REVIEW PAPER

Antivirals in medical biodefense

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Abstract

The viruses historically implicated or currently considered as candidates for misuse in bioterrorist events are poxviruses, filoviruses, bunyaviruses, orthomyxoviruses, paramyxoviruses and a number of arboviruses causing encephalitis, including alpha- and flaviviruses. All these viruses are of concern for public health services when they occur in natural outbreaks or emerge in unvaccinated populations. Recent events and intelligence reports point to a growing risk of dangerous biological agents being used for nefarious purposes. Public health responses effective in natural outbreaks of infectious disease may not be sufficient to deal with the severe consequences of a deliberate release of such agents. One important aspect of countermeasures against viral biothreat agents are the antiviral treatment options available for use in post-exposure prophylaxis. These issues were adressed by the organizers of the 16th Medical Biodefense Conference, held in Munich in 2018, in a special session on the development of drugs to treat infections with viruses currently perceived as a threat to societies or associated with a potential for misuse as biothreat agents. This review will outline the state-of-the-art methods in antivirals research discussed and provide an overview of antiviral compounds in the pipeline that are already approved for use or still under development.

Keywords Medical biodefense · Antiviral · BSL3/4 viral pathogens

Introduction

Antiviral compounds effective in infections caused by tropical and vector-borne viruses were a neglected topic of international antivirals research until very recently. A number of compounds are now in clinical trials, and very few have received regulatory approval, or have made it to the market.

This review refers to a presentation given by the authors at the 16th Medical Biodefense Conference held in Munich in 2018 and is intended to widen the view on the thematic issue with a special focus on biodefense-related aspects.

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Biodefense relevance

While infections with arthropod-borne and tropical viruses are fairly common in nature, severe outcomes are, with a few exceptions, very rare. Therefore, countermeasures against such unlikely events, especially in the developed world, are regarded as giving little or no return on investments and are sidelined by grant driven research and manufacturers. While this is a legitimate point of view for academia and the pharmaceutical industry, governments have to consider countermeasures against rare agents released, or threatened to be released deliberately by individuals or groups aiming to cause maximum societal disruption and chaos. For such events governments have to prepare credible countermeasures in order to be able to provide prophylaxis, isolation, and treatment for large numbers of exposed and infected individuals. The basis of all considerations on countermeasures and biothreat preparedness is an agent-related risk assessment, which includes numerous criteria like availability of stocks or samples for potential perpetrators, ease of handling, pathogenicity, transmission pathways, tenacity, availability of vaccines, antivirals, and others. This requires research into these countermeasures, including the development, testing

and stockpiling of vaccines and antiviral drugs, particularly for dangerous biological agents. This review will focus on viral agents that fit into this category, briefly discussing their relevance for public health and biodefense, mode of action, and give an overview of treatment options available or in the pipeline.

Public health relevance

Viral hemorrhagic fevers (VHFs) cause the highest mortality in human hosts among all known viral agents. Encephalitides and severe respiratory infections caused by a range of viruses are other diseases with often severe clinical outcomes. The recent emergence of such infections from geographical hotspots is mainly a consequence of the rapid development of ground and air transport. Vector-borne infections are also affected by climate change. Large-scale outbreaks were first described for Monkeypox virus in central Africa in the 1970s [117], while outbreaks of mosquito-borne Chikungunya virus [85] and Dengue virus infections in the Indian Ocean islands were seen mostly in the twenty-first century [124]. The historic Ebola outbreak in West Africa in 2013–2014, followed by a more recent one in the Republic of Congo with 1891 fatalities [37], has attracted extensive media attention. The rapid and uncontrolled spread of Ebola fever in Africa has been considered as a threat for the national security of developed countries with regard to the risk of imported cases but also for economic reasons. The Bundeswehr Institute of Microbiology (IMB) was involved in the international effort to contain Ebola fever in West Africa during the 2014–2016 outbreak [120]. The institute also runs a research program for antiviral drug development and hosts the biennial Medical Biodefense Conference (MBDC). Antiviral compounds and their possible role in biodefense were a special theme during the MBDC in 2018. The selection of topics with a focus on pox-, alpha- and flaviviruses was guided by the NATO AMedP-6 'Handbook on the medical aspects of nuclear, biological and chemical (NBC) defensive operations—Part II.' Smallpox, albeit eradicated in nature, continues to be perceived as a threat for several reasons, one of them being the risk that variola virus might be brought back with the methods of synthetic biology. Military forces and first responders in many countries were revaccinated in the early 2000s for fear that Iraq might have weaponized smallpox virus (which it had not, as was revealed later on). Emergency plans were developed to deal with a deliberate release. While no licensed drug was available at the time to treat infections with variola virus, a drug effective against orthopoxviruses, tecovirimat, has recently been approved by the United States Federal Drug Administration [55].

Smallpox as an exclusively human infection was eradicated by vaccination, but this is impossible for zoonoses like yellow fever, which has a number of non-human reservoir hosts. This is an important distinction, and in the case of an acute zoonotic viral infection, post-exposure antiviral treatment of the unvaccinated is a potentially lifesaving option in need of further development. Unfortunately, the public health repository of antiviral countermeasures for such infections is woefully small.
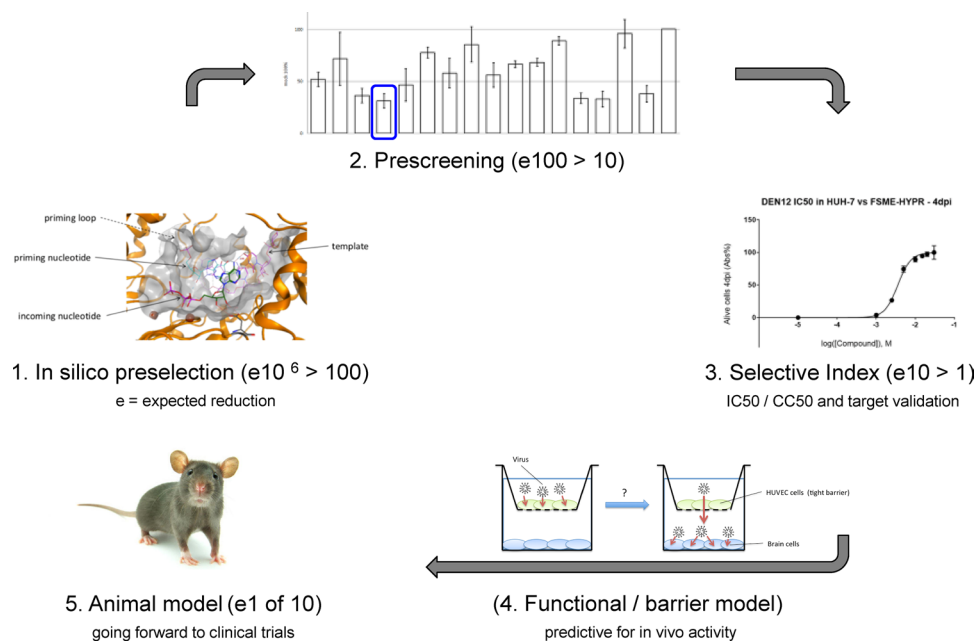
VHFs are caused by infection with RNA viruses. The standard of treatment for RNA virus infections where it shows efficacy, is ribavirin, developed in 1963 [32]. Where possible, early start of treatment of acute virus infections gives the best results and, in this context, accurate and rapid virus diagnosis is essential. The crucial role of a well-organized public health system and classic quarantine approaches was demonstrated in the recent Ebola outbreaks in West- and Central Africa. However, the need for new antiviral agents had generally been recognized and been reviewed by David Freestone as early as 1985 [47]. While many virus infections are asymptomatic, new or improved antiviral drugs are needed for the prevention and/or treatment of a number of significant conditions caused by viruses which at present cannot be controlled by alternative measures, including vector control, immunization and treatment with existing antiviral drugs. The need for specialized BSL-3/BSL-4 facilities with trained personnel for experiments with life viruses, and animal challenge, has further restricted research to a few high-security sites worldwide. As a result, there are no FDA-approved antivirals for Ebola or the causative viral agents of many other viral hemorrhagic fevers, viral encephalitides, and respiratory infections. Few therapeutic interventions are available except for supportive therapy.

