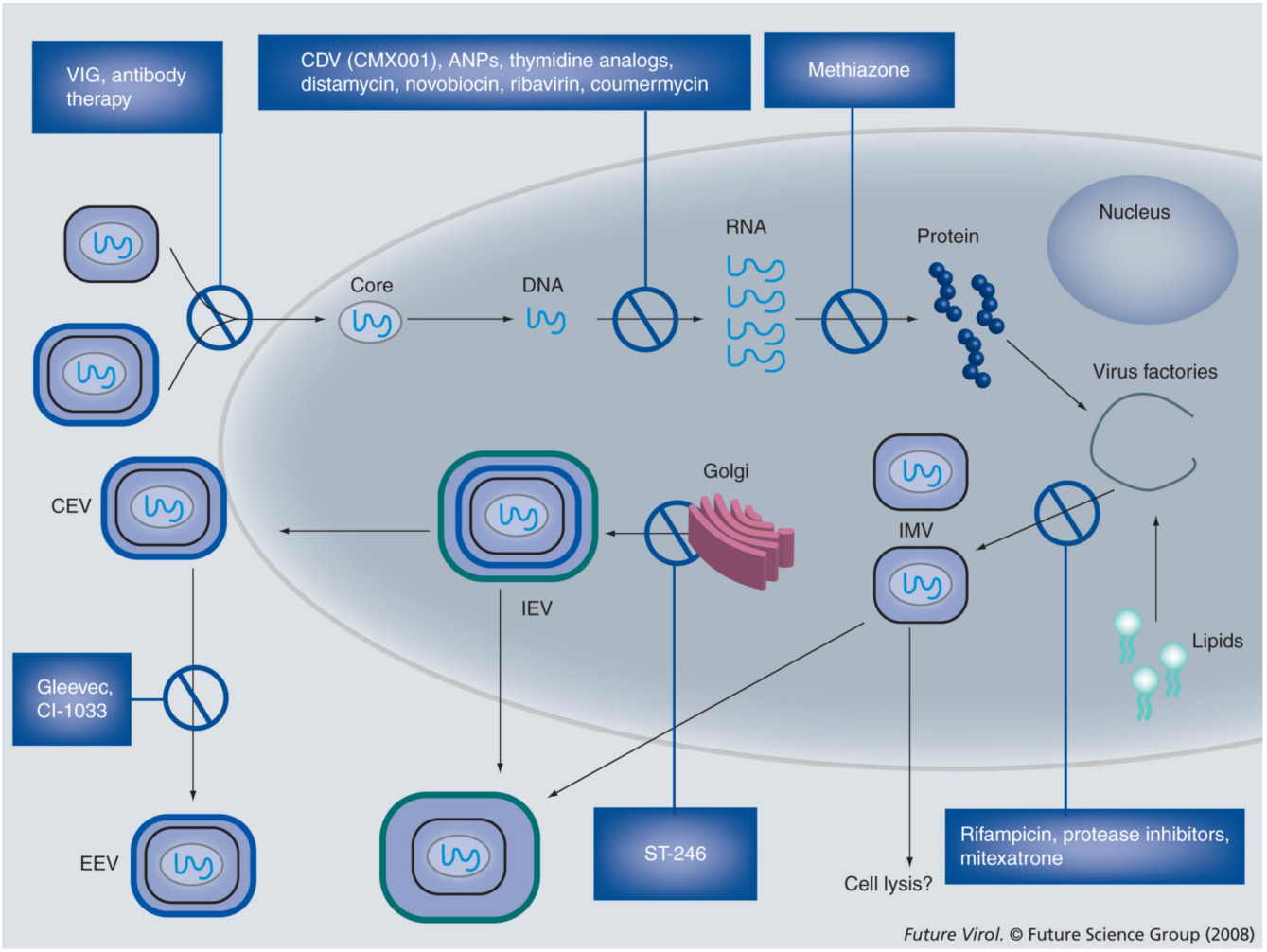

Figure 1. Orthopoxvirus therapeutics and prophylactics

The replication cycle of a typical orthopoxvirus is shown. The sites of action of many drugs having anti-orthopoxvirus activities are indicated.

