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Orthopoxvirus inhibitors that are active in animal models: an update from 2008 to 2012

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Abstract

Antiviral agents are being sought as countermeasures for the potential deliberate release of smallpox (variola) and monkeypox viruses, for the treatment of naturally acquired monkeypox virus infections, and as therapy for complications due to smallpox (live-attenuated vaccinia virus) vaccination or accidental infection after exposure to vaccinated persons. Reviews of the scientific literature spanning 1950–2008 have documented the progress made in developing small-animal models of poxvirus infection and identifying novel antiviral agents. Compounds of considerable interest include cidofovir, CMX001 and ST-246® (tecovirimat; SIGA Technologies, NY, USA). New inhibitors have been identified since 2008, most of which do not exhibit the kind of potency and selectivity required for drug development. Two promising agents include 4'-thioidoxuridine (a nucleoside analog) and mDEF201 (an adenovirus-vectored interferon). Compounds that have been effectively used in combination studies include vaccinia immune globulin, cidofovir, ST-246 and CMX001. In the future there may be an increase in experimental work using active compounds in combination.

Keywords

antiviral; camelpox; cowpox; drug combinations; ectromelia; monkeypox; rabbitpox; smallpox; vaccinia

The eradication of the smallpox (variola) virus in the early 1980s did not alleviate the threat of serious viral infection by deliberate release, and the emergence of the related monkeypox virus now accounts for serious natural infections [1–4]. Owing to these concerns, smallpox vaccination (with live-attenuated vaccinia virus) has continued to be performed in military personnel, in certain laboratory workers and in selected healthcare workers. Unfortunately, some of these vaccinations led to serious vaccine complications that were life-threatening [5–7]. In these situations, antiviral countermeasures appeared to be important for the infected individual's survival. Antiviral chemotherapy researchers have made dramatic strides since 1950 in identifying antiorthopoxvirus compounds and appropriate testing methods in animal models of disease. Two thorough reviews have been published on this topic, one covering the period 1950–2002 [8], and the other describing research performed from 2003 to early 2008 [9]. The present review constitutes an update covering the 5-year period 2008–2012. Topics addressed include newly reported or refined poxvirus animal

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models and compounds found active in animal models (inactive compounds tested during the period are not reviewed). A listing of published reviews related to these topics is also provided.

Newly reported or refined orthopoxvirus animal models used for antiviral studies

Historically, cowpox, ectromelia (mousepox), rabbitpox (related to vaccinia), monkeypox, vaccinia and variola major (human smallpox) have been used for animal infection models and antiviral experiments. During the period 2008–2012, a number of new models were developed (Table 1). Camelpox virus was found to infect athymic nude mice, representing the first small-animal model for this virus. The mice failed to gain weight during the infection but did not lose weight, and were euthanized when the weight of uninfected mice exceeded the weight of infected animals by 25% [10]. Camelpox virus may be attractive because it represents the closest relative to variola virus, although the severity of the infection model appears to be limited. The reported mouse models with ectromelia, cowpox and vaccinia viruses are variations of existing intranasal, intraperitoneal, or skin scarification infection models. Certain researchers prefer using ectromelia virus over cowpox and vaccinia because the virus is in its natural host (the mouse), and infections can be initiated by a very small virus inoculum (depending upon the strain of mouse used). Other investigators avoid ectromelia because of its potential to spread throughout a mouse colony.

Noteworthy are four small-animal models for monkeypox virus infection, using CAST/EiJ mice, STAT1-deficient (knockout) mice, African dormice or prairie dogs (Table 1). The most useful of these models may be the CAST/EiJ mice owing to their sensitivity to monkeypox virus infection at low virus challenge doses by multiple routes, immune competence, genetic homogeneity, available immunological reagents, and (importantly) commercial production availability. The monkeypox models were developed in response to an outbreak of monkeypox virus in humans in the USA that was acquired from infected prairie dogs purchased from a pet store that also sold certain species of exotic African rodents [11]. The African rodents were the source of the infection that transmitted to prairie dogs. As an alternative to using monkeypox virus infection of monkeys, researchers developed the marmoset model of cowpox virus (using a special strain designated as calpox virus) infection (Table 1). *Cynomolgus* macaques have also been infected by the intrabronchial route with the cowpox virus [12]. Both of the cowpox virus infection models in monkeys have the advantage over using the monkeypox virus in primates because they can be used at a lower biohazard safety level.