In the following sections we will give a summary of the antivirals session held during the MBDC 2018, as well as an overview of antiviral drug development methodologies and selected experimental antivirals designed for potential biothreat agents.

MBDC 2018: antivirals session

After an introduction on the chances and challenges encountered in the development of novel antivirals (Brancale—MBDC-2018-GO1), a discussion on the current conditions in UK/EU research networks, obstacles at the interface between research and industry, and preparedness for the treatment of infections with biodefense-related viruses followed. Further contributions outlined the methodical approach to antivirals design and biological evaluation (Fig. 1). Using examples from chemists present at the meeting, the structural approach (Step 1; Bassetto—MBDC-2018-GO1), based on in silico dynamic models of antivirals targets, i.e., small-molecule inhibitors of polymerases, proteases, methyltransferases, and ProTide-based improvements of antiviral nucleosides

Fig. 1 Methodical approach to antivirals design and biological evaluation

2. Prescreening (e100 > 10)

1. In silico preselection (e10 6 > 100)
e = expected reduction

3. Selective Index (e10 > 1)
IC50 / CC50 and target validation

5. Animal model (e1 of 10)
going forward to clinical trials

(4. Functional / barrier model)
predictive for in vivo activity

[98, 132], were explained in detail. The dynamic models are based on solved NMR structures of protein targets. The preselection of virtual candidate antiviral compounds in in silico models against viral protein targets reduces the number of compounds by four magnitudes (10^6 library $\rightarrow 10^2$ selected candidates). The compounds are then synthesized, shipped and compared at a standard concentration (10 μM at IMB) for comparative effectiveness and toxicity in organotypical cell lines against a panel of viruses of interest for the biodefense community, including alpha-, bunya-, filo-, flavi-, ortho-/paramyxo-, and poxviruses. Hit compounds with high efficacy and low toxicity are identified (Step 2). This is followed by IC_{50}/CC_{50} evaluation (Step 3) of emerging hit to lead compounds, aiming for selective indices > 30 in sensitive (e.g., Huh-7 hepatoma cells) and organotypical cell lines selected for the pathogenic traits of the viruses of interest (e.g., U138 glioblastoma cells for encephalitis viruses). This usually results in another reduction of candidate numbers by one to two magnitudes. To confirm drug targets, target validation is then carried out, either by the use of enzymatic assays for viral enzyme targets (Silvestri—MBDC-2018- GO3) or by induction of resistant virus strains showing resistance mutations in the antiviral target areas, as shown with tecovirimat (ST-246) for orthopoxviruses. This concludes the classical in vitro evaluation of antiviral drug candidates. The winnowing process up to this point leads to a reduction ratio of six magnitudes (10^6 to 1). If in vitro toxicity is minimal, the compounds go straight into pharmacokinetics testing (rodent models), and into animal models of viral infections (Step 5). Here a dramatic rate of attrition leads to only one out of ten compounds tested in animal models making it into phase I clinical studies [79].

To further select compounds prior to animal testing, complex infection models, including in vitro 3D models, are currently the focus of much research in the antivirals field [77]. Functional models of virus infection at barriers, and the effect of antivirals on the virus passing the barrier, give an indication of antiviral effects on typical viral pathogenesis, e.g., encephalitis viruses that are being tested on models of the blood brain barrier (Step 4; Hurler—MBDC-2018-GP1). A successful prediction by in vitro functional models of antivirals efficacy in vivo, particularly using primary human organotypic cells, would also result in a significant reduction of unsuccessful drug testing in animal models. The evaluation cycle described above follows the general considerations as outlined by Huggins et al. for Ebolavirus (EBOV) in 1999 [64], with the addition of in silico design with dynamic models for compound preselection, which had not yet been available at that time, and represents a methodical approach to antivirals design and development. This approach is used by groups active in the field and is also the basis of the 'Antivirals Platform' collaboration between Cardiff University and IMB into prophylaxis and treatment of infections caused by viral biothreat agents, which is funded by SER CYMRU/MRC and IMB's basic funding. The platform established comprises all steps from molecular design to in vitro testing in complex infection models. Talks at MBDC 2018 included different examples of this approach towards antiviral drug discovery: in silico design of small nucleosidic antivirals and prodrugs against arboviruses (Bassetto-MBDC-2018-GO2), Cima-4, Den-12, MB-124, tick borne encephalitis (TBEV) polymerase inhibitor nucleoside analogues with superior activity in the central nervous system (CNS) cells compared to sofosbuvir (Bugert-MBDC-2018-GO3), novel protease

153

inhibitors for Zika virus as surrogate virus for other flaviviruses using an enzymatic assay for target validation as well as a Zika mouse model (Silvestri-MBDC-2018-GO4), and BB4-D9, a dandelion natural extract antiviral against poxviruses (Zanetta-MBDC-2018-GO5). FDA approval of oral TPOXX® (Tecovirimat/ST-246®), a F13L morphogenesis inhibitor of orthopoxviruses, was reported in the poxvirus session (Grosenbach-MBDC-2018-HO2). Posters provided meaningful examples of the evaluation cycle, with contributions on live cell imaging of virus-infected cells for antivirals testing in a model of the blood brain barrier (Hurler-MBDC-2018-GP2), a novel polymerase-inhibiting CHIKV antiviral (MB-70, Hucke-MBDC-2018-GP4), a NS4a autophagy testing system for flaviviruses (Tscherne-MBDC-2018-GP5), and MoA studies on Cf2642 inhibiting macropinocytosis of measles and poxviruses for use as synergistic cell-targeting antiviral along with virus-specific compounds (Narayan-MBDC-2018-GP6).