ANP: Acyclic nucleoside phosphonate; CDV: Cidofovir; CEV: Cell-associated enveloped virus; EEV: Extracellular enveloped virus; IMV: Intracellular mature virus; VIG: Vaccinia immune globulin.

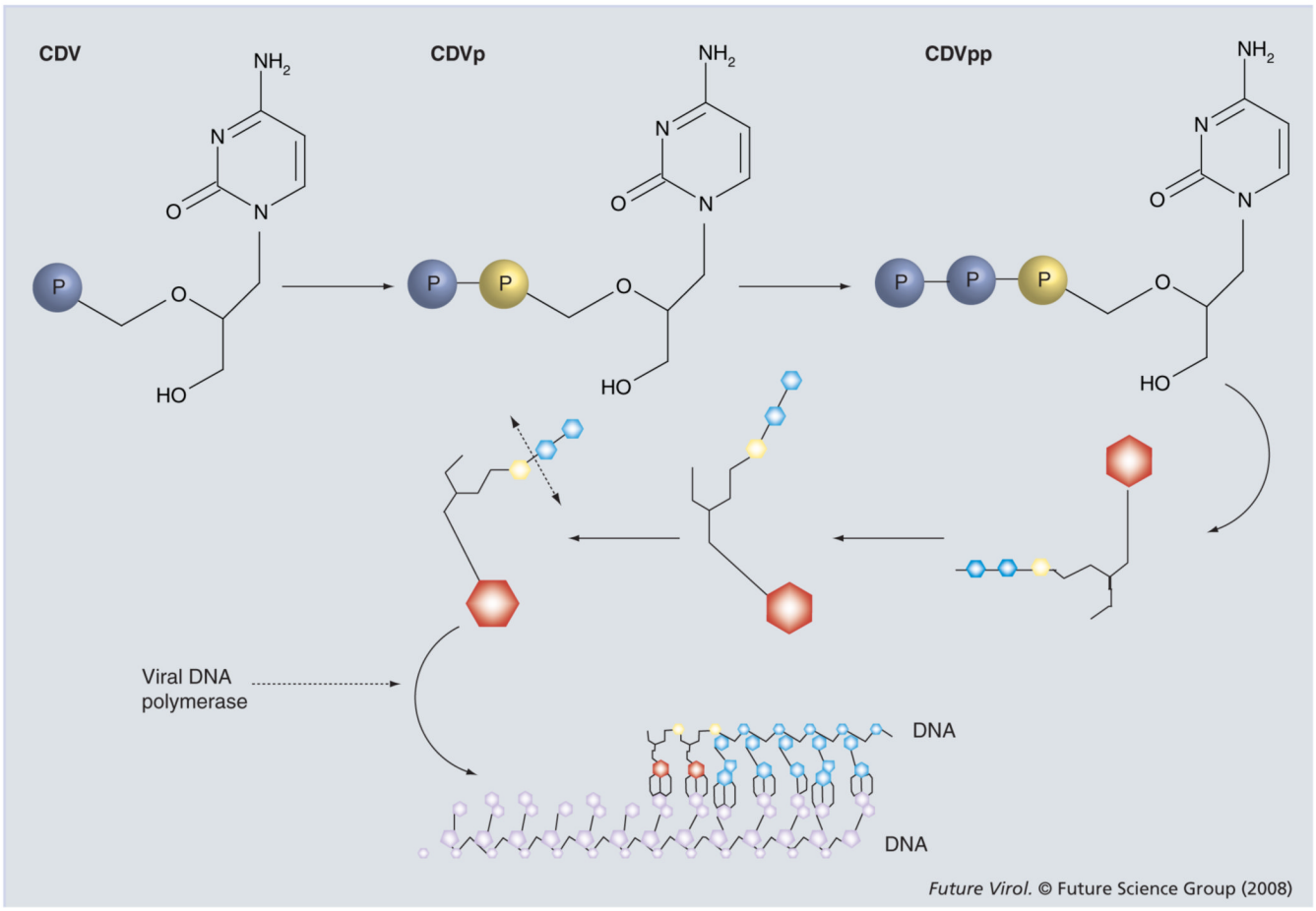

Figure 2. Mode of action of cidofovir

CDV is already phosphorylated once it enters the cell. Two further rounds of phosphorylation are required for it to become active in preventing DNA replication.

