Recent advances in the development of antiviral therapeutics for Rift Valley fever virus infection

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Rift Valley fever virus (RVFV) is a mosquito-borne bunyavirus endemic to sub-Saharan Africa and the Arabian Peninsula and the etiological agent of Rift Valley fever. Rift Valley fever is a disease of major public health and economic concern, affecting livestock and humans. In ruminants, RVFV infection is characterized by high mortality rates in newborns and near 100% abortion rates in pregnant animals. Infection in humans is typically manifested as a self-limiting febrile illness, but can lead to severe and fatal hepatitis, encephalitis, hemorrhagic fever or retinitis with partial or complete blindness. Currently, there are no specific treatment options available for RVFV infection. This review presents a summary of the therapeutic approaches that have been explored on the treatment of RVFV infection.

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Rift Valley fever virus (RVFV) is a mosquito-borne virus of the *Bunyaviridae* family (genus *Phlebovirus*) endemic throughout much of the African continent [1,2]. Vectored by mosquito species of the *Aedes*, *Culex* and *Mansonia* genera, RVFV is responsible for regular outbreaks in peridomestic herd animals, including cattle, goats and sheep, as well as causing spillover events into human populations [3–9]. In pregnant ruminants, RVFV infection typically causes high-rate abortions up to 100% or fetal malformations, while newborn lambs usually die because of acute hepatitis [1,10–12]. In addition to infecting vertebrate hosts, where it can be transmitted horizontally to farmers, healthcare personnel or family members of patients, RVFV is also likely maintained in the environment by transovarial transmission in *Aedes* spp. [13].

The emergence of RVFV in Egypt, the Arabian Peninsula and the Comoros Archipelago and Madagascar, highlights that the geographical range of RVFV may be increasing. This is a major concern, since RVF outbreaks outside endemic countries could cause serious agricultural and health problems [14]. Introduction of RVFV into non-endemic countries, where the mosquito vector is present, could potentially occur by the movement of infected mosquitoes, animals or even patients [15–19]. Indeed, the first human case of RVF has recently been imported from Angola to the People's Republic of China [20,21]. In addition, the intentional spread of RVFV is of concern in terms of national and global biosecurity. RVFV has, therefore, been classified as a Category A overlap select agent by Centers for Disease Control & Prevention and United States Department of Agriculture and handling of pathogenic RVFV requires biosafety level (BSL) 3 enhanced (3E) or BSL4 containment laboratories. The best approach to control RVF is effective vaccination of both humans and ruminants. Since the 1960s, major vaccine development strategies have been explored for RVFV, including but not limited to inactivated, live-attenuated, vectored and subunit vaccine candidates. Efficacy of those vaccine candidates has been evaluated in multiple animal models and typically demonstrated 90–100% protection. However, there are still no vaccines approved for human use in the USA and other countries. Detailed information on RVFV vaccines has been reviewed in [22–25].

Like all members of the *Bunyaviridae*, RVFV has a single-stranded tripartite genome composed of negative (L[arge]- and M[edium]-segments) or ambisense (S[mall]-segment) polarity [26]. The L-segment contains coding regions for the RNA-dependent RNA polymerase (L protein), and the M-segment encodes a nonstructural protein (NSm), a 78-kDa protein, and the two envelope glycoproteins Gn and Gc in a single open reading frame (ORF). The S-segment expresses the nucleoprotein (N) and nonstructural protein (NS). The L and N proteins are required for viral replication and transcription, and form the ribonucleoprotein (RNP) complex with the viral genome, which are packaged in lipid-enveloped viral particles. Assembly and budding of newly formed virions takes place at the Golgi complex inside cisternae, which is initiated by interaction of the glycoprotein cytoplasmic tails with the RNP complexes [27–30]. The two glycoproteins are arranged in the viral membrane into an icosahedral lattice and mediate attachment to and fusion with the host cellular membranes [31–33]. Entry into host cells is mediated through caveolin-1-mediated endocytosis. DC-SIGN [34,35], as well as other cell surface markers, including heparan sulfate [36], play a role during entry. Following internalization, Gc, a class II fusion protein [37,38], undergoes an acid-dependent conformational change, fusing the viral and host cellular membranes and allowing the viral genome segments to enter the cytoplasm and begin transcription [39]. Early after infection, during primary transcription, N and NSs mRNA are being transcribed. The NSs protein is considered to be the major virulence factor of RVFV, and plays a crucial role in evasion from the host innate immune response [40]. NSs inhibits the synthesis of IFN- β mRNA and effectively suppresses the innate immune response [40–42]. In addition, the NSs protein forms intranuclear filaments that are related to its proviral transcriptional and translational program [43–45]. The contribution of the NSm protein to RVFV virulence is less clear [46].

Pathogenesis & clinical symptoms in patients

Infections with RVFV can result in a wide range of clinical features, varying from an asymptomatic (30–60%), to a weeklong undifferentiated febrile illness, to the severe development of hemorrhagic fever, encephalitis, retinitis and potentially death, with an estimated overall case fatality rate between 0.5 and 2%. While the most frequent course of the disease is a self-limiting febrile illness, RVF outbreaks have been reported that involved tens of thousands of patients. During the 1977–1978 outbreak in Egypt, approximately 200,000 human cases with 600 deaths were estimated to have occurred [47,48], and an estimated 27,500 RVFV infections occurred during the 1997–1998 outbreak in the Garissa district of Kenya [49].

The majority of RVF cases are self-limiting and characterized by a mild often subclinical febrile illness, with an incubation time of typically 4–6 days [1,10,50–53]. Symptoms typically include severe chills, weakness, malaise, headache and dizziness [54,55]. Typically, patients experience a biphasic febrile phase, which is normally combined with a severe headache. Patients, on the other hand, who develop one of the severe forms of RVF (jaundice, hemorrhagic fever or neurological disease) are at increased risk of fatality [53,56]. Hemorrhagic manifestation (<1%) is typically characterized by a sudden onset of illness, including fever, throbbing headache, lethargy, malaise, vomiting, nausea and body pain. These patients may also present with a low blood pressure, diarrhea, jaundice, macular rash over the entire trunk, subconjunctival hemorrhage and bleeding from the gums and/or gastrointestinal mucosal membrane [55,57,58]. Infection of the liver is typically characterized by elevated aspartate aminotransferase, lactate dehydrogenase, alanine aminotransferase and reduction of platelet count and hemoglobin are typical [58,59]. Death can occur within 3–17 days after onset of these symptoms, and fatal RVF cases show diffuse hepatic and gastrointestinal necrosis [58,60]. RVF patients developing encephalitis (<1%) may present with a sudden onset of fever, rigor, retro-orbital and throbbing headache and bilateral retinal hemorrhage [55,61,62]. After onset of the illness, it has been described that patients eventually experience confusion, fatigue, malaise, stupor and coma, and temporary or permanent vision loss [61–63]. Patients with a developing RVF-induced encephalitis can also present with a decreased level of consciousness [61]. In certain cases, RVF patients can develop a retino- or maculopathy (0.5–2%), which can affect either one or both eyes and can occur either very early or months after infection [64–67]. It has been reported that patients can experience a blurry vision or even the loss of central vision. While in some cases a partial improvement in vision over time established [64,66–68], many patients will experience partial or complete blindness [64–67,69–71].

Antivirals for RVFV

Generally, antiviral treatments either target processes intrinsic to the virus, such as host cell binding or replication of the viral genome, or modulate host cellular processes important for the lifecycle of the virus. The former viral targeting paradigm has proven very effective in both HIV and HCV, which are both now largely controllable

Table 1. Summary of antiviral therapeutics.

Molecule	Mechanism of action	<i>In vitro</i> efficacy [EC ₅₀] [†]	<i>In vivo</i> efficacy	Ref.
Ribavirin ± poly(ICLC)	Nucleoside analog ± immunostimulant	78 µg/ml (Vero)	BALB/c mice; Swiss Webster; Golden Syrian hamster	[81–84, 104, 168]
Favipiravir (T-705, Avigan®) ± ribavirin	Nucleotide analog ± nucleoside analog	31 µM (Vero 76)	Golden Syrian hamster; Wistar-Furth rat	[104, 105]
6051, 7007	NP oligomerization inhibition	<0.4 µM (Vero E6)		[109]
Suramin	vRNA/N Interaction	22.3 µM (HEK 293T)		[110]
BCX4430	Adenosine analog inhibiting RNA polymerase	41.6 µM (HeLa)	C57/BL6 mice	[113, 114]
Six related compounds	Not known	2.1–7.0 µM (A549)		[115]
LJ001, JL-compound series	Membrane disruption	<0.5 µM (BHK-5)	BALB/c mice	[93, 118]
Sorafanib	RNA synthesis, egress	6.4 µM (Vero)	BALB/c mice	[119]
Bortezomib	Ubiquitin proteasome inhibitor	<0.01 µM (HSAEC)		[122]
FGI-106	Unknown host protein	800 nM (Vero E6)		[123]
Rapamycin	mTOR Inhibition	11 µM (H2.35)	BALB/c mice	[129]
17AAG, BAPT A-AM	HSP90 Inhibition	<10 µM (Vero)		[130]
5,6-dimethoxyindan-1-one	Undefined	0.73 µg/ml		[131]
1E7–03	PP1 inhibition	3.5 µM (HSAEC03)		[136]
25HC	Viral-cell membrane fusion block	<1 µM (HeLa)		[137]
GY4137 (H ₂ S-donor)	Reactive species scavenger	<5 mM (Vero)		[143]
Four signal transduction inhibitors	Block early stages of infection	4.7–36.4 µM (HeLa)		[145]
Four protease inhibitors	Proteasome system inhibition	13.6–61.5 µM (HeLa)		[146]
RNASEK	Internalization block	siRNA knockdown		[147]
SMER28	Autophagy inhibition	6.9 µM (U2OS)		[124]
NSC 62914	Reactive species scavenger	<10 µM (A549)	C57/BL6 mice	[151]
RNA aptamers	RNA binding inhibition	Not determined		[112]
Gc stem fragment	Peptide fusion inhibition	Not determined		[159]

[†] *In vitro* cell culture system used to evaluate antiviral activity against Rift Valley fever virus.

and manageable diseases [72,73]. These compounds exploit unique aspects of the viral lifecycle, such as viral DNA integration or replication of RNA genomes in combination to improve patient outcome and limit the development of viable escape mutations [74]. Host targeted antivirals function by modulating cellular processes, such as translation and lipid metabolism that are essential for viral replication. While these processes would also be necessary for viral replication, they could be modulated to slow viral replication and allow the host immune response to control viral infection without overt toxicity.