Antivirals: FDA approved and experimental

Complementing the recent review by De Clercq and Li [32] this section will focus on small-molecule antiviral compounds and discuss a selection of compounds that are either FDA approved or lately proved effective against viruses associated with a biothreat risk in in vitro experiments, animal or phase I–III clinical studies. Subsections give a brief overview of the viral agents in the order of relevance for biodefense, the FDA-approved treatment options, and antivirals in development, with top candidates highlighted in yellow in Table 1, which lists virus-specific compounds in the same order of relevance, detailing compound class, target and stage of development.

Poxviridae

Variola virus (smallpox virus), a member of the orthopoxvirus (OPV) genus of the family *poxviridae*, was used in the 18th century as a biological warfare agent by British and American forces in North America [36], and remains on the top of the list of biological threat agents for warfare or bioterrorism [35, 107]. Effective vaccines and FDA-approved antivirals exist and could be used to control a deliberate release. Variola virus (VariolaV), which only infects humans, was declared eradicated in 1980, after a global vaccination campaign. Handling of VariolaV requires BSL-4 containment. Virus stocks are officially kept in only two designated laboratories in Russia and the US. Monkeypox virus (BSL-3), a zoonotic agent causing sequelae similar to smallpox but less fatal, is endemic in central Africa (Democratic Republic of Congo; DRC); recent introductions to the UK were travel-related. Poxviruses are transmitted by contact infection and via the respiratory tract, causing a systemic infection in humans and animals. Smallpox virus infection leads to a fatal multiorgan failure syndrome within 7–14 days, in complicated cases with a hemorrhagic syndrome and CNS involvement. Smallpox has played a role in large-scale epidemics in history and its causative agent continues to be considered a potential biological warfare agent [35]. Orthopoxviruses (OPV) are ovoid-shaped enveloped viruses with Group I double-stranded (ds) DNA genomes, replicating via a virus-encoded DNA polymerase, an antivirals target, in the cytoplasm of infected cells [42]. Poxviruses enter cells by macropinocytosis, but a poxvirus-specific receptor is still elusive [100]. *Anti-poxvirus drugs*. One of the first effective drugs in clinical use as a parenteral treatment in severe OPV infections was cidofovir, a bisphosphonate developed at REGA, in Belgium [31, 35]) and FDA approved against human cytomegalovirus (HCMV). The ether lipid analogue **brincidofovir** (CMX001), a prodrug of cidofovir, has shown efficacy in small animal models and is awaiting FDA approval [26, 44, 56, 115, 116, 118, 139]. The F13L virus egress inhibitor **tecovirimat** (ST-246, TPOXX®) has been independently developed to treat smallpox infections and has been FDA approved since 2018. Tecovirimat has recently been used to treat non-human primates infected with variola, and humans exposed to OPV [55, 104, 118, 146]. Tecovirimat (TPOXX®) is currently stockpiled in the US and production for similar stockpiles in Europe is planned. Anti-poxvirus drugs effective in animal models are reviewed in more detail elsewhere [133]. Further candidate anti-poxvirus drugs include kinase inhibitors imatinib (Gleevec/STI-571; [122, 123]) and olomoucine [61], teramoprocol [119], mitoxandrone [5], the membrane targeting ddBCNA cf2642 [99], bisbenzimide derivatives [151], FC-6407, a OPV D4 processivity factor mimic [111], and a number of natural extracts that have shown interesting antiviral activity against OPV in in vitro infection models [27, 153] (Table 1).

Filoviridae

Filoviruses are category A select agents, World Health Organization risk group 4 pathogens, high on the list of potential biological threat agents [107] and their handling requires BSL-4 containment. In nature they infect primates, pigs and bats (free-tailed and fruit bats) and are transmitted to human hosts by exposure to infected bush meat and body fluids of human patients. Ebola and Marburg viruses (EBOV/MARV) cause severe viral hemorrhagic fevers with hematemesis, bloody diarrhea, prostration and case fatality rates of up to 90% within three days of infection. The EBOV envelope glycoprotein has been used in the VSV-EBOV vaccine, which is 70–100% effective preventing disease in exposed and vaccinated

Table 1 The table lists virus-specific compounds in the order of their relevance, detailing compound class, target and stage of development

Compound name	Virus/Target	Paper/ Author-Date	Regulatory Approval/ Dev. Stage
Poxviridae- VariolaV, other OPV (Baltimore Group I dsDNA) - section 3.1			
Tecovirimat (ST246, TPOXX)	OPV/ F13L - egress	Mucker 2013	FDA-appr. Orthopoxvirus
Cidofovir	OPV/ Pol	De Clerc 2002	FDA-appr. CMV Compassionate Use
Brincidofovir	OPV/ Pol	Parker 2008	IND
Gleevec (STI-571)	OPV/ kinases	Reeves 2005	FDA-appr. Cancer *in vitro*
Mitoxandrone	OPV/ unclear	Altmann 2012	FDA-appr. Cancer in vitro
Olomoucine II	OPV/ kinases	Holcakova 2010	in vitro
Terameprocol	OPV/ unclear	Pollara 2010	in vitro
ddBCNA-cf2642	OPV/ membranes, autophagy	McGuigan 2013	in vitro
Bis-benzimides	OPV/ DNA intercalators	Yakimovich 2017	in vitro
KPB-100/200	OPV/ unclear	Cryer 2017	in vitro
FC-6407	OPV/ D4	Nuth 2019	in vitro
BB4 D9	OPV/ unclear	Zanetta 2019	in vitro
Filoviridae - EBOV, MARV (Baltimore Group V ss-RNA) - section 3.2			
Remdesivir (GS-5734)	EBOV/ Pol	Warren 2016	IND in vitro Phase II clinical 2019
Favipiravir (T705)	EBOV/ Pol	Bixler 2018a	appr. in Japan - Influenza in vivo
Galidesivir (BCX4430)	RVFV/ Pol	Warren 2014 Taylor 2016	IND in vivo
CM-10-18	EBOV-MARV/ a Gluc. ER enzymes	Chang 2013	in vivo
FGI-106	EBOV/ entry	Aman 2009	in vitro
AR-12 (OSU 03012)	EBOV-MARV / PDK-1	Mohr 2015	in vitro
K11777	EBOV/ Prot	Zhou 2015	in vitro
Alphaviridae – CHIKV, EEEV, VEEV (Baltimore Group IV ss+RNA) - section 3.3			
Ribavirin	CHIKV/ Pol, GTP depletion, mutagenic	Abdelnabi 2015	FDA-appr. HCV; RSV in vivo
Sofosbuvir	CHIKV/ Pol	Ferreira 2019	FDA-appr. HCV in vitro
Favipiravir (T705)	CHIKV/ Pol	Abdelnabi 2017	appr. in Japan - Influenza in vivo
Suramin (Germanin™, Antrypol™)	CHIKV/ unclear	Kuo 2016	FDA-appr. antiparasitic in vivo