CDV: Cidofovir; P: Phosphate group.

Adapted from [69].

Table 1
Antiviral therapeutics to treat human orthopoxvirus infections

Antiviral	Mode of action	Comments	Limitations
Antibody therapy	Neutralization	• The efficacy of VIG has been shown to be largely dependent on anti-B5 antibodies • Humanized monoclonal anti-B5 antibodies have demonstrated higher efficacies than VIG	• Not tested in humans *in vivo*
ANPs (other) and their cyclic prodrugs (adefovir dipivoxil, (*S*)-HPMPA)	DNA replication	• Orally bioavailable nucleoside analogs (similar concept to CMX001) • Efficacy of adefovir dipivoxil has been demonstrated *in vitro* against VACV and CPXV • H961 protects SCID mice from VACV infection • (*S*)-HPMPA has good *in vivo* efficacy when covalently coupled to a lipid molecule (see CMX001)	• Incorporation into host DNA
ATA	Blocks cellular and viral factors	• ATA blocks the phosphorylation of extraceullular signal-regulated kinase 1-2, which is essential for OPV replication • It also inhibits the phosphotase activity of viral enzymes required for viral transcription	• *in vitro* data only
CDV	DNA replication	• An ANP analog of deoxycytidine • Highly efficacious against all OPVs • Currently licensed for the treatment of CMV retinitis in AIDS patients • Restricted to investigational new drug protocol for treatment of OPV when VIG treatment fails • OPVs resistant to CDV have been generated by serial passaging, however, these viruses have a loss of virulence *in vivo* suggesting that OPVs will not easily develop resistance to CDV	• Intravenous administration requires attentive clinical care • Nephrotoxicity • Incorporation into host DNA
CI-1033 (canertinib dihydrochloride) and U1026	EGF receptor kinase inhibitor (similar to Gleevec) and ERK inhibitor	• OPVs encode their own growth factor which is similar to EGF • CI-1033 inhibits this binding • CI-1033 protects mice against intranasal VACV challenge • CI-1033 has prolonged pan suppression of the receptor compared with other EGF receptor inhibitors, such as gefitinib and erlotinib	• CI-1033 was administered 6 h postinfection • Delayed intervention not demonstrated • Can cause diarrhea and skin rash • U1026 efficacy demonstrated *in vitro* only

Antiviral	Mode of action	Comments	Limitations
		• U1026 is an extracellular signal-regulated kinase inhibitor that inhibits VACV replication *in vitro*	
CMX001	DNA replication	• A modified CDV drug with a lipid hydrocarbon group attached ensures oral bioavailabilty • Currently in Phase I clincial trial for treatment of OPV infections • Cyclic ester prodrug enables efficient entry of CDV into the cell • Increased efficacy over CDV • Unlike CDV, nephrotoxicity is not associated with CMX001 • Delayed intervention has been demonstrated successfully in mice infected with various OPVs	• Incorporation into host DNA
Distamycin	Binds DNA thereby preventing transcription factor activity	• Inhibits transcription from the intermediate promoters for the three late transcription factors • Most major late proteins fail to accumulate in the presence of distamycin • Congocidine, netropsin and monopyrazole analogs have similar molecular structures and antiviral properties as distamycin	• *in vitro* data only
ETYA and BE755c	Inhibitors of the lipoxygenase pathway	• Inhibits OPV replication. • Shown to perturb virus-specific membranes	• *in vitro* data only
Gleevec (STI-571)	Inhibits Abl-family tyrosine kinases. Block EEV release	• Approved for the use of chronic myeloid leukemia • Has anti-OPV properties • Protects mice against intranasal VACV infection	• Gleevec was administered subcutaneously via an osmotic pump • Delayed intervention has not been demonstrated
IFN-γ mimetics	Activation of adaptive and innate immune response	• Have been shown to have good oral bioavailability and activity up to 2 days postinfection	• Efficacy data limited to VACV in mice
Methisazone	Viral RNA translation	• Derivative of anti-tuberculosis agent active against OPVs • This drug is the first demonstrated antiviral • Prophylactic efficacy against smallpox contacts has been demonstrated • No longer available	• Severe nausea and vomiting
Mitoxantrone	Approved anticancer drug	• Mitoxantrone blocks late stage viral assembly	• *in vitro* data only

