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Research progress on live attenuated vaccine against African swine fever virus

Le Liu, Xiangwei Wang, Ruoqing Mao, Yahua Zhou, Juanbin Yin, Yuefeng Sun **, Xiangping Yin *

State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, 730046, PR China

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ABSTRACT

African swine fever (ASF) is an acute, hemorrhagic and severe infectious disease caused by African swine fever virus (ASFV) in domestic pigs and various wild boars, with a mortality rate up to 100%. ASF was first discovered in 1921 in Kenya. ASFV has a large genome and complex immune escape mechanism creating difficulties in the production of vaccines. Recently, remarkable advances have been made in vaccine development all over the world especially in live-attenuated vaccine. This article aims to review the research progress of ASF attenuated live vaccines in order to provide a reference for the development of vaccines for this disease.

1. Introduction

African swine fever (ASF) is an acute, hemorrhagic infectious disease of swine caused by African swine fever virus (ASFV). It is an animal disease that must be reported to the World Organization for Animal Health (OIE) because it causes high morbidity and mortality [1]. ASFV is a large double-stranded DNA virus which belongs to the family Asfarviridae [2]. The structure of ASFV particles is icosahedral in symmetry and has a capsule. The electron microscope examination shows that the virus particles are shaped like hexagons, with a diameter of about 172–220 nm [3]. The genome of ASFV is about 170–190 kbp and encodes 150–200 kinds of proteins.

ASF was first discovered in Kenya in 1921 [4], after that it spread to other countries in Europe, America and Asia. Globally, ASF has caused huge economic losses for nearly 100 years. However, due to the complex immune protection and immune evasion mechanism, no effective and safe vaccine has been available till date. With the continuous development of biotechnology, researchers have made a breakthrough in developing ASF vaccine ranging from inactivated viruses, recombinant proteins/peptides, and DNA vaccines to live attenuated vaccine candidates.

The inactivated vaccine of ASFV was first tried to be developed, however, the protection was only 0–70% [5–7]. The subunit vaccine of ASFV based on p72, p54, p30, and p12 etc. showed a 0–60% protection efficiency [8–15]. The viral vector vaccines containing ASFV protective antigens (mostly poxvirus and adenovirus as carriers) show a protection efficiency of 0–50% [16–19]. Compared with this vaccine candidates, live attenuated vaccine shows a strong immune protection ability. This review focuses on the research progress of live attenuated vaccine of ASF in order to provide a reference for the research and development of ASF vaccine.

2. Live-attenuated vaccine

Live-attenuated vaccines, also known as attenuated vaccines, prepared by reducing the toxicity of the virulent strain through physical and chemical methods and by genetic modification (mostly deletion of specific genes)to lose the pathogenicity of the host but still have immunogenicity. ASFV live-attenuated vaccines can be divided into three categories: natural-attenuated strains, subculture-attenuated strains and recombinant-attenuated strains.

2.1. Natural-attenuated strains

Researchers have isolated some naturally attenuated strains of ASFV from soft ticks and chronically infected pigs [20,21], such as OUR T88/3 and NH/P68. The immune protection rate of these two ASFV natural attenuated strains varies from 66% to 100% against the challenge of homologous virulent strains depending on the challenge virus as well as the administration dose and route of administration. The results of

* Corresponding author.
** Corresponding author.
E-mail addresses: sunyuefeng@caas.cn (Y. Sun), yinxiangping@caas.cn (X. Yin).

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several sets of experiments provide useful data on the immune parameters involved in protection. Both antibodies and cytotoxic $CD8^+$ T cells have been shown to play an important role in the protection [22]. It was demonstrated that the natural attenuated strain NH/P68 (genotype I) is able to protect 100% against the virulent strain L60 (genotype I) and importantly, also against a heterologous challenge with Arm/07 (genotype II), although several side effects appeared in the vaccinated animals [23]Leitão et al. conducted longitudinal clinical and immunological studies by immunizing pigs with NH/P68 strains [24]: pigs with lesions developed, had viremia and fever at the late stage of infection, NK activity levels were close to control animals, and anti-ASFV specific antibody levels were higher. On the other hand, asymptomatic pigs had no viremia or fever on the 14th day after infection, and NK cell activity increased but the specific antiviral antibody concentration was relatively low throughout the experiment. Importantly, the latter group of pigs are resistant to attack by the highly virulent ASFV L60 isolate and can survive. And there were no signs of infection in clinical and serological tests. King K et al. used OUR T88/3 vaccine for the initial vaccination, while OUR T88/1 strain was used for booster immunity. The immunized pigs' protection rates ranged from 85.7% to 100% for Benin 97/1 and Uganda 1965 virulent strains challenged, respectively. This study demonstrated for the first time that the induction of cross-protective immunity can resist the challenge of non-homologous ASFV strains [25]. Similarly, Mulumba-Mfumu LK, etc. were first immunized with OUR T88/3 and then boosted with the virulent strain OUR T88/1 strain [26] and the results indicated that immunized animals were found to produce cross-protection against heterologous strains of ASFV type I. These results suggested that an effective vaccine could be developed to induce cross protection. However, natural attenuated strains may also cause many adverse reactions after immunization of animals. For example, after immunization with NH/P68 strains, 25%–47% of pigs exhibit chronic infection [24]. The pigs immunized with OUR T88/3 developed symptoms such as fever and joint swelling [26]. Sánchez-Cordón P J et al. used low-virulence ASFV genotype I isolate OURT88/3 [27] to inoculate pigs with different immunization doses and routes (intramuscular and intranasal routes). Pigs were immunized intranasally with OURT88/3 of 10^3 and 10^4 $TCID_{50}$, and then fully protected when challenged by the virulent type I OURT88/1 isolate, and the protected pigs had a smaller clinical response. High-dose vaccination (10^5 $TCID_{50}$) in this way induces chronic forms of ASF. On the other hand, pigs vaccinated intramuscularly at the same dose do not get better protection (protection rate 50%–66%). But the protected pigs are clinically asymptomatic, and the viral load in the blood is low or undetectable. Safety is the key issue for vaccine development. Therefore, even if the protection provided by intranasal vaccination is higher than that of intramuscular vaccination at the same dose, intramuscular immunization is the most feasible and safest way. Gallardo et al. [28] reported that a non-harmadsorbing (non-HAD) ASFV genotypeII, namely Lv/17/WB/Rie1 was isolated from a wild boar hunted in Latvia in 2017. The genome sequence of this strain was characterized by the presence of a single nucleotide deletion inside the EP402R gene, this deletion generating a truncated protein, which lacks most protein domains, making this a nonfunctional protein. Demostic pigs experimentally infected Infected pigs have non-specific clinical signs, some even asymptomatic, showing intermittent and weak viremia and high antibody response. Two months later, these animals were fully protected when exposed to other animals that infected with a relative virulent HAD genotype II ASFV. Although the number of experimental animals is small, the above results show that Lv17/WB/Rie1 has the potential to be a target for the development of live attenuated vaccines. Pérez-Núñez et al. [29] reported a non-biased methodology for viral DNA purification of extracellular ASFV virions combined with next-generation sequencing (NGS) revealed an expected heterogeneity within the genotype II Arm/07 stock. This approach is key for genetic characterization of ASFV stock and LAV prototypes, because several genetic factors, such as off-target modifications, parental contamination and the presence of possible subpopulations and/or minor viral variants in the stocks, which eventually could also play a role in the overall safety of LAVs. The results shown that there were two separate Arm/07/CBM/clones 2 and 4, which belonging to genotype II and I respectively, have been identified at the Arm/07 stock by NGS. According to their specific characteristics, specilay hemadsorption and immune response, two different strains display different degrees of virulence in animals, Arm/07/CBM/clones 4 show a more attenuated phenotype and would be a good canidated strain for future LAV development. Interestingly, a single mutation found at the N-terminal region of the EP402R gene (CD2v) of Arm/07/CBM/c4, further assessed by NGS and Sanger, induced a frame shift variant that shortened the N-terminal domain of CD2v. All in all, despite the ability to induce protective antibodies and T cell response, the side effects and safety issues of the naturally attenuated strain need to be considered. The natural-attenuated ASFV Vaccines strain are shown in Table 1.