Table 1 (continued)

Ivermectin	CHIKV/ unclear	Varghese 2016	FDA-anhelmintic in vitro
Mefenamic acid	CHIKV/ eIF4E dephosphorylation	Rothan 2016	FDA-cancer in vivo
Sorafenib	CHIKV, VEEV, EEEV/ eIF4E dephosphorylation	Lundberg 2018	FDA-cancer in vitro
Halofuginone	CHIKV/ Protyl tRNAse	Hwang 2019	Veterinary use in vitro
ML-336	VEEV, EEEV/ Nsp4	Jonsson 2019	in vivo
LL-37	VEEV/ entry	Ahmed 2019	in vitro
Compound 25	CHIKV/ nsP2	Bassetto 2013	in vitro
Prest-37, -392	VEEV/ nsP1 capping enzyme	Ferrera-Ramos 2019	in vitro
Baicalin	CHIKV/ unclear	Oo 2018	in vitro
Arenaviridae – LassaV, JuninV (Baltimore Group V ss-RNA) - section 3.4			
Ribavirin	LassaV/ Pol, GTP depletion, mutagenic	McCormick 1986	FDA-appr. HCV; RSV Compassionate use LassaF
Favipiravir (T705)	LassaV/ Pol	Rosenke 2018	appr. in Japan - Influenza in vivo
LHF 535	JuninV/ glycoprotein GP2	Madu 2018	in vivo
Bunyaviridae – CCHFV, RVFV, other PhleboV (Baltimore Group V ss-RNA) - section 3.5			
Ribavirin	CCHFV/ Pol, GTP depletion, mutagenic	van Eeden 1985	FDA- appr. HCV; RSV Compassionate use CCHF
Favipiravir (T705)	PhleboV, CCHFV/ Pol	Gowen 2010 Hawman 2018	appr. in Japan - Influenza in vivo
Galidesivir	RVFV/ Pol	Westover 2018	IND in vivo
2'-Fluoro-2'-deoxycytidine	PhleboV/ Pol	Smee 2018	in vivo
FGI-106	CCHFV +/ entry	Smith 2010	in vitro
Flaviviridae – TBEV, DENV, YFV + (Baltimore Group IV ss-RNA) section 3.6			
Ribavirin	YFV +/ Pol, GTP depletion, mutagenic	Malinoski 1990	FDA-appr. HCV; RSV Compassionate use YF

Table 1 (continued)

Sofosbuvir	ZikaV, YFV +/ Pol	Bullard-Feibelman 2017 De Freitas 2019	FDA-appr. HCV in vivo
Favipiravir (T705)	UsutuV/ Pol	Seguera Guerrero 2018	appr. in Japan - Influenza in vivo
Ivermectine	YFV +/ Helicase	Mastrangelo 2012	FDA-appr. antihelmintic in vitro
Bromocriptine	ZikaV/ Prot (Dopamine agonist)	Chan 2017	FDA-appr. Diabetes/ Parkinson in vitro
Erythrosin B	DENV +/ Prot	Li 2018	FDA-appr. food additive in vitro
Niclosamide	YFV +/ entry/fusion-translation	Mazzon 2019	FDA-appr. antihelmintic in vivo
Galidesivir (BCX4430)	TBEV, WNV / Pol	Eyer 2017	IND in vitro
AR-12 (OSU 03012)	ZikaV / PI3K-Akt pathway	Chan 2018	IND-NSAID in vitro
FGI-106	DENV / entry	Aman 2009	in vitro
3',5'-di-O- trityluridine	YFV, DENV / unclear	De Burghgraeve 2013	in vitro
ddBCNA-cf2642	ZikaV/ membranes, autophagy	Nolte 2016	in vitro
NITD008	DENV/ Pol	Milligan 2018	in vitro
K22	ZikaV +/ unclear	García-Nicolás 2018	in vitro
PBTZ 16	YFV, TBEV +/ Virus maturation	Cannalire 2019	in vitro
Orthomyxoviridae – Influenza virus (Baltimore Group V ss-RNA) - section 3.7			
Oeseltamivir, Zanamivir, Laninamivir, Peramivir	Influenza virus/ neuraminidase	Gubareva 2017	FDA–appr. Influenza
Baloxavir -Marboxil	Influenza virus/ cap dependent endonuclease (CEN)	Noshi 2018	FDA–appr. Influenza
Favipiravir (T705)	Influenza virus/ Pol	Furuta 2002 Baz 2018	appr. in Japan - Influenza
Haloxanide/Nitazoxanide	Influenza virus/ HA maturation	Tilmanis 2017	Phase III
Cycloheptathiophene	Influenza virus/ Pol	Nannetti 2019	in vitro

Table 1 (continued)

Paramyxoviridae – MeaslesV, NipahV + (Baltimore Group V ss-RNA) - section 3.8			
Ribavirin	MeaslesV +/ Pol, GTP depletion, mutagenic	Jeulin 2009	FDA-appr. HCV; RSV in vivo
ERDRP-0519	MeaslesV/ Pol	Krumm 2014	in vivo
Favipiravir (T705)	NipahV/ Pol	Dawes 2018	appr. in Japan - Influenza in vivo
Remdesivir (GS-5734)	NipahV +/ Pol	Lo 2017	in vitro
ddBCNA-cf2642	MeaslesV/ membranes autophagy	McGuigan 2013	in vitro
Droserone	(Measles virus)/ unclear	Lieberherr 2017	in vitro
4'-Azidocytidine (R1479) Balapiravir	NipahV +/ Pol	Hotard 2017	in vitro