Antiviral	Mode of action	Comments	Limitations
		• A viral protein and/or cellular topoisomerase II are the likely targets of the drug • It has no apparent impact on viral gene expression • A missense mutantion in vaccinia DNA ligase induces mitoxantrone resistance	
Novobiocin/coumermycin	Viral DNA inhibitors	• Drugs target viral topoisomerase but not host topoisomerase, thus preventing enzyme-DNA interactions	• Limited *in vivo* data available • Intravenous novobiocin was used to treat MRSA • It is no longer available
Prostaglandins (PGI_2)	Likely gene expression	• PGs are naturally occurring lipids • Depending on class, the PGs have opposite effects on VACV infection *in vivo* • Some reduce mortality (PGI_2) others increase mortality (e.g., PGA_1, PGE and PGA_2) • PGI 2 was delivered intraperitoneally every 12 h beginning 1 h postinfection and provided 100% protection compared with 50% survival in controls. No efficacy studies in other OPV systems	• No oral efficacy has been demonstrated • VACV doses had to be reasonably low for 100% survival • At higher doses, 100% of control mice died and 45% of treated mice survived
Ribavirin and ribavirin analogs	Inhibitor of inosine monophosphate dehydrogenase and immune system modulation	• A broad-spectrum, purine nucleoside antiviral • Mice infected intranasally with CPXV were protected by ribavirin when the innoculum dose was low • No delayed intervention studies demonstrated with OPV infections • Several analogs have increased antiviral activity over ribavirin *in vitro*	• Limited *in vivo* efficacy studies • Infection with high virus doses required several delayed CDV injections for efficacy in mice
Rifampin	Inhibition of mature virion formation	• *in vitro* studies only • Demonstration that cells degenerate after 3-4 days in the presence of 150 μg/ml • Likely has action against RNA metabolism - possibly against viral associated RNA polymerase	• Requires high doses - toxic
ST-246	Egress inhibitor	• Oral drug with low toxicity • Currently in Phase II clinical trials for treatment of OPV infections • Prevents OPVs from becoming extracellular but does not prevent virion formation	• Mutations in *F13L* gene or orthologs reduce drug efficacy

Antiviral	Mode of action	Comments	Limitations
		• Efficacious against many OPVs • Efficacious following delayed intervention	
Tetrapyrroles		• Porphyrins and phthalocyanines have an inhibitory effect on enveloped and nonenveloped VACV in cell culture	• *in vitro* data only
Thymidine analogs (*N*-methanocarbathymidine)	DNA replication	• Activity dependent on OPV thymidine kinase leading to inhibition of viral DNA synthesis • Treatment can be delayed by 2 days in mice infected with VACV • Oral bioavailability revealed that the drug provided good protection and reduced liver, spleen and kidney titers	• *in vivo* experiments have mainly focused on intraperitoneal delivery • Drug must be given frequently (daily) when given orally • Dependent on functional thymidine kinase gene
VIG	Neutralization	• Efficacy is low • Production is time-consuming • Poor stock availability	• Therapeutic effects of VIG are poorly demonstrated and controversial
β-defensin	Possibly due to the release of PG	• Produced by keratinocytes in response to injury or inflammation • Has been shown to reduce the expression of DNA-dependent RNA polymerase and viral plaques	• *in vitro* data only

ANP: Acyclic nucleoside phosphonate; ATA: Aurintricarboxylic acid; CDV: Cidofovir; CMV: Cytomegalovirus; CPXV: Cowpox virus; EEV: Extracellular enveloped virus; MRSA: Methicillin-resistant Staphylococcus aureus; OPV: Orthopoxvirus; PG: Prostaglandin; VIG: Vaccinia immune globulin; VACV: Vaccinia virus.