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Review

Antiviral agents against African swine fever virus

Erik Arabyan, Armen Kotsynyan, Astghik Hakobyan, Hovakim Zakaryan*

Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS, Yerevan, Armenia

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ABSTRACT

African swine fever virus (ASFV) is a significant transboundary virus that continues to spread outside Africa in Europe and most recently to China, Vietnam and Cambodia. Pigs infected with highly virulent ASFV develop a hemorrhagic fever like illness with high lethality reaching up to 100%. There are no vaccines or antiviral drugs available for the prevention or treatment of ASFV infections. We here review molecules that have been reported to inhibit ASFV replication, either as direct-acting antivirals or host-targeting drugs as well as those that act *via* a yet unknown mechanism. Prospects for future antiviral research against ASFV are also discussed.

1. Introduction

African swine fever (ASF) is one of the most important swine diseases due to its significant socioeconomic consequences for affected countries. It was first observed in Kenya in the early 20th century following the introduction into the country of European domestic swine. Montgomery (1921) described the disease as an entity distinct from classical swine fever. Shortly after Kenya, reports of ASF in South Africa and Angola followed (Steyn, 1928; Gago da Câmara, 1933). Since then, ASF were reported in many African countries, particularly in the sub-Saharan region, where it still remains within wild and domestic pig populations. It occurred in Europe (Portugal and Spain) in 1957 and 1960, and from there it spread to other European countries. However, ASF was eradicated from Europe in the mid-1990s, except for Sardinia, where it has been endemic since 1978 (Gallardo et al., 2015). In 2007, a second reintroduction of ASF to the European Continent occurred in Georgia from where it quickly spread to neighboring countries. ASF has been reported in Russia, Ukraine, Poland, Latvia, Lithuania, Estonia, Moldova, Czech Republic, Romania, Hungary and most recently in China, Vietnam and Cambodia. As a result of ASF outbreaks in the Russian and Eastern Europe, more than 800,000 pigs were slaughtered from 2014 to 2017 (Sánchez-Cordón et al., 2018). The value of exports of pork in Eastern Europe was reduced more than EUR 950 million (Cisek et al., 2016; Sánchez-Cordón et al., 2018; Chenais et al., 2019). Further spread and huge economic losses seems highly possible due to the lack of effective vaccines and control measures.

Since the first antiviral drug, idoxuridine, approved in 1963, great achievements have been made in the field of antiviral drug discovery. Today more than 90 antiviral drugs categorized into 13 functional groups are available for the treatment of human infectious diseases such as HIV and hepatitis B virus infections. Nevertheless, there is still no antiviral drug for more than 200 viruses affecting human populations worldwide (De Clercq and Li, 2016). Moreover, control of some animal viruses like ASFV by means of an antiviral therapy appears to be an attractive approach due to the lack of other control measures. Therefore, the development of antiviral agents for mass application in veterinary is as much important as the development of antiviral drugs against human viruses.

In this review, a general introduction to the replication cycle of African swine fever virus (ASFV) is presented, followed by an overview of antiviral agents with identified and unknown targets and currently available methods for antiviral drug testing. Future directions for developing antiviral agents against ASFV is also discussed.

2. Replication cycle of ASFV

ASFV is a large double-stranded DNA virus that belongs to the family *Asfarviridae*. In infected pigs, ASFV replicates in cells of the mononuclear phagocyte system, mostly in macrophages and monocytes. The ASFV replication cycle starts with viral attachment and entry into the host cell. Although the identity of cellular receptors involved in ASFV entry remains unknown, the receptor-dependent mechanism of viral entry, such as clathrin-mediated endocytosis has been reported (Galindo et al., 2015). There is also evidence that ASFV utilizes an alternative mechanism, macropinocytosis, when it enters into Vero cells (Sánchez et al., 2012). Within the endosome, ASFV undergoes conformational changes due to the low pH environment. Viral uncoating, the loss of outer membrane and protein capsid, occurs in multivesicular late endosomes, followed by the fusion of inner membrane with endosomes and the release of nude core to the cytosol (Hernáez et al.,

* Corresponding author at: Institute of Molecular Biology of NAS, Hasratyan 7, 0014, Yerevan, Armenia.
E-mail address: h_zakaryan@mb.sci.am (H. Zakaryan).

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2016). Incoming ASFV particles are then localized near the nucleus, where they form the viral factory. On this site, newly synthesized viral DNA and proteins are accumulated. According to their accumulation kinetics, four classes of ASFV transcripts are known. Transcription of *immediately early* and *early* genes starts immediately after infection and continues until the initiation of viral DNA synthesis. The transcription of two remaining classes, *intermediate* and *late* genes, occurs after DNA replication is initiated (Rodríguez and Salas, 2013). Finally, mature ASFV particles are transported from the viral factory to the cell surface *via* a microtubule-mediated mechanism, which depends on the capsid protein pE120R and the motor protein conventional kinesin (Andrés et al., 2001; Jouvenet et al., 2004). They exit the host cell by budding at the membrane, allowing extracellular virions to acquire an additional lipid membrane. At very late times of infection, ASFV causes cell lysis, which may represent an alternative mechanism of viral egress (Breese and DeBoer, 1966).

Based on their anti-ASFV mechanisms, potential antiviral agents can be divided into two groups: (1) inhibitors that directly act on ASFV by targeting the viral proteins (direct-acting antivirals), or (2) inhibitors that target cellular factors involved in ASFV replication (host-targeting antivirals). Since anti-ASFV drug discovery largely depends on the fundamental knowledge of virus replication, further research on the role of viral enzymes and host factors remains needed. In the following section, we will provide a brief review of laboratory methods to study antiviral agents against ASFV.

3. Methods for evaluation of antiviral compounds

3.1. Cells and viruses

For many years, ASFV studies were only performed on the natural target cells, porcine monocyte-macrophages purified from anticoagulanted-blood samples (peripheral blood mononuclear cells) (Enjuanes et al., 1976) or from pulmonary lavage of swine (Carrascosa et al., 1982). Both sources allow researchers to isolate up to 1×10^9 macrophages and use them immediately or store frozen for many years. These cells could be used to diagnose, grow and titrate the ASFV isolates obtained from outbreaks. However, the application of porcine monocyte/macrophages has some limitations such as variation among different lots of cells, impeding the reproducibility of experiments, or their inability to grow in culture, when a significant number of cells is required to perform molecular virology, biochemical and other studies. To overcome these obstacles, some ASFV isolates have been adapted to grow in different established cell lines like Vero, COS, and CV, all derived from African green monkey (*Cercopithecus aethiops*) (Hess et al., 1965; Hurtado et al., 2010). However, there are many studies in which the use of porcine cells is preferable. For example, the analysis of cytokine production by the host cells requires more natural environment, thereby encouraging the searching of porcine cell lines derived from a monocyte/macrophage lineage. Two porcine cell lines, IPAM and WSL, are currently used by different ASFV laboratories (de León et al., 2013). Although both cell lines are negative for some myeloid markers, they remain sensitive to different ASFV isolates.