Lead small-molecule drug candidates are highlighted in yellow

Appr. approved, *FDA* US Food and Drug Administration, *IND* FDA investigational drug, *NHP* non-human primates, *NSAID* non-steroidal anti-inflammatory drug, *Phase* clinical trial phase I to III, *Pol* viral polymerase, *Prot* viral protease, *Vs.* versus, + more viruses, not listed

individuals and has been approved in October 2019 in the EU as the world's first Ebola vaccine [19]. Filoviruses are filamentous enveloped viruses with Group V negative-sense single-stranded (ss) RNA genomes. The endosomal Nieman Pick C1 protein, also relevant in flavivirus infections [114] and the TIM-1 (HAVCR1) receptor on the surface of T cells, also relevant for hepatitis C virus (HCV) entry [73], are potential targets for antiviral drug development. *Anti-filovirus drugs*. While treatment recommendations are emphasizing intensive medical support if suitable clinical facilities and cohort isolation are available [15, 16], defense against the use of filoviruses as biological weapons would benefit from an effective virus-targeting therapy. There are currently no licensed antiviral drug treatments for filoviruses. However, in a recent multi-outbreak, multi-country study (PALM- "Together save lives") started in November 2018 in the DRC, two monoclonal antibodies (Mabs) emerged as giving the greatest chance to survive Ebolavirus infection. Zmapp, mAb114 and REGN-EB3 were compared to the small-molecule drug remdesivir [150]. The trial was stopped early with REGN-EB3 and mAb114 giving the greatest chance to survive Ebolavirus infection. The WHO recommends using these two Mabs for all further treatments [150]. Remdesivir (GS-5734;1-cyano-substituted adenosine nucleotide analogue), a nucleoside-analogue prodrug and lead compound of the small-molecule antivirals class, has been shown to inhibit EBOV in cell culture and in non-human primates likely by chain termination [144], but showed lower efficacy in the clinical trial compared to monoclonal antibody-based therapeutics. A good alternative, albeit not tested in the

DRC clinical trial, may be T705 (favipiravir; [48]), a repurposed drug synthesized by FUJIFILM-Toyam Chemical Co., licensed for use against influenza virus in Japan, and since found to be a broad-spectrum inhibitor of viral RNA polymerases [34, 49]. T705 and the related pyrazine-carboxamide compounds T-1105 and T-1106 have similar antiviral properties—see also section "Alphaviridae." FDA approval for use of favipiravir to treat filovirus infections is pending. Several animal pilot studies, most recently in non-human primates (NHP), have shown the efficacy of favipiravir [13, 14]). While extensively tested, ribavirin is not FDA approved for EBOV [65]. Other promising candidates (Table 1) are the FGI-106 entry inhibitor [8], CM-10-18-type glycan processing inhibitors, active against Marburg virus and Ebola virus in mouse models [25], a number of kinase inhibitors, including AR-12 (OSU-03012; [24, 102]), and K11777, a protease inhibitor developed for Chagas disease, which has additional activity against SARS-CoV and Ebola virus [154].

Alphaviridae

Alphaviruses are mosquito-borne viruses, but some can be effectively transmitted via the aerosol route from contaminated rodent feces. Rodents, birds and possibly marine species are maintenance reservoirs [43]. Alphaviruses can cause a number of diseases in humans, including Chikungunya fever, Eastern, Western and Venezuelan equine encephalitis. The handling of the respective viruses requires BLS-3 containment. Two type species, Venezuelan and Eastern Equine Encephalitis viruses (VEEV and EEEV), are considered

potential biological threat agents [107] with up to 70% mortality in unprotected populations [142] and represent category B select agents. While human infections with VEEV and EEEV are rare, sporadic and unpredictable but explosive epidemics caused by Chikungunya virus (CHIKV) have occurred in the last decade mainly in South-East Asia and in South America, Central America and the Caribbean, globally amounting to millions of cases. Autochthonous cases of Chikungunya fever have been reported in Italy [93]. Viremia with rashes and fever usually lead to death of cells lining joints, causing arthritis and joint pain. CHIKV infections of neurons can result in potentially fatal encephalitis. Fatal infections, mainly seen in human infants, are rare, but long-lasting polyarthralgia and encephalitis cause significant morbidity [95]. Alphaviruses are enveloped viruses with positive-sense ss-RNA genomes. Most experimental antivirals target the viral RNA polymerase. There are no licensed antiviral drugs against alphaviruses causing arthritis and encephalitis, and the treatment of infections is mainly supportive (anti-inflammatory drugs, glucocorticoids). *Anti-alphavirus drugs*. While pox- and filoviruses are highly lethal biological agents, alphavirus infections are rarely fatal, but can lead to large numbers of incapacitated individuals, due to severe arthralgias and headaches. In this sense, alphaviruses might be effective biological threat agents where incapacitation and saturation of medical care facilities are the goal of a perpetrator (incapacitating agents). Specific antivirals should be able to pass the blood brain barrier (BBB) to control postexposure encephalitis. Intravenous Ribavirin, which is FDA approved for HCV and respiratory syncytial virus (RSV) infection, does not pass the BBB, thus alleviating peripheral symptoms but not providing cure [1, 3]. Intranasal ribavirin may be more effective. Ribavirin resolves joint swelling in CHIKV [121], but has no activity against VEEV in vitro [45]. Sofosbuvir, an FDA-approved antiviral drug against HCV, which has been suggested for repurposing against various viruses, has been evaluated for in vitro activity against CHIKV [40]. Among the most promising novel compounds is the broad-spectrum antiviral candidate favipiravir (T-705), initially developed to treat human influenza, which shows a potent antiviral effect in small animal models. The drug is licensed in Japan, while FDA approval is pending [49]. An in vitro comparison between ribavirin and favipiravir revealed that efficacy is cell-type dependent [45]. Efficacy was also shown in a mouse model [2]. Other compounds of interest (Table 1) include drugs approved for other medical conditions and tested for repurposing. Those are the old antiparasitic suramin, which shows ameliorating effects against CHIKV infection in mice [82] and the anthelmintic ivermectin, which shows in vitro activity against a range of alphaviruses [141]. Compounds with known cellular targets include the cancer drugs mefenamic acid and sorafenib, inhibiting replication of CHIKV and other alphaviruses via

eIF4E dephosphorylation in vivo [90, 126], and halofuginone, a prolyl t-RNA synthetase inhibitor in veterinary use that is active in vitro against both alpha- and flaviviruses [66]. Also promising is the virus-specific antiviral ML336 that inhibits Nsp4 of VEEV and EEEV in vivo [72]. Less well-described compounds are LL-37 peptide, an alphavirus entry inhibitor in vitro [4], compound 25 that was identified in silico and optimized to inhibit CHIKV replication in vitro [9], Prest-37 and -392, with in vitro activity against VEEV nsP1 capping enzyme [41], and baicalin, which inhibits CHIKV replication in vitro by interfering with a cellular target [113].