In addition to the cited animal models used in antiviral studies, other refinements to monkeypox infection models have been made that may be used in future antiviral experiments. New aerosolization methods have been developed for more efficiently infecting nonhuman primates [13,14]. A monkeypox virus encoding green fluorescent protein has been developed and used to track the virus in *cynomolgus* macaques [15]. A review of experimental and natural infections of animals with monkeypox virus was recently published [16]. The limited availability and high cost of monkeys prohibit their widespread use in research. Nevertheless, these and the other previously described models [8,9] are important for establishing efficacy of new antiviral compounds under the US FDA's two-animal rule for drug development against orthopoxvirus infections [17].

Efficacy of antiviral compounds in animal models

Three of the most important antipoxvirus inhibitors that were discovered prior to 2008 are cidofovir, CMX001 (or hexadecyloxypropyl-cidofovir, an orally active prodrug form of

cidofovir), and ST-246[®] (tecovirimat; SIGA Technologies, NY, USA). Cidofovir is a nucleotide analog that converts to a cytidine triphosphate analog intracellularly, and as such, inhibits poxvirus DNA polymerase [18]. CMX001 converts to cidofovir *in vivo*, and thus has the same mode of action. ST-246 is a unique compound that blocks a late step in virus assembly by preventing intracellular envelope virus formation and subsequent virus egress from the cell [19,20]. This greatly restricts the spread of virus *in vivo*. Since their discovery and development, these compounds, along with vaccinia immune globulin, have been used to treat humans with serious complications from smallpox vaccinations, or who became seriously infected after exposure to smallpox vaccinees [5,21–23]. Additionally, human infections caused by the molluscum contagiosum poxvirus have been treated with cidofovir [24] and CMX001 [25]. Treatment of infections with immunosuppressive agents is not a viable approach, since orthopoxvirus infections are more severe in immunodeficient humans [6] and immunocompromised animals [26,27].

Between 2008 and 2012, research continued with cidofovir, CMX001 and ST-246 for studying the treatment of orthopoxvirus infections in animals. Investigators used these compounds as positive control agents for antiviral studies and as benchmark inhibitors in new animal infection models. The number of newly discovered anti-orthopoxvirus compounds since 2007 has been small, but there are some worth highlighting.

In indicating the treatment regimens that are reported in Tables 1–7, it is important to clarify what is meant in this review by pre-exposure prophylaxis, postexposure prophylaxis and therapy. Whether the start of compound administration occurs before, shortly after, or days after virus exposure, all of these regimens are considered ‘treatments’. Therapy refers to treatments initiated after virus exposure, but not so early that it might be considered postexposure prophylaxis. Pre-exposure prophylaxis or simply ‘prophylaxis’ will always indicate treatments initiated prior to virus challenge. Postexposure prophylaxis is a more nebulous term because there is no accepted standard as to how long after infection that treatments can be initiated before they are considered as therapy (or therapeutic). With orthopoxvirus infections in the various animal models, there is considerable virus replication occurring at 24 h after infection, suggesting that treatments initiated at that time or later constitute therapy. Tables 1–7 indicate the times of treatment initiation, and the reader can apply their own definition as to what constitutes postexposure prophylaxis versus therapy. The antiviral potency of the compound dictates how long after virus challenge that treatments can be initiated and still be efficacious.

Activity of cidofovir in animal models

Cidofovir has been the most widely studied compound for the treatment of orthopoxvirus infections in animals [8,9]. Due to poor oral bioavailability, the compound must be delivered parenterally for systemic treatment or else topically for treatment of orthopoxvirus lesions. Since 2007, cidofovir has been tested in numerous new animal infection models, including camelpox virus infections in athymic nude mice, cowpox virus upper respiratory tract infections in mice, rabbitpox virus infections in rabbits and severe combined immunodeficiency (SCID) mice scarified at the base of the tail and infected with vaccinia virus (Table 3). Notably, one experiment showed that a single treatment with cidofovir, administered as late as 6 days after infection, was effective against an ectromelia virus infection in mice.