To avoid the need for BSL-3 facilities, Vero-adapted ASFV BA71 V strain can be used. This avirulent strain has been obtained by adaptation to grow in Vero cells. It differs from its virulent parental ASFV BA71 strain by deletions in the 5′-end of the genome of 8238 nts, as well as by deletion of 2669 nts in the 3′-end (Rodríguez et al., 2015). These deletions with some other changes contribute to the attenuation of parental strain. ASFV BA71 V strain is unable to replicate in the porcine macrophage subsets and replicates less efficiently in porcine dendritic cells compared to other ASFV strains (Franzoni et al., 2017, 2018). However, ASFV enzymes involved in viral nucleotide metabolism, replication, transcription and repair are highly conserved among all strains. For example, the topoisomerase II enzyme of ASFV BA71 V is 96–100% identical or similar to other strains. Therefore, ASFV BA71 V is an excellent model for antiviral drug research and is widely used by different laboratories.

3.2. Methods to evaluate in vitro antiviral activity

Two assays, plaque and cell viability/cytopathic effect (CPE) reduction assays, are usually employed for the initial identification of molecules with antiviral activity against ASFV. Virus-induced CPE assay can be observed microscopically or quantified by colorimetric measurements using MTT, crystal violet stain or neutral red dye uptake methods. The advantage of colorimetric measurements is the possibility of both discovering new antiviral agents and evaluating their cytotoxic effects using the same method.

Recently, a new method for real-time monitoring of the CPE of ASFV has been reported (Burmakina et al., 2018). This method is based on microelectronic biosensor technology (xCELLigence® system developed by ACEA Inc.) allowing real time, label-free and non-invasive analysis of cellular changes including cell viability, proliferation and CPE. The application of this real-time novel system as a straightforward and efficient assay to measure the CPE has been demonstrated for several DNA and RNA viruses (Fang et al., 2011; Marlina et al., 2015; Piret et al., 2016). Burmakina et al. (2018) showed that the xCELLigence® system is able to detect measurable changes even though cells are infected with ASFV strains that do not develop clear CPE, indicating that this method can be utilized in those studies where CPE-based assays are not applicable.

4. Inhibitors with identified targets

4.1. Nucleoside analogues

Nucleosides and nucleotides play key roles in the replication and transcription of genetic information, and therefore they have been utilized in chemotherapy, antibacterial and antiviral therapeutics for many decades (Foss, 2000; Yssel et al., 2017). Nucleoside analogues possess one or more modifications thereby mimicking the structure of natural nucleosides enough to be incorporated into the DNA or RNA replication cycle (Fig. 1). The incorporation of nucleoside analogues into DNA or RNA may terminate chain elongation or lead to the accumulation of mutations. Nucleoside analogues can also act as inhibitors of cellular and viral enzymes such as ribonucleotide reductases and polymerases. The first antiviral drugs based on nucleoside analogues were developed in the 1960s and currently there are more than 25 approved therapeutic nucleosides against human viruses such as HIV (tenofovir, Viread™) (Ray et al., 2016) and hepatitis C virus (sofosbuvir, Sovaldi™) (Stedman, 2014).

The first nucleoside analogue reported to be active against ASFV is iododeoxyuridine which is used in cancer diagnosis and therapy (Haag et al., 1965; Berry and Kinsella, 2001). Gil-Fernández et al. (1979) showed that iododeoxyuridine at 100 μg/ml concentration completely inhibited ASFV infection in Vero cells, reducing ASFV yield by about 4 log. However, the authors noted that mock infected cells exposed to 100 μg/ml drug became rounded and smaller in size, suggesting that this concentration could be toxic for cells. At low concentrations iododeoxyuridine may lead to the development of ASFV persistence in Vero cells (Gil-Fernández and García-Villalón, 1988). After continuous treatment with iododeoxyuridine cells appeared to be "cured" (no virus was detected) and became more resistant to superinfections with the wild type virus.

(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ((S)-HPMPA) was first described in the mid-1980s as a broad-spectrum antiviral agent against DNA viruses such as HSV-1, vaccinia virus and adenoviruses (De Clercq et al., 1986). (S)-HPMPA along with other nucleosides like pyrazofurin and ribavirin has been tested against ASFV infection *in vitro*. This compound demonstrated the highest potency and greatest specificity (selectivity index (SI): 15,000), decreasing the viral

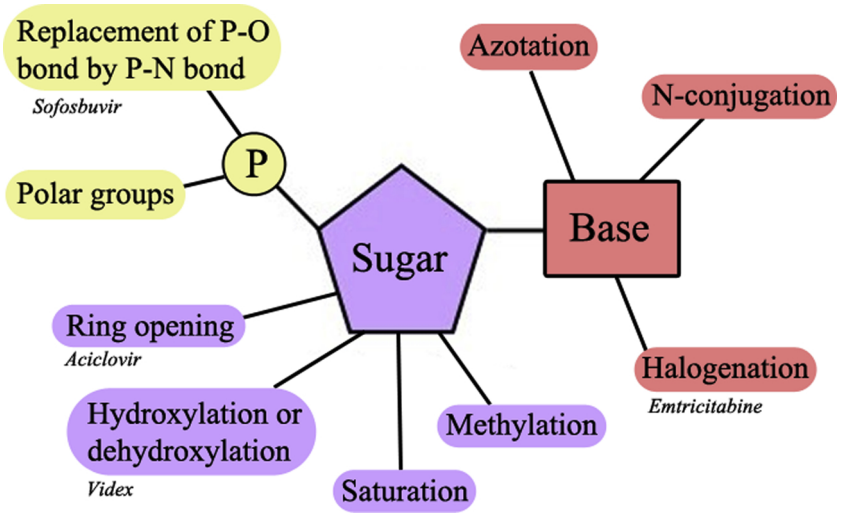

Fig. 1. General structural and chemical modifications of nucleotide. Some antiviral drugs are indicated in *Italics*.

titer by 5 log at 50 μg/ml concentration (Gil-Fernández and De Clercq, 1987a). Using dot hybridization technique, it has been shown that (S)-HPMPA may inhibit ASFV infection by disrupting viral DNA synthesis (Arzuza et al., 1988). These studies have been extended to a wide variety of phosphonylmethoxyalkylpurine and -pyrimidine derivatives related to (S)-HPMPA (Gil-Fernández et al., 1987b). Although tested compounds including (S)-HPMPC (Vistide®) appeared less potent than (S)-HPMPA, from a structural-functional viewpoint the (S)-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) nucleosides have shown promise as antiviral agents against ASFV.