2.2. Subculture-attenuated strains

Virulent strains can reduce the virulence by subculturing in the sensitive cells for multiple generations. The pathogenicity of ASFV gradually decreased, the immunogenicity and stability of the virus will decrease as virus passaged from the porcine bone marrow cells, Vero cells and COS-1 cell lines. In the 1960s, R.J. Manso and others attenuated ASFV by using porcine bone marrow cells and pigs were found to resist the attack of virulent strains after vaccinated with attenuated strains [28]. Later, in Spain and Portugal, field experiments by using attenuated strains had disastrous consequences. The immunized animals developed side effects such as pneumonia, miscarriage and death. Tabarés E et al. [29] compared the restriction enzyme cleavage maps of ASFV grown in pig leukocytes (strain E70 L6) and adaption in MS monkey kidney cells (strain E70MS14). The mapping data revealed that before adaptation to growth in MS cells, the size of the DNA from ASFV strain E70 L6 was l73 kbp and after adaptation it was only 156 kbp. The decrease in size was produced by deletions and additions mainly in the terminal regions of the genome. Afterwards, Krug et al. subcultured the ASFV-G strain in Vero cells and found that the replication ability of the virus in Vero cells increased and the virus virulence gradually decreased. The virulence was completely lost when it was passaged to the 110th generation. Pigs did not obtain corresponding immune protection after vaccination [30]. Then they subcultured the ASFV-G/V strain in Vero cells and performed whole genome sequencing [31]. As a result, it was found that large fragments of gene deletions and mutations appeared at the end of the genome of the ASFV-G/V strain. This mutation may be the reason for its adaptation to Vero cells, but it changed the antigenicity of ASFV-G/V and failed to induce the protective immune response.

Table 1
Natural-attenuated strains.

Strain	Virulence	Challenge	Protection	References
NH/P68	low	Heterologous strain L60	100%	Leitão et al., 2001
OUR T88/3	low	Heterologous strain Benin 97/1	85.7%	King et al., 2011
		Heterologous strain Uganda 1965	100%	
OUR T88/3	low	Homologous strain OURT88/1	100%	Mulumba-Mfumu et al., 2016
		Heterologous strain DRC 085/10	100%	
OUR T88/3	low	Homologous strain OURT88/1	50–100%	Sánchez-Cordón et al., 2017
Lv17/WB/Rie1	low	Homologous strain HAD Latvian ASFV	100%	Gallardo et al., 2019

2.3. Recombinant-attenuated strains

Using homologous recombination methods, knocking out viral virulence genes or immunosuppressive immune escape related genes can reduce viral virulence. The virulence genes of ASFV mainly include TK, UK, 9 GL and CD2v, the immune escape related genes mainly include MGF and A238L [33], and the inhibition of interferon (γ) gene A276R [34]. Abrams CC et al. deleted DP71L and DP96R gene from the OUR T88/3 genome to produce the recombinant virus OUR T88/3ΔDP2. Groups of 6 pigs were immunized with deletion virus OUR T88/3ΔDP2 or parental virus OUR T88/3 and challenged with virulent OUR T88/1 virus. The group of deletion virus OUR T88/3ΔDP2 provided 66% protection while the parental virus OUR T88/3 provided 100% protection [35]. The conclusion of this experiment is that after deleting the two genes DP71L and DP96R from the OUR T88/3 strain, its protective effect is reduced when the pigs are challenged by virulent strains after inoculation. Although deletion of DP71L and DP96R was unsuccessful at improving OUR T88/3 as a vaccine, an alternative vaccine approach may be to delete ASFV genes from a parental virulent strain of ASFV which may result in an attenuated recombinant virus which reduce side effects but induces effective protection. Gallardo C et al. immunized pigs with NH/P68 subcultured in PAM cells can resist the attack of the Arm07 virulent strain, but the constructed gene deletion strain NH/P68 △A276R cannot resist the attack of the Arm07 virulent strain [23]. This also confirms that A276R plays an important role in the immune protection of ASFV.

O'Donnell V et al. knocked out the gene 9 GL (B119L) [36] from the Georgia 2007/1 isolate (ASFV-G), which was a highly virulent virus isolate detected in the Canucasus region in 2007 and now spreading through the Canucasus region and Eastern Europe, to construct the recombinant virus ASFV-G-Δ9GL which showed limited replication in primary swine macrophages. They used 10^4 HAD_{50} of ASFV-G-Δ9GL intramuscularly to give pigs a virulence phenotype that induced lethal disease in pigs like the parental ASFV-G. Lower doses (10^2 to 10^3 HAD_{50}) of ASFV-G-Δ9GL did not induce a virulent phenotype in swine and when challenged protected pigs against disease. A dose of 10^2 HAD_{50} conferred partial protection when pigs were challenged at either 21 or 28 days post infection (dpi). A dose of 10^3 HAD_{50} conferred partial and complete protection at 21 and 28 dpi, respectively. Although attenuation was achieved, the protective dose and lethal dose were too similar. They also deleted another gene UK (DP96R) to construct a double-gene-deletion recombinant virus ASFV-G-Δ9GL/ΔUK [37] to enhance attenuation of the virus. Inoculation of pigs (i.m.) with high doses (10^6 HAD_{50}) did not cause disease. Inoculating pigs with a dose of 10^4 HAD_{50} can protect them from ASFV-G challenge. Furthermore, this study found that the presence of protection correlates with the appearance of antibodies against ASFV, but not with virus-specific circulating ASFV-specific IFN-γ-producing cells [37]. Reis AL et al. constructed the recombinant virus BeninΔDP148R [38] by deleting the gene DP148R. The virus's virulence in vivo is lower than that of the parental virus. Pigs survived after inoculation with BeninΔDP148R (only showed transient mild clinical symptoms immediately after immunization). After challenging with the parental virus, all pigs immunized by the intramuscular route survived, and all pigs immunized by the intranasal route survived except one. The surviving pigs had only mild symptoms or no clinical symptoms compared with the control pigs with acute ASF symptoms.