Activity of CMX001 in animal models

CMX001 has an advantage over cidofovir in being orally bioavailable, and it also exhibits reduced nephrotoxicity relative to the parent drug [28]. Between 2008 and 2012, CMX001

was evaluated in several ectromelia virus models in mice, in rabbits infected with rabbitpox virus and in a monkeypox virus mouse infection model (Table 3). Many of the effective treatment regimens for mice infected with ectromelia or monkeypox viruses involved a 10-mg/kg loading dose followed by 2.5 mg/kg/day given every other day through to 14 days of infection. A single treatment of 25 mg/kg was effective when given 5 days after ectromelia virus infection. CMX001 was also quite active in rabbits, with a 5-mg/kg dose given twice a day for 5 days protecting 75% of animals from death when administered as late as 5 days after infection (Table 3). As reported in a recent review, minimal efficacy was found for CMX001 against monkeypox virus infection in nonhuman primates [29]. This was attributed to poor pharmacokinetics of the inhibitor *in vivo*, leading to lower exposures to the active drug, cidofovir, than anticipated. Thus, the monkey model does not appear to be useful for studying antiviral treatments with CMX001. Recently the first pharmacokinetic and safety studies of CMX001 in humans were published [30].

Activity of ST-246 in animal models

ST-246 has primarily been used orally, although there is a human case treated both orally and topically [23]. During the period 2008–2012, ST-246 was shown to have efficacy in the treatment of ectromelia and vaccinia virus infections in mouse models, in rabbitpox virus infections in rabbits, in monkeypox virus in three animal species (mice, prairie dogs and monkeys) and in variola virus-infected monkeys (Table 4). Doses of the compound reported to be effective were generally in the 30–300 mg/kg/day range and were given for 14 days. In a study by Jordan and colleagues, complete protection from death of monkeys infected with monkeypox virus was found at a dose of 3 mg/kg/day when treatment began 3 days after infection (Table 4) [31]. Treatment of variola virus infection in monkeys was only performed at a 300 mg/kg/day dose; thus, it is not known whether lower doses would be efficacious.

Activities of other compounds in animal models

A number of other compounds have been tested and were found to be active to varying degrees in mouse infection models between 2008 and 2012 (Table 5). Four compounds (CSA-13 ceragenin, cyclic HPMPC, imiquimod and phosphonoacetic acid) were applied topically to treat cutaneous infections in mice, and reduced lesion formation and delayed the time to death. CSA-13 ceragenin is an antimicrobial peptide that has an antiproliferative effect on cancer cells by altering membrane permeability [32]. Cyclic HPMPC converts to cidofovir *in vivo* to inhibit viral DNA polymerase. Imiquimod is an immune-stimulatory agent that acts through Toll-like receptor 7 [33]. Phosphonoacetic acid inhibits viral DNA polymerases [34]. HPMP-5AzaC and HPMPDAP (both of which are structurally related to cidofovir and are DNA synthesis inhibitors) were effective by intra-peritoneal route against intranasal infections with camelpox and/or vaccinia viruses in mice. Thus, these compounds would not have any advantage over cidofovir because they require the same route of administration, and this is not advantageous compared with oral dosing. An approved drug (for other purposes), imatinib mesylate (Gleevec®; Novartis, NY, USA), an Abl-family tyrosine kinase inhibitor [35], was active against vaccinia in mice when delivered by osmotic pump. This method of delivery was required because of its short half-life in mice. An anticancer agent that blocks vaccinia virion assembly [36], mitoxantrone, dramatically extended the time to death in cowpox-infected mice by intraperitoneal treatment, but the majority of treated animals died from infection regardless. SOCS-1 mimetics (that act as cellular tyrosine kinase inhibitors) protected mice from lethal vaccinia virus infections by either oral or intraperitoneal treatment. Vaccinia immune globulin, administered intraperitoneally, was found to be effective in reducing lesion severity and prolonging the time to death in SCID mice infected with a vaccine strain of vaccinia virus.

The two most impressive compounds identified between 2008 and 2012 were 4'-thioidoxuridine (4'-thio-IDU; a nucleoside analog that inhibits poxvirus DNA synthesis [37]) and mDEF201 (an adenovirus-vectored IFN- α ; Table 5). 4'-thioIDU is a derivative of the antiviral drug IDU, and appears to be superior to IDU as an antipoxvirus agent, with activity seen by both oral and intraperitoneal treatments. IDU, administered systemically, was effective in delaying the time to death of SCID mice infected with vaccinia virus [38], but was ineffective against cowpox virus infections [39]. A wide range of doses of 4'-thioIDU was effective against vaccinia virus infections in mice, and treatments could be delayed by 4 days after virus exposure and still achieve protective benefits. The compound also appeared to be well tolerated by mice. mDEF201 is primarily active as a prophylactic agent. A single 10^7 PFU/mouse intranasal dose administered 56 days (8 weeks) preinfection was protective against lethal vaccinia virus infection. By contrast, it required a 10^8 PFU/mouse dose to protect mice when administered 24 h after infection. As mDEF201 acts by producing interferon, the interferon has to be produced early to shut down the virus so that viral anti-interferon proteins do not neutralize interferon activity [40].