Some adenosine analogues exert their antiviral effect through inhibition of S-adenosylhomocysteine hydrolase, a key enzyme in transmethylation reactions (Turner et al., 2000). Viruses with methylated caps at the 5'-end of mRNAs are significantly inhibited by adenosine analogues (De Clercq, 2009). Since ASFV synthesizes mRNAs with methylated cap structures, the adenosine analogues targeting the S-adenosylhomocysteine hydrolase have been shown to inhibit ASFV infection *in vitro* (Villalón et al., 1993). The most potent inhibitors were 3-deazaneplanocin A with SI of 3000, followed by c3DHCaA (SI = 2500), 4′β-vinyl-DHCaA (SI = 2000) and 6′β -fluoroaristeromycin (SI = 1250).

Rigid amphipathic fusion inhibitors (RAFIs) were initially reported as derivatives of uracil nucleosides with ability to inhibit infectivity of unrelated enveloped viruses, including HSV-1, IAV and HCV (St Vincent et al., 2010). Given the fact that these nucleosides have no effect on the non-enveloped viruses, their interaction with viral lipids has been proposed (Vigant et al., 2014). Since ASFV is an enveloped virus, we have tested the inhibitory activity of several RAFIs against ASFV. We found that 5-(Perylen-3-ylethynyl)-arabino-uridine (aUY11) and 5-(Perylen-3-ylethynyl)uracil-1-acetic acid (cm1UY11) (Fig. 2A) displayed a potent, dose-dependent inhibitory effect on ASFV infection in Vero cells and porcine macrophages (Hakobyan et al., 2018). The major antiviral effect was observed when both compounds were added at ASFV internalization stage (Fig. 2B). The titres were reduced from 6.5 ± 0.1 to 3.0 ± 0.8 log $TCID_{50}$/ml and 2.7 ± 0.1 log $TCID_{50}$/ml upon treatment with aUY11 and cm1UY11, respectively. These compounds also displayed a significant extracellular anti-ASFV activity. Although the main chemical properties required for antiviral activity and low cytotoxicity have been recently defined by Korshun and colleagues (Speerstra et al., 2018), further structure optimization is needed in order to overcome some limitations such as low solubility in water.

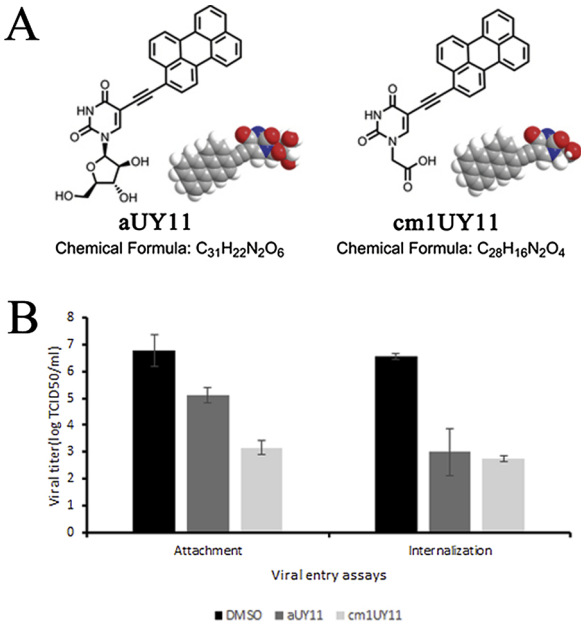

Fig. 2. RAFIs with anti-ASFV activity. (A) Chemical structures, 3D models (grey, carbon; white, hydrogen; red, oxygen; blue, nitrogen) and compositions. (B) Effect of RAFIs, aUY11 and cm1UY11, on ASFV entry. Viral titers were evaluated by CPE-based assay (Hakobyan et al., 2018).

4.2. Interferons

Interferons (IFNs) were first characterized over 60 years ago as soluble glycoproteins with potent antiviral activity (Isaacs et al., 1957). All mammals encode three groups of IFNs classified as type I, type II and type III according to their genetic, structural, and functional characteristics. IFNs activate JAK–STAT signaling, leading to the transcriptional induction of hundreds of IFN-stimulated genes (ISGs) such as directly acting antiviral proteins OAS, PKR and Mx (Lin and Young, 2014).

Early studies have demonstrated that the production of both virulent and non-virulent ASFV isolates was significantly inhibited by porcine IFN-gamma (Esparza et al., 1988). Co-treatment with IFN-alpha and IFN-gamma exerted a synergistic inhibition. Furthermore, a continuous treatment with IFN-alpha may cure Vero cells from lytic and persistent infections with ASFV (Paez et al., 1990). Involvement of different ISGs in anti-ASFV response remains poorly explored. Recently, Portugal et al. (2018) showed that the expression level of PKR, Mx1, ISG15 and Bst-2 genes was higher in cells infected with low virulent ASFV suggesting that highly virulent ASFV strains have evolved

mechanisms to evade IFN response (Frączyk et al., 2016). Although no precise inhibitory mechanisms have been proposed for ISGs mentioned above, other authors reported that the expression of human MxA but not MxB protein inhibited ASFV infection by 100-fold compared with that in control cells (Netherton et al., 2009). This inhibition of ASFV was linked to the recruitment of MxA protein to perinuclear viral assembly sites, where it interacted with ASFV particles similarly to those seen between MxA and different RNA viruses. Among other ISGs, the interferon-induced transmembrane proteins (IFITMs) known as entry inhibitors of a wide variety of enveloped RNA viruses (Zhao et al., 2019) were shown to reduce ASFV infectivity in Vero cells (Muñoz-Moreno et al., 2016). Particularly, IFITM2 and IFITM3 affected the viral entry/uncoating process through altering the endocytosis-mediated viral entry and cholesterol efflux.

4.3. Plant-derived compounds: genistein and genkwanin

Over the past decades plant-derived compounds have received increasing attention for their therapeutic potential. Furthermore, there are currently hundreds of modern drugs based on active compounds isolated from plants. Research interests in antiviral agents isolated from plants first arose in the 1950s, when the action of more than 200 plants against influenza A virus was evaluated in embryonated eggs (Chantrill et al., 1952). Today numerous plants and plant-derived compounds with antiviral properties are known (Zakaryan et al., 2017; Xu et al., 2017; Kaihatsu et al., 2018).

