

1 **VG D-19-00236 - Title: Antivirals in Medical Biodefense**

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9 **Abstract**

10 The viruses historically implicated or currently considered as candidates for misuse
11 in bioterrorist events are poxviruses, filoviruses, bunyaviruses, ortho- and paramyxoviruses
12 and a number of arboviruses causing encephalitis, including alphaviruses and flaviviruses.
13 All these viruses are of concern for public health services when they occur in natural
14 outbreaks or emerge in unvaccinated populations. However, there is also a generally
15 growing risk of dangerous biological agents being misused by the terror scene for
16 malevolent purposes as exemplified by recent events and as revealed by intelligence
17 reports. Public health responses commonly used in natural disasters and outbreaks of
18 infectious disease may not be sufficient to deal with the severe consequences of a deliberate
19 release of such agents. One important aspect of countermeasures against viral biothreat
20 agents is the availability of post-exposure prophylaxis based on a number of antiviral
21 treatment options. These issues had motivated the organizers of the 16th Medical
22 Biodefense Conference, held in Munich in 2018, to address aspects of antiviral research in
23 this particular context in a special session. Following this thematic approach our review
24 will provide an overview of antiviral compounds in the pipeline that are already approved
25 for use or still under development and which target agents currently perceived as a threat to
26 societies or associated with a potential for misuse as biothreat agents.

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

28 **1. Introduction**

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

33 **Biodefense relevance.** While infections with arthropod-borne and tropical viruses
34 are fairly common in nature, severe outcomes are usually rare. Therefore, countermeasures
35 against such unlikely events, especially in the developed world, are regarded as giving little
36 or no return on investments and are sidelined by grant driven research and manufacturers.
37 While this is a legitimate point of view for academia and the pharmaceutical industry,
38 governments have to consider countermeasures against rare agents released, or threatened
39 to be released deliberately by individuals or groups aiming to cause maximum societal
40 disruption and chaos. For such events governments have to prepare credible
41 countermeasures in order to be able to provide prophylaxis, isolation, and treatment for
42 large numbers of exposed and infected individuals. This requires research into these
43 countermeasures, including the development, testing and stockpiling of vaccines and
44 antiviral drugs, particularly for dangerous biological agents. This review will focus on viral
45 agents that fit into this category, briefly discussing their relevance for public health and
46 biodefense, mode of action, and give an overview of treatment options available or in the
47 pipeline. The basis of all considerations on countermeasures and biothreat preparedness is
48 an agent-related risk assessment, which includes numerous criteria like availability of
49 stocks or samples for potential perpetrators, ease of handling, pathogenicity, transmission
50 pathways, tenacity and others.

51 **Public health relevance.**

52 Viral hemorrhagic fevers (VHFs) cause the highest mortality in human hosts among
53 all known viral agents. Encephalitides and severe respiratory infections caused by a range
54 of viruses are other diseases with often severe clinical outcomes. The recent emergence of

55 such infections from geographical hotspots are mainly a consequence of the rapid
56 development of ground and air transport. Vector-borne infections are also affected by
57 climate change. Large scale outbreaks were first described for Monkeypox virus in central
58 Africa in the 70s (Petersen et al., 2019), while outbreaks of mosquito-borne Chikungunya
59 virus (Levi and Vignuzzi, 2019) and Dengue virus infections in the Indian Ocean islands
60 were seen mostly in the 21st century (Robert et al., 2019). The historic Ebola outbreak in
61 West-Africa in 2013-2014, followed by a more recent one in the Republic of Congo with
62 1891 fatalities (Dyer, DRC 2019), has attracted extensive media attention. The rapid and
63 uncontrolled spread of Ebola fever in Africa has been considered as a threat for the national
64 security of developed countries with regard to the risk of imported cases but also for
65 economic reasons. The Bundeswehr Institute of Microbiology (IMB) was involved in the
66 international effort to contain Ebola fever in West-Africa during the 2014-2016 outbreak
67 (Quick et al., 2016). The institute also runs a research program for antiviral drug
68 development and hosts the biennial Medical Biodefense Conference (MBDC). Antiviral
69 compounds and their possible role in biodefense were a special theme during the MBDC in
70 2018. The selection of topics with a focus on pox-, alpha- and flaviviruses was guided by
71 the NATO AMedP- 6 ‘Handbook on the medical aspects of nuclear, biological and
72 chemical (NBC) defensive operations –Part II’. Smallpox, albeit eradicated in nature, is
73 continuously perceived as a threat for several reasons, one of them being the risk that
74 variola virus might be brought back with the methods of synthetic biology. Military forces
75 and first responders in many countries were revaccinated in the early 2000s for fear that
76 Iraq might have weaponized smallpox virus (which it did not, as was revealed later on).
77 Emergency plans were developed to deal with a deliberate release. While no licensed drug
78 was available at the time to treat infections with variola virus, a drug effective against
79 orthopoxviruses, tecovirimat, has recently been approved by the United States Federal Drug
80 Administration (FDA; Grosenbach et al., 2018).