Current strategies for treatment of patients with RVF focus primarily on providing supportive care. While numerous groups have been focused on studying the molecular mechanisms involved in the lifecycle of RVFV, no US FDA-approved effective therapeutics are available at this time. This review will summarize the strategies and identified compounds that have been evaluated for their efficacy as antiviral therapeutics for RVFV infection. Compounds with antiviral efficacy in cell culture, as well as animal models, are summarized in Table 1.

Studies discussed in this review have primarily used two RVFV strains, pathogenic ZH501 and vaccine strain MP-12. RVFV ZH501 is a pathogenic strain that was isolated from a fatal human case during the first Egyptian RVF outbreak in 1977–1978. This strain is considered a select agent, and must be used at BSL3E or BSL4 containment. The MP-12 strain has been developed by serial plaque passages of the Egyptian ZH548 strain 12-times in the presence of a chemical mutagen, 5-fluorouracil. RVFV ZH548 was isolated from a febrile patient during the Egyptian outbreak in 1977–1978 [75]. RVFV MP-12 is not considered a select agent and can be handled at BSL2, simplifying screening for antiviral compounds in proof-of-principle studies.

Ribavirin

Ribavirin, a nucleoside analog, is one of the few drugs approved for treatment of selected viral hemorrhagic fevers [76–78]. Since its development in the 1980s, it was the first antiviral being tested in models of phlebovirus infection. In initial studies it showed high efficacy *in vitro* using immortalized cell lines isolated from African green monkey, Rhesus macaque and human cervical carcinoma against a diverse panel of RNA and DNA viruses with EC₅₀ values ranging from 1 to 1000 µg/ml, including an EC₅₀ of 80 µg/ml against RVFV ZH501 [79]. These studies were quickly extended to a mouse model of infection using the closely related phlebovirus, Punta Toro virus. Here, ribavirin treatment significantly impaired virus replication and severity of symptoms with 100% survival at 18.8 mg/kg subcutaneous (SC) daily administration, with initial treatment 4 hours before SC infection [80,81]. Reed *et al.* examined the efficacy of intraperitoneal (IP) ribavirin in an aerosol challenge model of pathogenic wildtype RVFV ZH501 in BALB/c mice after infection with 1000 plaque forming units (pfu) [82]. In this study, mice exposed by the aerosol route were not protected from RVFV infection, which has important implications for treatment of humans infected to aerosolized RVFV, such as in either a slaughterhouse environment or intentional release context [82]. IP administration of the immunostimulant poly(ICLC) in combination with ribavirin showed enhanced efficacy against SC delivered RVFV ZH501 infection (250 pfu) compared with ribavirin alone in infected Swiss–Webster mice [83]. Unfortunately, the side effect profile of ribavirin, most prominently inflammation and hemolytic anemia, has limited its use in clinical settings. Lipid encapsulation has been successfully used in reducing the side effects through administration of lower but more targeted doses, resulting in increased survival of infected mice [81,84].

Favipiravir

Favipiravir (also known as T-705 or Avigan®), originally developed by Toyama Chemical (Japan), is a nonnucleoside inhibitor in the influenza polymerase and has received approval for use against influenza viruses in Japan, and Phase III clinical studies have been completed in the USA [85–89]. Importantly, favipiravir has been demonstrated to be efficacious as a broad-spectrum antiviral against a wide panel of RNA viruses, including but not limited to filo-, arena-, paramyxo- and bunyaviruses [87,89–103]. Indeed, during the 2014–2016 west African Ebola outbreak, favipiravir was used under emergency protocols and in registered clinical trials. In initial *in vitro* studies, Gowen *et al.* demonstrated that favipiravir is highly active against bunyaviruses from different genera, including RVFV with an EC₅₀ of 32 µM [91]. Subsequently, efficacy of favipiravir was evaluated in a hamster model for RVFV ZH501 [104]. Treatment initiated 1 hour postinfection (HPI) with 200 mg/kg/day twice daily (BID) orally (PO) for 10 days protected 80% of SC infected animals with 30 pfu RVFV ZH501. Ribavirin (75 mg/kg/day, BID, PO) was included as a positive control and only resulted in 20% survival. Performing a delayed treatment study, it appeared that the therapeutic window for favipiravir in hamsters did not extend much beyond 6 HPI, where the overall survival was 60%. In contrast, delaying treatment with ribavirin until 24 HPI was highly effective at protecting all animals from acute hepatic infection; however, nearly all ribavirin-treated animals ultimately succumbed to late-onset encephalitis. Interestingly, favipiravir appeared to be more effective in protecting animals from both acute and late-onset encephalitis, but the treatment window was limited to approximately 6 HPI. Last, a synergistic effect in protecting against acute and late-onset encephalitis could be demonstrated (up to 40% survival) when infected hamsters were treated 24 HPI with both antivirals in combination in comparison to monotherapies with either drug at the concentrations tested [104]. In a separate study, using an aerosol exposure Wistar–Furth rat model, Caroline and colleagues could demonstrate that animals receiving favipiravir within 1 HPI at 100 mg/kg/day BID PO for 14 days were fully protected from lethal RVFV infection with 50 pfu. When treatment was initiated 48 HPI, survival was still at 92%. While surviving animals did not show any signs of clinical disease, rats that received favipiravir and died during the course of the study had indications for neurological disease with pathological changes in the brain in the form of meningitis and lymphocytic vasculitis [105].

Small molecules targeting viral components

The introduction of robotics and automation in the drug development pipeline has led to the regular publication of high compound number screens for diverse biological conditions. Through precise control of infection conditions, concentration and data analysis, single concentration and single replicate studies can be reliably interpreted as primary screens of efficacy. The design of primary assays must be carefully controlled to ensure biological relevance of the results, although secondary assays can be performed in relevant primary cell lines to add further stringency to these efforts. Many of the compounds and screens described below are based on such high-throughput screens

(HTS). Despite the power of HTS methods, it is imperative to evaluate compounds and libraries for their chemical character. Curcumin, a component of the spice and traditional medicine turmeric, is a promiscuous binder and highly reactive, which can cause false positives in screening assays [106]. Its highly reactive character can cause significant downstream effects but such highly reactive and nonspecific compounds may not be suitable for further development [106,107]. In RVFV (MP-12 and ZH501) infected cells (multiplicity of infection [MOI] = 3) curcumin inhibits the activation of the IKK- β 2 pathway and thereby decreases the release of extracellular viral genomes [107]. Rapid evaluation of RVFV viral replication in the absence of cytopathic effect can also be achieved using qPCR of cell culture supernatants from assay plates or lysed cells in secondary assays [108].

Broce *et al.* have developed a novel biochemical screen using purified RVFV N capsids capable of differentiating between poorly and highly ordered capsid structures [109]. Recombinant N is purified from wheat germ extract and will assemble into highly ordered filaments in solution. Using a modified sandwich ELISA and antibodies against the ordered N structure, they could differentiate between assembled N filaments and drug induced aberrant structures. Using this technology, a library of 10,000 compounds was screened, and two related compounds termed 6051 and 7007 with a 3,7-bis(dialkylamino)phenothiazine-5-ium backbone were identified. Activity of those compounds was evaluated in a plaque reduction assay against RVFV (MOI = 0.1), as well as Lassa Fever virus (family *Arenaviridae*) (MOI = 0.1), and resulted in EC₅₀s between <0.4 μ M and <5 μ M, respectively, with low toxicity profiles [109]. Using a biochemical screen that assays the interaction and binding of RVFV N to viral RNA (vRNA), suramin, an inhibitor of trypanosomiasis and onchocerciasis used in Africa, was identified. It can act early during the lifecycle of RVFV (MOI = 0.1) by disrupting vRNP complexes on entry but also late in disrupting the assembly of new vRNPs [110–112]. A challenge of drug development that uses a biochemical screen as the primary selection criteria is the translation of those results into cell culture systems, making this achievement particularly notable. BCX4430 is a nucleoside analog by BioCryst Pharmaceuticals, originally developed as a hepatitis C virus replication inhibitor, and has recently been repurposed for the treatment of Ebola virus infection [113,114]. BCX4430 is converted into the active triphosphate nucleotide in the liver or in liver-derived cell models by host kinases and serves as an ATP mimic, which is incorporated into newly synthesized mRNA or vRNA by the Ebola virus RNA-dependent RNA polymerase. Antiviral activity of BCX4430 could also be demonstrated for RVFV *in vitro* with an EC₅₀ in the low micromolar range and treatment of mice infected with a lethal dose of RVFV ZH501 resulted in increased survival (up to 60%) [113]. This compound has been moved into preliminary Phase I safety trials for several routes of administration and appears to be well tolerated [113,114].