Genistein is a soy-derived isoflavone and a typical example of a phytoestrogenic compound. It inhibits protein-tyrosine kinase and type II topoisomerase activity and is used as an antitumor and antineoplastic agent (Chae et al., 2019). Genistein has been reported to inhibit the infectivity of several DNA and RNA viruses including HSV-1, Ebola virus, HIV and rotaviruses (Lyu et al., 2005; Kolokoltsov et al., 2012; Sauter et al., 2014; Huang et al., 2015). It was recently shown that ASFV encodes for a type II topoisomerase which has an essential role during viral genome replication and transcription, suggesting that it could be as a possible target for antiviral drugs including genistein (Coelho et al., 2016; Freitas et al., 2016). Indeed, we reported that genistein disturbed ASFV infection at non-cytotoxic concentrations in Vero cells and porcine macrophages by targeting the viral topoisomerase II (Arabyan et al., 2018). The most significant anti-ASFV effect was observed when genistein was added to cells at middle-phase of infection (8 hpi), disrupting viral DNA replication. Furthermore, the single cell electrophoresis analysis revealed the presence of fragmented ASFV genomes in cells exposed to genistein, pointing that genistein interfered with viral type II topoisomerase. Molecular docking studies demonstrated that genistein may interact with four residues of the ATP-binding site of viral topoisomerase, Asn-144, Val-146, Gly-147 and Leu-148, showing more binding affinity (-4.62 kcal/mol) than ATP4- (-3.02 kcal/mol). Therefore, genistein may act as an ATP-competitive inhibitor in ASFV infection.

We have previously shown that apigenin, a naturally occurring plant flavone, exerts significant anti-ASFV activity (discussed in the next section). However, apigenin is almost insoluble in highly polar solvents like water and it usually occurs in derivative forms in plants. We screened several commercially available apigenin derivatives for their ability to inhibit ASFV infection *in vitro*. Among them, genkwanin, an O-methylated flavone abundant in the seeds of *Alnus glutinosa*, showed significant inhibition of ASFV, reducing viral titer from 6.5 ± 0.1 to 4.75 ± 0.25 log TCID/ml in a dose-dependent manner (IC50 = 2.9 μM and SI = 205.2) (Hakobyan et al., 2019). Our further experiments indicated that genkwanin was able to inhibit ASFV infection at entry and egress stages. Since both processes depend on microtubules (Carvalho et al., 1988; Jouvenet et al., 2004), and since apigenin has been reported to be a highly potent inhibitor of tubulin polymerization by targeting the colchicine-binding site (Choudhury et al., 2013), we supposed that genkwanin may act as an inhibitor of tubulin assembly, thereby disrupting the viral entry and egress. To test this hypothesis, we studied the interaction of genkwanin with tubulin using *in silico* approaches. Our results showed that genkwanin bound to the colchicine binding site (-31.6 kcal/mol). More specifically, Phe-240, Asn-254, Met-255 Thr-310, Ala-312 amino acids had highest contribution in the tubulin-genkwanin binding process. Based on these results, we suppose that genkwanin is able to inhibit the ASFV infection *in vitro* through disrupting the virus movement along microtubules.

4.4. Antibiotics

Although antibiotics are antimicrobial compounds active against bacterial infections, a limited number of antibiotics also possess antiviral activity. Rifampicin is used to treat some bacterial infections such as Legionnaires' disease (caused by *Legionella pneumophila*), *Mycobacterium avium* complex and leprosy (caused by *Mycobacterium leprae*). This antibiotic was shown to be effective against vaccinia virus and cytomegalovirus by targeting DNA-dependent RNA polymerase and blocking viral transcription (Halsted et al., 1972; Pennington and Follett, 1971). Similar results were reported for ASFV. Rifampicin reduced the virus titer from 1- to 5-log depending on the drug concentration, the multiplicity of infection and the time after infection (Dardiri et al., 1971). The inhibition of ASFV in PK-15 cells was greatest at a concentration of 200 ug/ml. However, cytotoxic effect was not quantitatively evaluated leaving a question open whether rifampicin at 200 ug/ml concentration is usable or not. A few years later, Salas and colleagues demonstrated that several rifamycin derivatives inhibited the DNA-dependent RNA polymerase of ASFV (Salas et al., 1983). They also reported that coumermycin A1, an inhibitor of type II topoisomerases, significantly inhibited RNA synthesis by ASFV, suggesting that other highly effective antibiotics targeting bacterial topoisomerases may represent potential antiviral drugs against ASFV. Thirty fluoroquinolones known for their inhibitory effect on topoisomerases were screened against ASFV (Mottola et al., 2013). Six fluoroquinolones reduced the cytopathic effect in ASFV-infected Vero cells. Moreover, after continuous (7 days) treatment with fluoroquinolones the culture supernatants were unable to infect new cells and no ASFV genomes were detected by conventional PCR.

4.5. Small interfering RNA and CRISPR/Cas9

Small interfering RNA (siRNA) is a class of double-stranded RNA molecules, usually up to 25 base pairs in length, with a great deal of therapeutic interest including antiviral applications (Levanova and Poranen, 2018). Keita et al. (2010) designed several siRNA in order to down regulate the targeted genes, A151R and B646 L encoding proteins essential for ASFV morphogenesis. Some siRNAs reduced the virus titer and RNA transcripts up to 4 log. However, the combination of multiple siRNAs targeting both genes did not improve the antiviral effect significantly compared with the use of individual siRNAs.

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 nuclease system is used for targeted and precise genome editing in eukaryotic cells. This system has also been harnessed to target viruses as a potential new antiviral strategy (Kennedy et al., 2015). Among these viruses, ASFV has been shown to be significantly inhibited by CRISPR/Cas9 system targeting codons 71–78 of the viral phosphoprotein p30 gene (CP204 L) (Hübner et al., 2018). The almost complete inhibition of spread and productive replication of both avirulent and virulent ASFV strains, BA71 V and Armenia, respectively, on WSL-gRp30 cells was considered to be because of specific inhibition by CRISPR/Cas9-mediated cleavage of the virus genome at the p30 gene locus. However, further studies have to determine the genetic barrier of ASFV to CRISPR/Cas9-based antiviral intervention.

5. Inhibitors with unknown targets

Some natural compounds were reported to inhibit the *in vitro* replication of ASFV by yet unknown mechanism. Sulfated polysaccharides lambda and kappa carrageenan, pentosan polysulfate, and fucoidan showed anti-ASFV activity in Vero cells (García-Villalón and Gil-Fernández, 1991). Lambda carrageenan was the most efficacious with a selectivity index of 120, followed by pentosan polysulfate with 30, kappa carrageenan 13.3 and fucoidan 10. All these polysaccharides exerted their antiviral activity when they were present during virus adsorption, with the exception of lambda and kappa carrageenan, which were also active when added immediately after the adsorption stage.

Treatment with lauryl gallate resulted in significant inhibition of the *in vitro* replication of ASFV in Vero cells (Hurtado et al., 2008). The addition of lauryl gallate before virus adsorption completely abolished ASFV infection in a one-step growth virus cycle. However, no anti-ASFV effect was observed when this drug was added after 5–8 h post-infection, thereby suggesting that it could target early events in ASFV infection.

Galindo et al. (2011) tested polyphenolic phytoalexins, resveratrol and oxyresveratrol, against ASFV infection in Vero cells. Both compounds at non-toxic concentrations achieved more than 90% reduction in viral titers, inhibiting viral DNA replication, late viral protein synthesis and viral factory formation. This antiviral activity was reported not only for chemically synthesized resveratrol and oxyresveratrol but also for oxyresveratrol extracted from natural sources such as mulberry twigs.