81 Smallpox as an exclusively human infection was eradicated by vaccination, but this
82 is impossible for zoonoses like yellow fever, which has a number of non-human reservoir
83 hosts. This is an important distinction, and in the case of an acute zoonotic viral infection,
84 post-exposure antiviral treatment of the unvaccinated is a potentially lifesaving option in
85 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 16th 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

115 antivirals targets, i.e. small molecule inhibitors of polymerases, proteases,
116 methyltransferases, and ProTide-based improvements of antiviral nucleosides (McGuigan
117 et al., 2010; Slusarczyk et al., 2018), were explained in detail. The dynamic models are
118 based on solved NMR structures of protein targets. The preselection of virtual candidate
119 antiviral compounds in in silico models against viral protein targets reduces the number of
120 compounds by four magnitudes (10^6 library \rightarrow 10^2 selected candidates). The
121 compounds are then synthesized, shipped and compared at a standard concentration ($10\mu\text{M}$
122 at IMB) for comparative effectiveness and toxicity in organotypical cell lines against a
123 panel of viruses of interest for the biodefense community, including alpha-, bunya-, filo-,
124 flavi-, ortho-/paramyxo-, and poxviruses. Hit compounds with high efficacy and low
125 toxicity are identified (Step 2). This is followed by $\text{IC}_{50}/\text{CC}_{50}$ evaluation (Step 3) of
126 emerging hit to lead compounds, aiming for selective indices > 30 in sensitive (e.g. Huh-7
127 hepatoma cells) and organotypical cell lines selected for the pathogenic traits of the viruses
128 of interest (e.g. U138 glioblastoma cells for encephalitis viruses). This usually results in
129 another reduction of candidate numbers by one to two magnitudes. To confirm drug targets,
130 target validation is then carried out, either by the use of enzymatic assays for viral enzyme
131 targets (Silvestri - MBDC-2018- GO3), or by induction of resistant virus strains showing
132 resistance mutations in the antiviral target areas, as shown with tecovirimat (ST-246) for
133 orthopoxviruses. This concludes the classical in vitro evaluation of antiviral drug
134 candidates. The winnowing process up to this point leads to a reduction ratio of six
135 magnitudes (10^6 to 1). If in vitro toxicity is minimal (generally over $50\mu\text{M}$), the
136 compounds go straight into pharmacokinetics testing (rodent models), and into animal
137 models of viral infections (Step 5). Here a dramatic rate of attrition leads to only one out of
138 ten compounds tested in animal models making it into phase I clinical studies (Kola et al.,
139 2004). To further select compounds prior to animal testing, complex infection models,
140 including in vitro 3D models, are currently the focus of much research in the antivirals field
141 (Koban et al., 2018). Functional models of virus infection at barriers, and the effect of
142 antivirals on the virus passing the barrier, give an indication of antiviral effects on typical
143 viral pathogenesis, e.g. encephalitis viruses that are being tested on models of the blood
144 brain barrier (Step 4; Hurler –MBDC-2018- GP1). A successful prediction by in vitro
145 functional models of antivirals efficacy in vivo, particularly using primary human

146 organotypic cells, would also result in a significant reduction of unsuccessful drug testing
147 in animal models. The evaluation cycle described above, follows the general considerations
148 as outlined by Huggins et al. for Ebolavirus (EBOV) in 1999 (Huggins et al., 1999), with
149 the addition of in silico design with dynamic models for compound preselection, which had
150 not yet been available at that time, and represents a methodical approach to antiviral
151 design and development. This approach is used by groups active in the field and is also the
152 basis of the ‘Antivirals Platform’ collaboration between Cardiff University and IMB into
153 prophylaxis and treatment of infections caused by viral biothreat agents, which is funded by
154 SER CYMRU/MRC and IMB’s basic funding. The platform established comprises all steps
155 from molecular design to in vitro testing in complex infection models. Talks at MBDC
156 2018 included different examples of this approach towards antiviral drug discovery: in
157 silico design of small nucleosidic antivirals and prodrugs against arboviruses (Bassetto-
158 MBDC-2018-GO2, Yates et al., 2019-), Cima-4, Den-12, MB-124, tick borne encephalitis
159 (TBEV) polymerase inhibitor nucleoside analogues with superior activity in the central
160 nervous system (CNS) cells compared to sofosbuvir (Bugert-MBDC-2018-GO3), novel
161 protease inhibitors for Zika virus as surrogate virus for other flaviviruses using an
162 enzymatic assay for target validation as well as a Zika mouse model (Silvestri-MBDC-
163 2018-GO4), and BB4-D9, a dandelion natural extract antiviral against poxviruses (Zanetta
164 –MBDC-2018-GO5). FDA approval of oral TPOXX® (Tecovirimat/ST-246®), a F13L
165 morphogenesis inhibitor of orthopoxviruses, was reported in the poxvirus session
166 (Grosenbach-MBDC-2018-HO2). Posters provided meaningful examples of the evaluation
167 cycle, with contributions on live cell imaging of virus-infected cells for antivirals testing in
168 a model of the blood brain barrier (Hurler-MBDC-2018-GP2), a novel polymerase-
169 inhibiting CHIKV antiviral (MB-70, Hucke-MBDC-2018-GP4), a NS4a autophagy testing
170 system for flaviviruses (Tscherne-MBDC-2018-GP5), and MoA studies on Cf2642
171 inhibiting macropinocytosis of measles and poxviruses for use as synergistic cell targeting
172 antiviral along with virus-specific compounds (Narayan-MBDC-2018-GP6).

173

174

175 **3. Antivirals - FDA approved and experimental**

176 Complementing the recent review by De Clercq and Li (De Clercq and Li, 2016)
177 this section will focus on small-molecule antiviral compounds and discuss a selection of
178 compounds that are either FDA approved or lately proved effective against viruses
179 associated with a biothreat risk in in vitro experiments, animal or phase I-III clinical
180 studies. Subsections give a brief overview of the viral agents in the order of relevance for
181 biodefense, the FDA-approved treatment options, and antivirals in development, with top
182 candidates highlighted in yellow in **Table 1**, which lists virus-specific compounds in the
183 same order of relevance, detailing compound class, target and stage of development.