While many viral screens examine either cell death or the release of viral particles (expressed as viral titer), assays utilizing recombinant viruses expressing fluorescent reporter genes can help increase the number of antivirals to be tested, as well as reduce testing time. Using a replication-competent recombinant RVFV (MOI = 3 for the initial screen) encoding red fluorescent protein instead of the NSs ORF, Islam and colleagues identified a subset of six compounds out of a library of 28,437 compounds total to be effective at reducing red fluorescent protein expression in a dose-dependent manner with EC₅₀ values in the low micromolar range (ranging from 2.1 to 7.0 μ M). Interestingly, each identified molecule belonged to a separate compound class, although all contained many cyclic motifs, which may present similar binding profiles to their target protein [115]. Future studies will be necessary to further characterize the mechanism of action, as well as *in vivo* efficacy.

Lipid-enveloped viruses represent a large group of pathogens known to cause severe disease in animals and humans. Targeting the fusion step between host cell and virus membranes during virus entry therefore represents a broad-spectrum approach which can ultimately be applied to multiple viruses [116]. One such class of compounds are amphiphilic thiazolidine derivatives, which are light-activated membrane-targeting singlet oxygen generators (also known as type II photosensitizers) [117,118]. Generated singlet oxygens then oxidize the C = C double bonds in unsaturated phospholipids, and ultimately result in a reduced fluidity of the membrane and increased positive curvature, which negatively affect virus-cell membrane fusion. The small molecule LJ001 is such a thiazolidine derivative and has been demonstrated to inhibit the entry of numerous enveloped viruses, including RVFV, with IC₅₀ values of $\leq$ 0.5 μ M, through lipid oxidation of viral membranes [93,118]. Based on structure–activity relationship optimization of LJ001 a new class of membrane-targeting compounds (oxazolidine-2,4-dithione derivatives) were synthesized, resulting in markedly enhanced antiviral activity [118]. Two of those compounds, JL118 and JL122 (administered IP), showed moderate activity in the RVFV mouse model after IP infection of 20 pfu. While the compounds significantly delayed time to death, they were not able to result in increased survival [118]. It was speculated that the moderate *in vivo* efficacy is limited by the depth of penetration of visible light into deeper tissue,

thus resulting in reduced levels of lipid oxidation. Future advances in photochemistry may help overcome those hurdles, ultimately advancing *in vivo* efficacy of these compounds [116].

Small molecules targeting host cellular components

In addition to screens focusing on the identification of small molecules designed to show direct antiviral effect, drug discovery efforts have increasingly focused on the modulation of host cellular processes that thereby inhibit the replication of the virus and subsequent pathology. A common strategy applied to numerous viruses has been the testing of FDA-approved compounds and to repurpose drugs with antiviral activity. This has the benefit of evaluating compounds that have already undergone extensive clinical testing for safety and ultimately to leverage the path for potential evaluation in an outbreak scenario. Importantly, Benedict *et al.* have performed such a screen and identified compounds with strong *in vitro* efficacy against a recombinant replication-competent RVFV MP-12 (MOI = 0.1) encoding luciferase gene instead of the NSs ORF in Vero CCL-81 cells. Positive hits included growth factor receptor inhibitors, compounds with antiparasitic function, calcium channel inhibitors and microtubule assembly inhibitors [119]. The strongest hit in their assay was Sorafenib, which blocked 93% of infection *in vitro*. However, when tested for efficacy *in vivo* after PO administration, an increasing survival of SC infected mice receiving 1000 pfu, as well as decreasing RVFV genome copies in target organs such as liver and spleen, could be observed but the results were not significant [119]. The ubiquitin proteasome pathway is required for the targeted degradation of intracellular proteins and an important modulator for the regulation of many fundamental cellular processes. Many viruses have evolved to utilize the ubiquitin proteasome pathway for their benefit and it is not surprising that proteasome inhibitors can display an antiviral effect [120]. Keck and colleagues, as well as unpublished data from our group, have demonstrated that Bortezomib, a first generation FDA-approved proteasome inhibitor for the treatment of multiple myeloma [121], significantly decreased the release of infectious RVFV particles at MOIs = 0.1 and higher, as well as intracellular viral genome production. Mechanistically, Bortezomib altered the interaction of the NSs host antagonist with the IFN- β repressor complex and altered ubiquitination across many cellular targets [122]. Host targeted drugs will require extensive work to elucidate the mechanism of action, especially when non-targeted compound libraries are used for primary screens. In the case of proteasome inhibitors, a careful balance between cytotoxicity and reduction in viral replication must be found at clinically achievable concentrations, because of the nature of those inhibitors as cancer therapeutics. While the intent of chemotherapy for aggressive cancers and antiviral therapy may appear discordant, host cell process targeting drugs may prove useful at lower doses for antiviral uses, as well as in single or short-term administration sufficient to slow down viral replication and to allow the host immune response to activate its own defense mechanisms. Aman and colleagues have identified a compound termed FGI-106 from a US Army Medical Research Institute of Infectious Diseases library of compounds with aromatic heterocycles and at least two ionizable groups that were hypothesized to likely target a host cellular pathway because of its broad inhibition across virus families [123]. The compound is efficacious against a variety of viruses of different families in cell culture in the mid to high nanomolar range, including RVFV (MOI = 1; EC₅₀ = 800 nM), HIV (MOI = 0.001; 150 nM), HCV (200 nM), dengue type 1–4 (MOI = 0.1; 400–900 nM) and Ebola virus (MOI = 1; 100 nM) and with minimal toxicity. The compound was further evaluated in a mouse model of lethal Ebola virus infection, with both infection (1000 pfu) and dosing performed via the IP route, that both reduced mortality and viral titer detected in kidney, spleen and liver; however, it has not been evaluated *in vivo* for RVFV at this time [123]. Other classes of inhibitors targeting different host cellular pathways, including modulators of the autophagy system, were identified and ongoing work in our laboratory is focusing on the impact of kinase inhibitors on viral replication [124,125]. Notably efficacious in many studies has been the BCR-abl kinase inhibitor imatinib, marketed under the trade name Gleevec, as a chemotherapeutic for chronic myelogenous leukemia and acute lymphocytic leukemia, but also strong efficacy in the reduction of poxvirus replication [126,127]. PKC-epsilon has also been implicated in early stages of RVFV infection, and as calcium channel inhibitors were identified by Benedict *et al.*, this may represent a conserved and targetable pathway [128]. Rapamycin, an inhibitor of the S6 kinase pathway, inhibits replication of both RVFV ZH501 and RVFV MP-12 *in vitro* (MOI = 5) via decreased phosphorylation of Akt and other components of the mTOR pathway. Rapamycin was also effective in rescuing 50% of mice from death and moderating weight loss in this model [129]. HSP90, an important protein folding chaperone, has been shown to associate with RVFV particles in mass spectrometry analysis. Further testing of clinically progressing HSP90 inhibitors, including 17-AAG and BAPT A-AM, can reduce RVFV (MOI = 0.1) replication significantly if applied early in infection *in vitro* at 10 μ M [130]. 5,6-dimethoxyindan-1-one derivatives display high efficacy against diverse viruses including HIV, VSV, Tacaribe and

RVFV MP-12, with an IC_{50} against MP-12 of 0.78 $\mu\text{g/ml}$ *in vitro*. The broad-spectrum activity implies that the compound may be targeting a host factor to successfully inhibit such divergent viruses, but the mechanism must be explored further [131].

Lipid-enveloped viruses, including RVFV, must make extensive use of host lipid membranes and membrane biogenesis machinery. RVFV in particular is sensitive to the action of the AMP kinase pathway, which can inhibit acetyl CoA carboxylase and thereby fatty acid synthesis, slowing RVFV genome replication and reducing viral release overall [132]. As kinases are responsible for transferring phosphate groups to target molecules, protein phosphatases perform the opposite reaction and remove them. Their interplay regulates many cell signaling functions. Protein phosphatase 1 (PP1), a promiscuous interactor with many signaling pathways, has been shown to modulate virus replication in RVFV, HIV, Ebola virus and other viruses [133–135]. 1E7-03 was identified as an inhibitor of PP1, which in cells infected with RVFV MP-12 (MOIs = 0.1 and 3) reduced the overall amount of viral protein, RNA and thus viral output [136]. The production of 25-hydroxycholesterol (25HC) inhibits RVFV ZH501 (MOI = 0.1) replication by blocking the fusion of viral and cellular membranes during infection. 25HC is a soluble form of cholesterol produced by the ISG cholesterol-25-hydroxylase following infection. Stimulators of this enzyme, or exogenous 25HC should be tested *in vivo* for antiviral activity [137].