Apigenin was reported as a potent *in vitro* inhibitor of ASFV infection (Hakobyan et al., 2016). Time-of-addition experiments showed that it was highly effective at the early stages of infection in Vero cells. The ASFV yield reduction was more than 99.99% (> 3 log) when apigenin was added at 1 hpi. However, no inhibitor effects on ASFV entry were observed. ASFV-specific protein synthesis, viral DNA replication as well as the formation of ASFV factories were diminished in the presence of apigenin. ASFV-infected cells continuously (up to 144 h post-infection) treated with apigenin did not display a virus-specific CPE. Mechanistic studies are currently ongoing to identify the antiviral targets through which apigenin inhibits ASFV.

Some marine microalgae extracts were also evaluated for their antiviral activity against ASFV. The aqueous extracts from *Porphyridium cruentum*, *Chlorella autotrophica* and *Ellipsoidon* sp., displayed a significant inhibition of the *in vitro* replication of ASFV in a dose-dependent manner (Fabregas et al., 1999). This inhibition could be due to sulfated polysaccharides since exocellular extracts from microalgae enriched with these compounds also reduced the ASFV infection in Vero cells.

6. Future perspectives

In addition to the re-emergence of ASFV in Europe, this disease is now ravaging China's pork industry resulting in more than 1.2 million pigs being slaughtered since 3 August 2018, when the first outbreak of ASFV was reported in China (Zhou et al., 2018). The worldwide spread of ASFV calls for action. Besides vaccine development, another pillar of the ASFV action plan should be the development of potent antiviral agents at reasonable costs. Although various types of active anti-ASFV agents have been reported, *in vivo* efficacy has not yet been evaluated for these compounds. For instance, (S)-HPMPA and its derivatives have never been tested *in vivo* against ASFV most probably due to the high costs of nucleosides. It is apparent that the major limitation of antiviral agents against animal diseases including ASF is the cost of antivirals that should not be more costly than animals raised to be farmed. For this reason, natural compounds like plant-derived flavonoids are at the center of our attention (Zakaryan et al., 2017). We believe that sufficient efforts should be executed to screen natural product libraries in order to discover new compounds with anti-ASFV activity. Since natural product libraries consist of hundreds of molecules, their screening requires high-throughput cell-based assays designed to identify the best hits inhibiting ASFV infection (Postnikova et al., 2018). Currently we are developing an *in vitro* cell-based (CPE) high-throughput screening assay which allows to screen large compound libraries using colorimetric readout.

The long and complex path leading to the development of an effective drug against ASFV can be greatly accelerated by computational *in silico* methods (Fig. 3). In this regard, the knowledge of structural and biochemical features of the proposed targets such as ASFV proteins is relevant for *in silico* screenings. However, in the absence of

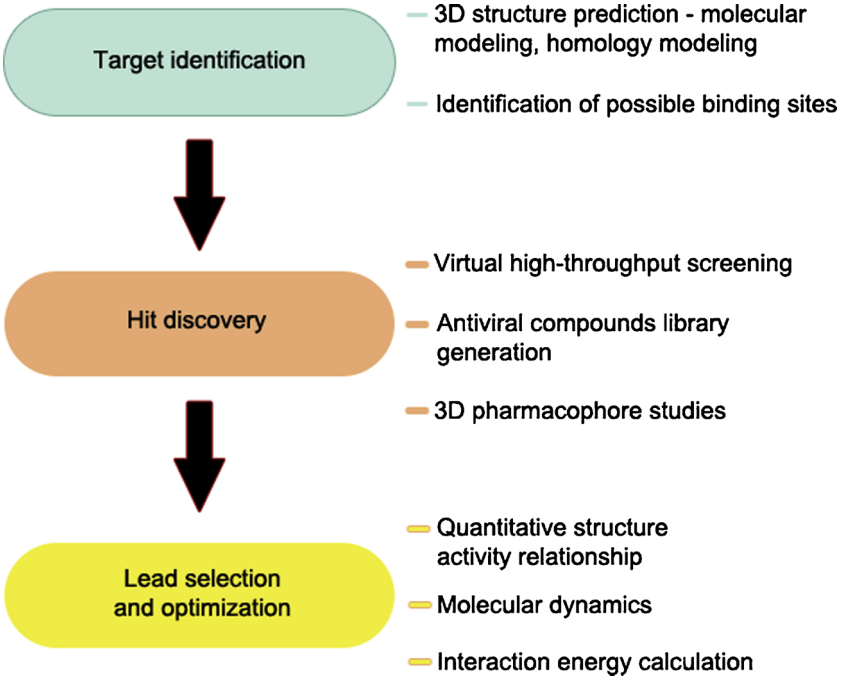

Fig. 3. Possible workflow of *in silico* approaches for ASFV drug discovery.

crystallographic data for ASFV proteins, some computer-modeling protocols like homology modeling can be used to predict protein structure and putative binding sites. Recently, we performed homology modeling in order to construct the viral topoisomerase II tertiary structure (Arabyan et al., 2018). It has allowed us to identify the ATP-binding site as an attractive target for further *in silico* screenings. We used a similar approach to construct 3D structure of ASFV ribonucleotide reductase (unpublished data) whose inhibition by hydroxyurea was shown to reduce the viral yield by more than 3 log (Cunha and Costa, 1992). Virtual high-throughput screening is currently ongoing. Finally, *in silico* methods can be used to target a host protein that is essential in the life cycle of ASFV. However, this strategy is still controversial since inhibiting the host factors may result in high toxicity. On the other hand, the selection of viral resistance, which is a major problem for any virus-specific drug, should be lower for a host-targeting antiviral. The feasibility of this approach will likely depend on which host factor is targeted and how ASFV depends on it. For instance, microtubules play an essential role in ASFV transport to the perinuclear area as well as in the formation of the viral factory (Muñoz-Moreno et al., 2015). Also, ASFV requires microtubules to exit the cell. Therefore, *in silico* screening of tubulin polymerization inhibitors may provide new hits for further antiviral evaluation.

In conclusion, we believe that with sufficient effort and time, it should be possible to develop safe and potent antiviral drugs against ASFV. Antiviral therapies may have a beneficial effect on the current situation with ASFV. Potent and safe antiviral drugs may provide immediate protection in the case of ASFV outbreak, thereby isolating the epidemic area and providing the designated authorities enough time to produce other countermeasures. However, the main challenge to the development of anti-ASFV drugs will be their final/market cost. This challenge must be pursued because antiviral drugs will be a crucial tool, together with effective vaccines, to stop the spread of ASFV that will probably remain a significant global threat for the near future. The ASFV research community should continue working hard to get positive results. Fortune favors the hard workers.

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References

Andrés, G., García-Escudero, R., Viñuela, E., Salas, M.L., Rodríguez, J.M., 2001. African swine fever virus structural protein pE120R is essential for virus transport from assembly sites to plasma membrane but not for infectivity. J. Virol. 75 (15), 6758–6768.