184

185 **3.1 Poxviridae**

186 Variola virus (smallpox virus), a member of the orthopoxvirus (OPV) genus of the
187 family *poxviridae*, was used in the 18th century as a biological warfare agent by British and
188 American forces in North America (Dixon, 2005), and remains on the top of the list of
189 biological threat agents for warfare or bioterrorism (NATO AMedP-6; Delaune et al.,
190 2017). Effective vaccines and FDA-approved antivirals exist and could be used to control a
191 deliberate release. Variola virus (VariolaV), which only infects humans, was declared
192 eradicated in 1980, after a global vaccination campaign. Handling of VariolaV requires
193 BSL-4 containment. Virus stocks are officially kept in only two designated laboratories in
194 Russia and the US. Monkeypox virus (BSL-3), a zoonotic agent causing sequelae similar to
195 smallpox but less fatal, is endemic in central Africa (Democratic Republic of Congo;
196 DRC), recent introductions to the UK were travel-related. Poxviruses are transmitted by
197 contact infection and via the respiratory tract, causing a systemic infection in humans and
198 animals. Smallpox virus infection leads to a fatal multiorgan failure syndrome within 7-14
199 days, in complicated cases with a hemorrhagic syndrome and CNS involvement. Smallpox
200 has played a role in large-scale epidemics in history and its causative agent continues to be
201 considered as a potential biological warfare agent (Delaune et al., 2017). **Orthopoxviruses**
202 **(OPV)** are ovoid-shaped enveloped viruses with Group I double stranded (ds) DNA
203 genomes, replicating via a virus-encoded DNA polymerase, an antivirals target, in the

204 cytoplasm of infected cells (Fields, 2013). Poxviruses enter cells by macropinocytosis, but
205 a poxvirus-specific receptor is still elusive (Mercer and Helenius, 2009). **Anti-poxvirus**
206 **drugs**. One of the first effective drugs in clinical use as a parenteral treatment in severe
207 OPV infections was **cidofovir**, a biphosphonate developed at REGA, in Belgium (De
208 Clercq, 2002; Delaune et al., 2017) and FDA approved against human cytomegalovirus
209 (HCMV). The ether lipid analogue **brincidofovir** (CMX001), a prodrug of cidofovir, has
210 shown efficacy in small animal models and is awaiting FDA approval (Parker et al.,
211 2008,2014; Trost et al., 2015; Chittick et al., 2017; Foster et al., 2017; Grossi et al., 2017;
212 Pires et al., 2018). The F13L virus egress inhibitor **tecovirimat** (ST-246, TPOXX®) has
213 been independently developed to treat smallpox infections and has been FDA-approved
214 since 2018. Tecovirimat has recently been used to treat nonhuman primates infected with
215 variola, and humans exposed to OPV (Mucker et al., 2013; Grosenbach et al., 2018; Pires et
216 al., 2018, Whitehouse et al., 2019). Tecovirimat (TPOXX®) is currently stockpiled in the
217 US and production for similar stockpiles in Europe is planned. Anti-poxvirus drugs
218 effective in animal models are reviewed in more detail elsewhere (Smee and Sidwell,
219 2003). Further candidate anti-poxvirus drugs include kinase inhibitors **imatinib**
220 (Gleevec/STI-571; Reeves et al., 2005 a,b) and **olomoucine** (Holcakova et al., 2010),
221 **terameprocol** (Pollara et al., 2010), **mitozandrone** (Altmann et al., 2012), the membrane
222 targeting **ddBCNA cf2642** (Mcguigan et al, 2013), **bisbenzimidazole** derivatives (Yakimovich
223 et al., 2017), **FC-6407**, a OPV D4 processivity factor mimic (Nuth et al., 2019), a number
224 of natural extracts that have shown interesting antiviral activity against OPV in in vitro
225 infection models (Cryer et al., 2017; Zanetta, 2019; **Table 1**).

226

227 **3.2 Filoviridae**

228 Filoviruses are category A select agents, World Health Organization risk group 4
229 pathogens, high on the list of potential biological threat agents (NATO AMedP-6), and
230 their handling requires BSL-4 containment. In nature they infect primates, pigs and bats
231 (free-tailed and fruit bats) and are transmitted to human hosts by exposure to infected bush
232 meat and body fluids of human patients. Ebola and Marburg viruses (EBOV/ MARV)

233 cause severe viral hemorrhagic fevers with hematemesis, bloody diarrhea, prostration and
234 case fatality rates of up to 90% within three days of infection. The EBOV envelope
235 glycoprotein has been used in the VSV-EBOV vaccine, which is 70–100% effective
236 preventing disease in exposed and vaccinated individuals and has been approved in October
237 2019 in the EU as the world’s first Ebola vaccine (Callaway E, 2019). **Filoviruses** are
238 filamentous enveloped viruses with Group V negative sense single stranded (ss) RNA
239 genomes. The endosomal Nieman Pick C1 protein, also relevant in flavivirus infections
240 (Osuna-Ramos et al., 2018) and the TIM-1 (HAVCR1) receptor on the surface of T cells,
241 also relevant for hepatitis C virus (HCV) entry (Kachko A. et al., 2018), are potential
242 targets for antiviral drug development. **Anti-filovirus drugs.** While treatment
243 recommendations are emphasizing intensive medical support if suitable clinical facilities
244 and cohort isolation are available (Bray and Paragas, 2002; Bray, 2003), defense against the
245 use of filoviruses as biological weapons would benefit from an effective virus-targeting
246 therapy. There are currently no licensed antiviral drug treatments for filoviruses. However,
247 in a recent multi-outbreak, multi-country study (PALM- “Together save lives”) started in
248 November 2018 in the DRC, two monoclonal antibodies (Mabs) emerged as giving the
249 greatest chance to survive Ebolavirus infection. **Zmapp, mAb114 and REGN-EB3** were
250 compared to the small molecule drug **remdesivir** (WHO, 2019). The trial was stopped
251 early with REGN-EB3 and mAb114 giving the greatest chance to survive Ebolavirus
252 infection. The WHO recommends, to use these two Mabs for all further treatments (WHO,
253 2019). **Remdesivir** (GS-5734; 1-cyano-substituted adenosine nucleotide analogue), a
254 nucleoside-analogue prodrug and lead compound of the small molecule antivirals class, has
255 been shown to inhibit EBOV in cell culture and in non-human primates likely by chain
256 termination (Warren et al., 2017), but showed lower efficacy in the clinical trial compared
257 to monoclonal antibody based therapeutics. A good alternative, albeit not tested in the DRC
258 clinical trial, may be **T705 (favipiravir; Furuta et al., 2002)**, a repurposed drug synthesized
259 by FUJIFILM-Toyam Chemical Co., licensed for use against influenza virus in Japan, and
260 since found to be a broad-spectrum inhibitor of viral RNA polymerases (Furuta et al., 2013,
261 Delang et al., 2018). T705 and the related pyrazinecarboxamide compounds T-1105 and T-
262 1106 have similar antiviral properties - see also section 3.3. (Alphaviruses). FDA approval
263 for use of favipiravir to treat filovirus infections is pending. Several animal pilotstudies,