Drug combination studies

It has become apparent from the studies of patients with progressive vaccinia infections that a single agent is insufficient for efficacy. These individuals have some degree of underlying immunosuppression that makes the infections very difficult to treat. Thus, two or more agents have been evaluated in combination, the choices being vaccinia immune globulin, cidofovir, CMX001 and ST-246 [21–23]. It is known from animal studies that severely immunosuppressed animals cannot be cured from their infections. However, the time to death can be delayed significantly by drug treatment [26,27,41]. Two combination chemotherapy studies have been conducted in animals during 2008–2012 (Table 6). In an ectromelia (IL-4 recombinant) virus model, it required the combination of CMX001 and ST-246 to render a survival benefit, with either compound alone being ineffective. CMX001 and ST-246 were previously shown to be synergistically active when used in combination to treat cowpox virus infections *in vivo* [42].

In a vaccination complication model in SCID mice, treatments of vaccinia immune globulin and cidofovir (applied either topically or systemically) were more effective than either agent alone (Table 6). In an extraordinary difficult human case, a military vaccinee with severe progressive vaccinia infection was treated parenterally with vaccinia immune globulin (341 vials used), orally (for 73 days) and topically (for 68 days) with ST-246, and orally (for 6 weeks) with CMX001, for a total of 75 days overall before his condition finally resolved [23]. In the latter weeks of treatment, the virus isolated from the patient became resistant to ST-246, although resolution of progressive vaccinia occurred anyway. This case demonstrated the need for continued discovery and development of novel antiorthopox-virus agents. More drug combination studies are also warranted to understand how to maximize treatment benefit. Systemically administered and topically applied compounds should be investigated in combination with vaccinia immune globulin, with the particular goal of understanding the minimal requirements of vaccinia immune globulin use (since this material cannot be manufactured in large quantity).

Review articles published on antiorthopoxvirus compounds

Fourteen review articles on antiorthopoxvirus agents and diseases were published during the period from 2008 to 2012 (Table 7). Of these, six publications dealt specifically with CMX001 and related nucleotide analogs. Three of the CMX001 articles reviewed aspects surrounding the preclinical development of the compound, two articles reviewed the treatment of rabbitpox virus infections and one focused on mouse infection studies. There

were four reviews that specifically covered ST-246, three of which discussed its discovery and early development, while the fourth dealt with treatment of rabbitpox virus infections. Two reviews covered all classes of poxvirus inhibitors (Table 7). Another review described the rabbitpox virus infection model and treatment of infections in rabbits. A final review highlighted the treatment of eczema vaccinatum in human patients with the four leading antiviral compounds (cidofovir, CMX001, ST-246 and vaccinia immune globulin).

Summary of progress made between 2008 & 2012

New and refined animal models for cowpox, ectromelia and vaccinia virus infections in mice are now available. An immunocompromised mouse model for camelpox virus infection was reported and small-animal models for monkeypox virus infections were made available using CAST/EiJ mice, STAT1-deficient mice, African dormice and prairie dogs. Further studies have been conducted with the top candidate anti-poxvirus agents cidofovir, CMX001, ST-246 and vaccinia immune globulin. A number of new substances have been identified that have antiviral activity in animals, most notable of which are 4'-thioIDU (therapeutically active) and mDEF201 (primarily a prophylactic agent). The use of active compounds in combination has led to more effective treatments of severe infections in immunocompromised animals, and this may lead to a better understanding of how to treat serious infections in humans. A number of reviews have been added to the scientific literature covering various aspects of orthopoxvirus antiviral research.

