**VG D-19-00236 - Title: 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, ortho- and paramyxoviruses and a number of arboviruses causing encephalitis, including alphaviruses and flaviviruses. All these viruses are of concern for public health services when they occur in natural outbreaks or emerge in unvaccinated populations. However, there is also a generally growing risk of dangerous biological agents being misused by the terror scene for malevolent purposes as exemplified by recent events and as revealed by intelligence reports. Public health responses commonly used in natural disasters and 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 is the availability of post-exposure prophylaxis based on a number of antiviral treatment options. These issues had motivated the organizers of the 16th Medical Biodefense Conference, held in Munich in 2018, to address aspects of antiviral research in this particular context in a special session. Following this thematic approach our review will provide an overview of antiviral compounds in the pipeline that are already approved for use or still under development and which target agents currently perceived as a threat to societies or associated with a potential for misuse as biothreat agents.

**Keywords:** Medical biodefense, antiviral, BSL3 / 4 viral pathogens.

## 1. Introduction

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

**Biodefense relevance.** While infections with arthropod-borne and tropical viruses are fairly common in nature, severe outcomes are usually 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. 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. 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 and others.

**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 are 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 70s (Petersen et al., 2019), while outbreaks of mosquito-borne Chikungunya virus (Levi and Vignuzzi, 2019) and Dengue virus infections in the Indian Ocean islands were seen mostly in the 21st century (Robert et al., 2019). The historic Ebola outbreak in West-Africa in 2013-2014, followed by a more recent one in the Republic of Congo with 1891 fatalities (Dyer, DRC 2019), 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 (Quick et al., 2016). 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, is continuously 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 did 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 (FDA; Grosenbach et al., 2018).

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

86 countermeasures for such infections is woefully small.

87 VHFs are caused by infection with RNA viruses. The standard of treatment for
88 RNA virus infections where it shows efficacy, is ribavirin, developed in 1963 (De Clercq
89 and Li, 2016). Where possible, early start of treatment of acute virus infections with
90 existing drugs gives the best results and, in this context, accurate and rapid virus diagnosis
91 is essential. The crucial role of a well-organized public health system and classic quarantine
92 approaches was demonstrated in the recent Ebola outbreaks in West- and Central Africa.
93 However, the need for new antiviral agents had generally been recognized and been
94 reviewed by David Freestone as early as 1985 (Freestone, 1985). While many virus
95 infections are asymptomatic, new or improved antiviral drugs are needed for the prevention
96 and/or treatment of a number of significant conditions caused by viruses which at present
97 cannot be controlled by alternative measures, including vector control, immunization and
98 treatment with existing antiviral drugs. The need for specialized BSL-3/BSL-4 facilities
99 with trained personnel for experiments with life viruses, and animal challenge, has further
100 restricted research to a few high-security sites worldwide. As a result, there are no FDA-
101 approved antivirals for Ebola or the causative viral agents of many other viral hemorrhagic
102 fevers, viral encephalitides, and respiratory infections. Few therapeutic interventions are
103 available except for supportive therapy.

104 In the following sections we will give a summary of the antivirals session held
105 during the 16$^{th}$ MBDC, as well as an overview of antiviral drug development
106 methodologies and selected experimental antivirals designed for potential biothreat agents.

107 **2. MBDC 2018 – Antivirals Session**

108 After an introduction on the chances and challenges encountered in the development
109 of novel antivirals (Brancale - MBDC-2018-GO1), a discussion on the current conditions in
110 UK/ EU research networks, obstacles at the interface between research and industry, and
111 preparedness for the treatment of infections with biodefense-related viruses followed.
112 Further contributions outlined the methodical approach to antiviral design and biological
113 evaluation (Fig. 1.). Using examples from chemists present at the meeting, the structural
114 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 (McGuigan et al., 2010; Slusarczyk et al., 2018), 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 -> 10^2 selected candidates). The compounds are then synthesized, shipped and compared at a standard concentration (10µM 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 (generally over 50 µM), 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 (Kola et al., 2004). 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 (Koban et al., 2018). 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 (Huggins et al., 1999), 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, Yates et al., 2019-), 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 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).

## 3. Antivirals - FDA approved and experimental

Complementing the recent review by De Clercq and Li (De Clercq and Li, 2016) 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.