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

272 **3.3 *Alphaviridae***

273 Alphaviruses are mosquito-borne viruses, but some can be effectively transmitted
274 via the aerosol route from contaminated rodent feces. Rodents, birds and possibly marine
275 species are maintenance reservoirs (Forrester et al., 2012). Alphaviruses can cause a
276 number of diseases in humans, including Chikungunya fever, Eastern, Western and
277 Venezuelan equine encephalitis. The handling of the respective viruses requires BLS-3
278 containment. Two type species, Venezuelan and Eastern Equine Encephalitis viruses
279 (VEEV and EEEV), are considered potential biological threat agents (NATO AMedP-6)
280 with up to 70% mortality in unprotected populations (Walton and Johnson, 1988) and
281 represent category B select agents. While human infections with VEEV and EEEV are rare,
282 sporadic and unpredictable but explosive epidemics caused by Chikungunya virus
283 (CHIKV) have occurred in the last decade mainly in South-East Asia and in South
284 America, Central America and the Caribbean, globally amounting to millions of cases.
285 Autochthonous cases of Chikungunya fever have been reported in Italy (Marano et al.,
286 2017). Viremia with rashes and fever usually lead to death of cells lining joints, causing
287 arthritis and joint pain. CHIKV infections of neurons can result in potentially fatal
288 encephalitis. Fatal infections, mainly seen in human infants, are rare, but long-lasting
289 polyarthralgia and encephalitis cause significant morbidity (Matusali et al., 2019).
290 **Alphaviruses** are enveloped viruses with positive-sense ss RNA genomes. Most
291 experimental antivirals target the viral RNA polymerase. There are no licensed antiviral
292 drugs against alphaviruses causing arthritis and encephalitis, and the treatment of infections
293 is mainly supportive (anti-inflammatory drugs, glucocorticoids). **Anti-alphavirus drugs.**

294 While pox- and filoviruses are highly lethal biological agents, alphavirus infections are
295 rarely fatal, but can lead to large numbers of incapacitated individuals, due to severe
296 arthralgias and headaches. In this sense, alphaviruses might be effective biological threat
297 agents where incapacitation and saturation of medical care facilities are the goal of a
298 perpetrator (incapacitating agents). Specific antivirals should be able to pass the blood brain
299 barrier (BBB) to control post-exposure encephalitis. Intravenous **Ribavirin**, which is FDA-
300 approved for HCV and respiratory syncytial virus (RSV) infection, does not pass the BBB,
301 thus alleviating peripheral symptoms but not providing cure (Abdelnabi et al., 2015).
302 Intranasal ribavirin may be more effective. Ribavirin resolves joint swelling in CHIKV
303 (Ravichandran and Manian, 2008), but has no activity against VEEV in vitro (Franco et al.,
304 2018). **Sofosbuvir**, an FDA-approved antiviral drug against HCV, which has been
305 suggested for repurposing against various viruses, has been evaluated for in vitro activity
306 against CHIKV (Ferreira et al., 2019). Among the most promising novel compounds is the
307 broad-spectrum antiviral candidate **favipiravir** (T-705), initially developed to treat human
308 influenza, which shows a potent antiviral effect in small animal models. The drug is
309 licensed in Japan, while FDA approval is pending (Furuta et al., 2013). An in vitro
310 comparison between ribavirin and favipiravir revealed that efficacy is cell-type dependent
311 (Franco et al., 2018). Efficacy was also shown in a mouse model (Abdelnabi et al., 2017).
312 Other compounds of interest (**Table 1**) include drugs approved for other medical conditions
313 and tested for repurposing. Those are the old antiparasitic **suramin**, which shows
314 ameliorating effects against CHIKV infection in mice (Kuo et al., 2016) and the
315 anthelmintic **ivermectin**, which shows in vitro activity against a range of alphaviruses
316 (Varghese et al., 2016). Compounds with known cellular targets include the cancer drugs
317 **mefenamic acid and sorafenib**, inhibiting replication of CHIKV and other alphaviruses
318 via eIF4E dephosphorylation in vivo (Rothan et al., 2016; Lundberg et al., 2018), and
319 **halofuginone**, a prolyl t-RNA synthetase inhibitor in veterinary use that is active in vitro
320 against both alpha- and flaviviruses (Hwang et al., 2019). Also promising is the virus-
321 specific antiviral **ML336** that inhibits Nsp4 of VEEV and EEEV in vivo (Jonsson et al.,
322 2019). Less well described compounds are **LL-37 peptide**, an alphavirus entry inhibitor in
323 vitro (Ahmed et al., 2019), **compound 25** that was identified in silico and optimized to
324 inhibit CHIKV replication in vitro (Bassetto et al., 2013), **Prest-37 and -392**, with in vitro

325 activity against VEEV nsP1 capping enzyme (Ferreira-Ramos et al., 2019), and **baicalin**,
326 which inhibits CHIKV replication in vitro by interfering with a cellular target (Oo et al.,
327 2018).