Arenaviridae

Arenaviruses (Lassa virus—Old World/Junin, Machupo virus—New World) can also cause viral hemorrhagic fevers and are therefore on the list of potential biological threat agents (NATO AMed P-6 [107]; Argentine—Bolivian hemorrhagic fevers). Handling of Lassa virus (LassaV) requires BSL-4 containment. Annual case numbers of Lassa fever (LassaF) are estimated to be between 100.000 and 300.000 in West Africa, but the true public health burden of LassaF is unknown, as are exact case numbers on New World arenavirus infections [147]. Transmitted by aerosolized rodent droppings, arenavirus infections start with a generalized flu-like illness and then cause a range of conditions from aseptic meningitis/encephalitis with choroid plexus infiltration (Lymphocytic Choriomeningitis Virus; LCMV) to potentially fatal hemorrhagic fevers (Lassa, Junin, Guanarito, Machupo, Sabia, and Whitewater Arroyo Virus), with case fatality rates over 30%. Recently a person-to-person transmission of Lassavirus in Germany [148] and an outbreak in Nigeria raised public health concerns. Arenaviruses are enveloped viruses incorporating ribosomes ('arena' in Latin for sand; 'sand'-like appearance of ribosomes in electron microscopy of virus particles, hence arenavirus), with a Group IV genome of two ambisense ss-RNA segments. They use the ubiquitously expressed alpha-dystroglycan as their cellular receptor, and their main cellular targets are antigen-presenting cells. *Anti-arenavirus drugs*. Ribavirin is used under compassionate use protocols for the treatment of LassaF [97, 112], while recently favipiravir was evaluated and found to enhance survival in cynomolgus (crab-eating) macaques [125]. A further interesting compound is LHF 535, an entry inhibitor targeting arenaviral GP2 [91].

Bunyaviridae

Human pathogenic bunyaviruses, particularly Hantaviruses and Crimean-Congo Hemorrhagic Fever Virus (CCHFV), can cause hemorrhagic fevers, and CCHFV is on the list of potential biological threat agents (NATO AMed P-6 [107].

Handling of these viruses requires BSL-3/BSL-4 containment. Bunyaviruses have a wide host range, including plants, ticks (Hyalomma ticks—CCHFV), insects (Culex—Rift Valley fever virus) and rodents (Hantaviruses), which also serve as transmission vectors. Humans are dead-end hosts, suffering fatal outcomes in the case of Crimean-Congo hemorrhagic fever (CCHF), as well as in hemorrhagic fever with renal syndrome (HFRS; Europe—South-East Asia; Puumala/Hantaan-type viruses) and hantavirus pulmonary syndrome (HPS; Americas; Sin Nombre-type viruses). The clinical outcome is linked to geographical context and the typical animal vector. While high case fatality rates were described with the Korean hantavirus types and with Sin Nombre-type viruses causing HPS in the Americas, the European situation indicates a high case load with HFRS, but less severe clinical outcomes (nephropathia epidemica), caused mainly by Puumala-type viruses [17, 76, 127] as reported by the European Center for Disease Control [38]. Bunyavirus infections are endemic, vector-borne infections. Normally they do not cause epidemics, with the exception of nosocomially transmitted CCHF. Thousands of cases usually occur only in hyperendemic situations over a longer period of time. Beginning with an initial generalized flu-like illness and fever which lasts for about 3 days, these infections can end in fatal hemorrhagic fever (CCHF, HFRS), and pulmonary syndrome (HPS) with a 1–40% case fatality rate depending on virus strain [71]. Bunyaviruses are enveloped viruses with bi- and tri-segmented ambisense ss-RNA Group IV genomes. Human cellular receptors include human beta 3 integrins, the main human cellular targets are macrophages and endothelial cells, and bunyaviruses replicate in the cytoplasm. No vaccines or licensed treatments are currently available. *Anti-bunyavirus drugs*. The focus towards the identification of antiviral agents has mostly been on CCHFV infections, which are common in endemic areas, but are either asymptomatic or cause a nonspecific febrile illness that does not require hospitalization or specific treatment. Few patients develop hypotension and hemorrhage, and medical management is then largely supportive, with volume replacement, and prevention of edema and inflammation [68]. Ribavirin has been used to treat CCHF patients under compassionate use protocols with some success since 1985 [140], especially if given early in the course of the infection, but many studies with apparently beneficial results lack controls. Recent randomized clinical trials were unable to show significant beneficial effects of ribavirin versus CCHFV [70, 78]. Further interesting candidates for virus-specific treatment (Table 1) include favipiravir (T-705), which has been evaluated against a number of phleboviruses (PhleboV) and to treat CCHFV infection in rodent models [53, 54, 59], galidesivir (BCX4430), effective against Rift Valley fever virus (RVFV) infection in a hamster model and investigated for use by the FDA [145],

2′-fluoro-2′-deoxycytidine (2FdC), which showed protective effects against infections with PhleboV in a rodent model [134], and the FGI-106 entry inhibitor [135].