### 3.1 *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 (Dixon, 2005), and remains on the top of the list of biological threat agents for warfare or bioterrorism (NATO AMedP-6; Delaune et al., 2017). 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 as a potential biological warfare agent (Delaune et al., 2017). **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 (Fields, 2013). Poxviruses enter cells by macropinocytosis, but a poxvirus-specific receptor is still elusive (Mercer and Helenius, 2009). **Anti-poxvirus drugs**. One of the first effective drugs in clinical use as a parenteral treatment in severe OPV infections was **cidofovir**, a biphosphononate developed at REGA, in Belgium (De Clercq, 2002; Delaune et al., 2017) 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 (Parker et al., 2008,2014; Trost et al., 2015; Chittick et al., 2017; Foster et al., 2017; Grossi et al., 2017; Pires et al., 2018). 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 nonhuman primates infected with variola, and humans exposed to OPV (Mucker etal., 2013; Grosenbach et al., 2018; Pires et al., 2018, Whitehouse et al., 2019). 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 (Smee and Sidwell, 2003). Further candidate anti-poxvirus drugs include kinase inhibitors **imatinib** (Gleevec/STI-571; Reeves et al., 2005 a,b**)** and **olomoucine** (Holcakova et al., 2010), **terameprocol** (Pollara et al., 2010), **mitozandrone** (Altmann et al., 2012), the membrane targeting **ddBCNA cf2642** (Mcguigan et al, 2013), **bisbenzimide** derivatives (Yakimovich et al., 2017), **FC-6407**, a OPV D4 processivity factor mimic (Nuth et al., 2019), a number of natural extracts that have shown interesting antiviral activity against OPV in in vitro infection models (Cryer et al., 2017; Zanetta, 2019; **Table 1**).

### 3.2 *Filoviridae*

Filoviruses are category A select agents, World Health Organization risk group 4 pathogens, high on the list of potential biological threat agents (NATO AMedP-6), 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 individuals and has been approved in October 2019 in the EU as the world's first Ebola vaccine (Callaway E, 2019). **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 (Osuna-Ramos et al., 2018) and the TIM-1 (HAVCR1) receptor on the surface of T cells, also relevant for hepatitis C virus (HCV) entry (Kachko A. et al., 2018), 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 (Bray and Paragas, 2002; Bray, 2003), 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** (WHO, 2019). The trial was stopped early with REGN-EB3 and mAb114 giving the greatest chance to survive Ebolavirus infection. The WHO recommends, to use these two Mabs for all further treatments (WHO, 2019). **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 (Warren et al., 2017), 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;** Furuta et al., 2002**),** 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 (Furuta et al., 2013, Delang et al., 2018). T705 and the related pyrazinecarboxamide compounds T-1105 and T-1106 have similar antiviral properties - see also section 3.3. (Alphaviruses). FDA approval for use of favipiravir to treat filovirus infections is pending. Several animal pilotstudies,

most recently in nonhuman primates (NHP), have shown the efficacy of favipiravir (Bixler et al., 2018a + b). While extensively tested, **ribavirin** is not FDA-approved for EBOV (Huggins, 1989). Other promising candidates (**Table 1**) are the **FGI-106** entry inhibitor (Aman et al., 2009), **CM-10-18** type glycan processing inhibitors, active against Marburg virus and Ebola virus in mice models (Chang et al., 2013), a number of kinase inhibitors, including **AR-12** (OSU-03012; Mohr et al., 2015; Chan et al., 2018), and **K11777**, a protease inhibitor developed for Chagas disease, which has additional activity against SARS-CoV and Ebola virus (Zhou et al., 2015).

### 3.3 *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 (Forrester et al., 2012). 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 (NATO AMedP-6) with up to 70% mortality in unprotected populations (Walton and Johnson, 1988) 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 (Marano et al., 2017). 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 (Matusali et al., 2019). **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 post-exposure 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 (Abdelnabi et al., 2015). Intranasal ribavirin may be more effective. Ribavirin resolves joint swelling in CHIKV (Ravichandran and Manian, 2008), but has no activity against VEEV in vitro (Franco et al., 2018). **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 (Ferreira et al., 2019). 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 (Furuta et al., 2013). An in vitro comparison between ribavirin and favipiravir revealed that efficacy is cell-type dependent (Franco et al., 2018). Efficacy was also shown in a mouse model (Abdelnabi et al., 2017). 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 (Kuo et al., 2016) and the anthelmintic **ivermectin**, which shows in vitro activity against a range of alphaviruses (Varghese et al., 2016). 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 (Rothan et al., 2016; Lundberg et al., 2018), and **halofuginone**, a prolyl t-RNA synthetase inhibitor in veterinary use that is active in vitro against both alpha- and flaviviruses (Hwang et al., 2019). Also promising is the virus-specific antiviral **ML336** that inhibits Nsp4 of VEEV and EEEV in vivo (Jonsson et al., 2019). Less well described compounds are **LL-37 peptide**, an alphavirus entry inhibitor in vitro (Ahmed et al., 2019), **compound 25** that was identified in silico and optimized to inhibit CHIKV replication in vitro (Bassetto et al., 2013), **Prest-37 and -392**, with in vitro

activity against VEEV nsP1 capping enzyme (Ferreira-Ramos et al., 2019), and **baicalin**, which inhibits CHIKV replication in vitro by interfering with a cellular target (Oo et al., 2018).