328

329 **3.4 Arenaviridae**

330 Arenaviruses (Lassa virus – Old World/ Junin, Machupo virus –New World) can
331 also cause viral hemorrhagic fevers and are therefore on the list of potential biological
332 threat agents (NATO AMed P-6; Argentine – Bolivian hemorrhagic fevers). Handling of
333 Lassa virus (LassaV) requires BSL-4 containment. Annual case numbers of Lassa fever
334 (LassaF) are estimated to be between 100.000 and 300.000 in West Africa, but the true
335 public health burden of LassaF is unknown, as are exact case numbers on New World
336 arenavirus infections (WHO Roadmap Neglected Tropical Diseases, 2012). Transmitted by
337 aerosolized rodent droppings, arenavirus infections start with a generalized flu-like illness
338 and then cause a range of conditions from aseptic meningitis/encephalitis with choroid
339 plexus infiltration (Lymphocytic Choriomeningitis Virus; LCMV) to potentially fatal
340 hemorrhagic fevers (Lassa, Junin, Guanarito, Machupo, Sabia, and White Water Arroyo
341 Virus), with case fatality rates over 30%. Recently a person-to-person transmission of
342 Lassa virus in Germany (WHO, 2016) and an outbreak in Nigeria raised public health
343 concerns. **Arenaviruses** are enveloped viruses incorporating ribosomes (‘arena’ is latin for
344 sand; ‘sand’-like appearance of ribosomes in electron microscopy of virus particles, hence
345 arenavirus), with a Group IV genome of two ambisense ss RNA segments. They use the
346 ubiquitously expressed alpha-dystroglycan as their cellular receptor, and their main cellular
347 targets are antigen-presenting cells. **Anti-arenavirus drugs. Ribavirin** is used under
348 compassionate use protocols for the treatment of LassaF (McCormick et al., 1986;
349 Ölschläger et al., 2011), while recently **favipiravir** was evaluated and found to enhance
350 survival in cynomolgus (crab-eating) macaques (Rosenke et al., 2018). A further interesting
351 compound is **LHF 535**, an entry inhibitor targeting arenaviral GP2 (Madu et al., 2018).

352

353 3.5 *Bunyaviridae*

354 Human pathogenic bunyaviruses, particularly Hantaviruses and Crimean-Congo
355 Hemorrhagic Fever Virus (CCHFV), can cause hemorrhagic fevers, and CCHFV is on the
356 list of potential biological threat agents (NATO AMed P-6). Handling of these viruses
357 requires BSL-3/BSL-4 containment. Bunyaviruses have a wide host range, including plants,
358 ticks (*Hyalomma* ticks - CCHFV), insects (*Culex* - Rift Valley fever virus) and rodents
359 (Hantaviruses), which also serve as transmission vectors. Humans are dead-end hosts,
360 suffering fatal outcomes in the case of Crimean-Congo hemorrhagic fever (CCHF), as well
361 as in hemorrhagic fever with renal syndrome (HFRS; Europe – South East Asia; Puumala/
362 Hantaan type viruses) and hantavirus pulmonary syndrome (HPS; Americas; Sin Nombre
363 type viruses). The clinical outcome is linked to geographical context and the typical animal
364 vector. While high case fatality rates were described with the Korean hantavirus types and
365 with Sin Nombre type viruses causing HPS in the Americas, the European situation
366 indicates a high case load with HFRS, but less severe clinical outcomes (nephropathia
367 epidemica), caused mainly by Puumala type viruses (Bugert et al., 1999; Klempa et al.,
368 2003; Schmidt-Chanasit et al., 2009; Report of the European Center for Disease Control
369 2016). Bunyavirus infections are endemic, vector-borne infections. Normally they do not
370 cause epidemics, with the exception of CCHF in case of nosocomial transmission.
371 Thousands of cases usually occur only in hyperendemic situations over a longer period of
372 time. Beginning with an initial generalized flu-like illness and fever which lasts for about
373 three days, these infections can end in fatal hemorrhagic fever (CCHF, HFRS), and
374 pulmonary syndrome (HPS) with a 1 – 40 % case fatality rate depending on virus strain
375 (Jonsson et al., 2008). **Bunyaviruses** are enveloped viruses with bi- and tri-segmented
376 ambisense ss RNA Group IV genomes. Human cellular receptors include human beta 3
377 integrins, the main human cellular targets are macrophages and endothelial cells, and they
378 replicate in the cytoplasm. No vaccines or licensed treatments are currently available. **Anti-**
379 **bunyavirus drugs.** The focus towards the identification of antiviral agents has been
380 mostly on CCHFV infections, which are common in endemic areas, but are either
381 asymptomatic or cause a non-specific febrile illness that does not require hospitalization or
382 specific treatment. Few patients develop hypotension and hemorrhage, and medical
383 management is then largely supportive, with volume replacement, and prevention of edema

384 and inflammation (Jabbari et al., 2012). **Ribavirin** has been used to treat CCHF patients
385 under compassionate use protocols with some success since 1985 (van Eeden et al., 1985),
386 especially if given early in the course of the infection, but many studies with apparently
387 beneficial results lack controls. Recent randomized clinical trials were unable to show
388 significant beneficial effects of ribavirin versus CCHFV (Koksal et al., 2010; Johnson et
389 al., 2018). Further interesting candidates for virus-specific treatment (**Table 1**) include
390 **favipiravir** (T-705), which has been evaluated against a number of phleboviruses
391 (PhleboV) and to treat CCHFV infection in rodent models (Gowen and Holbrook, 2008;
392 Gowen et al., 2010; Hawman et al., 2018), **galidesivir** (BCX4430), effective against Rift
393 Valley fever virus (RVFV) infection in a hamster model and investigated for use by the
394 FDA (Westover et al., 2018), 2'-fluoro-2'-deoxycytidine (**2FdC**), which showed
395 protective effects against infections with PhleboV in a rodent model (Smee et al., 2018),
396 and the **FGI-106** entry inhibitor (Smith et al., 2010).