O'Donnell V et al. specifically deleted 6 genes including MGF505, MGF360-1R, MGF360-12L, MGF360-13L, MGF360-14L, MGF505-2R and MGF505-3R from Georgia 2007/1 isolate (ASFV-G) to construct the recombinant virus ASFV-G-ΔMGF [39]. In primary pig macrophages, the replication efficiency of ASFV-G-ΔMGF is as high as the parental virus. After intramuscular inoculation of pigs, the replication efficiency of ASFV-G-ΔMGF is completely attenuated in vivo. After inoculation, the inoculated pigs can resist the challenge of the parental strain. Importantly, although it has a good protective effect, it also brings a series of safety problems after vaccination. To solve this problem, they constructed a new virus strain ASFV-G-Δ9G/ΔMGF [40] based on the deletion of all genes deleted in ASFV-G-Δ9GL and ASFV-G-ΔMGF. Compared with ASFV-G and ASFV-G-ΔMGF, the replication ability of ASFV-G-Δ9GL/ΔMGF in primary culture of porcine macrophages is reduced, but it is similar to ASFV-G-Δ9GL. ASFV-G-Δ9GL/ΔMGF were weakened even at high dose of 10^6 HAD_{50} when intramuscularly inoculated into pigs. Animals with infection doses from 10^2 to 10^6 HAD_{50} had no detectable virus levels in their blood at any time after infection, nor did they produce detectable levels of anti-ASFV antibodies. Importantly, ASFV-G-Δ9GL/ΔMGF failed to induce protection against challenge from the virulent parent ASFV-G. Therefore, this study recommended that when developing rationally designed ASFV vaccine strains, attention should be paid on genome manipulation. Reis et al. deleted the genes MGF360 (MGF360-10L, 11L, 12L, 13L, 14L) and MGF530/505 (MGF530/505-1R, 2R and 3R) and interrupting genes (MGF360-9L and MGF530/505-4R) from the genome of the ASFV Benin 97/1 isolate and constructed the deletion mutant BeninΔMGF [41]. The replication of BeninΔMGF in porcine macrophages is not much different from the parental virulent virus Benin 97/1 and the natural attenuated isolate OURT88/3, which has a similar deletion of MGF360 and 530/505 genes. Pigs were immunized and boosted with the BeninΔMGF, and then challenged with a lethal dose of Benin 97/1, all pigs were protected. In order to better define the safety and efficacy of this attenuated vaccine candidate and to understand protective mechanisms, Sánchez-Cordón P J et al. [42] extended previous studies by intramuscular immunisation of pigs with the deletion mutant BeninΔMFG at different doses (10^2, 10^3, 10^4 TCID50), together with intranasal immunisation at the 10^3 dose. The results show that the intramuscular route using high doses (10^4 $TCID_{50}$) is the best option for immunisation. From the data that both protected and non-protected pigs showed an increase of IFNγ and IL-10 in serum after immunisation, protective mechanisms might involve also other chemical mediators and cellular components. The above experiments deleted the genes in the ASFV multi-gene family MGF360 and MGF530/505 showed that these genes are involved in the regulation of type I interferon (IFN) response. Their data confirmed that these MGF360 and MGF530/505 genes play a role in inhibiting the induction of type I interferon [41].

Monteagudo P L et al. deleted the CD2v gene of the BA71 strain and constructed a recombinant virus BA71ΔCD2 [43], which highly attenuated the virulence of BA71 strain in vivo. Pigs inoculated with BA71ΔCD2 not only resisted the attack of the parental BA71, but also protected the pig from the attack of the heterologous E75 (two genotype I strains). In addition, pigs immunized with BA71ΔCD2 survived the challenge of Georgia 2007/1 (ASFV genotype II strain). The final conclusion is that after deletion of the CD2v gene in the BA71 strain, it can provide complete protection for both type I and type II ASFV and has cross-protection effect. Elisabeth Lopez et al. [43]extend previous results using BA71ΔCD2 as a tool trying to understand ASFV cross-protection, using phylogenetically distant ASFV strain, they firstly found that five out of six (83.3%) of the pigs immunized once with 10^6 PFU of BA71ΔCD2 survived the tick-bite challenge using Ornithodoros sp. soft ticks naturally infected with RSA/11/2017 strain (genotype XIX, clade D), secondly, the results shown that only two out of six (33.3%) survived the challenge with Ken 06. Bus (genotype IX, clade A), which is phylogenetically more distant to BA71ΔCD2 than the RSA/11/2017 strain. The results from this work confirm that live attenuated ASFV as tools to dissect the mechanisms involved in cross-protection, which is crucial for the future designing of vaccines for endemic areas with complex ASFV epidemiological situations.

Borca, O'Donnell et al. used ASFV Georgia/2010 as the parental virus through homologous recombination, replacing 10-1083 nucleotides in the coding region of 8DR (EP402R) with p72 GUS, thereby deleting the 8DR gene and obtaining a lack of 8DR Gene deletion virus [44]. Compiling the multi-step growth curve of the virus revealed that the growth kinetics of ASFV-G-Δ8DR was only slightly reduced compared to the parental ASFV-G virus. There were no significant