Arabyan, E., Hakobyan, A., Kotsinyan, A., Karalyan, Z., Arakelov, V., Arakelov, G., Nazaryan, K., Simonyan, A., Aroutiounian, R., Ferreira, F., Zakaryan, H., 2018. Genistein inhibits African swine fever virus replication in vitro by disrupting viral DNA synthesis. Antiviral Res. 156, 128–137.

Arzuza, O., García-Villalón, D., Tabarés, E., Gil-Fernández, C., De Clercq, E., 1988. Inhibition of African swine fever virus DNA synthesis by (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine. Biochem. Biophys. Res. Commun. 154 (1), 27–32.

Berry, S.E., Kinsella, T.J., 2001. Targeting DNA mismatch repair for radiosensitization. Semin. Radiat. Oncol. 11 (4), 300–315.

Breese, S.S.Jr., DeBoer, C.J., 1966. Electron microscope observations of African swine fever virus in tissue culture cells. Virology 28 (3), 420–428.

Burmakina, G., Bliznetsov, K., Malogolovkin, A., 2018. Real-time analysis of the cytopathic effect of African swine fever virus. J. Virol. Methods 257, 58–61.

Carrascosa, A.L., Santaren, J.F., Vinuela, E., 1982. Production and titration of African swine fever virus in porcine alveolar macrophages. J. Virol. Methods 3 (6), 303–310.

Carvalho, Z.G., De Matos, A.P., Rodrigues-Pousada, C., 1988. Association of African swine fever virus with the cytoskeleton. Virus Res. 11 (2), 175–192.

Chae, H.S., Xu, R., Won, J.Y., Chin, Y.W., Yim, H., 2019. Molecular targets of genistein and its related flavonoids to exert anticancer effects. Int. J. Mol. Sci. 16 (20) (10).

Chantrill, B.H., Coulthard, C.E., Dickinson, L., Inkley, G.W., Morris, W., Pyle, A.H., 1952. The action of plant extracts on a bacteriophage of Pseudomonas pyocyanea and on influenza A virus. J. Gen. Microbiol. 6, 74–84.

Chenais, E., Depner, K., Guberti, V., Dietze, K., Viltrop, A., Ståhl, K., 2019. Epidemiological considerations on African swine fever in Europe 2014-2018. Porcine Health Manag. 5, 6.

Choudhury, D., Ganguli, A., Dastidar, D.G., Acharya, B.R., Das, A., Chakrabarti, G., 2013. Apigenin shows synergistic anticancer activity with curcumin by binding at different sites of tubulin. Biochimie 95 (6), 1297–1309.

Cisek, A.A., Dąbrowska, I., Gregorczyk, K.P., Wyżewski, Z., 2016. African swine fever virus: a new old enemy of Europe. Ann. Parasitol. 62 (3), 161–167.

Coelho, J., Ferreira, F., Martins, C., Leitão, A., 2016. Functional characterization and inhibition of the type II DNA topoisomerase coded by African swine fever virus. Virology 493, 209–216.

Cunha, C.V., Costa, J.V., 1992. Induction of ribonucleotide reductase activity in cells infected with African swine fever virus. Virology 187 (1), 73–83.

Dardiri, A.H., Bachrach, H.L., Heller, E., 1971. Inhibition by rifampin of African swine fever virus replication in tissue culture. Infect. Immun. 4 (1), 34–36.

De Clercq, E., Li, G., 2016. Approved antiviral drugs over the past 50 years. Clin. Microbiol. Rev. 29 (3), 695–747.

De Clercq, E., Holy, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C., 1986. A novel selective broad-´spectrum anti-DNA virus agent. Nature 323, 464–467.

De Clercq, E., 2009. Another ten stories in antiviral drug discovery (part C): "Old" and "new" antivirals, strategies, and perspectives. Med. Res. Rev. 29 (4), 611–645.

de León, P., Bustos, M.J., Carrascosa, A.L., 2013. Laboratory methods to study African swine fever virus. Virus Res. 173 (1), 168–179.

Enjuanes, L., Carrascosa, A.L., Moreno, M.A., Vinuela, E., 1976. Titration of African swine fever (ASF) virus. J. Gen. Virol. 32 (3), 471–477.

Esparza, I., González, J.C., Viñuela, E., 1988. Effect of interferon-alpha, interferon-gamma and tumour necrosis factor on African swine fever virus replication in porcine monocytes and macrophages. J. Gen. Virol. 69 (12), 2973–2980.

Fabregas, J., García, D., Fernandez-Alonso, M., Rocha, A.I., Gómez-Puertas, P., Escribano, J.M., Otero, A., Coll, J.M., 1999. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antiviral Res. 44 (1), 67–73.

Fang, Y., Ye, P., Wang, X., Xu, X., Reisen, W., 2011. Real-time monitoring of flavivirus induced cytopathogenesis using cell electric impedance technology. J. Virol. Methods 173 (2), 251–258.

Foss, F.M., 2000. Combination therapy with purine nucleoside analogs. Oncology 14 (6), 31–35.

Frączyk, M., Woźniakowski, G., Kowalczyk, A., Bocian, Ł., Kozak, E., Niemczuk, K., Pejsak, Z., 2016. Evolution of African swine fever virus genes related to evasion of host immune response. Vet. Microbiol. 193, 133–144.

Franzoni, G., Graham, S.P., Giudici, S.D., Bonelli, P., Pilo, G., Anfossi, A.G., Pittau, M., Nicolussi, P.S., Laddomada, A., Oggiano, A., 2017. Characterization of the interaction of African swine fever virus with monocytes and derived macrophage subsets. Vet. Microbiol. 198, 88–98.

Franzoni, G., Graham, S.P., Sanna, G., Angioi, P., Fiori, M.S., Anfossi, A., Amadori, M., Dei Giudici, S., Oggiano, A., 2018. Interaction of porcine monocyte-derived dendritic cells with African swine fever viruses of diverse virulence. Vet. Microbiol. 216, 190–197.

Freitas, F.B., Frouco, G., Martins, C., Leitão, A., Ferreira, F., 2016. In vitro inhibition of African swine fever virus-topoisomerase II disrupts viral replication. Antiviral Res. 134, 34–41.

Gago da Câmara, N.J., 1933. AT História da peste suína em Angola. Pecuária, Anais dos Servic¸os Pecuários da Colónia de Angola 1, 25–40.

Galindo, I., Cuesta-Geijo, M.A., Hlavova, K., Muñoz-Moreno, R., Barrado-Gil, L., Dominguez, J., Alonso, C., 2015. African swine fever virus infects macrophages, the natural host cells, via clathrin- and cholesterol-dependent endocytosis. Virus Res. 200, 45–55.