397

398 **3.6 Flaviviridae**

399 Flaviviruses causing hemorrhagic fever or severe encephalitis (Omsk hemorrhagic
400 fever, Dengue and Yellow fever, Russian spring-summer encephalitis/ Tick Borne
401 Encephalitis (TBEV)) are listed as potential biological threat agents (NATO AMed P-6)
402 and handling requires BSL-3/BSL-4 containment. Flaviviruses are arthropod-borne viruses
403 that are endemic worldwide with virus/vector specific geographical distributions, causing
404 regular outbreaks and fatalities, with 30.000 cases/year through yellow fever in Africa
405 alone (Garske et al., 2014; WHO, 2018). Infections with flaviviruses can lead to
406 hemorrhagic fevers (Omsk hemorrhagic fever, yellow fever (YF) and dengue fever with
407 case fatality rates of up to 30%) or affect the CNS, causing encephalitis (e.g. Japanese
408 encephalitis, tick borne encephalitis with case fatality rates up to 20%, Zika and West Nile
409 encephalitis). Human-to-human transmission is not effective. Live vaccines against yellow
410 fever (17D) and Japanese Encephalitis (JE), a number of inactivated TBEV vaccines, and
411 most recently a live Dengue virus vaccine are available. **Flaviviruses** are a large family of
412 mosquito- or tick-transmitted enveloped viruses with a Group IV positive-sense single-

413 strand RNA genome, using G-protein coupled receptors for entry into host cells (Fields,
414 2013). **Anti- flavivirus drugs. Ribavirin** is an effective early treatment for yellow fever
415 under compassionate use protocols, but fails to improve survival of dengue infections in
416 non-human-primates (NHP; Malinoski et al., 1990; Monath, 2008). Out of a quite large
417 number of drugs investigated for repurposing against flaviviruses by the FDA (**Table 1**),
418 the most promising candidate is **sofosbuvir** (Bullard-Feibelman et al., 2017). Sofosbuvir
419 was initially developed and approved by FDA for treatment of hepatitis C. It shows activity
420 against a number of flaviviruses in vitro and in the mouse model (Mumtaz et al., 2017; de
421 Freitas et al., 2019). Further interesting candidates (13 compounds listed in **Table 1**) inhibit
422 the viral polymerase (Eyer et al., 2017; Segura Guerrero et al., 2018), NS2B/NS3 protease
423 and kinases (Chan et al., 2017; Chan et al., 2018), cell entry and membrane trafficking
424 (Nolte et al., 2016, Cannalire et al., 2019), and other flavivirus targets. The action and the
425 efficacy of most of these compounds in vivo are yet to be determined. The major
426 shortcoming of all candidates so far tested in animal models for the treatment of infections
427 with Usutu (UsutuV), Dengue (DENV) and Zika viruses (ZikaV) is their rather low
428 efficacy (Milligan et al., 2018; Chan et al., 2018).

429

430 **3.7 Orthomyxoviridae**

431 Orthomyxoviruses, in particular influenza viruses, although not on top of the list of
432 potential biological threat agents, are fast-moving airborne pathogens capable of causing
433 pandemics with significant mortality. Recombinant influenza viruses could be considered
434 as potential biological threat agents. Handling of avian influenza viruses and other
435 influenza viruses with high pathogenic potential require BSL-3 containment. Pandemic
436 influenza viruses type A are transmitted by the respiratory route to birds and mammals,
437 type B only from human to human, as well as via saliva, nasal secretions, feces and blood,
438 causing acute respiratory distress with potentially fatal outcomes in humans. In humans,
439 infection of the respiratory tract can lead to pneumonia, secondary pneumonia and
440 overwhelming immune responses, followed by multiorgan failure in rare cases.
441 Orthomyxoviruses are globally endemic, and cause sporadic outbreaks, rarely pandemics.