differences in the severity of the symptoms of pigs infected with ASFV-G-Δ8DR or parental ASFV-G by intramuscular or intranasal route (within the range of 10^2 to 10^4 $TCID_{50}$). However, the viremia of animals infected with ASFV-G-Δ8DR was reduced compared to animals infected with ASFV-G. This indicate that there is no direct correlation between the concentration of viral transmission and the severity and evolution of clinical disease. Recently, Chen et al. used the Pig/HLJ/2018ASFV strain isolated from China as the parent and used homologous recombination technology to construct a series of recombinant viruses with different gene deletions [45]. Through systematic pathogenicity, immunogenicity and immunoprotection tests conducted in pigs, a virus with 7 gene deletions (genes encoding MGF505-1R, MGF505-2R, MGF505-3R, MGF360-12L, MGF360-13L, MGF360-14L, and CD2v deleted, HLJ/18-7GD) was selected to meet the safety standards of live-attenuated vaccines. It can provide effective immune protection against the challenge of ASF virulent strains. Studies have shown that HLJ/18-7GD is a safe and effective ASFV vaccine and is expected to play an important role in controlling the spread of ASFV. Detail genetic characterization of both prototypes of LAVs and parental strains is essential for LAV generation, the LAV prototypes which have been extensively sequenced by NGS and which have not. For instance, the prototypes ASFV-G-Δ9GL, ASFV-G-ΔMGF or ASFV-G-Δ9GL/ΔMGF [36,39,40] have been completely sequence by NGS and the presence of extra off-target mutations has been ruled out. However, in the works describing the prototypes BeninΔDP148R or HLJ/18-7GD [38,45] only the absence of the targeted gene has been assessed and, hence, the involvement of off-target mutations cannot be ruled out. Then, Borca [46] and other researchers deleted the previously unidentified gene I177L by homologous recombination between the parental ASFV Georgia 2010 (ASFV-G)genome and the recombinant transfer vector to produce recombinant ASFV- G-ΔI177L. Pigs were inoculated with 10^2 to 10^6 HAD_{50} of ASFV-G-ΔI177L or 10^2 HAD_{50} parental ASFV-G virus intramuscularly (IM). All pigs infected with ASFV-G-ΔI177L were found clinically normal with low virus titers in the blood and strong virus-specific antibody responses. After 28 days, pigs infected with ASFV-G-ΔI177L were fully protected by challenge with the parental ASFV-G, even at the low infection dose of 10^2 HAD_{50}. Infecting pigs at a high dose of 10^6 HAD_{50} did not show any disease-related signs, indicating the safety of ASFV-G-ΔI177L as a potential vaccine candidate. In addition, the ASFV-G virus was isolated from only one pig vaccinated with a low dose of ASFV-G-ΔI177L. This indicates that the replication of the ASFV-G virus seems to be greatly restricted in pigs infected with ASFV-G-ΔI177L. Jingyuan Zhang et al. [47] constructed attenuated strain ASFV SY18ΔL7-11 that deleted L7L–L11L (containing L7L, L8L, L9R, L10L, and L11L)genes from ASFV SY18, the virulence was obviously dramatically reduced when compared the homologous parental virulent strain ASFV SY18. All 11 surviving pigs were completely protected against challenge with the parental ASFV SY18 on 28 days postinoculation (dpi). In view of SY18ΔL7-11 induce side effects, such as mild fever, it is necessary to further codelete one or more genes to improve its safety if a vaccine candidate is desired.

Borca MV et al. deleted the early virus protein L83L from the ASFV Georgia 2007/1 isolate, but the virus is not attenuated after infecting pigs. This experiment did not verify whether it provides protection after challenge [48]. Recent studies by Ramirez-Medina E and others have also shown that the deletion of the B119L, DP71L/NL and DP96R/UK genes of the ASFV Georgia 2007/1 strain reduces its replication efficiency, but the mutant cannot protect the immunized pigs from the parental virus challenge [49]. One thing is that the deletion of the TK gene in Georgia 2007/1 strain and Malawi strain attenuated the virus, but only Malawi strain induced a protective response in immunized pigs [43,50]. Therefore, it is speculated that the effect of gene deletion on the virus's ability to induce immune protection is strain-specific [50], so the rational development of candidate vaccines for live attenuated ASFV vaccines must be based on individual cases. The Recombinant-attenuated ASFV Vaccines strain are shown in Table 2.

The duration of immunity for any vaccine candidate is crucial. In the case of ASFV vaccine candidates, this issue has received little attention. Previous works shown that BeninΔMGF LAV prototype and the natural attenuated prototype OURT 88/3 (and may also apply to other LAV prototypes) produced high percentage of protection when challenge was inoculated shortly (20–40 days) after immunization, Sánchez-Cordón et al. [42] reported that the duration of immunity and the immune responses induced over a duration of 130 days, the results shown that after a single intramuscular immunization of domestic pigs with the OURT88/3 isolate or BeninΔMGF virus, animals were not protected against challenge with the virulent Benin 97/1 ASFV genotype I isolate at day 130 postimmunization. The levels of regulatory T cells and IL-10 were elevated at the end of the experiment, suggesting that this absence of long-term protection seemed to correlate with regulatory T cells and IL10 production.

2.4. Cell lines for production of LAVS

Since the ASFV mainly infects the primary cultures of porcine macrophages and monocytes derived from peripheral blood or alveolar macrophages, therefore, these cell culture systems are used in laboratories for biological and immunological studies of ASFV. However, the primary cells would be unlikely to be used in vaccine production due to issues of difficulty reproducibility, lot-to-lot variation, laborious and costly cell extraction and animal welfare concerns. At present, monkey cell lines, such as Vero or MS cells were used for biological studies, production, and purification of the adapted virus [51,52]. However, the adaptation of ASFV has always resulted in genomic changes especially in the variable regions at both ends, which reducing the virus replication in pigs such that protection is not achieved [32]. Hence, sustainable cell

Table 2
Recombinant-attenuated strains.

Strain	Virulence	Deleted genes	Challenge	Protection	References
OUR T88/3	low	DP71L and DP96R	Homologous strain OURT88/1	100%	Abrams et al., 2013
Georgia 2007/1	high	B119L(9 GL)	Homologous strain Georgia 2007/1	100%	O'Donnell et al., 2015
Georgia 2007/1	high	DP96R(UK) and B119L(9 GL)	Homologous strain Georgia 2007/1	100%	Vivian et al., 2016
Benin 97/1	high	DP148R	Homologous strain Benin 97/1	100%	Reis AL et al., 2017
Georgia 2007/1	high	MGF505/360(6)	Homologous strain Georgia 2007/1	100%	O'Donnell et al., 2015
Georgia 2007/1	high	MGF505/360 and B119L(9 GL)	Homologous strain Georgia 2007/1	100%	O'Donnell et al., 2016
Benin 97/1	high	MGF505/530/360	Homologous strain Benin 97/1	100%	Reis et al., 2016
Benin 97/1	high	MGF505/360	Homologous strain Benin 97/1	100%	Sánchez-Cordón et al., 2018
BA71	high	EP402R(CD2v)	Heterologous strain Georgia 2007/1	100%	Monteagudo et al., 2017
Georgia 2010	high	EP402R(CD2v)	Homologous strain Georgia 2010	100%	Borca et al., 2020
HLJ/2018	high	MGF505/360(6) and EP402R(CD2v)	Homologous strain HLJ/2018	100%	Chen et al., 2020
Georgia 2010	high	I177L	Homologous strain Georgia 2010	100%	Borca et al., 2020
Georgia 2007/1	high	L83L	do not verify	do not verify	Borca et al., 2018
Georgia 2007/1	high	B119L, DP71L and DP96R	Homologous strain Georgia 2007/1	0	Ramirez-Medina et al., 2019
NH/P68	low	A276R	Heterologous strain virulent Arm07	0	C. Gallardo et al., 2018

