สวทช NSTDA

NAC2023
18th NSTDA Annual Conference
การประชุมวิชาการประจำปี สวทช. ครั้งที่ ๑๘

Hope and Challenges in ASF vaccine development

Challika Kaewborisuth (D.V.M., Ph.D.)
Virology and Cell Technology Research Team (AVCT), BIOTEC, NSTDA

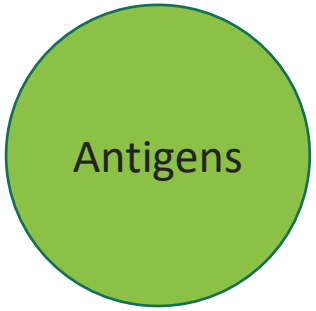
28-31
มีนาคม 2566

What is in a vaccine?

A biological product that can be used to safely induce an immune response that confers protection against infection and/or disease on subsequent exposure to a pathogen

Produced synthetically

Pathogen



One or more protein antigens

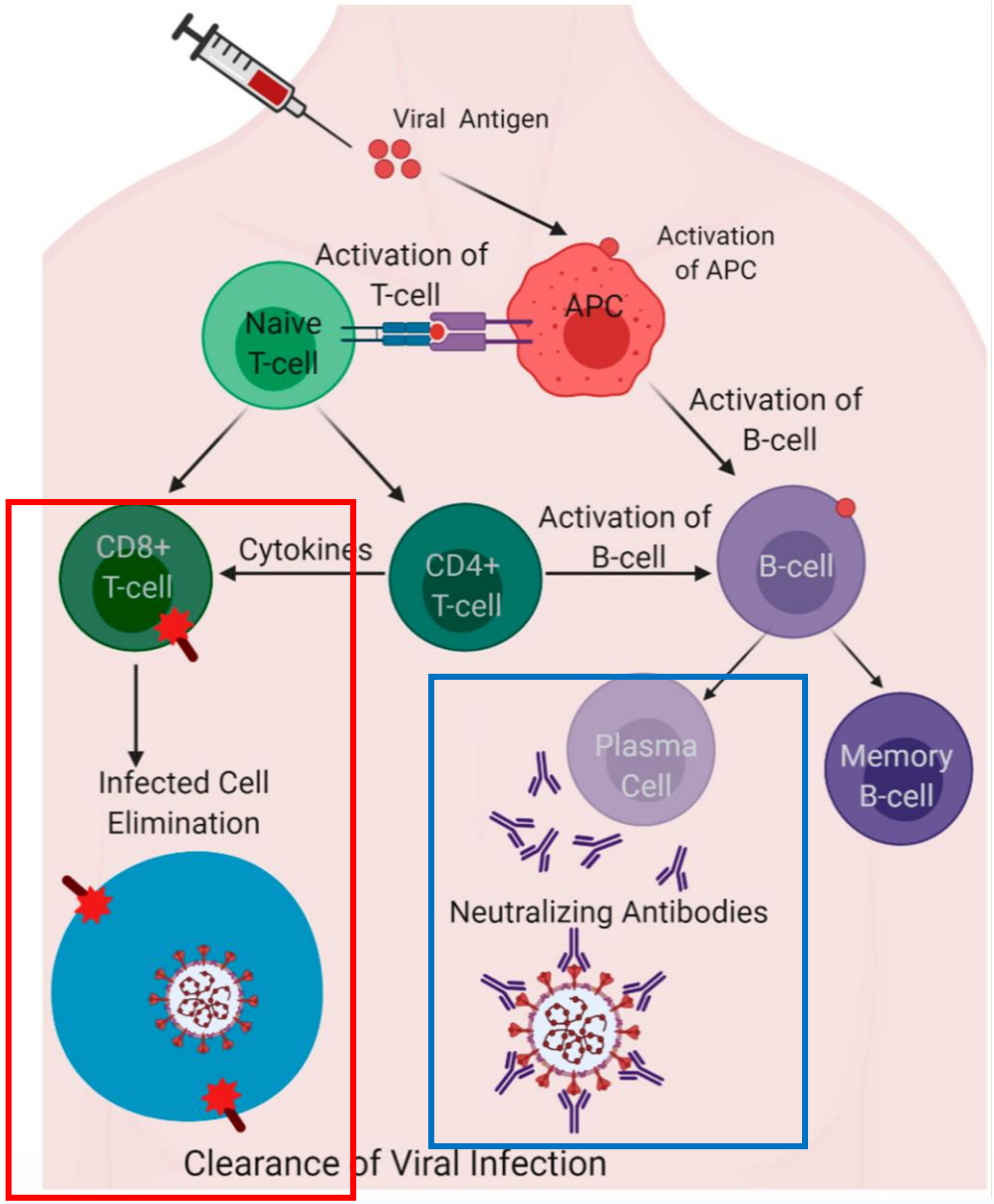
Induce immune responses that provide protection