Future perspective

For this analysis it is important to understand that more than one type of orthopoxvirus infection is being targeted; thus, several treatment approaches will need to be taken. An imminent clinical need is the treatment of vaccination complications, since severe infections have occurred in the recent past [21–23] and will most likely occur in the future. The second important need is the treatment of naturally occurring monkeypox virus infections. History has shown that these infections not only occur in Africa [43], but also in unexpected parts of the world (e.g., in the USA) [11,16]. The third scenario is the least likely to occur but one that would have the gravest consequences, that of deliberate smallpox or monkeypox virus release in a bioterrorist or biowarfare attack. Regarding this last scenario, the FDA held a meeting on 14–15 December 2011 to discuss pathways for developing antismallpox therapies. The consensus of the panel was that the three most appropriate models for compound evaluations are ectromelia virus in mice, rabbitpox virus in rabbits and monkeypox virus in monkeys. Members of the panel were concerned about the cost of running experiments in these models (particularly in the larger animal species). The panel also discussed what should be appropriate experimental parameters, such as times of initiation of therapy in the various models, endpoints for determining benefit, viral challenge route, inoculum size, viral strains to use and other parameters. Based upon these discussions, it is anticipated that future research will focus on these three models, and that studies will be designed to answer the questions raised by the 2011 FDA panel. Indeed, the study by Parker *et al.* published in 2012 investigating ectromelia virus infection in hairless mice was performed in light of the FDA panel's recommendations [44].

As the treatment of naturally occurring monkeypox virus infections is a concern, particularly in Africa, the new CAST/EiJ mouse model will probably be the model of choice to evaluate the efficacy of new agents, since it is considerably more cost effective than performing primate studies. The CAST/EiJ model has many advantages over the three other monkeypox models noted in Table 1.

Regarding treatment of vaccination complications, the logical choice of virus to use is one or more strains of vaccinia virus, with studies performed in immunocompromised mice (to model severe infections in humans with naive or impaired immune systems). A particular emphasis will be to explore drug combination regimens that may lower the amount of intravenous vaccinia immune globulin required for treatment, as has been preliminarily explored by Fisher *et al.* in a tail lesion model [26]. The importance of performing future research in this area is emphasized by the treatment of a recent military vaccinee, who was administered 341 vials of vaccinia immune globulin in conjunction with ST-246 and CMX001 treatments [23]. This single case caused a significant depletion of the US stockpile of this material. This clinical case illustrates another direction of future research, that of drug combination studies (with or without the use of vaccinia immune globulin). There are a number of newer inhibitors with different modes of antiviral action that have been identified (Table 6). Some of these agents will be tested in combination with cidofovir, ST-246 and/or CMX001. Since drug resistance has already been identified in a patient treated for several weeks with ST-246 [23], the US government will likely continue to solicit drug discovery research in order to identify new, promising inhibitors of orthopoxvirus infections.

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Executive summary

New animal models

- The majority of new animals models represent refinements of models that existed prior to 2008.
- A mouse model for camelpox virus infection was described for the first time in 2010.
- New small-animal models for monkeypox virus infections were described, with infection of CAST/EiJ mice being perhaps the most important.
- New models of cowpox virus infections in monkeys were described. These can be used in a biosafety level 2 environment.

Further studies of known active antiviral compounds under development

- A considerable number of studies have been performed to evaluate and develop cidofovir, CMX001 and ST-246[®] (SIGA Technologies, NY, USA) as antiorthopoxvirus agents.
- Cidofovir, CMX001, and ST-246 have been used in combination with vaccinia immune globulin to treat experimental animals and/or humans.

Evaluations of other compounds for antiviral activity

- A number of previously known antiviral substances were evaluated in orthopoxvirus animal models during 2008–2012.
- Certain host-directed compounds with antiviral properties were evaluated, among them tyrosine kinase inhibitors.
- mDEF201 (an adenovirus-vectored IFN- α) was reported as a highly effective prophylactic agent.
- 4'-thio-idoxuridine has perhaps the best therapeutic potential for treating orthopoxvirus infections, and is effective orally and systemically.

Table 1

New or further-refined animal models used for evaluating antiviral agents against poxviruses, 2008–2012.