lines that are susceptible to the infection of ASFV are in great demand. Earlier reports suggested that the CD163was associated with the susceptibility of cells to ASFV in vitro, subsequent studies showed that monocytes that didn't express CD163 could also be infected. Gene-edited pigs deleting CD163 were fully susceptible to infection by ASFV. Monocytes and macrophages from these pigs were also susceptible to infection confirming that CD163 is not required [53]. Paula L. Monteagudo et al. [43] reported that ASFV BA71 grown in COS-1 cells without significantly losing its virulence, no significant differences were observed in vivo after inoculation of pigs with different doses of BA71, grown either in COS-1 cells (BA71-Cos) or in PAMs (BA71). The genetically modified LAV, the BA71ΔCD2 produced in COS-1 cells, yielded an effective vaccine able to protects in a dose-dependent manner against BA71 homologous lethal challenge, meanwhile, BA71ΔCD2 confers protection against heterologous E75 lethal challenge, which correlating with the induction of cross-reactive $CD8^+$ T cells. Lately, COS-7 (an SV40 T-antigen-transformed epithelial green monkey cell line), was used as a model for the infection, since it allowed for productive ASFV infection [54]. Nevertheless, the use of a host-derived cell system is needed to maintain a more natural environment for studies of cell-host interaction and immune response. Raquel Portugala et al. [55] reported a porcine macrophage cell line, Zuckerman macrophage-4 (ZMAC-4) derived from foetal pig lung macrophages, which was susceptible to infection of eight different ASFV field isolates and supported their growth with similar kinetics which were observed in primary porcine macrophage cultures. Although the virus developed HAD in the ZAMC-4 cell culture, however, it did not develop CPEs after infection. Sánchez et al. [56] established four porcine cell lines, IPAM-WT, IPAM-CD163, CΔ2+ and WSL, were compared to porcine alveolar macrophage (PAM) in terms of surface marker phenotype, susceptibility to ASFV infection and virus production. The results shown that with the exception of WSL, all cell lines showed more efficient production of the naturally attenuated strain NHV/P68 but not of the virulent strains E70 and Armenia/07. The level of infection and virus production in IPAM-WT and CΔ2+ was much lower than in PAM, whereas WSL was able to sustain ASFV infection albeit with several differences compared with PAM. Kentaro Masujin et al. [57] reported that the immortalized porcine cell lines, IPKM cells, from primary porcine kidney macrophages by introducing SV40 large T antigen (SV40LT) and porcine telomerase reverse transcriptase (pTERT) genes with modified lentiviral vectors. The cells can facilitate high levels ($>10^{7.0}$ TCID50/mL) of viral replication of ASFV, and hemadsorption reactions and cytopathic effects were observed as with porcine alveolar macrophages when inoculated with virulent field isolates: Armenia 07, Kenya 05/Tk-1, and Espana75. These results suggested that IPKM may be a valuable tool for the isolation, replication, and genetic manipulation of ASFV in both basic and applied ASF research. It will play an important role in further understanding of virus knowledge and supporting the development of vaccines.

3. Conclusions

Since the first report in Kenya in 1921, ASF has a history of nearly 100 years. The development of ASFV vaccine has made great progress in recent decades, however, an efficacious, safe ASFV vaccine does currently not exist. This review highlighted recent discoveries in the development of ASF live-attenuated vaccine. There are several issues to make it clear before the attenuated vaccine be available for commercial. On the one hand, the existing candidate live-attenuated vaccines should be used for more in vivo experiments to determine their biological safety and protection effect against non-homologous wild strains. On the other hand, identifying a suitable cell line is a priority to live-attenuated vaccine production. Besides, with an in-depth understanding of the interaction between ASFV and host, more virulence genes, genes involved in immune escape and protective antigen genes can be screened for the development of live viral vector vaccines, subunit vaccines and nucleic acid vaccines.

Author contributions

Author Le Liu contributed to the conceptualization, writing, review, and editing. Authors Xiangwei Wang, Ruoqing Mao, Yahua Zhou, Juanbin Yin, Yuefeng Sun and Xiangping Yin contributed to the editing.

Declaration of competing interest

The author declare no conflict of interest.