Prevention of infection

A reduction in disease severity

A decreased rate of hospitalization

Development of and access to new vaccines

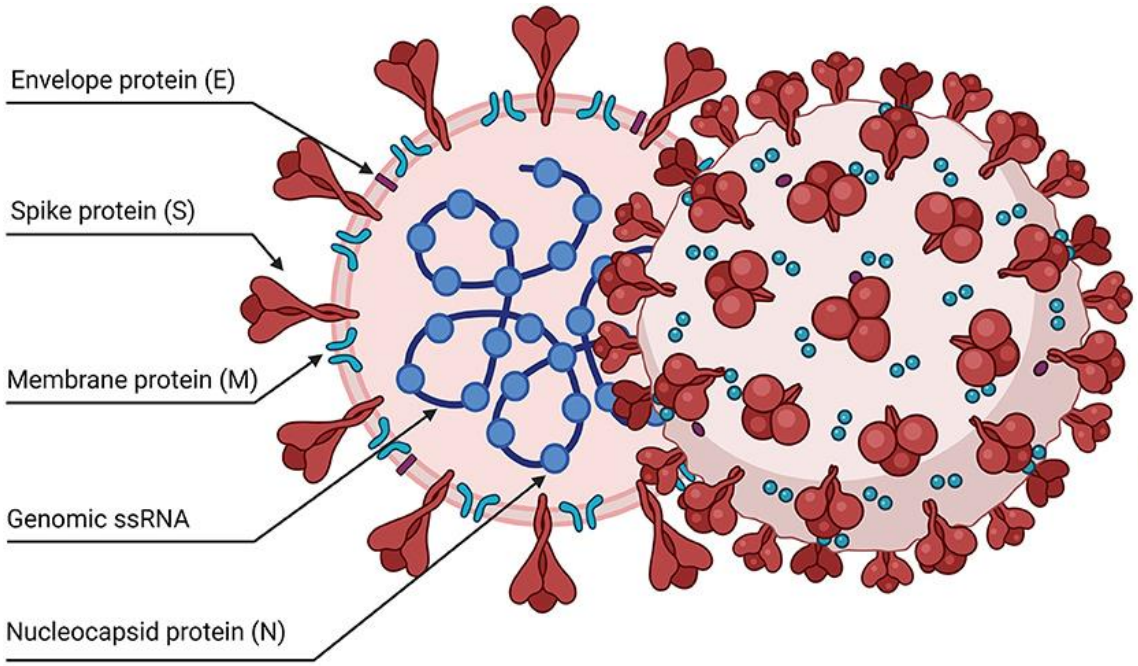


Jane et al., 2020

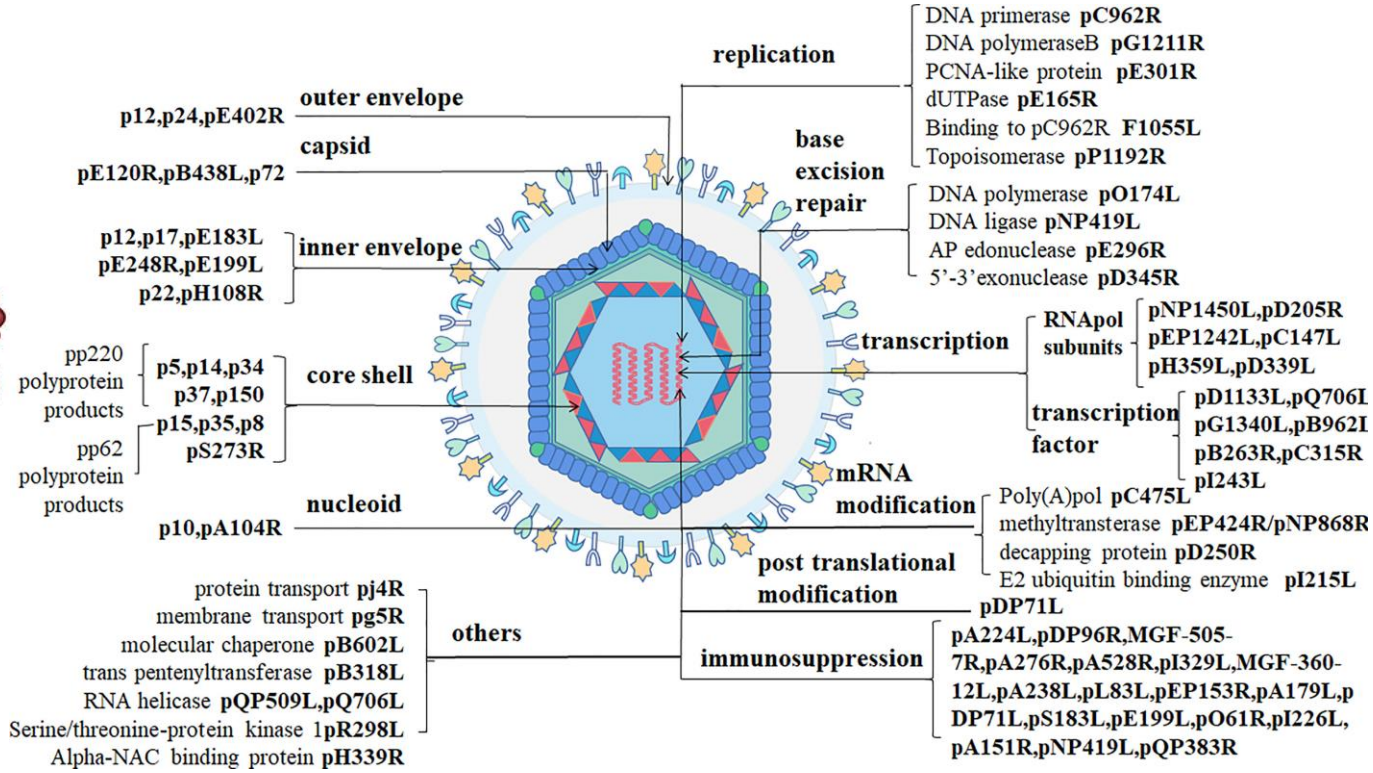
1

The difficulties in ASF vaccine research and development

SARS-CoV-2

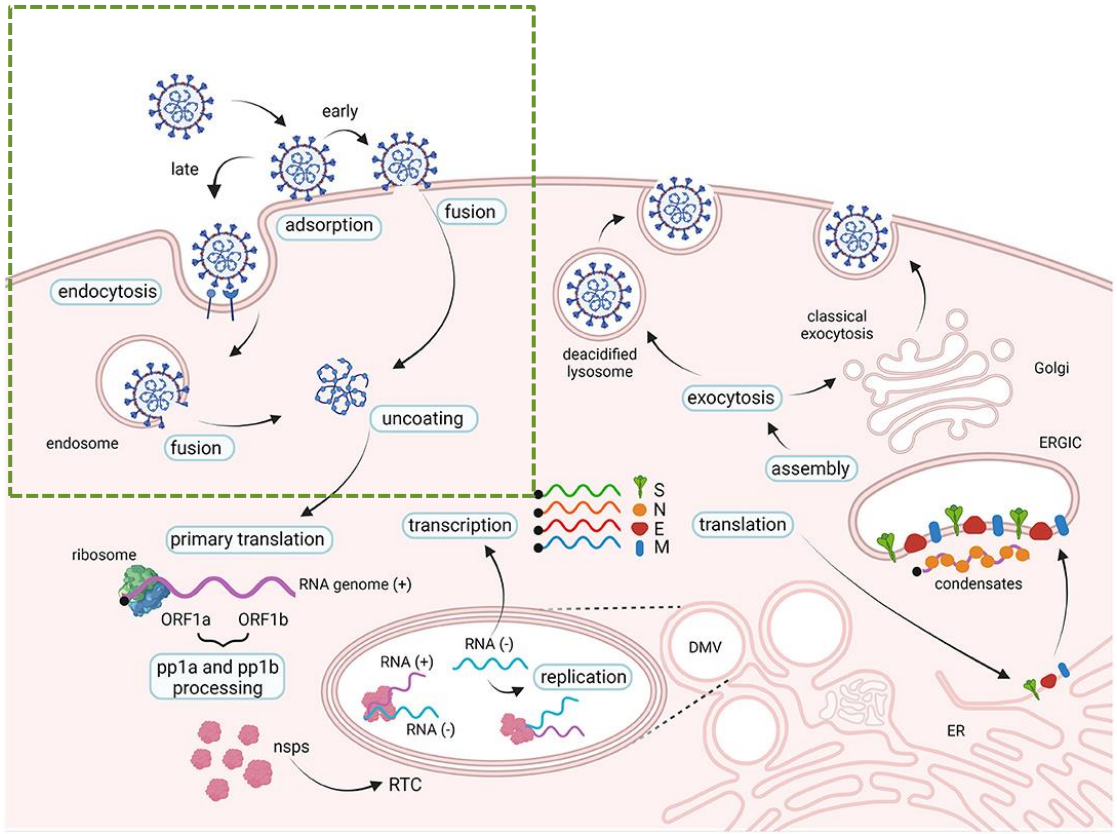


ASFV



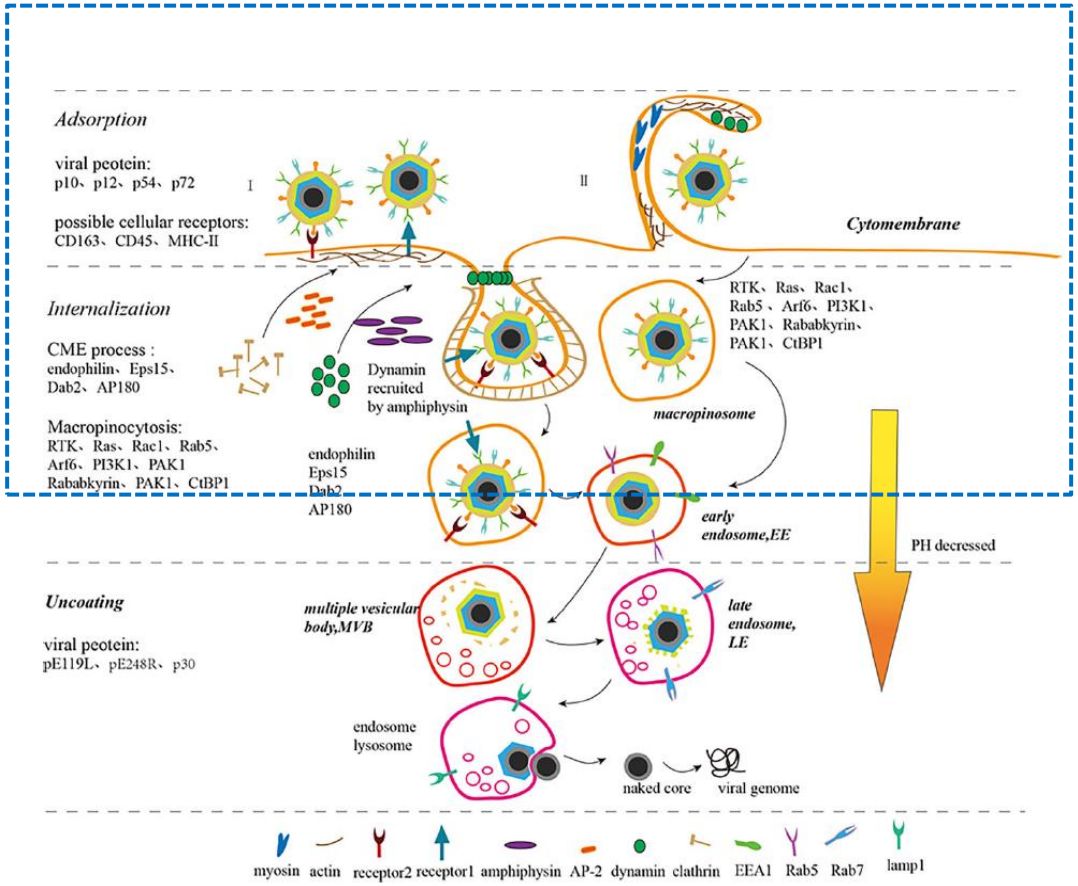
Wang et al., 2021

SARS-CoV-2



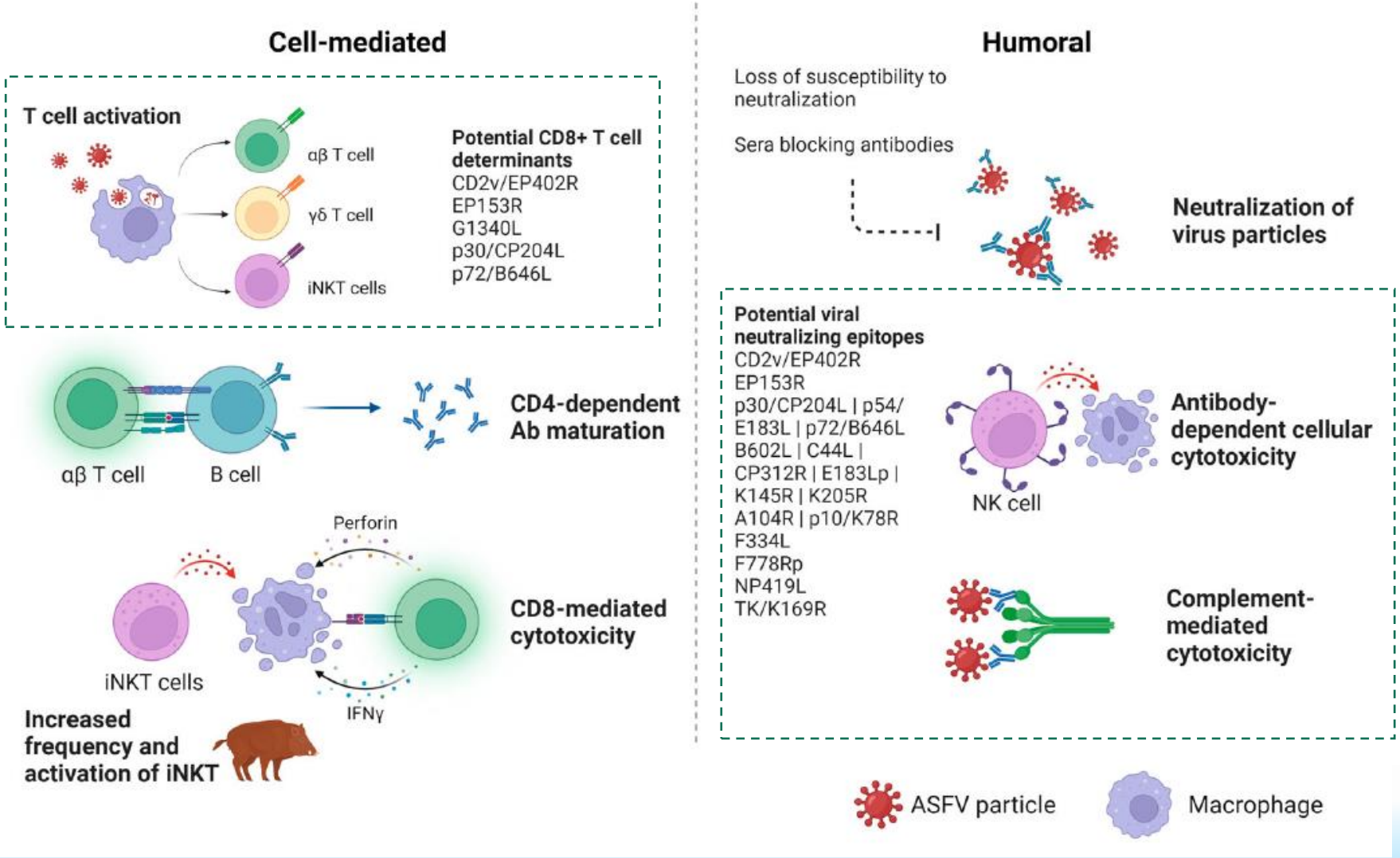
Pizzato et al., 2022

ASFV



Wang et al., 2021

Immune response to ASFV infection



Urbano and Ferreira, 2022

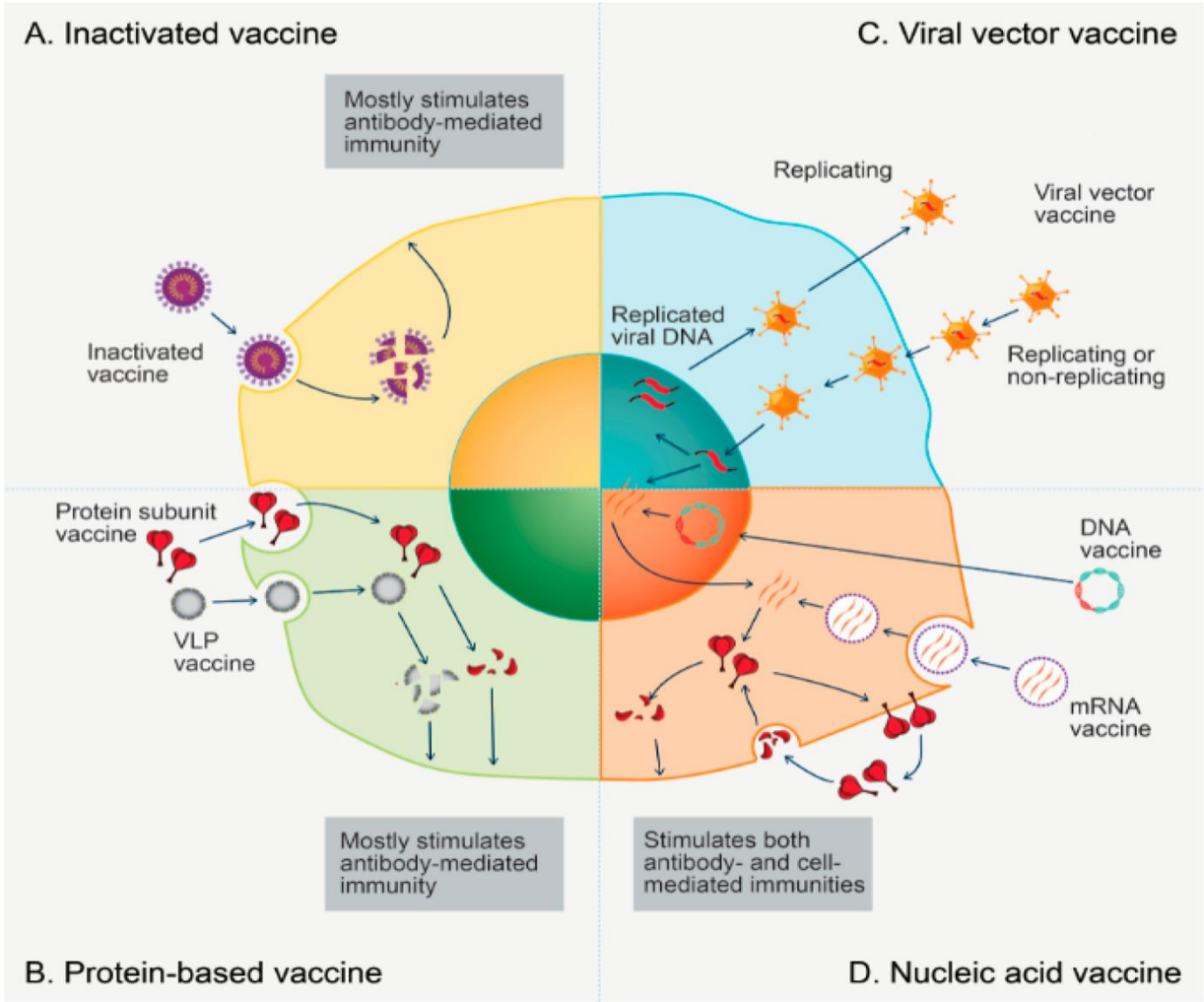
The difficulties in ASF vaccine research and development

Large ASFV genome,170 -193kbp and large size of ASFV virion, 200nm.	
Complexity of ASFV particle only partially known.	
Scarce knowledge related to virulence genes and immune protection.	**Unknown role for most of the genes**
Gaps in knowledge on virus–host interactions.	
Not fully neutralizing antibodies.	**Unknown protective immune mechanisms**
Gaps in knowledge regarding immune mechanisms of protection.	
Genetic variation among ASFV isolates.	**Unknown protective virus antigens**
Gaps in knowledge regarding **cross protection** among ASFV isolates.	**No commercial vaccine**