Animal model	Virus	Inoculation route	Principal infection (refinement of model)	Ref.
Mouse (athymic nude)	Camelpox	Intranasal	Systemic	[10]
Mouse (BALB/c)	Cowpox	Intranasal (small volume)	Upper respiratory tract	[45]
Mouse (C57BL/6)	Ectromelia	Intranasal	Systemic (less severe infection) [†]	[46]
Mouse (hairless SKH-1)	Ectromelia	Intranasal	Systemic (includes whole body rash)	[44]
Mouse (albino C57BL/6 or BALB/c)	Vaccinia–Luc [‡]	Intranasal and intraperitoneal	Pneumonitis and systemic (visual <i>in vivo</i> tracking of the infection)	[47,48]
Mouse (CAST/EiJ)	Monkeypox	Intraperitoneal and intranasal	Systemic	[49,50]
Mouse (SCID)	Vaccinia	Scarification at base of tail	Cutaneous, followed by systemic	[26]
Mouse (STAT1-deficient)	Monkeypox	Intranasal	Pneumonitis and systemic	[51]
African dormouse	Monkeypox	Intraperitoneal	Systemic	[52]
Prairie dog	Monkeypox	Intranasal and intradermal	Systemic	[53]
Marmoset	Cowpox [§]	Intranasal and intravenous	Pneumonitis, skin lesions	[54]
Monkey	Cowpox	Intrabronchial	Systemic	[12]

[†] Compared with more severe infections of A/Ncr mice, resulting in more effective antiviral treatments in C57BL/6 mice.

[‡] Vaccinia virus containing a luciferase gene.

[§] New cowpox virus strain, named calpox virus, isolated from New World monkeys.

Table 2

Activities of cidofovir in animal models of poxvirus infections, 2008–2012.

Virus and animal model	Effective treatment regimen	Ref.
Camelpox: in. (systemic) in athymic nude mice	50 mg/kg/day ip., daily for 3 days starting on the day of infection	[10]
Cowpox: low volume in. (upper respiratory tract infection) in mice	100 mg/kg/day ip., daily for 2 days starting 1 day after infection	[45]
Ectromelia: in. (systemic) in mice	2.5–100 mg/kg ip., one treatment only, given as late as 6 days postinfection depending upon dose	[55]
Rabbitpox: aerosol (systemic) in rabbits	0.5, 1 and 1.75 mg/kg by aerosol or 10 mg/kg iv. daily for 3 days starting immediately postinfection	[56]
Vaccinia: scarified base of tail (skin lesion and systemic) in severe combined immunodeficiency mice	50 mg/kg/day ip. on days 7, 10, 13, 16 and 19; or 1% cream topically on days 7–20 postinfection	[26]

The type of viral infection induced is given in parentheses.

in.: Intranasal; ip.: Intraperitoneal; iv.: Intravenous.

Table 3

Activities of CMX001 in animal models of poxvirus infections, 2008–2012.

Virus and animal model	Effective treatment regimen	Ref.
Ectromelia: in. (systemic) in mice	25 mg/kg p.o., one time only, as late at 5 days postinfection	[57]
Ectromelia: in. or aerosol (systemic) in mice	10 mg/kg p.o. and 2.5 mg/kg every other day from days 2–14; 1, 2, or 4 mg/kg/day p.o. for 5 days; and 1.25–5 mg/kg/day for 14 days; each regimen started at 4 h postinfection	[58]
Ectromelia: in. (systemic) in C57BL/6 mice compared with A/Ncr mice	10 mg/kg p.o. and 2.5 mg/kg every other day from days 2–14 (treatments could be delayed up to 6 days postinfection in C57BL/6 mice)	[46]
Ectromelia: in. (systemic) in C57BL/6 and hairless SKH-1 mice	20 mg/kg/day p.o. every 3 days for four treatments, starting as late as 5 days after infection in C57BL/6 mice; or 25 mg/kg p.o. followed by 2.5 mg/kg/day every 3 days for three treatments, starting as late as 3 days postinfection	[44]
Rabbitpox: id. (systemic) in rabbits	1 or 5 mg/kg p.o. twice daily for 3 days, starting 1 day preinfection; 2 or 5 mg/kg/day p.o. for 5 days, starting 1 day postinfection; and 5 mg/kg p.o. twice daily for 5 days, starting as late as 5 days postinfection	[59]
Rabbitpox: id. (systemic) in rabbits	20 mg/kg/day p.o. given one, two or three times (every other day), starting 3 or 4 days postinfection	[60]
Monkeypox: in. (systemic) in STAT1-deficient mice	10 mg/kg p.o. and 2.5 mg/kg every other day from days 2–14, starting at 4 h postinfection	[51]

The type of viral infection induced is given in parentheses.

id.: Intradermal; in.: Intranasal; p.o.: Oral

Table 4

Activities of ST-246[®] (SIGA Technologies, NY, USA) in animal models of poxvirus infections, 2008–2012.