Hydrogen sulfide (H_2S) is a gaseous intracellular signal transducer involved in regulation of inflammation, apoptosis, oxidative stress and cell cycle, to name a few [138]. Due to its many important physiological functions, H_2S has become a candidate for therapeutic intervention, primarily of diseases involving inflammatory reactions [139]. Delivery of exogenous H_2S can be achieved, for example, by synthetic H_2S -releasing compounds, such as GYY4137 [140]. GYY4137 has been demonstrated to inhibit replication of the paramyxoviruses respiratory syncytial virus (MOI = 1), metapneumovirus (MOI = 1) and Nipah virus (MOI = 0.01) [141]. In a respiratory syncytial virus mouse model, intranasal (IN) administered GYY4137 resulted in reduction of viral titers following IN infection with 10^7 pfu, in the lung and airway inflammation, and in an overall improved disease outcome [142]. In a recent publication, the broad-spectrum antiviral activity of GYY4137 was demonstrated against RVFV and Crimean–Congo hemorrhagic fever virus, as well as influenza virus, Ebola virus and far-eastern subtype tick-borne encephalitis flavivirus [143]. In the case of RVFV (MOI = 1), viral titers and levels of viral RNA were significantly reduced *in vitro* compared with nontreated cells. The antiviral therapeutic efficacy of GYY4137 *in vivo* will still need to be demonstrated.

While generalized high-throughput assays rely on reporter viruses or assays with simple readouts, such as fluorescence or luminescence, the increase in computing power and miniaturization has allowed for imaging based solutions. In these assays, individual culture plate wells are imaged in which cells are expressing a variety of fluorophores to distinguish individual cells from each other, as well as subcellular structures [144]. This can allow for more detailed observation of the phenotype of virus infection in a specific cell type. These data, combined with a more nuanced view of the percentage of infected cells, can provide more granular information on the actual effect of the compound under study and its effect on viral replication. This approach has been taken to evaluate the efficacy of a signal transduction inhibitor library in addition to a protease inhibitor library against both RVFV vaccine strain MP-12 as well as pathogenic ZH501 (both MOI = 1) strain *in vitro* [145,146]. From the protease inhibitor library, four compounds were identified with efficacies between 13 and 61 μM in HeLa cells, as well as small airway epithelial cell primary cell cultures. These compounds exhibited diverse mechanisms of inhibition, from Golgi trafficking defects, to mitotic arrest and microtubule depolymerization. Signal transduction inhibitors demonstrated broad antiviral activity at both the level of viral entry and replications, but not assembly or release of infectious particles.

Further work demonstrating the importance of other cellular factors could also illuminate new targets for anti-RVFV drug development. RNASEK (ribonuclease κ) has been shown to be involved in the internalization step of human cells by viruses that enter through an acid-dependent pathway, such as RVFV, West Nile virus and influenza virus [147]. Depletion of human RNASEK by siRNA significantly reduced RVFV (MOI = 1) infection of human U2OS cells. Importantly, inhibition of the RNASEK pathway does not significantly alter the normal uptake of cellular metabolites, making it a possible target for broad-spectrum antiviral development, which need to be demonstrated in future *in vivo* efficacy studies [147]. Conversely, as autophagy pathways are essential for RVFV replication, they could be further upregulated to support antiviral effects. Work by Moy and colleagues has shown that this pathway is deeply conserved in animal phylogeny, and therefore may also represent a broadly applicable antiviral target through the TLR MyD88 and the induction of autophagy. SMER28, a novel mTOR independent modulator of autophagy, inhibits RVFV (MOI = 1) replication with an IC_{50} of 6.9 μM [124]. In a whole genome

siRNA screen, the Wnt/ β -Catenin pathway was demonstrated to be an essential factor in RVFV infection, possibly through cell cycle modulation, and inhibitors of this pathway could be used as RVFV antivirals or probes [148]. Modulation of such basic cellular processes must be closely evaluated, but short treatment courses and careful evaluation of side effects may make treatment and development worthwhile. Particularly, if the compound can be shown efficacious against multiple neglected or emerging diseases.

Cells and organisms must protect themselves against the deleterious effects of oxidation by reactive species generated both by insult and by normal cellular processes. Oxidative stress as a result of viral infection has been shown to be a contributor to pathogenesis [149]. Indeed, Narayanan *et al.* demonstrated that the antioxidant enzyme superoxide dismutase 1 is downregulated at early time points after infection with RVFV, resulting in an increase of reactive oxygen species, as well as apoptosis in those cells [150]. Based on those data, antioxidants could potentially have a therapeutic effect on controlling RVFV replication, as well as improve survival of infected cells. An HTS performed by Panchal *et al.* identified a compound, NSC 62914, which acts as a scavenger of reactive oxygen species and exhibited an EC_{50} of 5.2 μ M against Ebola virus (MOI = 5) infection in Vero 76 cells [151]. In addition, this compound had antiviral activity with similar EC_{50} s against the related Marburg virus, but also RVFV (MOI = 1), Venezuelan Equine Encephalitis Virus (MOI = 1) and Lassa virus (MOI = 0.1), which led the authors to speculate that NSC 62914 is targeting a cellular pathways that is commonly used by those viruses. Importantly, a prophylactic effect could be demonstrated in the C57BL/6 mouse model (dosing and infection (1000 pfu) was performed via the IP route) for filoviruses, resulting in 80% survival in the Ebola virus and 90% survival in the Marburg virus challenge models when administered 1 hour prior to infection. In a postexposure setting, survival was reduced to 50% in the Ebola virus model when administered 1 day postinfection. Evaluation for efficacy against RVFV in an animal model still needs to be explored.

Nonsmall-molecule inhibitors

RNA aptamers are RNA oligonucleotides that are capable of binding with high affinity and specificity to specific targets. Ellenbecker *et al.* characterized the RNA-binding properties of the RVFV N protein as a potential target for development of antiviral strategies [111,112]. Specifically, they selected RNA aptamers that inhibit the formation of vRNA-N complexes, and could therefore potentially be utilized as sensitive sensors for future drug screening approaches.

The RVFV glycoprotein Gc is a class II fusion protein, and mediates the fusion step of the viral envelope with the host cellular membrane, allowing the viral genome to be released in the host cellular cytoplasm [38]. Disrupting this crucial step during the lifecycle of RVFV will likely prevent establishment of a successful infection, as has been demonstrated for other viruses such as HIV [152,153], dengue virus [154–157] and henipaviruses [158]. Koehler *et al.* designed a library of peptides analogous to the RVFV Gc glycoprotein nontransmembrane domain regions and demonstrate that peptides targeting the Gc's stem region (amino acids 449–468) being able to inhibit an RVFV-pseudotyped VSV-luc reporter virus [159]. Interestingly, they identified a peptide termed RVFV-6, which targets amino acids 450–468, not only to inhibit RVFV infection, but also infection of other viruses including Andes virus and Ebola virus, both using class I fusion proteins, as well as VSV using a class III fusion protein. Structural modeling of the different viral glycoproteins suggests that all display similar structures in their membrane proximal regions that can be modeled as highly amphipathic α -helices. Future studies must evaluate the inhibitory efficacy of this fusion-inhibiting peptide in relevant animal models.

Conclusion & future perspective

Rift Valley fever is a severe veterinary disease of livestock that also causes moderate-to-severe illness in humans. Since its discovery more than 80 years ago, RVF has broadened its geographic distribution throughout continental Africa, to the Arabian Peninsula, as well as Madagascar. Of additional concern is the presence of potentially competent mosquito vector in the Americas and Europe [19,160,161], potentially allowing RVF to emerge in new locations and cause substantial morbidity and mortality in humans and economic losses for the livestock industry [14]. Furthermore, travel-associated cases of human RVF have occurred previously [162,163]. The WHO recommended RVFV to be listed among the top emerging pathogens likely to cause severe outbreaks in the near future, and for which few or no medical countermeasures exist [164–166]. To control epizootics and to prevent the transmission into the human population in high-risk areas, vaccination of livestock is critical [167]. Symptoms of the mild form of RVF can be treated with medication for fever and pain relief; however, no specific and approved therapeutics are available for patients presenting with severe symptoms [14]. While there has been intensive research on the general

aspects of the lifecycle of RVFV and its pathogenicity, development and characterization of antivirals has been limited. Although robust rodent and nonhuman primate animal models for RVFV have been developed, only a very limited number of promising antivirals have been evaluated *in vivo* thus far. This might be due to dearth of animal BSL3E or BSL4 biocontainment facilities (even though multiple screens have utilized the BSL2 vaccine strain MP-12) or even potentially the bias against negative data publication from performed *in vivo* studies.

From the currently available data, promising different approaches can be further investigated, such as blocking virus entry with peptide fusion inhibitors [159], or interfering with viral replication with either nonnucleoside inhibitors (favipiravir) [104,105] or nucleoside analogs (BCX4430) [113]. Some of those approaches also have shown broad-spectrum activity against other viruses. Host targeting antivirals, specifically repurposing of FDA-approved compounds, such as proteasome inhibitors [122], have the potential to greatly improve antiviral therapy and potentially minimize the development of resistance mutations, as well as making use of the vast amount of safety and toxicology data already available for approved drugs. The fact that RVF can result in three severe clinical outcomes (hemorrhagic fever, retinitis or encephalitis), antiviral treatments will need to be developed capable of targeting the liver, eyes and/or CNS. While systemically delivered antivirals (e.g., favipiravir) might be effective in treating hepatic manifestation of RVF, poor penetration across the blood–brain barrier may not prevent late-onset neurological complications [104,105] and must be considered during medical management of patients.

Executive summary

Rift Valley fever virus

- Rift Valley fever virus (RVFV) is an important mosquito-borne veterinary and human pathogen that can cause severe and fatal illness.
- There is a significant concern that RVFV can emerge in non-endemic countries, including Europe and the Americas, which would affect human health and the livestock industry.