Muñoz-Pérez et al., 2021
Borca, 2020

2

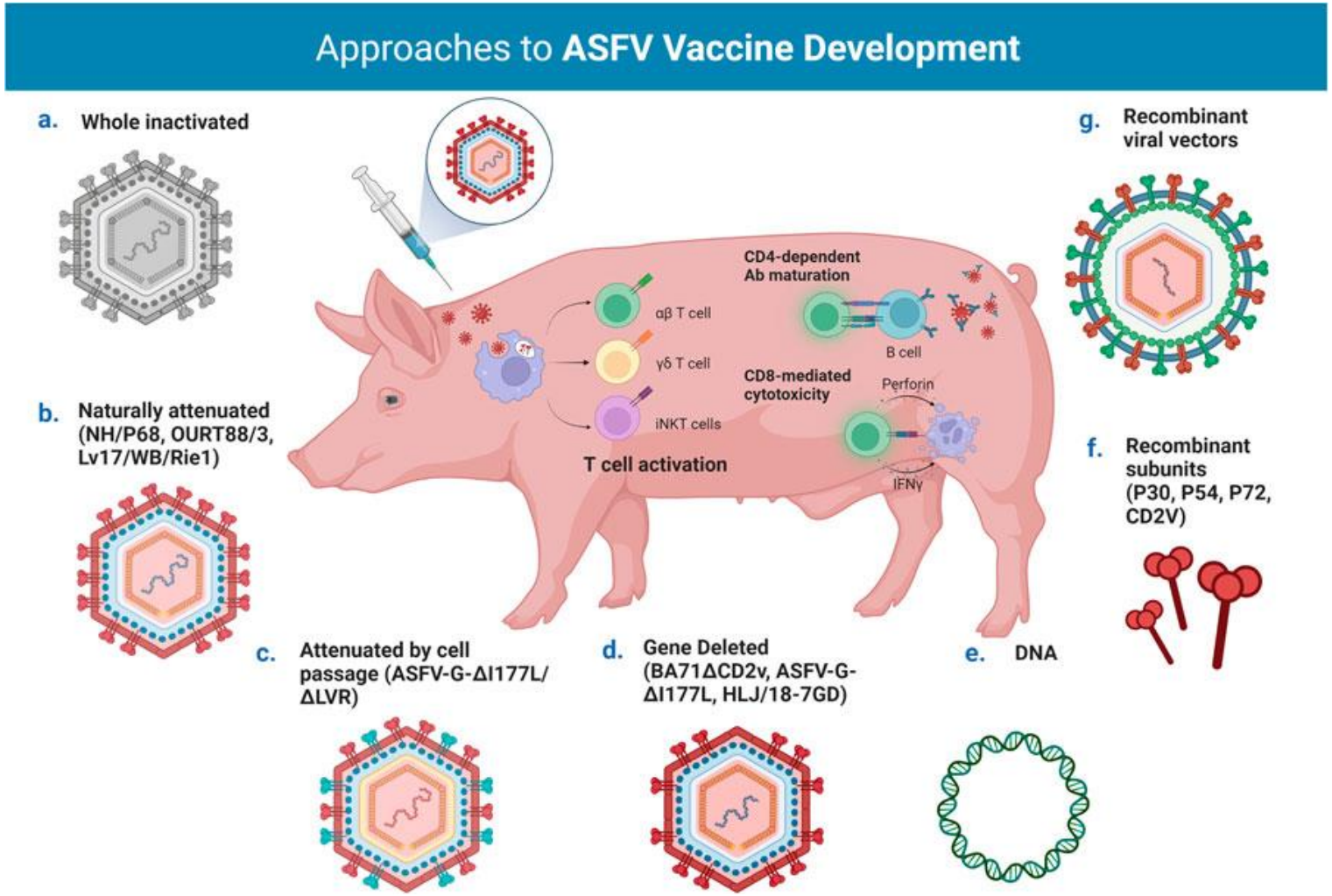
ASF vaccine research and development



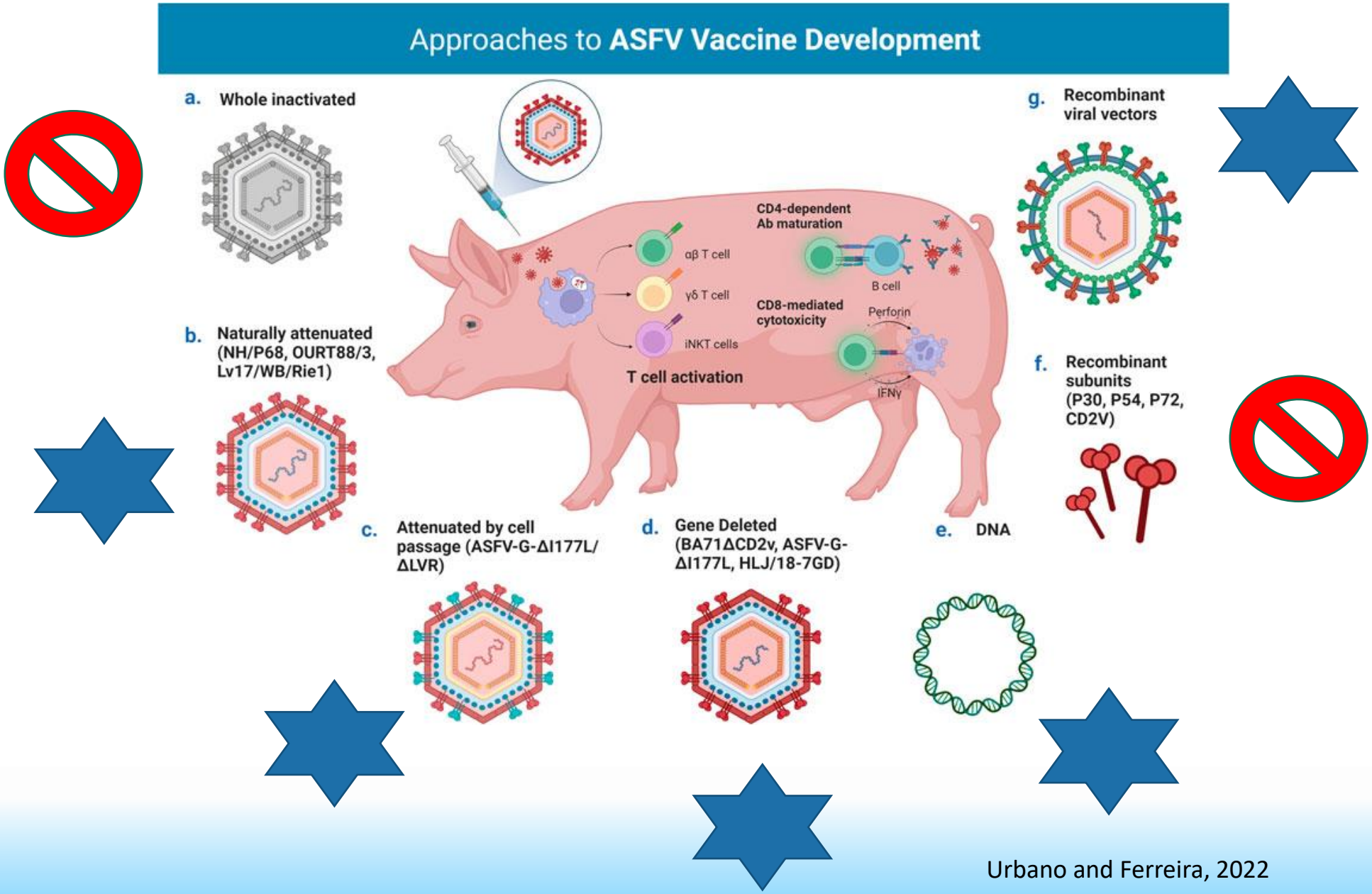
Live-attenuated vaccine

Synthetic peptide vaccine

Li et al., 2021

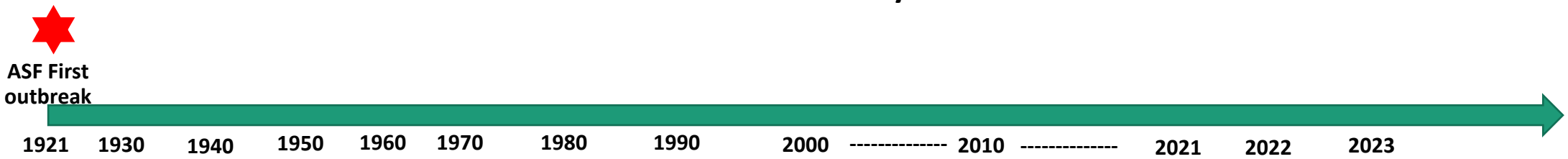


Urbano and Ferreira, 2022



Urbano and Ferreira, 2022

ASF vaccine History



Attenuated ASF vaccine (Cell culture passage)

Porcine bone marrow culture leukocytes and kidney cells

Partial protection against homologous ASFV
No cross-protection to heterologous ASFV

Side effects:
pneumonia, locomotor difficulties, skin ulcers, tumor and death

Persistent viremia
Carriers of the disease
Chronic forms

Inactivated ASF vaccine

Organ extracts treated with heat and chemicals
Glutaraldehyde-fixed infected cells

No - Partial protection

The importance of cellular response was highlighted

Subunit ASF vaccine

DNA, Protein, virus vector

No - Partial protection

Naturally attenuated ASF vaccine

Ornithodoros ticks
Wild boar

Partial – full protection

fever, skin necrotic lesions and the swelling of joints

CD8+ T cells played a specific role in combating viraemia and protection

Gene-deleted attenuated ASF vaccine

Partial – full protection

Inactivated ASF vaccine

Attenuated ASF vaccine (Cell culture passage)

Subunit ASF vaccine

Safety of repeated administration, overdoses, the genetic stability of the vaccine candidate and the characterizing of long-term shedding animals

Type of vaccine candidate		Percentage of studies
Live attenuated vaccines (LAV)		69%
LAV based on naturally attenuated isolates	24%	
LAV based on cell passages	12%	
LAV based on deleting specific genes	64%	
Inactivated virus vaccines		4%
Subunit protein vaccines		6%
DNA vaccines		6%
Virus vectored vaccines		6%
Combined vaccination strategy		9%

Muñoz-Pérez et al., 2021

3

Subunit, DNA and virus-vectored vaccines

Vaccine strategy	Vaccine candidate (ASFV isolate)	Vector/System	Challenge (ASFV isolate)	Percentage of protection	Reference
Inactivated virus vaccines	Pol16/DP/OUT21	-	Homologous (Pol16/DP/OUT21)	0%	(Cadenas-Fernández et al., 2021)
	Armenia08	-	Homologous (Armenia08)	0%	(Blome, Gabriel, & Beer, 2014)
Subunit protein vaccines	E70	Synthetic peptides	Homologous (E70)	0%	(Ivanov et al., 2011)
	Pr4	Baculovirus	Homologous (Pr4)	0%	(Neilan et al., 2004)
	E75	Baculovirus	Homologous (E75)	100%	(Barderas et al., 2001)
DNA vaccines	E75	DNA expression library	Homologous (E75, E75CV1)	50-60%	(Lacasta et al., 2014)
	E75	pCMV	Homologous (E75)	0-33%	(Argilaguet et al., 2012)
	E75	pCMV	Homologous (E75)	0%	(Argilaguet et al., 2011)
Virus vectored vaccines	Georgia 2007/1	Adenovirus	Heterologous (Arm07)	0%	(Cadenas-Fernández et al., 2020)
	Georgia 2007/1	Adenovirus	Homologous (Georgia2007/1)	20-56%	(Lokhandwala et al., 2019)
	E75	BacMam	Homologous (E75)	67%	(Argilaguet et al., 2013)
Combined vaccination strategy	OURT88/3, Benin97/1	r Ad prime + MVA boost	Homologous (OURT88/1)	0-100%	(Goatley et al., 2020)
	OURT88/3	r Ad prime + MVA boost	Homologous (OURT88/1)	0%	(Netherton et al., 2019)
	Georgia 2001/1, Ba71V	DNA + protein	Heterologous (Arm07)	0%	(Sunwoo et al., 2019)
	Georgia 2007/1	DNA prime + r VACV boost	Homologous (Georgia2007/1)	0%	(Jancovich et al., 2018)

Muñoz-Pérez et al., 2021

Subunit, DNA and virus-vectored vaccines

- **Gene targets**
  - B- and T-cell immunogenic major structural protein families (e.g., viral proteins comprising or associated with the complex viral envelope and capsid shell),
  - nonstructural proteins associated with viral replication and assembly
  - a few proteins of predicted but unknown function

- Structural proteins p54, p30, p72 and the hemagglutinin CD2v, and these proteins have traditionally been the main targets of vaccine strategies

Table 1. Antigen-based African swine fever virus (ASFV) vaccines evaluated in the swine model.

Vaccine Type	ASFV Target Protein (Strain)	Number of Immunizations; Dose, Adjuvant	Specific/Neutralizing Antibodies	T Cell Response	Challenge Strain; Dose	Clinical Outcome
Baculovirus-expressed proteins	CD2v (E75CV)	3×; 0.5–1 × 10^7 HAU + Freund's adjuvant	Yes; No	NA	E75; 4 × 10^2	100% protection, n = 3/3
Baculovirus-expressed proteins	p30, p54, p54 + p30 (E75)	3×; 100 µg + Freund's adjuvant	Yes; Yes	NA	E75; 5 × 10^2	50% protection, n = 3/6
Baculovirus-expressed proteins	p54/p30 chimera (E75)	5×; 100 µg + Freund's adjuvant	Yes; Yes	NA	E75; 5 × 10^2	100% protection, n = 2/2
Baculovirus-expressed proteins	p54 + p30 + p72 + p22 (Pr4)	4×; 200 µg + Freund's adjuvant	Yes; Yes	NA	Pr4; 10^4	Slight delay of clinical disease and viremia; No protection, (n = 0/6)
HEK cell-expressed proteins	p72, p54, p12 (Georgia 2007/1)	2×; 200 µg/antigen + TS6 adjuvant	Yes; NA	Some	NA	NA

p30 also referred to as p32; CD2v also referred to as HA = hemagglutinin; NA = not available.

Table 2. DNA-based ASFV vaccines evaluated in the swine model.

Vaccine Type	ASFV Target Protein (Strain)	Number of Immunizations; Dose	Specific/Neutralizing Antibodies	T Cell Response	Challenge Strain; Dose	Clinical Outcome
DNA (pCMV)	p54/p30 fusion (E75)	3×; 600 µg	No; NA	No	E75; 10^4	No protection, (n = 0/4; n = 0/4)
DNA (pCMV)	SLA-II/p54/p30 fusion (E75)	3×; 600 µg	Yes; No	Yes	E75; 10^4	No protection, (n = 0/4)
DNA (pCMV)	sHA/p54/p30 fusion (E75)	3× and 4×; 600 µg	Yes; No	Yes	E75; 10^4	No protection, (n = 0/6)
DNA (pCMV)	Ub/sHA/p54/p30 fusion (E75)	2× and 4×; 600 µg	Not detectable	Yes	E75; 10^4	Partial protection, (2 immunizations, n = 2/6; 4 immunizations, n = 1/6)
DNA expression library	80 ORFs fragments fused with Ub (Ba71V)	2×; 600 µg	Yes-after challenge; NA	Yes-after challenge	E75; 10^4	60% protection, (n = 6/10)

p30 also referred to as p32; CD2v also referred to as HA = hemagglutinin; sHA = extracellular/soluble domain; Ub = cellular ubiquitin; SLAII = swine leukocyte antigen class I molecule; pCMV = plasmid under cytomegalovirus promotor; ORFs = open reading frames; NA = not available.

Gaudreault et al., 2019

Table 3. Virus vector-based ASFV vaccines evaluated in the swine model.

Vaccine Type	ASFV Target Protein (Strain)	Number of Immunizations; Dose, Adjuvant	Specific/Neutralizing Antibodies	T Cell Response	Challenge Strain; Dose	Clinical Outcome
BacMam	sHA/p54/p30 fusion (E75)	3×; 10^7 PFU	No (only after challenge); No	Yes	E75; 2x sublethal challenge 10^2	Partial protection, (n = 4/6)
Adenovirus	p30+p54+pp62+p72 (Georgia 2007/1)	2×; 10^{10} or 10^{11} per Ad5-antigen + adjuvants	Yes; NA	Yes	NA	NA
Adenovirus	A151R+B119L+B602L+ EP402RΔPRR+B438L+K205R+A104R (Georgia 2007/1)	2×; 10^{11} per Ad5-antigen + adjuvant	Yes; NA	Yes	NA	NA
Vaccinia virus Ankara	p72, C-type Lectin, CD2v (Georgia 2007/1)	2×; rVACV-ASFV 10^7 $TCID_{50}$	No; NA	Yes	NA	NA
Alphavirus RPs	p30, p54, p72, sHA/72 (Ba71V)	3×: 2-4.5 × 10^7 RPs	Yes; NA	NA	NA	NA

p30 also referred to as p32; CD2v also referred to as HA = hemagglutinin; sHA = extracellular/soluble domain; rVACV = recombinant vaccinia virus; RPs = replicon par
NA = not available.

Table 4. Combination and heterologous prime-boost ASFV vaccine strategies.