442 **Orthomyxoviruses** are enveloped viruses with a negative-sense segmented ssRNA
443 genome. The viral RNA polymerase has a high error rate of 1/10000. Vaccines are
444 composed of HA/NA subunits (purified from inactivated virions), purified subunits from
445 recombinant sources, or live/attenuated strains of the endemic strains/subtypes of influenza
446 A virus (currently H1N1 and H3N2), as well as those of influenza B viruses (Fields, 2013).
447 **Anti-orthomyxovirus drugs.** FDA-approved neuraminidase inhibitors **oseltamivir**
448 (Tamiflu®), **zanamivir** (Relenza®), **laninamivir** (Inavir®), and **peramivir** have marginal
449 clinical benefits only when given early but may be useful in severe infections requiring
450 hospitalization/ mechanical ventilation (Gubareva et al., 2017). In 2018 **baloxavir -**
451 **marboxil** (Xofluza®), an inhibitor of the viral cap-dependent endonuclease (CEN;
452 influenza virus polymerase PA subunit), was approved by the FDA for the treatment of
453 acute, uncomplicated influenza among patients aged 12 years or older (Noshi et al., 2018,
454 Koszalka et al., 2019). **Favipiravir** developed and approved in Japan specifically for
455 treatment of influenza virus infections, and its combination with neuraminidase inhibitors
456 was shown to be effective in a mouse model (Furuta et al., 2002, Baz et al., 2018). Further
457 interesting candidates are: **haloxanide/nitazoxanide**, thiazolide compounds that were
458 originally developed as anti-parasitic agents, but were shown to inhibit influenza virus
459 hemagglutinin maturation and intracellular trafficking of viral components in infected cells
460 and that are now in clinical trials (Tilmanis et al., 2017; La Frazia et al., 2018) as well as
461 **cycloheptathiophene-3-carboxamide**, which interferes with the polymerase PA-PB1
462 subunits of influenza virus (Nannetti et al., 2019). Alicyclic amines/aminoadamantanes
463 **amantadine and rimantadine**, first described in 1985 as M2 protein blockers (Hay et al.,
464 1985; H⁺ channel/viroporin; only type A viruses) are not recommended anymore for
465 clinical use (WHO/ US), due to rapid induction of viral resistance mutations: 100% of
466 clinical isolates are resistant). A 2014 Cochrane review found no evidence for efficacy or
467 safety of amantadine for the treatment of influenza A (Alves Galvao et al., 2014). However,
468 their structures may still be useful as scaffolds for the design of future M2 inhibiting drugs.

469

470

471 3.8 *Paramyxoviridae*

472 *Paramyxoviridae* are fast-moving airborne pathogens infecting animals and
473 humans. Hendra (HeV) and Nipah (NiV) viruses, in the genus *Henipavirus*, are considered
474 zoonotic agents in Australia (horses) and South-East Asia (pigs), respectively. Both viruses
475 may be able to infect other domesticated mammals, and there is a real concern in the
476 veterinary and biodefense communities about spill-over infections and the high fatality rate
477 in humans (632 human NiV cases: 59% case fatality; Ang et al 2018; Singh et al. 2019).
478 Henipaviruses have so far not caused global epidemics, but due to a high percentage of
479 severe outcomes, as well as lack of vaccines or treatments, HeV and NiV are designated
480 biosafety level (BSL-4) agents (Nannetti et al., 2019). They are currently not on the NATO
481 AMed P-6 list of biological threat agents but their potential as agents for bioterrorism has
482 been discussed (Lam 2003; Luby 2013). Other Paramyxoviruses causing diseases in
483 animals are: canine distemper virus (CDV), endemic in Europe (dogs/humans; Beineke et
484 al., 2015), Newcastle disease virus affecting birds, and rinderpest virus infecting cattle.
485 Human parainfluenza viruses and respiratory syncytial virus (RSV) are major causes of
486 bronchiolitis, bronchitis and pneumonia in infants and children. Measles (morbilli, rubeola)
487 caused by measles virus (MeaslesV) was responsible for around 733,000 deaths globally in
488 2000 (CDC, 2009), mostly due to viral pneumonia, secondary bacterial infections due to
489 immune suppression (B cell tropism), and encephalitides (inclusion body encephalitis
490 (MIBE); subacute sclerosing panencephalitis (SSPE)). A very successful vaccine
491 (MeaslesV strain Edmonston) has been used with the goal to eradicate measles in 2010
492 (Holzmann et al., 2016). However, anti-vaccine movements have led to the loss of herd
493 immunity and the reemergence of measles in many developed countries (Dahl, 1986;
494 Fraser-bell, 2019). **Paramyxoviruses** are a family of enveloped viruses with a negative
495 sense ss RNA genome (mononegavirales) replicating in the cytoplasm (Fields, 2013). **Anti-**
496 **paramyxovirus drugs. Ribavirin** administered with cyclodextrin has been shown to be
497 effective in a mouse model for measles encephalitis (Jeulin et al., 2009). A very promising
498 candidate antiviral against measles is **ERDRP-0519**, which has been shown effective
499 against canine distemper virus in a ferret model (Krumm et al., 2014), however early
500 resistance development has been described (Kalbermatter et al., 2019). **Favipiravir** has a
501 protective effect against Nipah virus infections in the hamster model (Dawes et al., 2018),

502 **remdesivir** inhibits a number of paramyxoviruses in vitro (Lo et al., 2017). ddBCNAs (see
 503 section 3.1 and 3.6; McGuigan et al., 2013) and the plant extract **naphthoquinone**
 504 **droserone** have anti-measles activities in vitro (Lieberherr et al., 2017). The nucleoside
 505 analogue 4'-azidocytidine (R1479; balapiravir) was developed to inhibit HCV (Nelson et
 506 al. 2012), paramyxoviruses, and filoviruses in vitro (Hotard, et al, 2017), but showed low
 507 efficacy and high toxicity in hepatitis C patients in early clinical trials (Nelson et al.,
 508 2012).

509

510 **Table 1**

511

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 <i>in vitro</i>
Mitoxandrone	OPV/ unclear	Altmann 2012	FDA-appr. Cancer <i>in vitro</i>
Olomoucine II	OPV/ kinases	Holcakova 2010	<i>in vitro</i>
Terameprocol	OPV/ unclear	Pollara 2010	<i>in vitro</i>
ddBCNA-cf2642	OPV/ membranes, autophagy	McGuigan 2013	<i>in vitro</i>
Bis-benzimidides	OPV/ DNA intercalators	Yakimovich 2017	<i>in vitro</i>
KPB-100/200	OPV/ unclear	Cryer 2017	<i>in vitro</i>
FC-6407	OPV/ D4	Nuth 2019	<i>in vitro</i>
BB4 D9	OPV/ unclear	Zanetta 2019	<i>in vitro</i>
Filoviridae - EBOV, MARV (Baltimore Group V ss-RNA) - section 3.2			
Remdesivir (GS-5734)	EBOV/ Pol	Warren 2016	IND <i>in vitro</i> Phase II clinical trial DRC- 2018- 2019
Favipiravir (T705)	EBOV/ Pol	Bixler 2018a	appr. in Japan - Influenza <i>in vivo</i>