Pathogenesis & clinical symptoms in patients

- RVFV infection can range from a self-limiting febrile illness to severe hepatitis, hemorrhagic fever, encephalitis or retinitis.
- There are no approved vaccines or therapeutics for RVF in humans.

Antivirals for RVFV

- Ribavirin has demonstrated *in vitro* activity, but only limited efficacy *in vivo*.
- The broad-spectrum antiviral favipiravir protects rodents against acute hepatitis, but does not protect from late-onset encephalitis.
- Treatment with the broad-spectrum nucleoside analog BCX4430 resulted in increased survival in the mouse model.
- Multiple small molecules targeting viral components have demonstrated activity in cell culture.
- Compounds targeting host cellular components or pathways, such as the ubiquitin proteasome system, autophagy system, kinases and oxidative stress responses, have demonstrated antiviral efficacy against RVFV *in vitro*.
- Peptide fusion inhibitors targeting the RVFV envelope Gc glycoprotein have promising activity to block virus entry.

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References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

- 1 Daubney R, Hudson JR, Garnham PC. Enzootic hepatitis or rift valley fever. An undescribed virus disease of sheep cattle and man from East Africa. *J. Pathol. Bacteriol.* 34(4), 545–579 (1931).
- 2 Bird BH, Ksiazek TG, Nichol ST, Maclachlan NJ. Rift Valley fever virus. *J. Am. Vet. Med. Assoc.* 234(7), 883–893 (2009).
- 3 Linthicum KJ, Britch SC, Anyamba A. Rift Valley fever: an emerging mosquito-borne disease. *Annu. Rev. Entomol.* 61, 395–415 (2016).

- 4 Fafetine JM, Coetzee P, Mubemba B *et al.* Rift Valley fever outbreak in livestock, Mozambique, 2014. *Emerg. Infect. Dis.* 22(12), 2165–2167 (2016).
- 5 El Mamy AB, Lo MM, Thiongane Y *et al.* Comprehensive phylogenetic reconstructions of Rift Valley fever virus: the 2010 northern Mauritania outbreak in the *Camelus dromedarius* species. *Vector Borne Zoonotic Dis.* 14(12), 856–861 (2014).
- 6 Sow A, Faye O, Ba Y *et al.* Rift Valley fever outbreak, southern Mauritania, 2012. *Emerg. Infect. Dis.* 20(2), 296–299 (2014).
- 7 Hassan OA, Ahlm C, Sang R, Evander M. The 2007 Rift Valley fever outbreak in Sudan. *PLoS Negl. Trop. Dis.* 5(9), e1229 (2011).
- 8 Archer BN, Weyer J, Paweska J *et al.* Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. *S. Afr. Med. J.* 101(4), 263–266 (2011).
- 9 Jost CC, Nzietchueng S, Kihu S *et al.* Epidemiological assessment of the Rift Valley fever outbreak in Kenya and Tanzania in 2006 and 2007. *Am. J. Trop. Med. Hyg.* 83(2 Suppl.), 65–72 (2010).
- 10 Findlay GM, Daubney R. The virus of Rift Valley fever or enzootic hepatitis. *Lancet* 218(5651), 1350–1351 (1931).
- 11 Easterday BC. Rift Valley fever. *Adv. Vet. Sci.* 10, 65–127 (1965).
- 12 Daubney R, Hudson JR. Rift Valley fever. *Lancet* 1, 611–612 (1932).
- 13 Linthicum KJ, Davies FG, Kairo A, Bailey CL. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an interepizootic period in Kenya. *J. Hyg. (Lond.)* 95(1), 197–209 (1985).
- 14 Britch SC, Linthicum KJ, The Rift Valley Fever Working Group. Developing a research agenda and a comprehensive national prevention and response plan for Rift Valley fever in the United States. *Emerging Infect. Dis.* 13(8), e1 (2007).
- 15 Golnar AJ, Kading RC, Hamer GL. Quantifying the potential pathways and locations of Rift Valley fever virus entry into the United States. *Transbound. Emerg. Dis.* doi:10.1111/tbed.12608 (2017) (Epub ahead of print).
- 16 Golnar AJ, Turell MJ, Labeaud AD, Kading RC, Hamer GL. Predicting the mosquito species and vertebrate species involved in the theoretical transmission of Rift Valley fever virus in the United States. *PLoS Negl. Trop. Dis.* 8(9), e3163 (2014).
- 17 House JA, Turell MJ, Mebus CA. Rift Valley fever: present status and risk to the western hemisphere. *Ann. NY Acad. Sci.* 653, 233–242 (1992).
- 18 Kasari TR, Carr DA, Lynn TV, Weaver JT. Evaluation of pathways for release of Rift Valley fever virus into domestic ruminant livestock, ruminant wildlife, and human populations in the continental United States. *J. Am. Vet. Med. Assoc.* 232(4), 514–529 (2008).
- 19 Rolin AI, Berrang-Ford L, Kulkarni MA. The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. *Emerg. Microbes Infect.* 2(12), e81 (2013).
- 20 Liu J, Sun Y, Shi W *et al.* The first imported case of Rift Valley fever in China reveals a genetic reassortment of different viral lineages. *Emerg. Microbes Infect.* 6(1), e4 (2017).
- 21 Liu W, Sun FJ, Tong YG, Zhang SQ, Cao WC. Rift Valley fever virus imported into China from Angola. *Lancet Infect. Dis.* 16(11), 1226 (2016).
- 22 Ikegami T. Rift Valley fever vaccines: an overview of the safety and efficacy of the live-attenuated MP-12 vaccine candidate. *Expert Rev. Vaccines* 16(6), 601–611 (2017).
- 23 Indran SV, Ikegami T. Novel approaches to develop Rift Valley fever vaccines. *Front. Cell Infect. Microbiol.* 2, 131 (2012).
- 24 Ikegami T, Makino S. Rift valley fever vaccines. *Vaccine* 27(Suppl. 4), D69–D72 (2009).
- 25 Kortekaas J. One health approach to Rift Valley fever vaccine development. *Antiviral Res.* 106, 24–32 (2014).
- 26 Schmaljohn C, Nichol ST. Bunyaviridae In: *Fields Virology*, Knipe DM, Howley PM, Griffin DE *et al.* (Eds). Lippincott, Williams & Wilkins Philadelphia, PA, USA, 1741–1789 (2007).
- 27 Strandin T, Hepojoki J, Vaheri A. Cytoplasmic tails of bunyavirus Gn glycoproteins – could they act as matrix protein surrogates? *Virology* 437(2), 73–80 (2013).
- 28 Pettersson RF, Andersson A, Melin L. Mapping a retention signal for Golgi localization of a viral spike protein complex. *Cold Spring Harb. Symp. Quant. Biol.* 60, 147–155 (1995).
- 29 Piper ME, Sorenson DR, Gerrard SR. Efficient cellular release of Rift Valley fever virus requires genomic RNA. *PLoS ONE* 6(3), e18070 (2011).
- 30 Carnec X, Ermonval M, Kreher F, Flamand M, Bouloy M. Role of the cytosolic tails of Rift Valley fever virus envelope glycoproteins in viral morphogenesis. *Virology* 448, 1–14 (2014).
- 31 Sherman MB, Freiberg AN, Holbrook MR, Watowich SJ. Single-particle cryo-electron microscopy of Rift Valley fever virus. *Virology* 387(1), 11–15 (2009).
- 32 Freiberg AN, Sherman MB, Morais MC, Holbrook MR, Watowich SJ. Three-dimensional organization of Rift Valley fever virus revealed by cryoelectron tomography. *J. Virol.* 82(21), 10341–10348 (2008).
- 33 Huiskonen JT, Overby AK, Weber F, Grunewald K. Electron cryo-microscopy and single-particle averaging of Rift Valley fever virus: evidence for GN-GC glycoprotein heterodimers. *J. Virol.* 83(8), 3762–3769 (2009).