Galindo, I., Hernáez, B., Berná, J., Fenoll, J., Cenis, J.L., Escribano, J.M., Alonso, C., 2011. Comparative inhibitory activity of the stilbenes resveratrol and oxyresveratrol on African swine fever virus replication. Antiviral Res. 91 (1), 57–63.

Gallardo, M.C., Reoyo, A.T., Fernández-Pinero, J., Iglesias, I., Muñoz, M.J., Arias, M.L., 2015. African swine fever: a global view of the current challenge. Porcine Health Manag. 1, 21.

García-Villalón, D., Gil-Fernández, C., 1991. Antiviral activity of sulfated polysaccharides against African swine fever virus. Antiviral Res. 15 (2), 139–148.

Gil-Fernández, C., De Clercq, E., 1987a. Comparative efficacy of broad-spectrum antiviral agents as inhibitors of African swine fever virusreplication in vitro. Antiviral Res. 7 (3), 151–160.

Gil-Fernández, C., García-Villalón, D., De Clercq, E., Rosenberg, I., Holý, A., 1987b. Phosphonylmethoxyalkylpurines and -pyrimidines as inhibitors of African swine fever virusreplication in vitro. Antiviral Res. 8 (5–6), 273–281.

Gil-Fernández, C., García-Villalón, D., 1988. A model virus-cell system to study the persistence of African swine fever virus. Arch. Virol. 100 (3–4), 161–169.

Gil-Fernández, C., Páez, E., Vilas, P., Gancedo, A.G., 1979. Effect of disodium phosphonoacetate and iododeoxyuridine on the multiplication of African swine fever virus in vitro. Chemotherapy 25 (3), 162–169.

Haag, J., Lareanaudie, B., Gonzalvo, F.R., 1965. Peste porcine Africaine, action de la 5-iodo-2′- desoxyuridine sur la culture du virus in vitro. Bull. Off. Int. Epizoot. 63, 717–722.

Hakobyan, A., Arabyan, E., Avetisyan, A., Abroyan, L., Hakobyan, L., Zakaryan, H., 2016. Apigenin inhibits African swine fever virus infection in vitro. Arch. Virol. 161 (12), 3445–3453.

Hakobyan, A., Arabyan, E., Kotsinyan, A., Karalyan, Z., Sahakyan, H., Arakelov, V., Nazaryan, K., Ferreira, F., Zakaryan, H., 2019. Inhibition of African swine fever virus infection by genkwanin. Antiviral Res. 167, 78–82.

Hakobyan, A., Galindo, I., Nañez, A., Arabyan, E., Karalyan, Z., Chistov, A.A., Streshnev, P.P., Korshun, V.A., Alonso, C., Zakaryan, H., 2018. Rigid amphipathic fusion inhibitors demonstrate antiviral activity against African swine fever virus. J. Gen. Virol.