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References

[1] M.C. Gallardo, A. de la T. Reoyo, J. Fernández-Pinero, I. Iglesias, M.J. Muñoz, M. L. Arias, African swine fever: a global view of the current challenge, Porc Health Manag 1 (2015) 21, https://doi.org/10.1186/s40813-015-0013-y.
[2] E.C. Anderson, G.H. Hutchings, N. Mukarati, P.J. Wilkinson, African swine fever virus infection of the bushpig (Potamochoerus porcus) and its significance in the epidemiology of the disease, Vet. Microbiol. 62 (1998) 1–15, https://doi.org/10.1016/S0378-1135(98)00187-4.
[3] Y. Revilla, D. Pérez-Núñez, J.A. Richt, African swine fever virus biology and vaccine Approaches, in: Advances in Virus Research, Elsevier, 2018, pp. 41–74, https://doi.org/10.1016/bs.aivir.2017.10.002.
[4] R. Eustace Montgomery, On A form of swine fever occurring in British East Africa (Kenya Colony), J. Comp. Pathol. Ther. 34 (1921) 159–191, https://doi.org/10.1016/S0368-1742(21)80031-4.
[5] W. Bommeli, U. Kihm, F. Ehrensperger, Federal Vaccine Inst, Sep, Preliminary Study on Immunization of Pigs against African Swine Fever, 1983.
[6] A.J. Forman, R.C. Wardley, P.J. Wilkinson, The immunological response of pigs and Guinea pigs to antigens of African swine fever virus, Arch. Virol. 74 (1982) 91–100, https://doi.org/10.1007/BF01314703.
[7] S. Blome, C. Gabriel, M. Beer, Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation, Vaccine 32 (2014) 3879–3882, https://doi.org/10.1016/j.vaccine.2014.05.051.
[8] M.G. Barderas, F. Rodríguez, P. Gómez-Puertas, M. Avilés, F. Beitia, C. Alonso, J. M. Escribano, Antigenic and immunogenic properties of a chimera of two immunodominant African swine fever virus proteins, Arch. Virol. 146 (2001) 1681–1691, https://doi.org/10.1007/s007050170056.
[9] G. Burmakina, A. Malogolovkin, E.R. Tulman, L. Zsak, G. Delhon, D.G. Diel, N. M. Shobogorov, Yu.P. Morgunov, S.Yu. Morgunov, G.F. Kutish, D. Kolbasov, D. L. Rock, African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever, J. Gen. Virol. 97 (2016) 1670–1675, https://doi.org/10.1099/jgv.0.000490.
[10] P. Gómez-Puertas, F. Rodríguez, J.M. Oviedo, A. Brun, C. Alonso, J.M. Escribano, The African swine fever virus proteins p54 and p30 are involved in two distinct steps of virus Attachment and both Contribute to the antibody-mediated protective immune response, Virology 243 (1998) 461–471, https://doi.org/10.1006/viro.1998.9068.
[11] J. Lopera-Madrid, J.E. Osorio, Y. He, Z. Xiang, L.G. Adams, R.C. Laughlin, W. Mwangi, S. Subramanya, J. Neilan, D. Brake, T.G. Burrage, W.C. Brown, A. Clavijo, M.A. Bounpheng, Safety and immunogenicity of mammalian cell derived and Modified Vaccinia Ankara vectored African swine fever subunit antigens in swine, Vet. Immunol. Immunopathol. 185 (2017) 20–33, https://doi.org/10.1016/j.vetimm.2017.01.004.
[12] M.V. Murgia, M. Mogler, A. Certoma, D. Green, P. Monaghan, D.T. Williams, R.R. R. Rowland, N.N. Gaudreault, Evaluation of an African swine fever (ASF) vaccine strategy incorporating priming with an alphavirus-expressed antigen followed by boosting with attenuated ASF virus, Arch. Virol. 164 (2019) 359–370, https://doi.org/10.1007/s00705-018-4071-8.
[13] J.G. Neilan, L. Zsak, Z. Lu, T.G. Burrage, G.F. Kutish, D.L. Rock, Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection, Virology 319 (2004) 337–342, https://doi.org/10.1016/j.virol.2003.11.011.
[14] J.M. Argilaguet, E. Pérez-Martín, M. Nofrarías, C. Gallardo, F. Accensi, A. Lacasta, M. Mora, M. Ballester, I. Galindo-Cardiel, S. López-Soria, J.M. Escribano, P. A. Reche, F. Rodríguez, DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies, PloS One 7 (2012), e40942, https://doi.org/10.1371/journal.pone.0040942.
[15] S.-Y. Sunwoo, D. Pérez-Núñez, I. Morozov, E. Sánchez, N. Gaudreault, J. Trujillo, L. Mur, M. Nogal, D. Madden, K. Urbaniak, I. Kim, W. Ma, Y. Revilla, J. Richt, DNA-