- 34 Harmon B, Schudel BR, Maar D *et al*. Rift Valley fever virus strain MP-12 enters mammalian host cells via caveola-mediated endocytosis. *J. Virol.* 86(23), 12954–12970 (2012).
- 35 Lozach PY, Kuhbacher A, Meier R *et al*. DC-SIGN as a receptor for Phleboviruses. *Cell Host Microbe* 10(1), 75–88 (2011).
- 36 De Boer SM, Kortekaas J, De Haan CA, Rottier PJ, Moormann RJ, Bosch BJ. Heparan sulfate facilitates Rift Valley fever virus entry into the cell. *J. Virol.* 86(24), 13767–13771 (2012).
- 37 Garry CE, Garry RF. Proteomics computational analyses suggest that the carboxyl terminal glycoproteins of Bunyaviruses are class II viral fusion protein (beta-penitrenes). *Theor. Biol. Med. Model.* 1, 1–10 (2004).
- 38 Dessau M, Modis Y. Crystal structure of glycoprotein C from Rift Valley fever virus. *Proc. Natl Acad. Sci. USA* 110(5), 1696–1701 (2013).
- 39 De Boer SM, Kortekaas J, Spel L, Rottier PJ, Moormann RJ, Bosch BJ. Acid-activated structural reorganization of the Rift Valley fever virus Gc fusion protein. *J. Virol.* 86(24), 13642–13652 (2012).
- 40 Vialat P, Billecocq A, Kohl A, Bouloy M. The S segment of Rift Valley fever *Phlebovirus* (*Bunyaviridae*) carries determinants for attenuation and virulence in mice. *J. Virol.* 74(3), 1538–1543 (2000).
- 41 Ly HJ, Ikegami T. Rift Valley fever virus NSs protein functions and the similarity to other Bunyavirus NSs proteins. *Virol. J.* 13, 118 (2016).
- 42 Ikegami T, Narayanan K, Won S, Kamitani W, Peters CJ, Makino S. Dual functions of Rift Valley fever virus NSs protein: inhibition of host mRNA transcription and post-transcriptional downregulation of protein kinase PKR. *Ann. NY Acad. Sci.* 1171(Suppl. 1), E75–E85 (2009).
- 43 Le May N, Mansuroglu Z, Leger P *et al*. A SAP30 complex inhibits IFN-beta expression in Rift Valley fever virus infected cells. *PLoS Pathog.* 4(1), e13 (2008).
- 44 Swanepoel R, Blackburn NK. Demonstration of nuclear immunofluorescence in Rift Valley fever infected cells. *J. Gen. Virol.* 34(3), 557–561 (1977).
- 45 Yadani FZ, Kohl A, Prehaud C, Billecocq A, Bouloy M. The carboxy-terminal acidic domain of Rift Valley fever virus NSs protein is essential for the formation of filamentous structures but not for the nuclear localization of the protein. *J. Virol.* 73(6), 5018–5025 (1999).
- 46 Bird BH, Albarino CG, Nichol ST. Rift Valley fever virus lacking NSm proteins retains high virulence *in vivo* and may provide a model of human delayed onset neurologic disease. *Virology* 362(1), 10–15 (2007).
- 47 Meegan JM. The Rift Valley fever epizootic in Egypt 1977–78. 1. Description of the epizootic and virological studies. *Trans. R Soc. Trop. Med. Hyg.* 73(6), 618–623 (1979).
- 48 Hoogstraal H, Meegan JM, Khalil GM, Adham FK. The Rift Valley fever epizootic in Egypt 1977–78. 2. Ecological and entomological studies. *Trans. R Soc. Trop. Med. Hyg.* 73(6), 624–629 (1979).
- 49 Woods CW, Karpati AM, Grein T *et al*. An outbreak of Rift Valley fever in northeastern Kenya, 1997–98. *Emerg. Infect. Dis.* 8(2), 138–144 (2002).
- 50 Mundel B, Gear J. Rift Valley fever; I. The occurrence of human cases in Johannesburg. *S. Afr. Med. J.* 25(44), 797–800 (1951).
- 51 Joubert JD, Ferguson AL, Gear J. Rift Valley fever in South Africa: 2. The occurrence of human cases in the orange free state, the north-western Cape province, the western and southern Transvaal. A. Epidemiological and clinical findings. *S. Afr. Med. J.* 25(48), 890–891 (1951).
- 52 Strausbaugh LJ, Laughlin LW, Meegan JM, Watten RH. Clinical studies on Rift Valley fever, Part I. Acute febrile and hemorrhagic-like diseases. *J. Egypt. Public Health Assoc.* 53(3–4), 181–182 (1978).
- 53 Madani TA, Al-Mazrou YY, Al-Jeffri MH *et al*. Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. *Clin. Infect. Dis.* 37(8), 1084–1092 (2003).
- 54 Findlay GM, Daubney R. The virus of rift valley fever or enzootic hepatitis. *Lancet* 221, 1350–1351 (1931).
- 55 Kahlon SS, Peters CJ, Leduc J *et al*. Severe Rift Valley fever may present with a characteristic clinical syndrome. *Am. J. Trop. Med. Hyg.* 82(3), 371–375 (2010).
- 56 Laughlin LW, Meegan JM, Strausbaugh LJ, Morens DM, Watten RH. Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. *Trans. R. Soc. Trop. Med. Hyg.* 73(6), 630–633 (1979).
- 57 Yassin W. Clinico-pathological picture in five human cases died with Rift Valley fever. *J. Egypt. Public Health Assoc.* 53(3–4), 191–193 (1978).
- 58 Swanepoel R, Manning B, Watt JA. Fatal Rift Valley fever of man in Rhodesia. *Cent. Afr. J. Med.* 25(1), 1–8 (1979).
- 59 Al-Hazmi M, Ayoola EA, Abdurahman M *et al*. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. *Clin. Infect. Dis.* 36(3), 245–252 (2003).
- 60 Abdel-Wahab KS, El Baz LM, El-Tayeb EM, Omar H, Ossman MA, Yasin W. Rift Valley fever virus infections in Egypt: pathological and virological findings in man. *Trans. R. Soc. Trop. Med. Hyg.* 72(4), 392–396 (1978).
- 61 Alrajhi AA, Al-Semari A, Al-Watban J. Rift Valley fever encephalitis. *Emerg. Infect. Dis.* 10(3), 554–555 (2004).

- 62 Maar SA, Swanepoel R, Gelfand M. Rift Valley fever encephalitis. A description of a case. *Cent. Afr. J. Med.* 25(1), 8–11 (1979).
- 63 Van Velden DJ, Meyer JD, Olivier J, Gear JH, McIntosh B. Rift Valley fever affecting humans in South Africa: a clinicopathological study. *S. Afr. Med. J.* 51(24), 867–871 (1977).
- 64 El-Shinnawi BM, Sobhy F, El-Zawahry A. Laboratory findings in some cases of Rift Valley fever: histopathological haematological, and biochemical. *J. Egypt. Public Health Assoc.* 53(3–4), 187–189 (1978).
- 65 Deutman AF, Klomp HJ. Rift Valley fever retinitis. *Am. J. Ophthalmol.* 92(1), 38–42 (1981).
- 66 Siam AL, Meegan JM, Gharbawi KF. Rift Valley fever ocular manifestations: observations during the 1977 epidemic in Egypt. *Br. J. Ophthalmol.* 64(5), 366–374 (1980).
- 67 Siam AL, Meegan JM. Ocular disease resulting from infection with Rift Valley fever virus. *Trans. R Soc. Trop. Med. Hyg.* 74(4), 539–541 (1980).
- 68 Schrire L. Macular changes in Rift Valley fever. *S. Afr. Med. J.* 25(50), 926–930 (1951).
- 69 Freed I. Rift Valley fever in man, complicated by retinal changes and loss of vision. *S. Afr. Med. J.* 25(50), 930–932 (1951).
- 70 Ayoub M, Barhoma G, Zaghlol I. Ocular manifestations of Rift Valley fever. *Bull. Ophthalmol. Soc. Egypt* 71(75), 125–133 (1978).
- 71 Al-Hazmi A, Al-Rajhi AA, Abboud EB *et al.* Ocular complications of Rift Valley fever outbreak in Saudi Arabia. *Ophthalmology* 112(2), 313–318 (2005).
- 72 Behara R, Reau N. Updates on hepatitis C virus therapy in the direct-acting antiviral era. *Curr. Opin. Gastroenterol.* 33(3), 115–119 (2017).
- 73 Maina G, Mill J, Chaw-Kant J, Caine V. A systematic review of best practices in HIV care. *J. HIV AIDS Soc. Serv.* 15(1), 114–126 (2016).
- 74 Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb. Perspect. Med.* 2(4), a007161 (2012).
- 75 Caplen H, Peters CJ, Bishop DH. Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. *J. Gen. Virol.* 66(Pt 10) 2271–2277 (1985).
- 76 Kilgore PE, Ksiazek TG, Rollin PE *et al.* Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin. Infect. Dis.* 24(4), 718–722 (1997).
- 77 McCormick JB, King IJ, Webb PA *et al.* Lassa fever. Effective therapy with ribavirin. *N. Engl. J. Med.* 314(1), 20–26 (1986).
- 78 Monath TP. Treatment of yellow fever. *Antiviral Res.* 78(1), 116–124 (2008).
- 79 Kirsi JJ, North JA, McKernan PA *et al.* Broad-spectrum antiviral activity of 2-beta-D-ribofuranosylselenazole-4-carboxamide, a new antiviral agent. *Antimicrob. Agents Chemother.* 24(3), 353–361 (1983).
- 80 Sidwell RW, Huffman JH, Barnett BB, Pifat DY. *In vitro* and *in vivo* Phlebovirus inhibition by ribavirin. *Antimicrob. Agents Chemother.* 32(3), 331–336 (1988).
- 81 Kende M, Alving CR, Rill WL, Swartz GM Jr, Canonico PG. Enhanced efficacy of liposome-encapsulated ribavirin against Rift Valley fever virus infection in mice. *Antimicrob. Agents Chemother.* 27(6), 903–907 (1985).
- **Provides early data on antiviral intervention during Rift Valley fever virus (RVFV) infection in animal models.**
- 82 Reed C, Lin K, Wilhelmsen C *et al.* Aerosol exposure to Rift Valley fever virus causes earlier and more severe neuropathology in the murine model, which has important implications for therapeutic development. *PLoS Negl. Trop. Dis.* 7(4), e2156 (2013).
- 83 Kende M, Lupton HW, Rill WL, Gibbs P, Levy HB, Canonico PG. Ranking of prophylactic efficacy of poly(ICLC) against Rift Valley fever virus infection in mice by incremental relative risk of death. *Antimicrob. Agents Chemother.* 31(8), 1194–1198 (1987).
- 84 Peters CJ, Reynolds JA, Slone TW, Jones DE, Stephen EL. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. *Antiviral Res.* 6(5), 285–297 (1986).
- 85 Furuta Y, Takahashi K, Kuno-Maekawa M *et al.* Mechanism of action of T-705 against influenza virus. *Antimicrob. Agents Chemother.* 49(3), 981–986 (2005).
- 86 Kiso M, Takahashi K, Sakai-Tagawa Y *et al.* T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. *Proc. Natl Acad. Sci. USA* 107(2), 882–887 (2010).
- 87 Furuta Y, Takahashi K, Shiraki K *et al.* T-705 (favipiravir) and related compounds: novel broad-spectrum inhibitors of RNA viral infections. *Antiviral Res.* 82(3), 95–102 (2009).
- 88 Sleeman K, Mishin VP, Deyde VM, Furuta Y, Klimov AI, Gubareva LV. *In vitro* antiviral activity of favipiravir (T-705) against drug-resistant influenza and 2009 A(H1N1) viruses. *Antimicrob. Agents Chemother.* 54(6), 2517–2524 (2010).
- 89 Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. *Antiviral Res.* 100(2), 446–454 (2013).
- 90 Safronetz D, Rosenke K, Westover JB *et al.* The broad-spectrum antiviral favipiravir protects guinea pigs from lethal Lassa virus infection post-disease onset. *Sci. Rep.* 5, 14775 (2015).
- 91 Gowen BB, Wong MH, Jung KH *et al.* *In vitro* and *in vivo* activities of T-705 against arenavirus and Bunyavirus infections. *Antimicrob. Agents Chemother.* 51(9), 3168–3176 (2007).