Flaviviridae

Flaviviruses causing hemorrhagic fever or severe encephalitis (Omsk hemorrhagic fever, Dengue and Yellow fever, Russian spring–summer encephalitis/Tick Borne Encephalitis (TBEV)) are listed as potential biological threat agents (NATO AMed P-6 [107]) and handling requires BSL-3/BSL-4 containment. Flaviviruses are arthropod-borne viruses that are endemic worldwide with virus/vector specific geographical distributions, causing regular outbreaks and fatalities, with 30.000 cases/year through yellow fever in Africa alone [51, 149]. Infections with flaviviruses can lead to hemorrhagic fevers (Omsk hemorrhagic fever, yellow fever (YF) and dengue fever with case fatality rates of up to 30%) or affect the CNS, causing encephalitis (e.g., Japanese encephalitis, tick borne encephalitis with case fatality rates up to 20%, Zika and West Nile encephalitis). Human-to-human transmission is not effective. Live vaccines against yellow fever (17D) and Japanese Encephalitis (JE), a number of inactivated TBEV vaccines, and most recently a live Dengue virus vaccine are available. Flaviviruses are a large family of mosquito- or tick-transmitted enveloped viruses with a Group IV positive-sense single-strand RNA genome, using G-protein coupled receptors for entry into host cells [42]. *Anti-flavivirus drugs*. Ribavirin is an effective early treatment for yellow fever under compassionate use protocols, but fails to improve survival of dengue infections in non-human primates (NHP; [92, 103]. Out of a quite large number of drugs investigated for repurposing against flaviviruses by the FDA (Table 1), the most promising candidate is sofosbuvir [18]. Sofosbuvir was initially developed and approved by FDA for treatment of hepatitis C. It shows activity against a number of flaviviruses in vitro and in the mouse model [33, 105]. Further interesting candidates (13 compounds listed in Table 1) inhibit the viral polymerase [39, 128]), NS2B/NS3 protease and kinases [23, 24]), cell entry and membrane trafficking [20, 109], and other flavivirus targets. The action and the efficacy of most of these compounds in vivo are yet to be determined. The major shortcoming of all candidates so far tested in animal models for the treatment of infections with Usutu (UsutuV), Dengue (DENV) and Zika viruses (ZikaV) is their rather low efficacy [24, 101].

Orthomyxoviridae

Orthomyxoviruses, in particular influenza viruses, although not on top of the list of potential biological threat agents, are fast-moving airborne pathogens capable of causing pandemics with significant mortality. Recombinant influenza

viruses could be considered as potential biological threat agents. Handling of avian influenza viruses and other influenza viruses with high pathogenic potential requires BSL-3 containment. Pandemic influenza viruses type A are transmitted by the respiratory route to birds and mammals, type B only from human to human, as well as via saliva, nasal secretions, feces and blood, causing acute respiratory distress with potentially fatal outcomes in humans. In humans, infection of the respiratory tract can lead to pneumonia, secondary pneumonia and overwhelming immune responses, followed by multiorgan failure in rare cases. Orthomyxoviruses are globally endemic, and cause sporadic outbreaks, rarely pandemics. Orthomyxoviruses are enveloped viruses with a negative-sense segmented ss-RNA genome. The viral RNA polymerase has a high error rate of 1/10,000. Vaccines are composed of HA/NA subunits (purified from inactivated virions), purified subunits from recombinant sources, or live/attenuated strains of the endemic strains/subtypes of influenza A virus (currently H1N1 and H3N2), as well as those of influenza B viruses [42]. *Anti-orthomyxovirus drugs*. FDA-approved neuraminidase inhibitors oseltamivir (Tamiflu®), zanamivir (Relenza®), laninamivir (Inavir®), and peramivir have marginal clinical benefits only when given early but may be useful in severe infections requiring hospitalization/mechanical ventilation [57]. In 2018 baloxavir-marboxil (Xofluza®), an inhibitor of the viral cap-dependent endonuclease (CEN; influenza virus polymerase PA subunit), was approved by the FDA for the treatment of acute, uncomplicated influenza among patients aged 12 years or older [80, 110]. Favipiravir developed and approved in Japan specifically for treatment of influenza virus infections, and its combination with neuraminidase inhibitors was shown to be effective in a mouse model [10, 48]. Further interesting candidates are haloxanide/nitazoxanide, thiazolide compounds that were originally developed as antiparasitic agents, but were shown to inhibit influenza virus hemagglutinin maturation and intracellular trafficking of viral components in infected cells and that are now in clinical trials [83, 138] as well as cycloheptathiophene-3-carboxamide, which interferes with the polymerase PA-PB1 subunits of influenza virus [106]. Alicyclic amines/aminoadamantanes amantadine and rimantadine, first described in 1985 as M2 protein blockers ([60]; H + channel/viroporin; only type A viruses), are not recommended anymore for clinical use (WHO/US), due to rapid induction of viral resistance mutations: 100% of clinical isolates are resistant. A 2014 Cochrane review found no evidence for efficacy or safety of amantadine for the treatment of influenza A [6]. However, their structures may still be useful as scaffolds for the design of future M2 inhibiting drugs.

Paramyxoviridae

Paramyxoviridae are fast-moving airborne pathogens infecting animals and humans. Hendra (HeV) and Nipah (NiV) viruses, in the genus *Henipavirus*, are considered zoonotic agents in Australia (horses) and South-East Asia (pigs), respectively. Both viruses may be able to infect other domesticated mammals, and there is a real concern in the veterinary and biodefense communities about spill-over infections and the high fatality rate in humans (632 human NiVcases: 59% case fatality [7, 131]. Henipaviruses have so far not caused global epidemics, but due to a high percentage of severe outcomes, as well as lack of vaccines or treatments, HeV and NiV are designated biosafety level (BSL-4) agents [106]. They are currently not on the NATO AMed P-6 list of biological threat agents but their potential as agents for bioterrorism has been discussed [84, 89]. Other Paramyxoviruses causing diseases in animals are canine distemper virus (CDV), endemic in Europe (dogs/humans; [11]), Newcastle disease virus affecting birds, and rinderpest virus infecting cattle. Human parainfluenza viruses and respiratory syncytial virus (RSV) are major causes of bronchiolitis, bronchitis and pneumonia in infants and children. Measles (morbilli, rubeola) caused by measles virus (MeaslesV) was responsible for around 733,000 deaths globally in 2000 [22], mostly due to viral pneumonia, secondary bacterial infections due to immune suppression (B cell tropism), and encephalitides [inclusion body encephalitis (MIBE); subacute sclerosing panencephalitis (SSPE)]. A very successful vaccine (MeaslesV strain Edmonston) has been used with the goal to eradicate measles in 2010 [62]. However, anti-vaccine movements have led to the loss of herd immunity and the reemergence of measles in many developed countries [28, 46]. Paramyxoviruses are a family of enveloped viruses with a negative-sense ss-RNA genome (mononegavirales) replicating in the cytoplasm [42]. *Anti-paramyxovirus drugs*. Ribavirin administered with cyclodextrin has been shown to be effective in a mouse model for measles encephalitis [69]. A very promising candidate antiviral against measles is ERDRP-0519, which has been shown effective against canine distemper virus in a ferret model [81]; however, early resistance development has been described [74]. Favipiravir has a protective effect against Nipah virus infections in the hamster model [29], and remdesivir inhibits a number of paramyxoviruses in vitro [88]. ddBCNAs (see sections "Poxviridae "and "Flaviviridae"; [99]) and the plant extract naphthoquinone droserone have anti-measles activities in vitro [87]. The nucleoside-analogue 4′-azidocytidine (R1479; balapiravir) was developed to inhibit HCV [108], paramyxoviruses, and filoviruses in vitro [63], but showed low efficacy and high toxicity in hepatitis C patients in early clinical trials [108].