Virus and animal model	Effective treatment regimen	Ref.
Ectromelia: in. (systemic) in C57BL/6 mice	100 mg/kg/day p.o. daily for 14 days, starting as late as 6 days postinfection	[44]
Monkeypox: iv. (systemic) in monkeys	300 mg/kg/day p.o. once daily for 14 days, starting 1 or 3 days postinfection	[61]
Monkeypox: iv. (systemic) in monkeys	3–100 mg/kg/day p.o. once daily for 14 days, starting 3 days postinfection	[31]
Monkeypox: in. (systemic) in STAT1-deficient mice	100 mg/kg p.o. for 10 days starting at 4 h postinfection	[51]
Monkeypox: in. (systemic) in prairie dogs	30 mg/kg/day p.o. for 14 days, starting either on day 0, 3 or 8 postinfection	[62]
Rabbitpox: aerosol (systemic)	40 mg/kg/day p.o. for 14 days, starting 1 h postexposure or as late as 3 days postinfection	[63]
Vaccinia: in. (respiratory) in mice	100 mg/kg/day p.o. for at least 5 days, starting just before infection; or daily for 14 days, starting up to 3 days postinfection	[64]
Vaccinia: in. (respiratory and systemic) in various immunodeficient mice	100 mg/kg/day p.o. for 14–21 days, starting on the day of infection or 3 days after virus challenge	[65]
Vaccinia – Cantagalo virus strain: tail scarification (lesions)	100 mg/kg/day p.o. for 7 days, starting 4 h postinfection	[66]
Variola: iv. (systemic) in monkeys	300 mg/kg/day p.o. once daily for 14 days, starting 1 or 3 days postinfection	[61]

The type of viral infection induced is given in parentheses.

in.: Intranasal; iv.: Intravenous; p.o.: Oral.

Table 5

Activities of other compounds in animal models of poxvirus infections, 2008–2012.

Compound	Virus and animal model	Effective treatment regimen	Ref.
CSA-13 ceragenin	Vaccinia: cutaneous (lesions) on the backs of shaved SCID mice	Topical cream (unspecified concentration) applied daily, starting 2 h after virus exposure	[67]
Cyclic HPMP	Vaccinia: cutaneous (lesions and systemic) in immunosuppressed hairless mice	1% topical cream applied twice daily for 7 days, starting 5 days after virus challenge	[68]
HPMP-5AzaC	Vaccinia: in. (pneumonitis) in mice	50 mg/kg ip. once daily on days 0, 1 and 2 of the infection	[69]
HPMPDAP	Vaccinia: in. (pneumonitis) in mice	50 mg/kg ip. once daily on days 0, 1 and 2 of the infection	[69]
HPMPDAP	Camelpox: in. (systemic) in athymic nude mice	50 mg/kg ip. once daily on days 0, 1 and 2 of the infection	[10]
Imatinib mesylate (Gleevec® Novartis, NY, USA)	Vaccinia: in. (pneumonitis) in mice	200 mg/kg/day by osmotic pump implanted either 24 h prior to infection or at the time of infection	[70]
Imiquimod	Vaccinia: cutaneous (lesions and systemic) in immunosuppressed hairless mice	1% topical cream applied once daily every other day or every 3 days for four treatments, starting as late as 4 days postinfection	[71]
mDEF201 (adenovirus-vectored IFN- α)	Vaccinia: in. (pneumonitis) in mice	All treatments were single in. doses: 10^7 PFU of vector per mouse in. 56 days prior to virus challenge; 10^5 – 10^7 PFU/mouse at 24 h preinfection; or 10^8 PFU/mouse at 24 h postinfection	[72]
Mitoxantrone	Cowpox: in. (pneumonitis) in mice	0.25 and 0.5 mg/kg administered once ip. at 24 h postinfection	[73]
Phosphonoacetic acid	Vaccinia: cutaneous (lesions and systemic) in immunosuppressed hairless mice	1% topical cream applied twice daily for 7 days, starting 5 days after virus challenge	[68]
SOCS-1 mimetics (tyrosine kinase inhibitors)	Vaccinia: in. (pneumonitis) in mice	50–200 μ g/mouse ip. on days –2, –1 and 0 relative to virus challenge, or 500–1000 μ g p.o., on the same days	[74]
4'-thio-idoxuridine	Cowpox and vaccinia: in. (pneumonitis) in mice	3–100 mg/kg/day ip. and p.o., starting as late as 4 days postinfection	[37]
Vaccinia immune globulin	Vaccinia: tail scarification (lesions and systemic) in SCID mice	10 mg/mouse ip. every 3–5 days, starting as late as 7 days postinfection	[26]

The type of viral infection induced is given in parentheses.

in.: Intranasal; ip.: Intraperitoneal; p.o.: Oral; SCID: Severe combined immunodeficiency.