### 3.4 *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; 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 (WHO Roadmap Neclected Tropical Diseases, 2012). 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 White Water Arroyo Virus), with case fatality rates over 30%. Recently a person-to-person transmission of Lassavirus in Germany (WHO, 2016) and an outbreak in Nigeria raised public health concerns. **Arenaviruses** are enveloped viruses incorporating ribosomes (‘arena’ is 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 (McCormick et al., 1986; Ölschläger et al., 2011), while recently **favipiravir** was evaluated and found to enhance survival in cynomolgus (crab-eating) macaques (Rosenke et al., 2018). A further interesting compound is **LHF 535**, an entry inhibitor targeting arenaviral GP2 (Madu et al., 2018).

### *3.5 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). 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 (Bugert et al., 1999; Klempa et al., 2003; Schmidt-Chanasit et al., 2009; Report of the European Center for Disease Control 2016). Bunyavirus infections are endemic, vector-borne infections. Normally they do not cause epidemics, with the exception of CCHF in case of nosocomial transmission. 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 three 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 (Jonsson et al., 2008). **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 they replicate in the cytoplasm. No vaccines or licensed treatments are currently available. **Anti-bunyavirus drugs**. The focus towards the identification of antiviral agents has been mostly on CCHFV infections, which are common in endemic areas, but are either asymptomatic or cause a non-specific 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 (Jabbari et al., 2012). **Ribavirin** has been used to treat CCHF patients under compassionate use protocols with some success since 1985 (van Eeden et al., 1985), 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 (Koksal et al., 2010; Johnson et al., 2018). 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 (Gowen and Holbrook, 2008; Gowen et al., 2010; Hawman et al., 2018), **galidesivir** (BCX4430), effective against Rift Valley fever virus (RVFV) infection in a hamster model and investigated for use by the FDA (Westover et al., 2018), 2′-fluoro-2′-deoxycytidine (**2FdC**), which showed protective effects against infections with PhleboV in a rodent model (Smee et al., 2018), and the **FGI-106** entry inhibitor (Smith et al., 2010).

### *3.6 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) 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 (Garske et al., 2014; WHO, 2018). 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 (Fields, 2013). **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; Malinoski et al., 1990; Monath, 2008). Out of a quite large number of drugs investigated for repurposing against flaviviruses by the FDA (**Table 1**), the most promising candidate is **sofosbuvir** (Bullard-Feibelman et al., 2017). 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 (Mumtaz et al., 2017; de Freitas et al., 2019). Further interesting candidates (13 compounds listed in **Table 1**) inhibit the viral polymerase (Eyer et al., 2017; Segura Guerrero et al., 2018), NS2B/NS3 protease and kinases (Chan et al., 2017; Chan et al., 2018), cell entry and membrane trafficking (Nolte et al., 2016, Cannalire et al., 2019), 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 (Milligan et al., 2018; Chan et al., 2018).

### 3.7 *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 require 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 ssRNA genome. The viral RNA polymerase has a high error rate of 1/10000. 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 (Fields, 2013). **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 (Gubareva et al., 2017). 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 (Noshi et al., 2018, Koszalka et al., 2019). **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 (Furuta et al., 2002, Baz et al., 2018). Further interesting candidates are: **haloxanide**/**nitazoxanide**, thiazolide compounds that were originally developed as anti-parasitic 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 (Tilmanis et al., 2017; La Frazia et al., 2018) as well as **cycloheptathiophene**-3-carboxamide, which interferes with the polymerase PA-PB1 subunits of influenza virus (Nannetti et al., 2019). Alicyclic amines/aminoadamantanes **amantadine and rimantadine**, first described in 1985 as M2 protein blockers (Hay et al., 1985; 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 (Alves Galvao et al., 2014). However, their structures may still be useful as scaffolds for the design of future M2 inhibiting drugs.