Synergy through combination and the use of broad-spectrum antivirals

Combination treatments with antiviral compounds using different modes of action (MoA) are further increasing efficacy and, by means of individual dose reduction, allow for lower toxicity of the individual compounds. This exploits possible synergies between synthetic small molecules and natural extracts, virus-specific and broad-spectrum agents, and cell-targeting compounds. The use and potential benefits of multidrug cocktails, mainly reduction of resistance mutation and toxicity through dose reduction, have been pointed out by many authors, including in the context of yellow fever treatment [103]. Examples for synergistic effects in combinations of antiviral compounds with similar or different MoA are ribavirin with vitamin A in measles infections [12], ribavirin with favipiravir in Zika virus infections [75], and ribavirin with mefenamic acid in infections with Chikungunya virus [126]. Antiviral drug combinations may also be a way to deal with emerging antiviral drug resistance [74].

Broad-spectrum antivirals on the other hand show significant activity against several members of the same or distinct virus families, allowing the empirical treatment of severe viral infections prior to positive diagnosis of the viral agent. Leading examples are at his point the pyrazine-carboxamide compounds T-705 (favipiravir; [2, 7, 48]), T-1105 and T-1106, which are broad-spectrum viral RNA polymerase inhibitors, initially developed for the treatment of influenza virus, and found effective against bunyaviruses [21, 54, 59], alphaviruses [1], filoviruses [13] arenaviruses [125], paramyxoviruses [29], and flaviviruses [128]. A favipiravir resistance mechanism in influenza virus has been described [52]. Other potential broad-spectrum agents are remdesivir (GS-5734), another RNA polymerase inhibitor [137] active against filo-, corona-, and paramyxoviruses [88, 129, 130], FGI-106 with inhibitory activity against filo-, bunya-, and flaviviruses [8], galidesivir (BCX4430) with activity against filo-, bunya-, and flaviviruses [39, 143, 145], N^4-hydroxycytidine (NHC) inhibiting influenza-, paramyxo-, flavi-, corona-, as well as alphaviruses [152], and 2′fluoro-2′-deoxycytidine (2′-FdC), which was reported to inhibit various viruses in vitro, including Borna virus, HCV, Lassa virus, certain herpes viruses, and which also inhibits influenza viruses in mice [134]. Previously thought as a one-family-broad-spectrum compound, sofosbuvir (Sovaldi™, Soforal™) has in vitro and in vivo activity against several members of the family flaviviridae, and has most recently been shown to be effective against Chikungunya virus [40]. Natural product antivirals are single molecule natural compounds or complex mixtures of organic molecules (e.g., plant extracts) with antiviral activity. Natural product antivirals frequently exhibit broad-spectrum antiviral activity and often a single active compound cannot be identified in extracts [27].

Treatment of viral hemorrhagic fevers (VHF) with ribavirin

Viral hemorrhagic fevers (VHFs) cause the highest mortality in human hosts of all known viral agents and treatment options are a serious concern both in public health and in biodefense scenarios [67]. If specific antiviral treatment options are not available, supportive care is the mainstay of clinical interventions in VHF, including hemodynamic, hematological, pulmonary and neurological support treatments. Treatment with corticosteroids, vasoactive substances, hemodialysis, and mechanical ventilation saves the patients with the worst clinical symptoms. The only currently widely available antiviral drug, ribavirin, is not approved by the FDA for intravenous application in VHF and is used under compassionate use protocols only. Intravenous ribavirin reduces mortality of HFRS if combined with hemodialysis and both morbidity and mortality in the case of Lassa fever (LassaF). Ribavirin (Copegus™, Rebetol™, Virazole® ICN/Valeant (IND)) is used for the treatment of infections with African arenaviruses (Lujo- and Lassa fever) and bunyaviruses (HFRS, Crimean-Congo fever, and Rift Valley fever). However, intravenous ribavirin does not show any benefits for the treatment of any of the VHFs caused by filoviruses, or in infections with RNA viruses causing severe encephalitis [15, 67].

Conclusion

Antiviral drug development is determined by the virus life cycle, both the steps of viral replication per se and the cellular processes supporting viral replication. The action of antivirals targeting a viral replication step may be augmented by an antiviral hitting a different viral target or a cell process, or secondary effects via drug metabolism, resulting in synergy. Most antivirals in the experimental pipeline are either small molecules designed from scaffolds, mostly nucleoside analogues, or natural extracts/complex organic active compounds derived from extracts. The stages of antiviral drug development begin with in silico design and go via testing in single cell types (organotypic cell lines or primary cells) to determine IC50/CC50 = SI, and complex infection models to animal models, clinical trials, and eventually regulatory approval/market. A major hindrance to antivirals development is that of many compounds that show activity in vitro only very few are effective in animal models. Development may also stop for lack of interest and funding. Human organoids/complex in vitro infection models (e.g., barrier models) may provide a bridge to predict activity in clinical trials.

There are only a small number of antivirals with regulatory approval to treat virus infections, some of which have already been described to select for drug resistant strains. A number of drugs with antiviral activities which are approved for other conditions are being evaluated for repurposing, but the number of compounds currently in the experimental pipeline for clinical testing is small. Consequently, while there are treatment options, they may not be available in sufficient quantity in a biological threat situation. Therefore, research in identification, development, clinical testing and the stockpiling of approved antivirals in sufficient quantities must be a priority for the government actors put in charge of a credible response to deliberate releases of some of the biological agents discussed here. It is well known that even the threat of a biological attack would cause mass hysteria with concomitant economic disruption. Only timely preparation underlined by visible infrastructure, stockpiles of drugs and vaccines, and well-considered emergency plans will allow governments to give the necessary assurances when needed, to avoid negative outcomes [58]. Ideally, research on novel antivirals should also be a priority for research funding and pharmaceutical companies. As long as this is not the case, government funding and research in government-funded laboratories in collaboration with specialized university research groups organized in antiviral platforms have to step into the breach, when considerations of market performance and public health priorities are focusing resources elsewhere.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest, particularly, no recommendations regarding priority development of drugs or preferred use are made, except in the context of regulatory approval.

Research involving human and animal rights In this review article, research involving human participants and/or animals is reported and cited.

Informed consent Informed consent was required as per instructions to authors of the respective publishing journals.

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