protein vaccination strategy does not protect from challenge with African swine fever virus Armenia 2007 strain, Vaccines 7 (2019) 12, https://doi.org/10.3390/vaccines7010012.
[16] J.M. Argilaguet, E. Pérez-Martín, S. López, M. Goethe, J.M. Escribano, K. Giesow, G.M. Keil, F. Rodríguez, BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus, Antivir. Res. 98 (2013) 61–65, https://doi.org/10.1016/j.antiviral.2013.02.005.
[17] J.K. Jancovich, D. Chapman, D.T. Hansen, M.D. Robida, A. Loskutov, F. Craciunescu, A. Borovkov, K. Kibler, L. Goatley, K. King, C.L. Netherton, G. Taylor, B. Jacobs, K. Sykes, L.K. Dixon, Immunization of pigs by DNA prime and recombinant vaccinia virus boost to identify and Rank African swine fever virus immunogenic and protective proteins, J. Virol. 92 (2018), https://doi.org/10.1128/JVI.02219-17 e02219-17,/jvi/92/8/e02219-17.atom.
[18] S. Lokhandwala, S.D. Waghela, J. Bray, C.L. Martin, N. Sangewar, C. Charendoff, R. Shetti, C. Ashley, C.-H. Chen, L.R. Berghman, D. Mwangi, P.J. Dominowski, D. L. Foss, S. Rai, S. Vora, L. Gabbert, T.G. Burrage, D. Brake, J. Neilan, W. Mwangi, Induction of Robust immune responses in swine by using a Cocktail of adenovirus-vectored African swine fever virus antigens, Clin. Vaccine Immunol. 23 (2016) 888–900, https://doi.org/10.1128/CVI.00395-16.
[19] C.L. Netherton, L.C. Goatley, A.L. Reis, R. Portugal, R.H. Nash, S.B. Morgan, L. Gault, R. Nieto, V. Norlin, C. Gallardo, C.-S. Ho, P.J. Sánchez-Cordón, G. Taylor, L.K. Dixon, Identification and immunogenicity of African swine fever virus antigens, Front. Immunol. 10 (2019) 1318, https://doi.org/10.3389/fimmu.2019.01318.
[20] M. Arias, A. de la Torre, L. Dixon, C. Gallardo, F. Jori, A. Laddomada, C. Martins, R. M. Parkhouse, Y. Revilla, F, J.-M. Rodriguez, Sanchez-vizcaino, Approaches and perspectives for development of African swine fever virus vaccines, Vaccines 5 (2017) 35, https://doi.org/10.3390/vaccines5040035.
[21] S. Gil, N. Sepúlveda, E. Albina, A. Leitão, C. Martins, The low-virulent African swine fever virus (ASFV/NH/P68) induces enhanced expression and production of relevant regulatory cytokines (IFNα, TNFα and IL12p40) on porcine macrophages in comparison to the highly virulent ASFV/L60, Arch. Virol. 153 (2008) 1845–1854, https://doi.org/10.1007/s00705-008-0196-5.
[22] C.A.L. Oura, M.S. Denyer, H. Takamatsu, R.M.E. Parkhouse, In vivo depletion of CD8+ T lymphocytes abrogates protective immunity to African swine fever virus, J. Gen. Virol. 86 (2005) 2445–2450, https://doi.org/10.1099/vir.0.81038-0.
[23] C. Gallardo, E.G. Sánchez, Daniel Pérez-Núez, M. Nogal, Y. Revilla, African swine fever virus (ASFV) protection mediated by NH/P68 and NH/P68 recombinant live-attenuated viruses, Vaccine 36 (2018) 2694–2704, https://doi.org/10.1016/j.vaccine.2018.03.040.
[24] A. Leitão, C. Cartaxeiro, R. Coelho, B. Cruz, R.M.E. Parkhouse, F.C. Portugal, J. D. Vigário, C.L.V. Martins, The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response, J. Gen. Virol. 82 (2001) 513–523, https://doi.org/10.1099/0022-1317-82-3-513.
[25] K. King, D. Chapman, J.M. Argilaguet, E. Fishbourne, E. Hutet, R. Cariolet, G. Hutchings, C.A.L. Oura, C.L. Netherton, K. Moffat, G. Taylor, M.-F. Le Potier, L. K. Dixon, H.-H. Takamatsu, Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunisation, Vaccine 29 (2011) 4593–4600, https://doi.org/10.1016/j.vaccine.2011.04.052.
[26] L.K. Mulumba-Mfumu, L.C. Goatley, C. Saegerman, H.-H. Takamatsu, L.K. Dixon, Immunization of African indigenous pigs with attenuated genotype I African swine fever virus OURT88/3 induces protection against challenge with virulent strains of genotype I, Transbound Emerg Dis 63 (2016) e323–e327, https://doi.org/10.1111/tbed.12303.
[27] P.J. Sánchez-Cordón, D. Chapman, T. Jabbar, A.L. Reis, L. Goatley, C.L. Netherton, G. Taylor, M. Montoya, L. Dixon, Different routes and doses influence protection in pigs immunised with the naturally attenuated African swine fever virus isolate OURT88/3, Antivir. Res. 138 (2017) 1–8, https://doi.org/10.1016/j.antiviral.2016.11.021.
[28] C. Gallardo, A. Soler, I. Rodze, R. Nieto, C. Cano-Gómez, J. Fernandez-Pinero, M. Arias, Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017, Transbound Emerg Dis 66 (2019) 1399–1404, https://doi.org/10.1111/tbed.13132.
[29] D. Pérez-Núñez, E. Castillo-Rosa, G. Vigara-Astillero, R. García-Belmonte, C. Gallardo, Y. Revilla, Identification and isolation of two different subpopulations within African swine fever virus Arm/07 stock, Vaccines 8 (2020), https://doi.org/10.3390/vaccines8040625.
[30] J. Manso-Ribeiro, J.L. Nunes-Petisca, F. Lopez-Frazao, M. Sobral, Vaccination against ASF 24 (1963) 450–459. http://www.researchgate.net/publication/285727344_Vaccination_against_ASF. (Accessed 17 July 2020).
[31] E. Tabarés, I. Olivares, G. Santurde, M.J. Garcia, M.E. Carnero, African swine fever virus DNA: deletions and additions during adaptation to growth in monkey kidney cells, Arch. Virol. 97 (1987) 333–346, https://doi.org/10.1007/BF01314431.
[32] P.W. Krug, L.G. Holinka, V. O'Donnell, B. Reese, B. Sanford, I. Fernandez-Sainz, D. P. Gladue, J. Arzt, L. Rodriguez, G.R. Risatti, M.V. Borca, The progressive adaptation of a Georgian isolate of African swine fever virus to Vero cells leads to a gradual attenuation of virulence in swine corresponding to major modifications of the viral genome, J. Virol. 89 (2015) 2324–2332, https://doi.org/10.1128/JVI.03250-14.
[33] E.R. Tulman, G.A. Delhon, B.K. Ku, D.L. Rock, African swine fever virus, Virology Monographs Die Virusforschung Innzeldarstellungen 9 (2009) 43–87, https://doi.org/10.1007/BF01311692.
[34] S. Correia, S. Ventura, R.M. Parkhouse, Identification and utility of innate immune system evasion mechanisms of ASFV, Virus Res. 173 (2013) 87–100, https://doi.org/10.1016/j.virusres.2012.10.013.
[35] C.C. Abrams, L. Goatley, E. Fishbourne, D. Chapman, L. Cooke, C.A. Oura, C. L. Netherton, H.-H. Takamatsu, L.K. Dixon, Deletion of virulence associated genes from attenuated African swine fever virus isolate OUR T88/3 decreases its ability to protect against challenge with virulent virus, Virology 443 (2013) 99–105, https://doi.org/10.1016/j.virol.2013.04.028.
[36] V. O'Donnell, L.G. Holinka, P.W. Krug, D.P. Gladue, J. Carlson, B. Sanford, M. Alfano, E. Kramer, Z. Lu, J. Arzt, B. Reese, C. Carrillo, G.R. Risatti, M.V. Borca, African swine fever virus Georgia 2007 with a deletion of virulence-associated gene 9GL (B119L), when administered at low doses, leads to virus attenuation in swine and induces an effective protection against homologous challenge, J. Virol. 89 (2015) 8556–8566, https://doi.org/10.1128/JVI.00969-15.
[37] O'Donnell Vivian, R. Guillermo, Risatti, G. Lauren, Holinka, W. Peter, Simultaneous deletion of the9GLandUKGenes from the African swine fever virus Georgia 2007 isolate offers increased safety and protection against homologous challenge, J. Virol. (2016), https://doi.org/10.1128/jvi.01760-16.
[38] A.L. Reis, L.C. Goatley, T. Jabbar, Deletion of the African swine fever virus gene DP148R does not reduce virus replication in culture but reduces virus virulence in pigs and induces high levels of protection against challenge, J. Virol. 1 (2017), https://doi.org/10.1128/jvi.01428-17 e01760–16.
[39] V. O'Donnell, L.G. Holinka, D.P. Gladue, B. Sanford, P.W. Krug, X. Lu, J. Arzt, B. Reese, C. Carrillo, G.R. Risatti, M.V. Borca, African swine fever virus Georgia isolate harboring deletions of MGF360 and MGF505 genes is attenuated in swine and confers protection against challenge with virulent parental virus, J. Virol. 89 (2015) 6048–6056, https://doi.org/10.1128/JVI.00554-15.
[40] V. O'Donnell, L.G. Holinka, B. Sanford, P.W. Krug, J. Carlson, J.M. Pacheco, B. Reese, G.R. Risatti, D.P. Gladue, M.V. Borca, African swine fever virus Georgia isolate harboring deletions of 9GL and MGF360/505 genes is highly attenuated in swine but does not confer protection against parental virus challenge, Virus Res. 221 (2016) 8–14, https://doi.org/10.1016/j.virusres.2016.05.014.
[41] A.L. Reis, C.C. Abrams, L.C. Goatley, C. Netherton, D.G. Chapman, P. Sanchez-Cordon, L.K. Dixon, Deletion of African swine fever virus interferon inhibitors from the genome of a virulent isolate reduces virulence in domestic pigs and induces a protective response, Vaccine 34 (2016) 4698–4705, https://doi.org/10.1016/j.vaccine.2016.08.011.
[42] P.J. Sánchez-Cordón, T. Jabbar, M. Berrezaie, D. Chapman, A. Reis, P. Sastre, P. Rueda, L. Goatley, L.K. Dixon, Evaluation of protection induced by immunisation of domestic pigs with deletion mutant African swine fever virus BeninΔMGF by different doses and routes, Vaccine 36 (2018) 707–715, https://doi.org/10.1016/j.vaccine.2017.12.030.
[43] P.L. Monteagudo, A. Lacasta, E. López, L. Bosch, J. Collado, S. Pina-Pedrero, F. Correa-Fiz, F. Accensi, M.J. Navas, E. Vidal, M.J. Bustos, J.M. Rodríguez, A. Gallei, V. Nikolin, M.L. Salas, F. Rodríguez, BA71ΔCD2: a new recombinant live attenuated African swine fever virus with cross-protective Capabilities, J. Virol. 91 (2017), https://doi.org/10.1128/JVI.01058-17 e01058-17.
[44] M.V. Borca, V. O'Donnell, L.G. Holinka, G.R. Risatti, E. Ramirez-Medina, E. A. Vuono, J. Shi, S. Pruitt, A. Rai, E. Silva, L. Velazquez-Salinas, D.P. Gladue, Deletion of CD2-like gene from the genome of African swine fever virus strain Georgia does not attenuate virulence in swine, Sci. Rep. 10 (2020) 494, https://doi.org/10.1038/s41598-020-57455-3.
[45] W. Chen, D. Zhao, X. He, R. Liu, Z. Wang, X. Zhang, F. Li, D. Shan, H. Chen, J. Zhang, L. Wang, Z. Wen, X. Wang, Y. Guan, J. Liu, Z. Bu, A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs, Sci. China Life Sci. 63 (2020) 623–634, https://doi.org/10.1007/s11427-020-1657-9.
[46] M.V. Borca, E. Ramirez-Medina, E. Silva, E. Vuono, A. Rai, S. Pruitt, L.G. Holinka, L. Velazquez-Salinas, J. Zhu, D.P. Gladue, Development of a highly effective African swine fever virus vaccine by deletion of the I177L gene results in sterile immunity against the current epidemic Eurasia strain, J. Virol. 94 (2020) e02017–e02019, https://doi.org/10.1128/JVI.02017-19. /jvi/94/7/JVI.02017-19.atom.
[47] J. Zhang, Y. Zhang, T. Chen, J. Yang, H. Yue, L. Wang, X. Zhou, Y. Qi, X. Han, J. Ke, S. Wang, J. Yang, F. Miao, S. Zhang, F. Zhang, Y. Wang, M. Li, R. Hu, Deletion of the L7L-L11L genes Attenuates ASFV and induces protection against homologous challenge, Viruses 13 (2021), https://doi.org/10.3390/v13020255.
[48] M.V. Borca, V. O'Donnell, L.G. Holinka, E. Ramírez-Medina, B.A. Clark, E. A. Vuono, K. Berggren, M. Alfano, L.B. Carey, J.A. Richt, G.R. Risatti, D.P. Gladue, The L83L ORF of African swine fever virus strain Georgia encodes for a non-essential gene that interacts with the host protein IL-1β, Virus Res. 249 (2018) 116–123, https://doi.org/10.1016/j.virusres.2018.03.017.
[49] E. Ramirez-Medina, E. Vuono, V. O'Donnell, L.G. Holinka, E. Silva, A. Rai, S. Pruitt, C. Carrillo, D.P. Gladue, M.V. Borca, Differential effect of the deletion of African swine fever virus virulence-associated genes in the induction of attenuation of the highly virulent Georgia strain, Viruses 11 (2019) 599, https://doi.org/10.3390/v11070599.
[50] B. Sanford, L.G. Holinka, V. O'Donnell, P.W. Krug, J. Carlson, M. Alfano, C. Carrillo, P. Wu, A. Lowe, G.R. Risatti, D.P. Gladue, M.V. Borca, Deletion of the thymidine kinase gene induces complete attenuation of the Georgia isolate of African swine fever virus, Virus Res. 213 (2016) 165–171, https://doi.org/10.1016/j.virusres.2015.12.002.
[51] P. de León, M.J. Bustos, A.L. Carrascosa, Laboratory methods to study African swine fever virus, Virus Res. 173 (2013) 168–179, https://doi.org/10.1016/j.virusres.2012.09.013.
[52] A.L. Carrascosa, M.J. Bustos, P. de Leon, Methods for growing and titrating African swine fever virus: field and laboratory samples (Chapter 26), Curr Protoc Cell Biol (2011), https://doi.org/10.1002/0471143030.cb2614s53. Unit 26.14.