Vaccine Type	ASFV Target Protein (Strain)	Number of Immunizations; Dose, Adjuvant	Specific/Neutralizing Antibodies	T Cell Response	Challenge Strain; Dose	Clinical Outcome
Combination						
DNA–Protein	Combinations of DNA and protein: p15, p30, p35, p54, p72, CD2v, CP312R, g5R (Georgia 2007/1; Ba71V)	3×; 100 µg per DNA, 100 µg protein + ISA25 adjuvant	Yes; Yes	Some	NA	NA
DNA–Protein	Proteins: p15, p35, p54, p17; DNA: CD2v, p72, p54, p30, p17 (Georgia 2007/1; Ba71V)	3×; 100 µg per DNA, 100 µg protein + ISA25 adjuvant	Yes; No	Some	Armenia 2007; 360 HAU	No protection; disease enhancement
Heterologous Prime-Boost						
DNA prime + vaccinia virus boost	47 antigens (Georgia 2007/1)	Prime 2×: 10 µg pCMV-DNA + CpG oligo adjuvant; Boost 2×: 10^8 PFU rVACV-ASFV	Yes; No	Yes	Georgia 2007/1; 10^4	No protection; reduced viral load, higher clinical scores
Vaccinia virus prime + protein boost	p72, C-type Lectin, CD2v (Georgia 2007/1)	Prime: rVACV-ASFV 10^7 $TCID_{50}$; Boost: 200 µg/antigen + TS6 adjuvant	NA	Yes	NA	NA
Alphavirus RP prime + live attenuated ASFV boost	p30 (Ba71V) + OURT88/3	Prime 2×: 2-4.5 × 10^7 RPs; Boost: 10^4 $TCID_{50}$ OURT88/3	Yes; Yes	NA	NA	NA

p30 also referred to as p32; CD2v also referred to as HA = hemagglutinin; sHA = extracellular/soluble domain; rVACV = recombinant vaccinia virus; RPs = replicon par
NA = not available.

Gaudreault et al., 2019

Subunit, DNA and virus-vectored vaccines

- **Proof-of-concept:** clinical study designs (e.g., relatively high vaccine doses, multiple immunizations, unsafe adjuvants, ASFV low challenge doses) impractical for direct transition to a regulatory product development program.

How to identify the antigens?

frontiers in Immunology

ORIGINAL RESEARCH
published: 19 June 2019
doi: 10.3389/fimmu.2019.01318

Check for updates

Identification and Immunogenicity of African Swine Fever Virus Antigens

Christopher L. Netherton[1], Lynnette C. Goatley[1], Ana Luisa Reis[1], Raquel Portugal[1], Rachel H. Nash[1], Sophie B. Morgan[1], Lynden Gault[2], Raquel Nieto[3], Veronica Norlin[2], Carmina Gallardo[3], Chak-Sum Ho[2†], Pedro J. Sánchez-Cordón[1†], Geraldine Taylor[1] and Linda K. Dixon[1]*

[1] The Pirbright Institute, Woking, United Kingdom, [2] Gift of Life Michigan Histocompatibility Laboratory, Ann Arbor, MI, United States, [3] European Union Reference Laboratory for ASF, Centro de Investigación en Sanidad Animal-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain

Screening Peptides Pools Against ASFV Immune Lymphocytes by IFNg ELIspot

SLA^{dd} (DD) inbred pigs,
SLA^{cc} CC inbred pigs

OUR T88/3

OUR T88/1

Georgia 2007/1

Babraham
SLA^{dd} (DD) inbred pigs

OUR T88/3

OUR T88/1

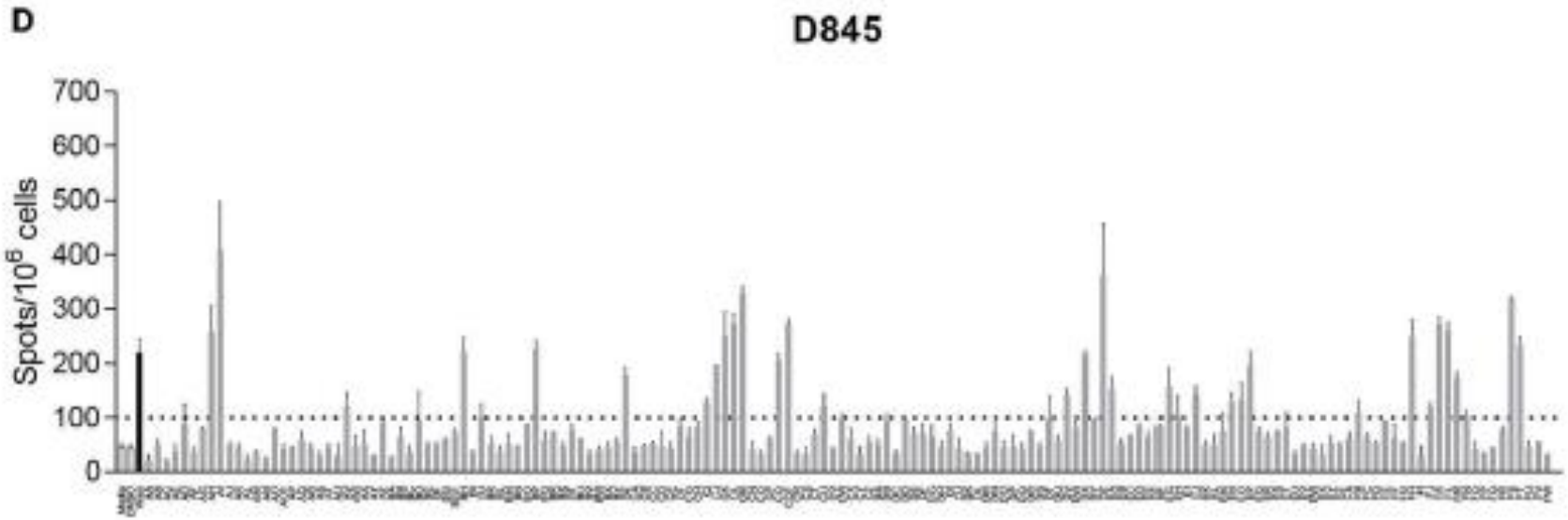
Splenocytes

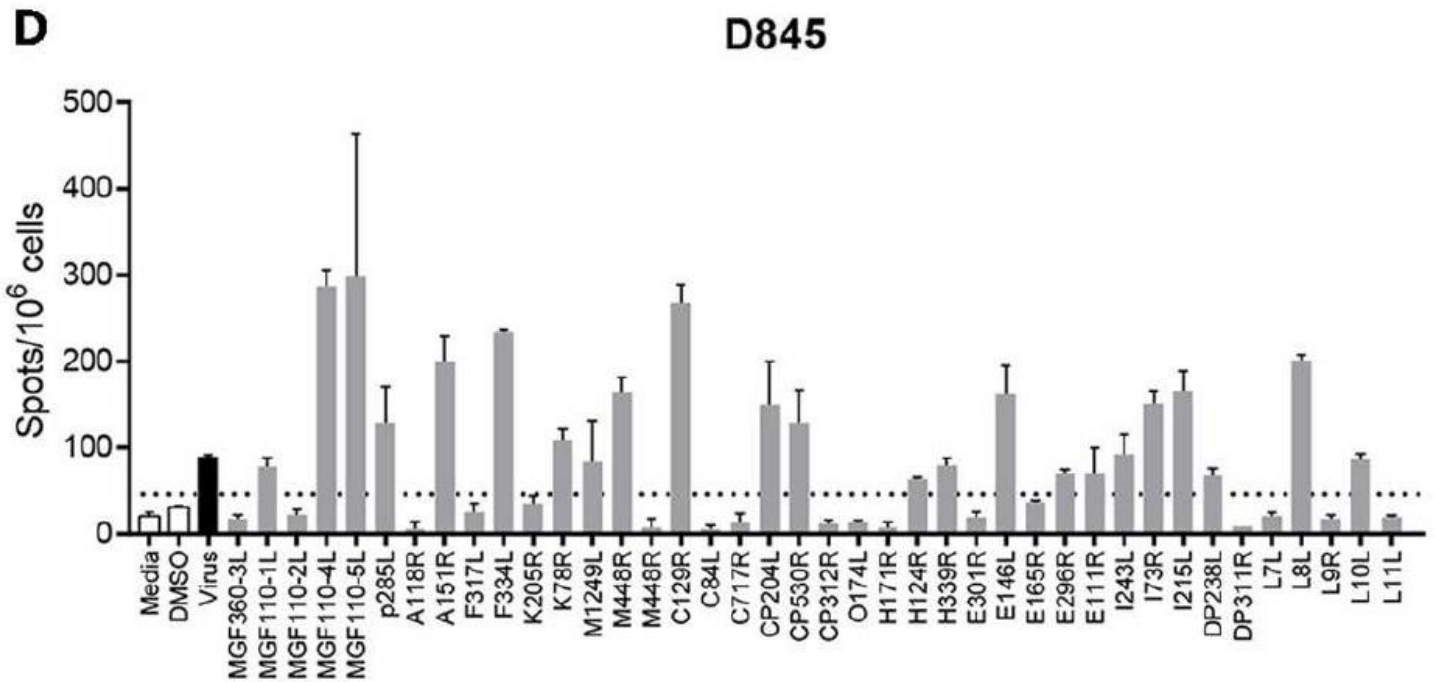
3,647 peptides in 156 pools corresponding to 129 ORFs

IFNg ELISpot response

161 peptides in 9 pools corresponding to another 4 ORFs

Netherton et al., 2019





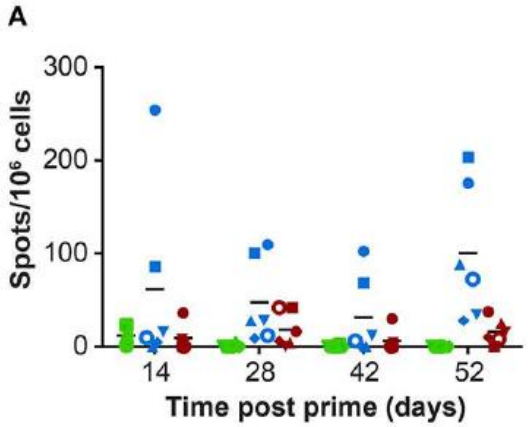
Netherton et al., 2019

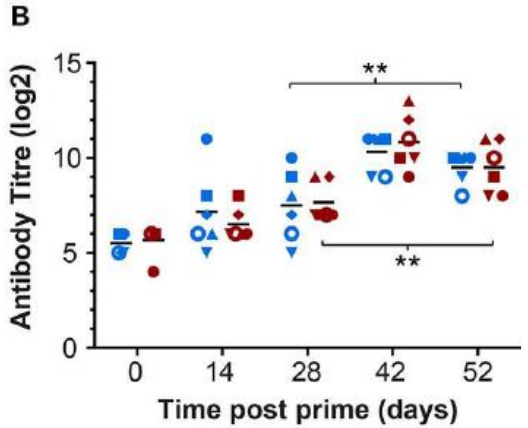
TABLE 1 | Pools of peptides that induced a significant IFNγ response in lymphocytes from at least one ASFV-immune pig.