Table 6

Compounds used in combination for treatment of orthopoxvirus virus infections in animals, 2008–2012.

Compounds	Virus and animal model	Effective treatment regimen	Ref.
CMX001 and ST-246 [®] (SIGA Technologies, NY, USA)	Ectromelia (IL-4 recombinant): in. (systemic) in mice	CMX001 (4 mg/kg/day p.o. for 14 days) plus ST-246 [®] (100 mg/kg/day p.o. for 14 days); each compound was ineffective when used alone	[75]
Vaccinia immune globulin and cidofovir	Vaccinia: tail scarification (lesions and systemic) in SCID mice	10 mg/mouse of vaccinia immune globulin ip. every 3–5 days starting 7 days after infection; combined with 50 mg/kg/day of cidofovir ip. on days 7, 10, 13, 16 and 19; or with 1% cidofovir cream topically on days 7–20 postinfection	[26]

The type of viral infection induced is given in parentheses.

in.: Intranasal; ip.: Intraperitoneal; p.o.: Oral; SCID: Severe combined immunodeficiency.

Table 7

Published reviews on orthopoxvirus diseases and their treatment, 2008–2012.

Virus	Model	Compound(s)	Subject of review	Ref.
Orthopoxviruses	Not specified	ST-246® (SIGA Technologies, NY, USA)	Insights into the discovery and development of the compound	[76]
Orthopoxviruses	Mouse, monkey	ST-246	Early development, pharmacology and pharmacokinetics, limited mouse infection studies	[20]
Cowpox, ectromelia, rabbitpox, vaccinia	Mouse, rabbit	CMX001 and related compounds	State-of-the-art use of these compounds to treat orthopoxvirus, herpesvirus, adenovirus and other DNA virus infections	[77]
Cowpox, ectromelia, monkeypox, vaccinia, variola	Mouse, ground squirrel, rabbit, monkey	All classes of inhibitors	Historical perspective of the development of antiviral agents against poxviruses	[78]
Cowpox, ectromelia, vaccinia	Mouse	CMX001	Discussion of the early development of the inhibitor as an antiorthopoxvirus agent	[79]
Cowpox, ectromelia, monkeypox, vaccinia, variola	Mouse, ground squirrel, rabbit, monkey	ST-246	Discovery and development of the compound for the treatment of poxvirus infections	[20]
Cowpox, vaccinia	Mouse	CMX001 (and related compounds), ST-246	Review of mouse infection studies involving treatment with these compounds	[80]
Cowpox, ectromelia, rabbitpox, monkeypox, vaccinia	Mouse, rabbit, monkey	CMX001	Development of the inhibitor with respect to antiviral activity, pharmacokinetics and toxicology	[29]
Rabbitpox	Rabbit	Cidofovir, ST-246, thiosemicarbazone, hyperimmune serum	Description of the aerosol rabbitpox virus infection model and summarizes antiviral studies with the four compounds	[81]
Rabbitpox	Rabbit	ST-246	Description of the rabbitpox virus infection model, and highlights the prior treatment studies with ST-246	[82]
Rabbitpox	Rabbit	CMX001	Summary of studies using CMX001 to treat the infection prophylactically, with new prophylactic and therapeutic studies added	[59]
Rabbitpox	Rabbit	CMX001	Summary of studies using CMX001 to treat the infection therapeutically and with new therapeutic studies added	[60]
Cowpox, ectromelia, monkeypox, vaccinia, variola	Mouse, monkey	All classes of inhibitors	Targets for the development of new antiorthopoxvirus agents and modes of antiviral action of inhibitors	[83]
Vaccinia	Human patients	Cidofovir, CMX001, ST-246, vaccinia immune globulin	Eczema vaccinatum complications and treatment of patients with the four inhibitors	[7]