- 92 Jochmans D, Van Nieuwkoop S, Smits SL, Neyts J, Fouchier RA, Van Den Hoogen BG. Antiviral activity of favipiravir (T-705) against a broad range of Paramyxoviruses *in vitro* and against human metapneumovirus in hamsters. *Antimicrob. Agents Chemother.* 60(8), 4620–4629 (2016).
- 93 Wolf MC, Freiberg AN, Zhang T *et al.* A broad-spectrum antiviral targeting entry of enveloped viruses. *Proc. Natl Acad. Sci. USA* 107(7), 3157–3162 (2010).
- 94 Safronetz D, Falzarano D, Scott DP, Furuta Y, Feldmann H, Gowen BB. Antiviral efficacy of favipiravir against two prominent etiological agents of hantavirus pulmonary syndrome. *Antimicrob. Agents Chemother.* 57(10), 4673–4680 (2013).
- 95 Mendenhall M, Russell A, Juelich T *et al.* T-705 (favipiravir) inhibition of arenavirus replication in cell culture. *Antimicrob. Agents Chemother.* 55(2), 782–787 (2011).
- 96 Mendenhall M, Russell A, Smee DF *et al.* Effective oral favipiravir (T-705) therapy initiated after the onset of clinical disease in a model of arenavirus hemorrhagic fever. *PLoS Negl. Trop. Dis.* 5(10), e1342 (2011).
- 97 Julander JG, Shafer K, Smee DF, Morrey JD, Furuta Y. Activity of T-705 in a hamster model of yellow fever virus infection in comparison with that of a chemically related compound, T-1106. *Antimicrob. Agents Chemother.* 53(1), 202–209 (2009).
- 98 Julander JG, Smee DF, Morrey JD, Furuta Y. Effect of T-705 treatment on western equine encephalitis in a mouse model. *Antiviral Res.* 82(3), 169–171 (2009).
- 99 Rocha-Pereira J, Jochmans D, Dallmeier K, Leyssen P, Nascimento MS, Neyts J. Favipiravir (T-705) inhibits *in vitro* norovirus replication. *Biochem. Biophys. Res. Commun.* 424(4), 777–780 (2012).
- 100 Furuta Y, Takahashi K, Fukuda Y *et al.* *In vitro* and *in vivo* activities of anti-influenza virus compound T-705. *Antimicrob. Agents Chemother.* 46(4), 977–981 (2002).
- 101 Gowen BB, Smee DF, Wong MH *et al.* Treatment of late stage disease in a model of arenaviral hemorrhagic fever: T-705 efficacy and reduced toxicity suggests an alternative to ribavirin. *PLoS ONE* 3(11), e3725 (2008).
- 102 Gowen BB, Julander JG, London NR *et al.* Assessing changes in vascular permeability in a hamster model of viral hemorrhagic fever. *Viol. J.* 7, 240 (2010).
- 103 Oestereich L, Rieger T, Neumann M *et al.* Evaluation of antiviral efficacy of ribavirin, arbidol, and T-705 (favipiravir) in a mouse model for Crimean–Congo hemorrhagic fever. *PLoS Negl. Trop. Dis.* 8(5), e2804 (2014).
- 104 Scharton D, Bailey KW, Vest Z *et al.* Favipiravir (T-705) protects against peracute Rift Valley fever virus infection and reduces delayed-onset neurologic disease observed with ribavirin treatment. *Antiviral Res.* 104, 84–92 (2014).
- **Describes the antiviral efficacy of broad-spectrum antiviral favipiravir in the hamster model. In addition, combinatorial effect of favipiravir with ribavirin is studied to limit CNS infection by RVFV.**
- 105 Caroline AL, Powell DS, Bethel LM, Oury TD, Reed DS, Hartman AL. Broad spectrum antiviral activity of favipiravir (T-705): protection from highly lethal inhalational Rift Valley fever. *PLoS Negl. Trop. Dis.* 8(4), e2790 (2014).
- **Evaluates the antiviral effect of favipiravir in an aerosol exposure rat model.**
- 106 Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin. *J. Med. Chem.* 60(5), 1620–1637 (2017).
- 107 Narayanan A, Kehn-Hall K, Senina S *et al.* Curcumin inhibits Rift Valley fever virus replication in human cells. *J. Biol. Chem.* 287(40), 33198–33214 (2012).
- 108 Garcia S, Crance JM, Billecocq A *et al.* Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. *J. Clin. Microbiol.* 39(12), 4456–4461 (2001).
- 109 Broce S, Hensley L, Sato T *et al.* Biochemical and biophysical characterization of cell-free synthesized Rift Valley fever virus nucleoprotein capsids enables *in vitro* screening to identify novel antivirals. *Biol. Direct* 11, 25 (2016).
- 110 Ellenbecker M, Lanchy JM, Lodmell JS. Inhibition of Rift Valley fever virus replication and perturbation of nucleocapsid-RNA interactions by suramin. *Antimicrob. Agents Chemother.* 58(12), 7405–7415 (2014).
- 111 Ellenbecker M, Lanchy JM, Lodmell JS. Identification of Rift Valley fever virus nucleocapsid protein-RNA binding inhibitors using a high-throughput screening assay. *J. Biomol. Screen.* 17(8), 1062–1070 (2012).
- 112 Ellenbecker M, Sears L, Li P, Lanchy JM, Lodmell JS. Characterization of RNA aptamers directed against the nucleocapsid protein of Rift Valley fever virus. *Antiviral Res.* 93(3), 330–339 (2012).
- 113 Warren TK, Wells J, Panchal RG *et al.* Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. *Nature* 508(7496), 402–405 (2014).
- **Treatment with the broad-spectrum nucleoside analog BCX4430 resulted in increased survival in the mouse model.**
- 114 Taylor R, Kotian P, Warren T *et al.* BCX4430 – a broad-spectrum antiviral adenosine nucleoside analog under development for the treatment of Ebola virus disease. *J. Infect. Public Health* 9(3), 220–226 (2016).
- 115 Islam MK, Baudin M, Eriksson J *et al.* High-throughput screening using a whole-cell virus replication reporter gene assay to identify inhibitory compounds against Rift Valley fever virus infection. *J. Biomol. Screen.* 21(4), 354–362 (2016).
- **High-throughput screen using reporter assay to increase identification of antiviral compounds outside high containment facilities.**