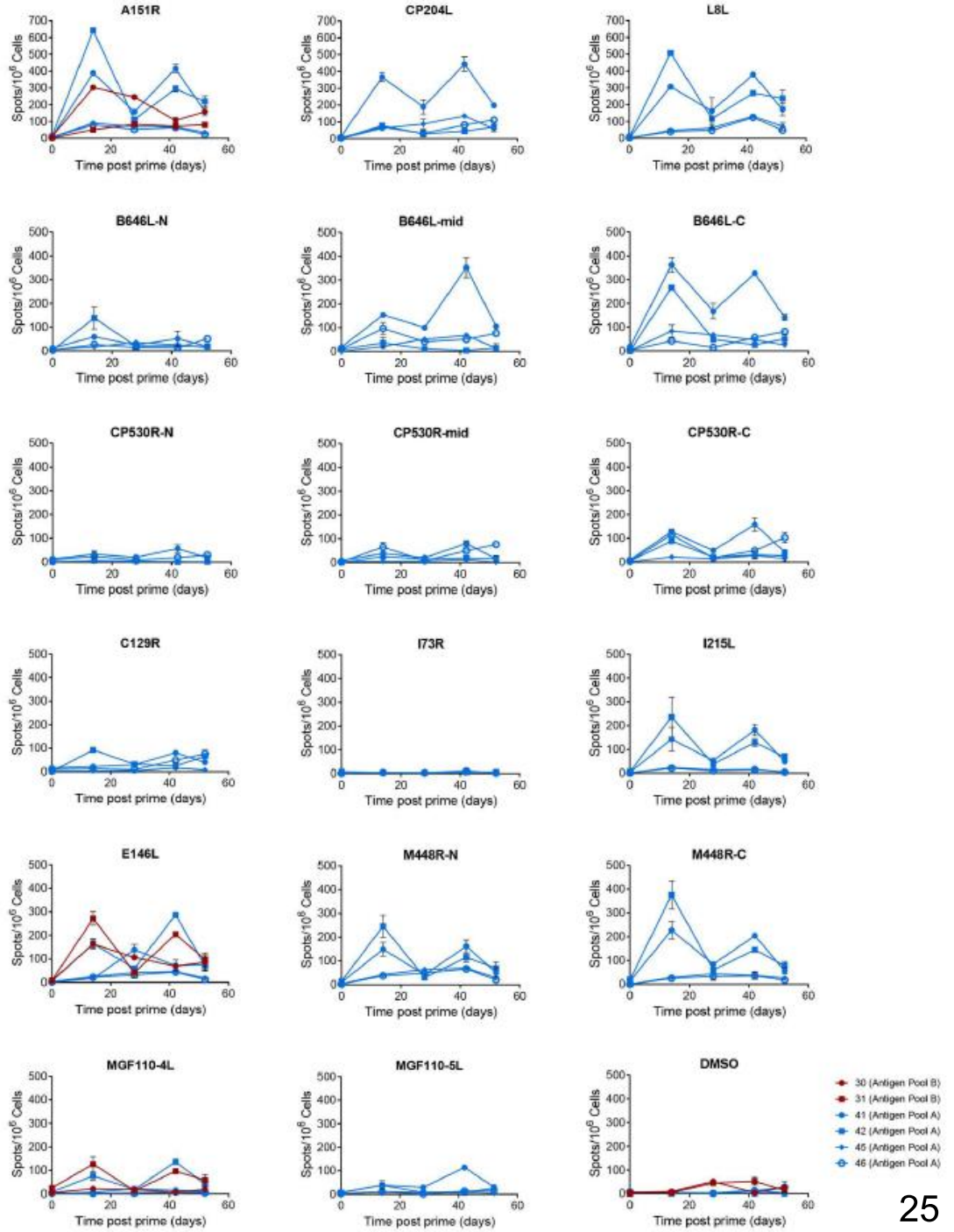
	DD Minipig[a]		CC Minipig[b]		Babraham[c]		Total	
Gene Name	**# pigs**	**%**	**# pigs**	**%**	**# pigs**	**%**	**# pigs**	**%**
KP177R	0	0	0	0	1	50	1	10
MGF110-1L	0	0	0	0	2	100	2	20
MGF110-4L	5	100	0	0	2	100	7	70
MGF110-5L	4	80	0	0	0	0	4	40
MGF300-1L	1	20	0	0	0	0	1	10
285L	1	20	0	0	0	0	1	10
A151R	5	100	0	0	1	50	6	60
F334L	3	60	0	0	0	0	3	30
K78R	0	0	1	33.3	2	100	3	30
K205R	0	0	2	66.7	0	0	2	20
M1249L	4	80	0	0	0	0	4	40
M448R	5	100	0	0	0	0	5	50
C257L	2	40	0	0	0	0	2	20
C475L	1	20	0	0	2	100	3	30
C129R	4	80	0	0	0	0	4	40
C962R	1	20	0	0	0	0	1	10
B646L*	4	100	NT	NT	0	0	4	66.7
CP204L	5	100	1	33.3	2	100	8	80
CP530R	4	80	3	100	2	100	9	90
CP312R	4	80	2	66.7	2	100	8	80
O174L	1	20	0	0	0	0	1	10
NP419L	1	20	0	0	0	0	1	10
NP868R	0	0	1	33.3	0	0	1	10
H359L	0	0	0	0	2	100	2	20
H339R	1	20	0	0	2	100	3	30
E146L	5	100	0	0	0	0	5	50
E184L	0	0	0	0	2	100	2	20
E165R	0	0	0	0	2	100	2	20
E296R	2	40	0	0	0	0	2	20
E248R	0	0	0	0	1	50	1	10
I243L	1	20	0	0	0	0	1	10
I73R	5	100	3	100	2	100	10	100
I215L	5	100	0	0	0	0	5	50
DP238L	1	20	0	0	1	50	2	20
MGF360-16R	1	20	0	0	0	0	1	10
MGF505-11L	0	0	0	0	2	100	2	20
L8L	5	100	0	0	0	0	5	50
L10L	1	20	0	0	2	100	3	30

Recombinant replication deficient adenoviruses (rAd) and modified vaccinia Ankara (MVA) expressing codon-optimized ASFV genes expressing each of the 18 selected ORFs were generated.

Netherton et al., 2019







The vaccine did not protect animals from severe disease but did reduce viremia following ASFV challenge.

Did not identify a consistent correlation between the number of interferon (IFN)γ-secreting cells and reduced viremia

Netherton et al., 2019

vaccines

MDPI

Article

A Pool of Eight Virally Vectored African Swine Fever Antigens Protect Pigs against Fatal Disease

Lynnette C. Goatley [1], Ana Luisa Reis [1], Raquel Portugal [1], Hannah Goldswain [1], Gareth L. Shimmon [1], Zoe Hargreaves [1], Chak-Sum Ho [2], María Montoya [1,†], Pedro J. Sánchez-Cordón [1,‡], Geraldine Taylor [1], Linda K. Dixon [1] and Christopher L. Netherton [1,*]

[1] The Pirbright Institute, Ash Road, Pirbright, Surrey GU24 0NF, UK; lynnette.goatley@pirbright.ac.uk (L.C.G.); ana.reis@pirbright.ac.uk (A.L.R.); raquel.portugal@pirbright.ac.uk (R.P.); hannah.gold@outlook.com (H.G.); gareth.shimmon@gmail.com (G.L.S.); zoe.hargreaves@pirbright.ac.uk (Z.H.); maria.montoya@cib.csic.es (M.M.); Pedro.Sanchez-Cordon@apha.gov.uk (P.J.S.-C.); geraldine.taylor@pirbright.ac.uk (G.T.); linda.dixon@pirbright.ac.uk (L.K.D.)

[2] Gift of Hope Organ and Tissue Donor Network, Itasca, IL 60143, USA; sho@giftofhope.org

* Correspondence: christopher.netherton@pirbright.ac.uk; Tel.: +44-(0)-1483-232-441

† Present address: Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain.

‡ Present address: Present address: Animal and Plant Health Agency, Addlestone KT15 3NB, UK.

Goatley et al., 2020

Female Landrace Large white pigs

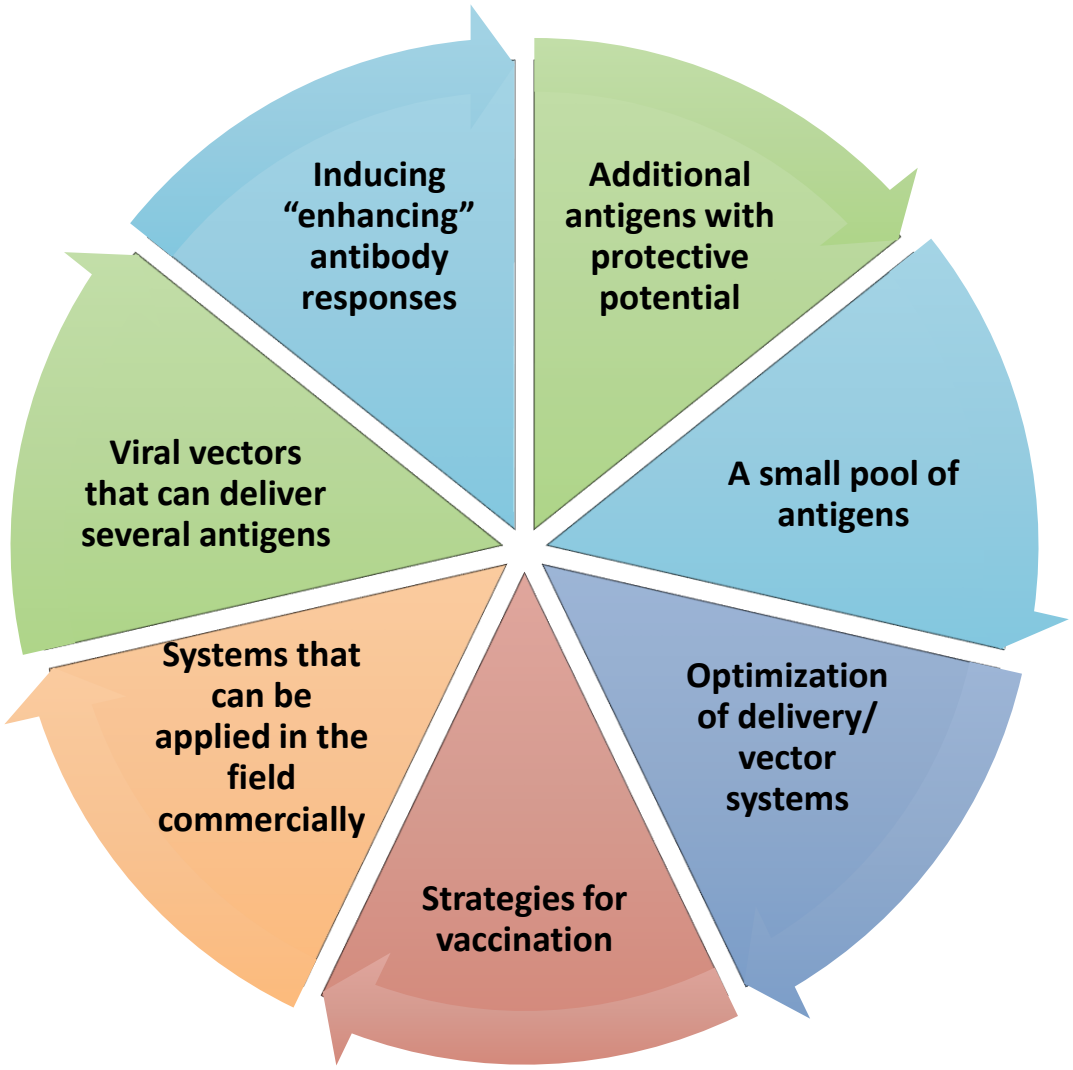
Table 1. Composition of Antigen Pools. The individual HA-tagged ASFV genes in each pool and the dose of each individual vector used in the prime and/or boost are indicated. Genes highlighted in bold were administered by rAd prime and MVA boost whereas those shown in italics by rAd prime and rAd boost.