[53] L. Popescu, N.N. Gaudreault, K.M. Whitworth, M.V. Murgia, J.C. Nietfeld, A. Mileham, M. Samuel, K.D. Wells, R.S. Prather, R.R.R. Rowland, Genetically edited pigs lacking CD163 show no resistance following infection with the African swine fever virus isolate, Georgia 2007/1, Virology 501 (2017) 102–106, https://doi.org/10.1016/j.virol.2016.11.012.

[54] C. Hurtado, M.J Bustos, A.L Carrascosa, The use of COS-1 cells for studies of field and laboratory African swine fever virus samples, J. Virol Methods 164 (2010), https://doi.org/10.1016/j.jviromet.2009.11.030.

[55] R. Portugal, L.C Goatley, R. Husmann, F.A Zuckermann, L.K Dixon, A porcine macrophage cell line that supports high levels of replication of OURT88/3, an attenuated strain of African swine fever virus, Emerg. Microb. Infect. 9 (2020), https://doi.org/10.1080/22221751.2020.1772675.

[56] E.J Sánchez, E. Riera, M. Nogal, C. Gallardo, P. Fernández, R. Bello-Morales, J. A López-Guerrero, C.G Chitko-McKown, J.A Richt, Y. Revilla, Phenotyping and susceptibility of established porcine cells lines to African Swine Fever Virus infection and viral production, Sci. Rep. 7 (2017), https://doi.org/10.1038/s41598-017-09948-x.

[57] K. Masujin, T. Kitamura, K. Kameyama, K. Okadera, T. Nishi, T. Takenouchi, H. Kitani, T. Kokuho, An immortalized porcine macrophage cell line competent for the isolation of African swine fever virus, Sci. Rep. 11 (2021), https://doi.org/10.1038/s41598-021-84237-2.