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
<i>Alphaviridae</i> – 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
Ivermectin	CHIKV/ unclear	Varghese 2016	FDA- anthelmintic 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
<i>Arenaviridae</i> – 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
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. antihelminthic 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. antihelminthic 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
<i>Orthomyxoviridae</i> – 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
<i>Paramyxoviridae</i> – 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

512

513 **Table 1 Legend**

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

516 Abbreviations:

517 Appr.: approved

518 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

548 viral RNA polymerase inhibitors, initially developed for the treatment of influenza virus,
549 and found effective against bunyaviruses (Gowen et al., 2010; Caroline et al., 2014;
550 Hawman et al., 2018), alphaviruses (Abdelnabi et al., 2015), filoviruses (Bixler et al.,
551 2018a) arenaviruses (Rosenke et al., 2018), paramyxoviruses (Dawes et al., 2018), and
552 flaviviruses (Seguera-Guerrero, 2018). A favipiravir resistance mechanism in influenza
553 virus has been described (Goldhill et al, 2018). Other potential broad-spectrum agents are:
554 **remdesivir** (GS-5734), another RNA polymerase inhibitor (Tchesnokov et al., 2019)
555 active against filo-, and paramyxoviruses (Lo et al., 2017), **FGI-106** with inhibitory activity
556 against filo-, bunya-, and flaviviruses (Aman et al., 2009), **galidesivir (BCX4430)** with
557 activity against filo-, bunya-, and flaviviruses (Warren et al., 2014; Eyer et al., 2017;
558 Westover et al., 2018) and 2'-fluoro-2'-deoxycytidine (**2'-FdC**), which was reported to
559 inhibit various viruses in vitro, including Borna virus, HCV, Lassa virus, certain herpes
560 viruses, and which also inhibits influenza viruses in mice (Smee et al., 2018). Previously
561 thought as a one-family-broad-spectrum compound, **sofosbuvir** (Sovaldi™, Soforal™) has
562 in vitro and in vivo activity against several members of the family flaviviridae, and has
563 most recently been shown to be effective against Chikungunya virus (Ferreira et al., 2019).
564 Natural product antivirals are single molecule natural compounds or complex mixtures of
565 organic molecules (e.g. plant extracts) with antiviral activity. Natural product antivirals
566 frequently exhibit broad spectrum antiviral activity and often a single active compound
567 cannot be identified in extracts (Cryer et al., 2017).

568

569 **3.10 Treatment of viral hemorrhagic fevers (VHF) with ribavirin**

570 Viral hemorrhagic fevers (VHFs) cause the highest mortality in human hosts of all
571 known viral agents and treatment options are a serious concern both in public health and in
572 biodefense scenarios (Ippolito et al., 2012). If specific antiviral treatment options are not
573 available, supportive care is the mainstay of clinical interventions in VHF, including
574 haemodynamic, haematological, pulmonary and neurological support treatments. Treatment
575 with corticosteroids, vasoactive substances, hemodialysis, and mechanical ventilation saves
576 the patients with the worst clinical symptoms. The only currently widely available antiviral

577 drug, ribavirin, is not approved by the FDA for intravenous application in VHF and is used
578 under compassionate use protocols only. Intravenous ribavirin reduces mortality of HFRS
579 if combined with hemodialysis and both morbidity and mortality in the case of Lassa fever
580 (LassaF). Ribavirin (Copegus™, Rebetol™, Virazole ® ICN / Valeant (IND)) is used for
581 the treatment of infections with African arenaviruses (Lujjo- and Lassa fever) and
582 bunyaviruses (HFRS, Crimean Congo fever, and Rift Valley fever). However, intravenous
583 ribavirin does not show any benefits for the treatment of any of the VHFs caused by
584 filoviruses, or in infections with RNA viruses causing severe encephalitis (Bray and
585 Paragas, 2002; Ippolito et al., 2012).

586

587 **4. Conclusions**

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

602 There is only a small number of antivirals with regulatory approval to treat virus
603 infections, some of which have already been described to select for drug resistant strains. A
604 number of drugs with antiviral activities which are approved for other conditions are being
605 evaluated for repurposing, but the number of compounds currently in the experimental

606 pipeline for clinical testing is small. Consequently, while there are treatment options, they
607 may not be available in sufficient quantity in a biological threat situation. Therefore,
608 research in identification, development, clinical testing and the stockpiling of approved
609 antivirals in sufficient quantities, must be a priority for the government actors put in charge
610 of a credible response to deliberate releases of some of the biological agents discussed here.
611 It is well known that even the threat of a biological attack would cause mass hysteria with
612 concomitant economic disruption. Only timely preparation underlined by visible
613 infrastructure, stockpiles of drugs and vaccines, and well considered emergency plans will
614 allow governments to give the necessary assurances when needed, to avoid negative
615 outcomes (Hawley and Eitzen, 2001). Ideally, research on novel antivirals should also be a
616 priority for research funding and pharmaceutical companies. As long as this is not the case,
617 government funding and research in government-funded laboratories in collaboration with
618 specialized university research groups organized in antivirals platforms have to step into
619 the breach, when considerations of market performance and public health priorities are
620 focusing resources elsewhere.

621

622 **5. Acknowledgements**

623 We thank Dr Romano Silvestri for thoughtful comments and suggestions, and
624 members of the Brancale/Bugert antivirals platform for critical reading of the manuscript.

625

626 **6. Declarations**

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

632

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