Experiment	**1**		**2**	
Antigen Pool	**A**	**B**	**A**	**C**
Genes	*B602L* *B646L* *CP204L* *E183L* *E199L* *EP153R* *F317L* *MGF505-5R*	*B602L* *EP153R* *EP364R* *F317L* *I329L* *MGF360-11L* *MGF505-4R* *MGF505-5R*	*B602L* *B646L* *CP204L* *E183L* *E199L* *EP153R* *F317L* *MGF505-5R*	*B646L* *CP204L* *E199L* *F317L* *MGF505-5R*
rAd Dose (IU)	5×10^9	5×10^9	1.5×10^{10}	1.5×10^{10}
MVA Dose (pfu)	7.5×10^7	7.5×10^7	2×10^8	2×10^8

There was a no detectable correlate(s) of immune protection

Goatley et al., 2020

How to improve subunit ASF vaccine



4

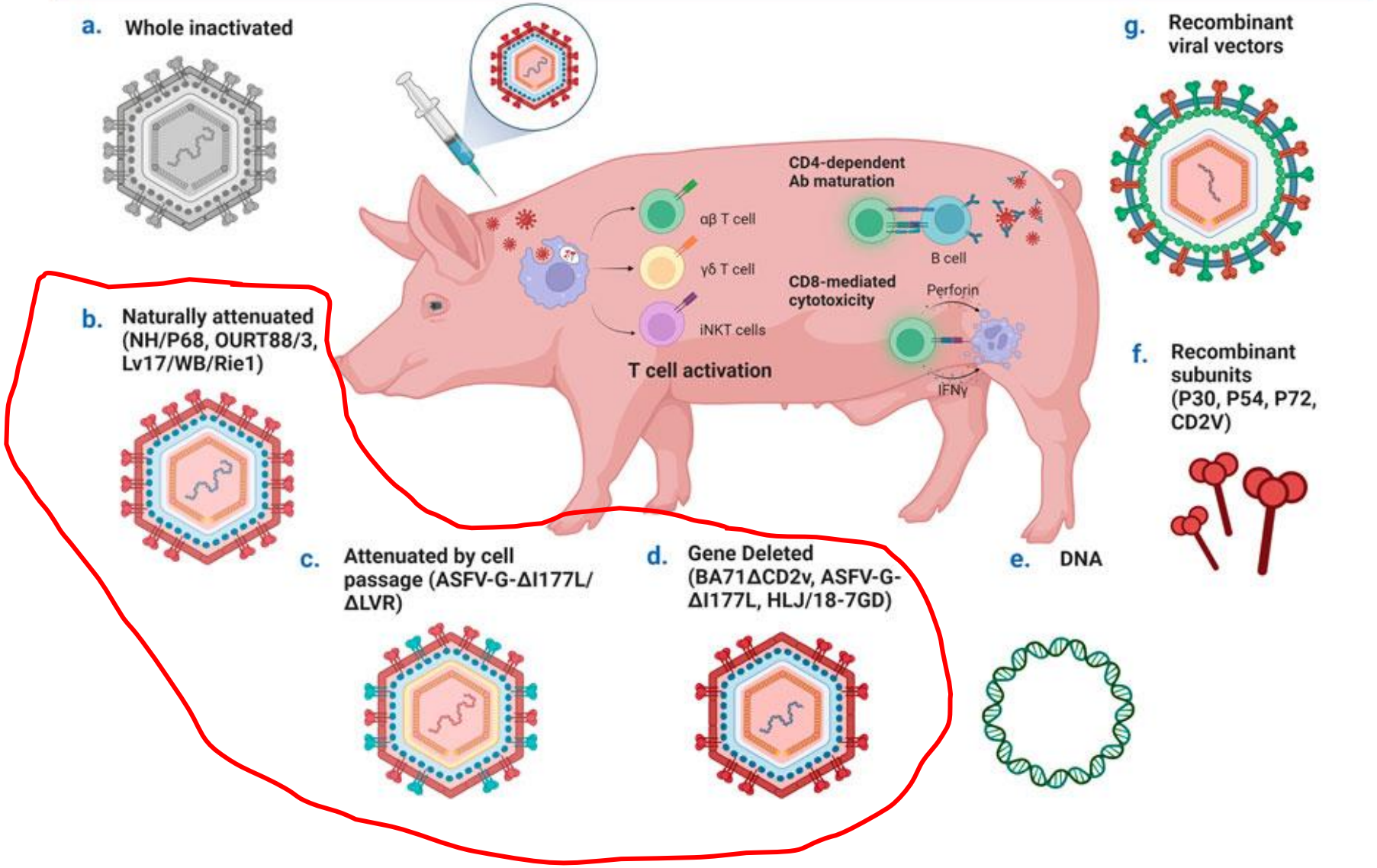
Live attenuated vaccines (LAVs)

Live attenuated vaccines (LAVs)

- LAVs can successfully replicate within the host
- Mimic natural infection thereby triggering both humoral and cellular pathways
- Do not require adjuvants with co-stimulatory activity to enhance the magnitude and quality of the immune response
- Elicit mucosal IgA antibodies

- May regain pathogenicity, causing the spread of disease
- They have the potential to cause post-vaccination reactions and side effects

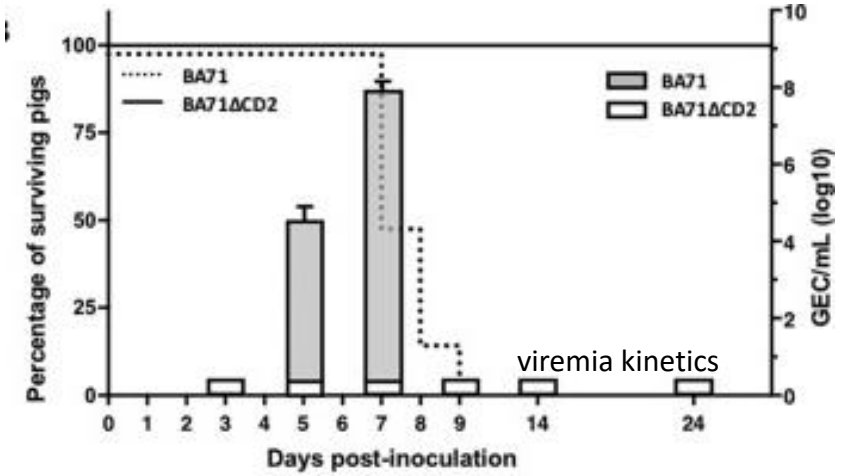
Approaches to **ASFV Vaccine Development**

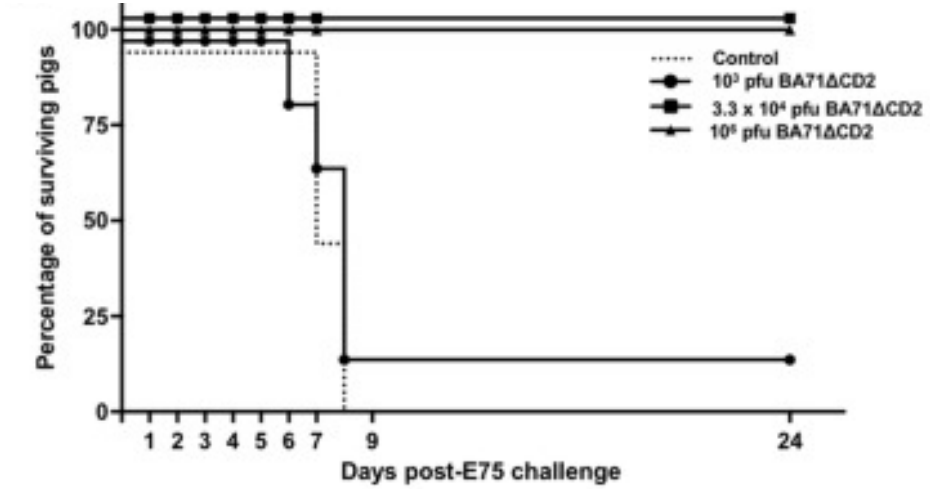


Gene deleted vaccines	SY18ΔL7-11	L7L, L8L, L9R, L10L, L11L	Homologous (SY18)	100%
	ASFV-G-Δ I177L	I177L	Homologous (Georgia 2010)	100%
	HLJ/18-7GD	MGF360, MGF505, EP402R (CD2)	Homologous (HLJ/18)	100%
	ASFV-G-Δ9GL	B119L (9GL)	Homologous (ASFV-G)	100%
	ASFV-G-Δ9GL/ΔCD2v	B119L (9GL), EP402R (CD2)	Homologous (ASFV-G)	0%
	ASFV-G-Δ9GL/ΔCD2v/ΔEP153R	B119L (9GL), EP402R (CD2), EP153R	Homologous (ASFV-G)	0%
	BA71ΔCD2	EP402R (CD2)	Heterologous (RSA/11/2017, Ken06.Bus)	17-83%
	BA71ΔCD2 + BA71 boost	EP402R (CD2)	Heterologous (Ken06.Bus)	100%
	OURT88/3ΔI329L	I329L	Homologous (OURT88/1)	33%
	BeninΔMGF	MGF 360, MGF 505, MGF 530	Homologous (Benin 97/1)	0%
	ASFV-GΔ9GL/ΔNL/ΔUK	B119L (9GL), DP71L (NL), DP96R (UK)	Homologous (ASFV-G)	0%
	NH/P68	-	Homologous (L60) followed by heterologous (Arm07)	100%
	NH/P68ΔA238L	A238L		0%
	NH/P68ΔA224L	A224L		50%
	NH/P68ΔEP153R	EP153R		0%
	NH/P68	-	Heterologous (Arm07)	33-100%
	NH/P68ΔA238L	A238L		40%
	NH/P68ΔA276R	A276R		0%
	BeninΔMGF	MGF 360, MGF 505, MGF 530	Homologous (Benin 97/1)	50-83%
	BA71ΔCD2	EP402R (CD2)	Homologous (BA71) and heterologous (E75, Georgia 2007/1)	17-100%
	ASFV-G-Δ9GL/ΔUK	B119L (9GL), DP96R (UK)	Homologous (Georgia 2007)	20-100%
	BeninΔDP148R	DP148R	Homologous (Benin 97/1)	83-100%
	PretΔ9GL	B119L (9GL)	Homologous (Pretoriuskop/96/4)	40-80%
	ASFV-G-Δ9GL/MGF	B119L (9GL), MGF 360, MGF 5050	Homologous (Georgia 2007)	0%
	BeninΔMGF	MGF 360, MGF 505, MGF 530	Homologous (Benin 97/1)	100%
	ASFV-G/V-ΔTK	TK	Homologous (ASFV-G)	0%
	ASFV-G-ΔMGF	MGF 360, MGF 5050	Homologous (Georgia 2007)	100%
	ASFV-G-Δ9GLv	B119L (9GL)	Homologous (Georgia 2007)	40-100%
	OURT88/3ΔDP2	DP2	Homologous (OURT88/1)	67%
	MALΔ9GL	B119L (9GL)	Homologous (Malawi)	100%