### 3.8 *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; Ang et al 2018; Singh at al. 2019). 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 (Nannetti et al., 2019). 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 (Lam 2003; Luby 2013). Other Paramyxoviruses causing diseases in animals are: canine distemper virus (CDV), endemic in Europe (dogs/humans; Beineke et al., 2015), 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 (CDC, 2009), 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 (Holzmann et al., 2016). However, anti-vaccine movements have led to the loss of herd immunity and the reemergence of measles in many developed countries (Dahl, 1986; Fraser-bell, 2019). **Paramyxoviruses** are a family of enveloped viruses with a negative sense ss RNA genome (mononegavirales) replicating in the cytoplasm (Fields, 2013). **Anti-paramyxovirus drugs**. **Ribavirin** administered with cyclodextrin has been shown to be effective in a mouse model for measles encephalitis (Jeulin et al., 2009). A very promising candidate antiviral against measles is **ERDRP-0519**, which has been shown effective against canine distemper virus in a ferret model (Krumm et al., 2014), however early resistance development has been described (Kalbermatter et al., 2019). **Favipiravir** has a protective effect against Nipah virus infections in the hamster model (Dawes et al., 2018),

**remdesivir** inhibits a number of paramyxoviruses in vitro (Lo et al., 2017). ddBCNAs (see section 3.1 and 3.6; McGuigan et al., 2013) and the plant extract **naphthoquinone droserone** have anti-measles activities in vitro (Lieberherr et al., 2017). The nucleoside analogue 4'-azidocytidine (R1479; balapiravir) was developed to inhibit HCV (Nelson et al. 2012), paramyxoviruses, and filoviruses in vitro (Hotard, et al, 2017), but showed low efficacy and high toxicity in hepatitis C patients in early clinical trials (Nelson et al., 2012).

**Table 1**

| **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<br>in vitro<br>Phase II clinical trial DRC- 2018-2019 |
| Favipiravir (T705) | EBOV/ Pol | Bixler 2018a | appr. in Japan - Influenza<br>in vivo |

| | | | |
|---|---|---|---|
| Galidesivir (BCX4430) | RVFV/ Pol | Warren 2014<br>Taylor 2016 | IND<br>in vivo |
| CM-10-18 | EBOV-MARV/<br>a Gluc.<br>ER enzymes | Chang 2013 | in vivo |
| FGI-106 | EBOV/ entry | Aman 2009 | in vitro |
| AR-12 (OSU 03012) | EBOV-MARV /<br>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;<br>RSV<br>in vivo |
| Sofosbuvir | CHIKV/ Pol | Ferreira 2019 | FDA-appr. HCV<br>in vitro |
| Favipiravir (T705) | CHIKV/ Pol | Abdelnabi 2017 | appr. in Japan -<br>Influenza<br>in vivo |
| Suramin (Germanin™, Antrypol™) | CHIKV/ unclear | Kuo 2016 | FDA-appr.<br>antiparasitic<br>in vivo |
| Ivermectin | CHIKV/ unclear | Varghese 2016 | FDA-<br>anhelmintic<br>in vitro |
| Mefenamic acid | CHIKV/ eIF4E dephosphorylation | Rothan 2016 | FDA-cancer<br>in vivo |
| Sorafenib | CHIKV, VEEV, EEEV/<br>eIF4E<br>dephosphorylation | Lundberg 2018 | FDA-cancer<br>in vitro |
| Halofuginone | CHIKV/<br>Protyl tRNAse | Hwang 2019 | Veterinary use<br>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/<br>Pol, GTP depletion, mutagenic | McCormick 1986 | FDA-appr. HCV;<br>RSV<br>Compassionate use LassaF |

| | | | |
|---|---|---|---|
| Favipiravir (T705) | LassaV/ Pol | Rosenke 2018 | appr. in Japan - Influenza<br>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<br>Compassionate use CCHF |
| Favipiravir (T705) | PhleboV, CCHFV/ Pol | Gowen 2010<br>Hawman 2018 | appr. in Japan - Influenza<br>in vivo |
| Galidesivir | RVFV/ Pol | Westover 2018 | IND<br>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 +/<br>Pol, GTP depletion, mutagenic | Malinoski 1990 | FDA-appr. HCV; RSV<br>Compassionate use YF |
| Sofosbuvir | ZikaV, YFV +/ Pol | Bullard-Feibelman 2017<br>De Freitas 2019 | FDA-appr. HCV<br>in vivo |
| Favipiravir (T705) | UsutuV/ Pol | Seguera Guerrero 2018 | appr. in Japan - Influenza<br>in vivo |
| Ivermectine | YFV +/ Helicase | Mastrangelo 2012 | FDA-appr. antihelmintic<br>in vitro |
| Bromocriptine | ZikaV/ Prot (Dopamine agonist) | Chan 2017 | FDA-appr. Diabetes/ Parkinson<br>in vitro |
| Erythrosin B | DENV +/ Prot | Li 2018 | FDA-appr. food additive<br>in vitro |
| Niclosamide | YFV +/ entry/fusion-translation | Mazzon 2019 | FDA-appr. antihelmintic<br>in vivo |
| Galidesivir (BCX4430) | TBEV, WNV / Pol | Eyer 2017 | IND<br>in vitro |
| AR-12 (OSU 03012) | ZikaV / PI3K-Akt pathway | Chan 2018 | IND-NSAID<br>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 |
| ***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 |
| | | | |