99 (1), 148–156.
Halsted, C.C., Minnefor, A.B., Lietman, P.S., 1972. Inhibition of cytomegalovirus by rifampin. J. Infect. Dis. 125 (5), 552–555.
Hernáez, B., Guerra, M., Salas, M.L., Andrés, G., 2016. African swine fever virus undergoes outer envelope disruption, capsid disassembly and inner envelope fusion before core release from multivesicular endosomes. PLoS Pathog. 12 (4), e1005595.
Hess, W.R., Cox, B.F., Heuschele, W.P., Stone, S.S., 1965. Propagation and modifica-tion of African swine fever virus in cell cultures. Am. J. Vet. Res. 26, 141–146.
Huang, H., Liao, D., Liang, L., Song, L., Zhao, W., 2015. Genistein inhibits rotavirus replication and upregulates AQP4 expression in rotavirus-infected Caco-2 cells. Arch. Virol. 160 (6), 1421–1433.
Hübner, A., Petersen, B., Keil, G.M., Niemann, H., Mettenleiter, T.C., Fuchs, W., 2018. Efficient inhibition of African swine fever virus replication by CRISPR/Cas9 targeting of the viral p30 gene (CP204L). Sci. Rep. 8 (1), 1449.
Hurtado, C., Bustos, M.J., Sabina, P., Nogal, M.L., Granja, A.G., González, M.E., Gónzalez-Porqué, P., Revilla, Y., Carrascosa, A.L., 2008. Antiviral activity of lauryl gallate against animal viruses. Antivir. Ther. (Lond.) 13 (7), 909–917.
Hurtado, C., Bustos, M.J., Carrascosa, A.L., 2010. The use of COS-1 cells for studies of field and laboratory African swine fever virus samples. J. Virol. Methods 164 (1-2), 131–134.
Isaacs, A., Lindenmann, J., Valentine, Rc., 1957. Virus interference. II. Some properties of interferon. Proc. R. Soc. Lond., B, Biol. Sci. 147, 268–273.
Jouvenet, N., Monaghan, P., Way, M., Wileman, T., 2004. Transport of African swine fever virus from assembly sites to the plasma membrane is dependent on microtubules and conventional kinesin. J. Virol. 78 (15), 7990–8001.
Kaihatsu, K., Yamabe, M., Ebara, Y., 2018. Antiviral mechanism of action of Epigallocatechin-3-O-gallate and its fatty acid esters. Molecules 23 (10).
Keita, D., Heath, L., Albina, E., 2010. Control of African swine fever virus replication by small interfering RNA targeting the A151R and VP72 genes. Antivir. Ther. (Lond.) 15 (5), 727–736.
Kennedy, E.M., Kornepati, A.V.R., Mefferd, A.L., Marshall, J.B., Tsai, K., Bogerd, H.P., Cullen, B.R., 2015. Optimization of a multiplex CRISPR/Cas system for use as an antiviral therapeutic. Methods 91, 82–86.
Kolokoltsov, A.A., Adhikary, S., Garver, J., Johnson, L., Davey, R.A., Vela, E.M., 2012. Inhibition of Lassa virus and Ebola virus infection in host cells treated with the kinase inhibitors genistein and tyrphostin. Arch. Virol. 157 (1), 121–127.
Levanova, A., Poranen, M.M., 2018. RNA interference as a prospective tool for the control of human viral infections. Front. Microbiol. 9, 2151.
Lin, F.C., Young, H.A., 2014. Interferons: success in anti-viral immunotherapy. Cytokine Growth Factor Rev. 25, 369–376.
Lyu, S.Y., Rhim, J.Y., Park, W.B., 2005. Antiherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro. Arch. Pharm. Res. 28 (11), 1293–1301.
Marlina, S., Shu, M.H., AbuBakar, S., Zandi, K., 2015. Development of a Real-Time Cell Analysing (RTCA) method as a fast and accurate screen for the selection of chikungunya virus replication inhibitors. Parasit. Vectors 8, 579.
Montgomery, R.E., 1921. On a form of swine fever occurring in British East Africa (Kenya Colony). J. Comp. Pathol. 34 (3/4), 159–191.
Mottola, C., Freitas, F.B., Simões, M., Martins, C., Leitão, A., Ferreira, F., 2013. In vitro antiviral activity of fluoroquinolones against African swine fever virus. Vet. Microbiol. 165 (1-2), 86–94.
Muñoz-Moreno, R., Cuesta-Geijo, M.Á., Martínez-Romero, C., Barrado-Gil, L., Galindo, I., García-Sastre, A., Alonso, C., 2016. Antiviral role of IFITM proteins in african swine fever virus infection. PLoS One 11 (4), e0154366.
Muñoz-Moreno, R., Galindo, I., Cuesta-Geijo, M.Á., Barrado-Gil, L., Alonso, C., 2015. Host cell targets for African swine fever virus. Virus Res. 209, 118–127.
Netherton, C.L., Simpson, J., Haller, O., Wileman, T.E., Takamatsu, H.H., Monaghan, P., Taylor, G., 2009. Inhibition of a large double-stranded DNA virus by MxA protein. J. Virol. 83 (5), 2310–2320.
Paez, E., Garcia, F., Gil Fernandez, C., 1990. Interferon cures cells lytically and persistently infected with African swine fever virus in vitro. Arch. Virol. 112 (1–2), 115–127.
Pennington, T.H., Follett, E.A., 1971. Inhibition of poxvirus maturation by rifamycin derivatives and related compounds. J. Virol. 7 (6), 821–829.
Piret, J., Goyette, N., Boivin, G., 2016. Novel method based on RealTime cell analysis for drug susceptibility testing of herpes simplex virus and human cytomegalovirus. J. Clin. Microbiol. 54 (8), 2120–2127.
Portugal, R., Leitão, A., Martins, C., 2018. Modulation of type I interferon signaling by African swine fever virus (ASFV) of different virulence L60 and NHV in macrophage host cells. Vet. Microbiol. 216, 132–141.
Postnikova, E., Cong, Y., DeWald, L.E., Dyall, J., Yu, S., Hart, B.J., Zhou, H., Gross, R., Logue, J., Cai, Y., Deiuliis, N., Michelotti, J., Honko, A.N., Bennett, R.S., Holbrook, M.R., Olinger, G.G., Hensley, L.E., Jahrling, P.B., 2018. Testing therapeutics in cell-based assays: factors that influence the apparent potency of drugs. PLoS One 13 (3), e0194880.
Ray, A.S., Fordyce, M.W., Hitchcock, M.J., 2016. Tenofovir alafenamide: a novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. Antiviral Res. 125, 63–70.
Rodríguez, J.M., Moreno, L.T., Alejo, A., Lacasta, A., Rodríguez, F., Salas, M.L., 2015. Genome sequence of african swine fever virus BA71, the virulent parental strain of the nonpathogenic and tissue-culture adapted BA71V. PLoS One 10 (11), e0142889.
Rodríguez, J.M., Salas, M.L., 2013. African swine fever virus transcription. Virus Res. 173 (1), 15–28.
Salas, M.L., Kuznar, J., Viñuela, E., 1983. Effect of rifamycin derivatives and coumermycin A1 on in vitro RNA synthesis by African swine fever virus. Brief report. Arch. Virol. 77 (1), 77–80.
Sánchez, E.G., Quintas, A., Pérez-Núñez, D., Nogal, M., Barroso, S., Carrascosa, Á.L., Revilla, Y., 2012. African swine fever virus uses macropinocytosis to enter host cells. PLoS Pathog. 8 (6), e1002754.
Sánchez-Cordón, P.J., Montoya, M., Reis, A.L., Dixon, L.K., 2018. African swine fever: a re-emerging viral disease threatening the global pig industry. Vet. J. 233, 41–48.
Sauter, D., Schwarz, S., Wang, K., Zhang, R., Sun, B., Schwarz, W., 2014. Genistein as antiviral drug against HIV ion channel. Planta Med. 80 (8–9), 682–687.
Speerstra, S., Chistov, A.A., Proskurin, G.V., Aralov, A.V., Ulashchik, E.A., Streshnev, P.P., Shmanai, V.V., Korshun, V.A., Schang, L.M., 2018. Antivirals acting on viral envelopes via biophysical mechanisms of action. Antiviral Res. 149, 164–173.
St Vincent, M.R., Colpitts, C.C., Ustinov, A.V., Muqadas, M., Joyce, M.A., Barsby, N.L., Epand, R.F., Epand, R.M., Khramyshev, S.A., Valueva, O.A., Korshun, V.A., Tyrrell, D.L., Schang, L.M., 2010. Rigid amphipathic fusion inhibitors, small molecule antiviral compounds against enveloped viruses. Proc. Natl. Acad. Sci. U S A. 107 (40), 17339–17344.
Stedman, C., 2014. Sofosbuvir, a NS5B polymerase inhibitor in the treatment of hepatitis C: a review of its clinical potential. Ther. Adv. Gastroenterol. 7, 131–140.
Steyn, D.G., 1928. Preliminary report on a South African virus disease amongst pigs. 13th and 14th Reports of the Director of Veterinary Education and Research.
Turner, M.A., Yang, X., Yin, D., Kuczera, K., Borchardt, R.T., Howell, P.L., 2000. Structure and function of S-adenosylhomocysteine hydrolase. Cell Biochem. Biophys. 33 (2), 101–125.
Vigant, F., Hollmann, A., Lee, J., Santos, N.C., Jung, M.E., Lee, B., 2014. The rigid amphipathic fusion inhibitor dUY11 acts through photosensitization of viruses. J. Virol. 88 (3), 1849–1853.
Villalón, M.D., Gil-Fernández, C., De Clercq, E., 1993. Activity of several S-adenosylhomocysteine hydrolase inhibitors against African swine fever virusreplication in Vero cells. Antiviral Res. 20 (2), 131–144.
Xu, J., Xu, Z., Zheng, W., 2017. A review of the antiviral role of green tea catechins. Molecules 22 (8).
Yssel, A.E.J., Vanderleyden, J., Steenackers, H.P., 2017. Repurposing of nucleoside- and nucleobase-derivative drugs as antibiotics and biofilm inhibitors. J. Antimicrob. Chemother. 72 (8), 2156–2170.
Zakaryan, H., Arabyan, E., Oo, A., Zandi, K., 2017. Flavonoids: promising natural compounds against viral infections. Arch. Virol. 162 (9), 2539–2551.
Zhao, X., Li, J., Winkler, C.A., An, P., Guo, J.T., 2019. IFITM genes, variants, and their roles in the control and pathogenesis of viral infections. Front. Microbiol. 9, 3228.
Zhou, X., Li, N., Luo, Y., Liu, Y., Miao, F., Chen, T., Zhang, S., Cao, P., Li, X., Tian, K., Qiu, H.J., Hu, R., 2018. Emergence of african swine fever in China, 2018. Transbound. Emerg. Dis. 65 (6), 1482–1484.