- 116 Vigant F, Santos NC, Lee B. Broad-spectrum antivirals against viral fusion. *Nat. Rev. Microbiol.* 13(7), 426–437 (2015).
- 117 Hollmann A, Castanho MA, Lee B, Santos NC. Singlet oxygen effects on lipid membranes: implications for the mechanism of action of broad-spectrum viral fusion inhibitors. *Biochem. J.* 459(1), 161–170 (2014).
- 118 Vigant F, Lee J, Hollmann A *et al.* A mechanistic paradigm for broad-spectrum antivirals that target virus-cell fusion. *PLoS Pathog.* 9(4), e1003297 (2013).
- **Broad-spectrum antiviral targeting the lipid envelope of RNA viruses.**
- 119 Benedict A, Bansal N, Senina S *et al.* Repurposing FDA-approved drugs as therapeutics to treat Rift Valley fever virus infection. *Front. Microbiol.* 6, 676 (2015).
- 120 Randow F, Lehner PJ. Viral avoidance and exploitation of the ubiquitin system. *Nat. Cell Biol.* 11(5), 527–534 (2009).
- 121 Chen D, Frezza M, Schmitt S, Kanwar J, Dou QP. Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. *Curr. Cancer Drug Targets* 11(3), 239–253 (2011).
- 122 Keck F, Amaya M, Kehn-Hall K, Roberts B, Bailey C, Narayanan A. Characterizing the effect of Bortezomib on Rift Valley Fever virus multiplication. *Antiviral Res.* 120, 48–56 (2015).
- **Evaluation of first US FDA-approved proteasome inhibitor as antiviral for RVFV infection.**
- 123 Aman MJ, Kinch MS, Warfield K *et al.* Development of a broad-spectrum antiviral with activity against Ebola virus. *Antiviral Res.* 83(3), 245–251 (2009).
- 124 Moy RH, Gold B, Molleston JM *et al.* Antiviral autophagy restricts Rift Valley fever virus infection and is conserved from flies to mammals. *Immunity* 40(1), 51–65 (2014).
- 125 Atkins C, Evans CW, Nordin B *et al.* Global human-kinase screening identifies therapeutic host targets against influenza. *J. Biomol. Screen.* 19(6), 936–946 (2014).
- 126 Dyall J, Coleman CM, Hart BJ *et al.* Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. *Antimicrob. Agents Chemother.* 58(8), 4885–4893 (2014).
- 127 Reeves PM, Bommarius B, Lebeis S *et al.* Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. *Nat. Med.* 11(7), 731–739 (2005).
- 128 Filone CM, Hanna SL, Caino MC, Bambina S, Doms RW, Cherry S. Rift valley fever virus infection of human cells and insect hosts is promoted by protein kinase C epsilon. *PLoS ONE* 5(11), e15483 (2010).
- 129 Bell TM, Espina V, Senina S *et al.* Rapamycin modulation of p70 S6 kinase signaling inhibits Rift Valley fever virus pathogenesis. *Antiviral Res.* 143, 162–175 (2017).
- 130 Nuss JE, Kehn-Hall K, Benedict A *et al.* Multi-faceted proteomic characterization of host protein complement of Rift Valley fever virus virions and identification of specific heat shock proteins, including HSP90, as important viral host factors. *PLoS ONE* 9(5), e93483 (2014).
- 131 Patil SA, Patil V, Patil R, Beaman K, Patil SA. Identification of novel 5,6-dimethoxyindan-1-one derivatives as antiviral agents. *Med. Chem.* doi:10.2174/1573406413666170330094822 (2017) (Epub ahead of print).
- 132 Moser TS, Schieffer D, Cherry S. AMP-activated kinase restricts Rift Valley fever virus infection by inhibiting fatty acid synthesis. *PLoS Pathog.* 8(4), e1002661 (2012).
- 133 Ilinykh PA, Tigabu B, Ivanov A *et al.* Role of protein phosphatase 1 in dephosphorylation of Ebola virus VP30 protein and its targeting for the inhibition of viral transcription. *J. Biol. Chem.* 289(33), 22723–22738 (2014).
- 134 Modrof J, Muhlberger E, Klenk HD, Becker S. Phosphorylation of VP30 impairs ebola virus transcription. *J. Biol. Chem.* 277(36), 33099–33104 (2002).
- 135 Nekhai S, Jerebtsova M, Jackson A, Southerland W. Regulation of HIV-1 transcription by protein phosphatase 1. *Curr. HIV Res.* 5(1), 3–9 (2007).
- 136 Baer A, Shafagati N, Benedict A *et al.* Protein phosphatase-1 regulates Rift Valley fever virus replication. *Antiviral Res.* 127, 79–89 (2016).
- **Targeting host processes may allow for the development of antivirals with reduced likelihood of resistance.**
- 137 Liu SY, Aliyari R, Chikere K *et al.* Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. *Immunity* 38(1), 92–105 (2013).
- 138 Olas B. Hydrogen sulfide in signaling pathways. *Clin. Chim. Acta* 439, 212–218 (2015).
- 139 Wu D, Hu Q, Zhu Y. Therapeutic application of hydrogen sulfide donors: the potential and challenges. *Front. Med.* 10(1), 18–27 (2016).
- 140 Rose P, Dymock BW, Moore PK. GYY4137, a novel water-soluble, H₂S-releasing molecule. *Methods Enzymol.* 554, 143–167 (2015).
- 141 Li H, Ma Y, Escaffre O *et al.* Role of hydrogen sulfide in paramyxovirus infections. *J. Virol.* 89(10), 5557–5568 (2015).
- 142 Ivanciuc T, Sbrana E, Ansar M *et al.* Hydrogen sulfide is an antiviral and antiinflammatory endogenous gasotransmitter in the airways. Role in respiratory syncytial virus infection. *Am. J. Respir. Cell Mol. Biol.* 55(5), 684–696 (2016).
- 143 Ibazhanov N, Escaffre O, Freiberg AN, Garofalo RP, Casola A. Broad-range antiviral activity of hydrogen sulfide against highly pathogenic RNA viruses. *Sci. Rep.* 7, 41029 (2017).

- 144 Boutros M, Heigwer F, Laufer C. Microscopy-based high-content screening. *Cell* 163(6), 1314–1325 (2015).
- 145 Mudhasani R, Kota KP, Retterer C *et al.* High-content image-based screening of a signal transduction pathway inhibitor small-molecule library against highly pathogenic RNA viruses. *J. Biomol. Screen.* 20(1), 141–152 (2015).
- 146 Mudhasani R, Kota KP, Retterer C, Tran JP, Whitehouse CA, Bavari S. High content image-based screening of a protease inhibitor library reveals compounds broadly active against Rift Valley fever virus and other highly pathogenic RNA viruses. *PLoS Negl. Trop. Dis.* 8(8), e3095 (2014).
- **Automated screening technologies that provide phenotypic information on the status of the cell culture during infection can provide more in depth preliminary data than basic cell viability assays.**
- 147 Hackett BA, Yasunaga A, Panda D *et al.* RNASEK is required for internalization of diverse acid-dependent viruses. *Proc. Natl Acad. Sci. USA* 112(25), 7797–7802 (2015).
- 148 Harmon B, Bird SW, Schudel BR, Hatch AV, Rasley A, Negrete OA. A genome-wide RNA interference screen identifies a role for WNT/beta-catenin signaling during Rift Valley fever virus infection. *J. Virol.* 90(16), 7084–7097 (2016).
- 149 Camini FC, Da Silva Caetano CC, Almeida LT, De Brito Magalhaes CL. Implications of oxidative stress on viral pathogenesis. *Arch. Virol.* 162(4), 907–917 (2017).
- 150 Narayanan A, Popova T, Turell M *et al.* Alteration in superoxide dismutase 1 causes oxidative stress and p38 MAPK activation following RVFV infection. *PLoS ONE* 6(5), e20354 (2011).
- 151 Panchal RG, Reid SP, Tran JP *et al.* Identification of an antioxidant small-molecule with broad-spectrum antiviral activity. *Antiviral Res.* 93(1), 23–29 (2012).
- 152 Kilby JM, Hopkins S, Venetta TM *et al.* Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat. Med.* 4(11), 1302–1307 (1998).
- 153 Wild C, Greenwell T, Matthews T. A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell-cell fusion. *AIDS Res. Hum. Retroviruses* 9(11), 1051–1053 (1993).
- 154 Hrobowski YM, Garry RF, Michael SF. Peptide inhibitors of dengue virus and West Nile virus infectivity. *Virol. J.* 2, 49 (2005).
- 155 Schmidt AG, Yang PL, Harrison SC. Peptide inhibitors of flavivirus entry derived from the E protein stem. *J. Virol.* 84(24), 12549–12554 (2010).
- 156 Schmidt AG, Yang PL, Harrison SC. Peptide inhibitors of dengue-virus entry target a late-stage fusion intermediate. *PLoS Pathog.* 6(4), e1000851 (2010).
- 157 Costin JM, Jenwitheesuk E, Lok SM *et al.* Structural optimization and *de novo* design of dengue virus entry inhibitory peptides. *PLoS Negl. Trop. Dis.* 4(6), e721 (2010).
- 158 Porotto M, Carta P, Deng Y *et al.* Molecular determinants of antiviral potency of paramyxovirus entry inhibitors. *J. Virol.* 81(19), 10567–10574 (2007).
- 159 Koehler JW, Smith JM, Ripoll DR *et al.* A fusion-inhibiting peptide against Rift Valley fever virus inhibits multiple, diverse viruses. *PLoS Negl. Trop. Dis.* 7(9), e2430 (2013).
- **Peptide fusion inhibitors targeting the RVFV envelope Gc glycoprotein have promising activity to block virus entry.**
- 160 Chevalier V, Pepin M, Plee L, Lancelot R. Rift Valley fever – a threat for Europe? *Euro Surveill.* 15(10), 19506 (2010).
- 161 Hartley DM, Rinderknecht JL, Nipp TL *et al.* Potential effects of Rift Valley fever in the United States. *Emerg. Infect. Dis.* 17(8), e1 (2011).
- 162 Rift Valley fever – China: ex Angola. www.promedmail.org/post/20160725.4368585
- 163 Rift Valley fever, human – France: ex Zimbabwe (Mashonaland east). www.promedmail.org/direct.php?id=916603
- 164 First report. www.promedmail.org/direct.php?id=916603
- 165 WHO. Workshop on prioritization of pathogens : blueprint for R&D preparedness and response to public health emergencies due to highly infectious pathogens. (2015).
- 166 WHO publishes list of top emerging diseases likely to cause major epidemics. www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/
- 167 Rift Valley fever – France ex Mali, 2015. www.promedmail.org/post/20160506.4207036
- 168 Bird BH, Nichol ST. Breaking the chain: Rift Valley fever virus control via livestock vaccination. *Curr. Opin. Virol.* 2(3), 315–323 (2012).
- 169 Kende M. Prophylactic and therapeutic efficacy of poly(I, C)-LC against Rift Valley fever virus infection in mice. *J. Biol. Response Mod.* 4(5), 503–511 (1985).