**Table 1 Legend**

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

Abbreviations:

Appr.: approved

FDA: US Food and Drug Administration

519 IND (FDA investigational drug)
520 NHP: non-human primates
521 NSAID: nonsteroidal anti-inflammatory drug
522 Phase: clinical trial phase I to III
523 Pol: viral polymerase
524 Prot: viral protease
525 Vs.: versus
526 Yellow highlight: lead small molecule drug candidate
527 +: more viruses, not listed
528

529 **3.9 Synergy through combination and the use of broad-spectrum antivirals**

530 Combination treatments with antiviral compounds using different modes of action
531 (MoA) are further increasing efficacy and, by means of individual dose reduction, allow for
532 lower toxicity of the individual compounds. This exploits possible synergies between
533 synthetic small-molecules and natural extracts, virus-specific and broad-spectrum agents,
534 and cell-targeting compounds. The use and potential benefits of multidrug cocktails, mainly
535 reduction of resistance mutation and toxicity through dose reduction, have been pointed out
536 by many authors, including in the context of yellow fever treatment (Monath, 2008).
537 Examples for synergistic effects in combinations of antiviral compounds with similar or
538 different MoA are ribavirin with vitamin A in measles infections (Bichon et al., 2017),
539 ribavirin with favipiravir in Zika virus infections (Kim et al., 2018), and ribavirin with
540 mefenamic acid in infections with Chikungunya virus (Rothan et al., 2016). Antiviral drug
541 combinations may also be a way to deal with emerging antiviral drug resistance
542 (Kalbermatter et al., 2019).

543 Broad-spectrum antivirals on the other hand show significant activity against
544 several members of the same or distinct virus families, allowing the empirical treatment of
545 severe viral infections prior to positive diagnosis of the viral agent. Leading examples are at
546 his point the pyrazine-carboxamide compounds **T-705** (**favipiravir**; Furuta et al., 2002;
547 Abdelnabi et al., 2017, Delang et al., 2018), T-1105 and T-1106, which are broad-spectrum

viral RNA polymerase inhibitors, initially developped for the treatment of influenza virus, and found effective against bunyaviruses (Gowen et al., 2010; Caroline et al., 2014; Hawman et al., 2018), alphaviruses (Abdelnabi et al., 2015), filoviruses (Bixler et al., 2018a) arenaviruses (Rosenke et al., 2018), paramyxoviruses (Dawes et al., 2018), and flaviviruses (Seguera-Guerrero, 2018). A favipiravir resistance mechanism in influenza virus has been described (Goldhill et al, 2018). Other potential broad-spectrum agents are: **remdesivir** (GS-5734), another RNA polymerase inhibitor (Tchesnokov et al., 2019) active against filo-, and paramyxoviruses (Lo et al., 2017), **FGI-106** with inhibitory activity against filo-, bunya-, and flaviviruses (Aman et al., 2009), **galidesivir (BCX4430)** with activity against filo- , bunya-, and flaviviruses (Warren et al., 2014; Eyer et al., 2017; Westover et al., 2018) 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 (Smee et al., 2018). 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 (Ferreira et al., 2019). 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 (Cryer et al., 2017).

### 3.10 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 (Ippolito et al., 2012). If specific antiviral treatment options are not available, supportive care is the mainstay of clinical interventions in VHF, including haemodynamic, haematological, 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 (Bray and Paragas, 2002; Ippolito et al., 2012).

## 4. Conclusions

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 is 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 (Hawley and Eitzen, 2001). 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 antivirals platforms have to step into the breach, when considerations of market performance and public health priorities are focusing resources elsewhere.

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## 6. Declarations

The authors declare no conflict of interest, particulary, no recommendations regarding priority development of drugs or preferred use are made, except in the context of regulatory approval. In this review article, research involving Human Participants and/or Animals is reported and cited. Informed consent was required as per instructions to authors of the respective publishing journals.

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