Deletion of the CD2v/EP402R gene (also known as 8DR) from **the genotype I BA71 isolate**

- attenuated the virus
- BA71ΔCD2v strain conferred protection against challenge with homologous and heterologous virulent viruses

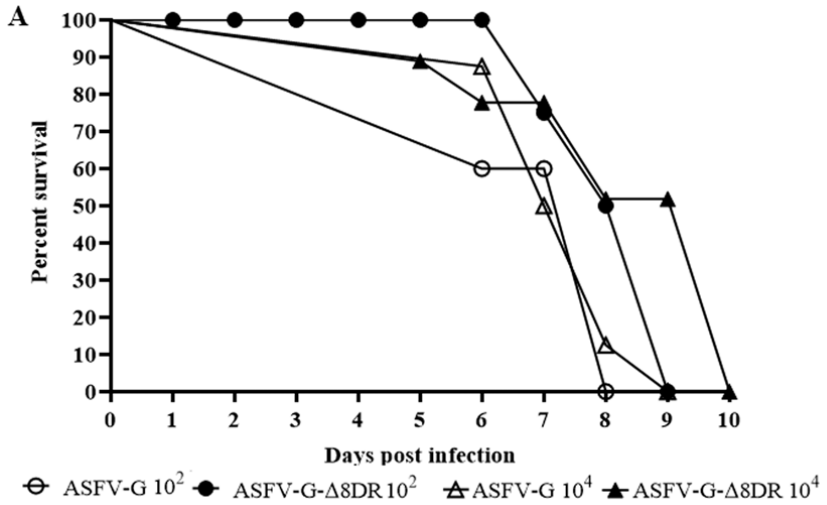




Monteagudo et al., 2017

The genotype II Georgia2010 isolate, ASFV-G-Δ8DR

- did not significantly alter the virulence of the virus
- produced clinical disease indistinguishable from that induced by the virulent parental strain



Borca et al., 2020

LAV candidates

Genes	Strains	Genotype	Minimal Protective Dose	Route	Challenge	Gene Function	References
A137R	Georgia2007/1	II	102HAD50	IM	Georgia2007/1	Unknown	[152]
I226R	SY18	II	102HAD50	IM	Georgia2007/1	Unknown	[270]
L7L-L11L	SY18	II	103HAD50	IM	SY18	Unknown	[271]
MGF505/360 and EP402R	HLJ/18	II	103HAD50 105HAD50	IM ON	HLJ/18	Hemadsorbing and inhibition of type I	[242]
EP402R	Ba71V	I	104HAD50	IM	Ba71V E75 Georgia2007/1	Interferon responses Hemadsorbing	[13]
I177L	Georgia2007/1	II	102HAD50 106HAD50	IM ON	Georgia2007/1 Georgia2007/1	Unknown	[15,16]

More safety and cross-protective ability tests are needed for the commercial vaccine production of LAVs because ASFV consists of duplications, mutations or deletions of certain sequences in the genome of different genotypes, which may lead to changes in virulence.

Li et al., 2022

ASF-LAV: Challenges

- **The absence of international standard guidelines for the assessment of ASF vaccine purity, potency, safety, and efficacy.**

- **Safety evaluation**
  - Long studies with many animals to assess the degree and stability of attenuation
  - Assays be implemented that distinguish attenuated from both fully virulent and partially virulent strains to assess reversion

- **An environmental risk assessment**
  - The possibility of shedding of vaccine organisms following administration

Urbano and Ferreira, 2022

ASF-LAV: Challenges

- Experiments with pigs require strict biosafety level 3 (BSL3) biocontainment laboratories, tend to be extremely expensive, and are also environmentally and ethically difficult

- Increasing biosafety tests for ASF LAV prototypes should be mandatory

Urbano and Ferreira, 2022

ASF-LAV: Challenges

- Detection of new ASF viruses that may arise from LAV vaccine strain and naturally circulating wild-type virus recombination

- A DIVA strategy is essential.
  - A multiplex real-time PCR targeting both wild-type ASFV strain and the deleted gene(s) of the LAV
  - The detection of antibodies induced by the deleted gene-encoded protein by enzyme-linked immunosorbent assay (ELISA)

Urbano and Ferreira, 2022

Directions for ASF LAV

- Vaccine registration
  - safety and immunogenicity ; a range of doses safety of repeated administration and overdose
- Genetic stability of LAVs during culture in vitro and pig passage in vivo
- The selection of targeted virulence genes to be deleted
- The availability of a licensed cell line to grow the LAVs for vaccine production
- Lack of recombination in vaccination experiments with wild type challenge virus
- Establishment of a pipeline for evaluation of LAV candidates
  - Selection at the stage of cell culture
  - Identifying correlates of in vitro pathogenesis
  - Ability to induce protection

Arias et al., 2017

Further research to improve ASF vaccines

Virus biology and virus–host interactions at all levels

A better knowledge of the **virus structural proteins**, particularly those on the virion surface

Better knowledge of **virus entry mechanisms, including the cell receptor(s) on pig macrophages**

The functions of **ASFV proteins**, in particular those **that inhibit the host's defenses**

Preliminary in vitro tests are absolutely necessary

Identification of the correlates of immune protection in vitro as well as in vivo

Mechanisms by which the natural hosts, including bushpig and warthog, resist ASFV disease

Oral vaccine for wild boar populations

ASF vaccine candidates

Short term

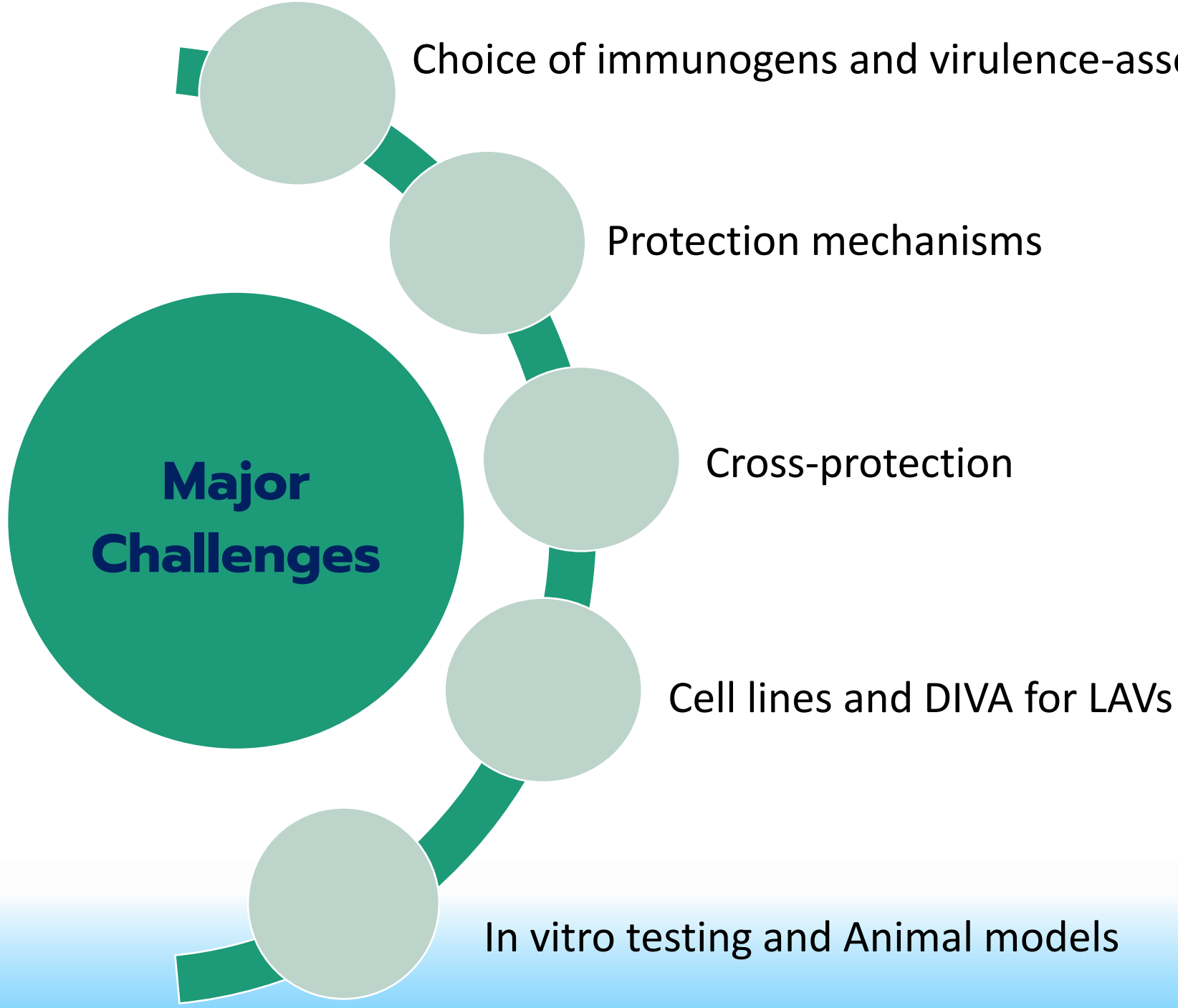
LAVs

Medium term

LAVs with the increased safety

Long term

Subunit


Major Challenges
Choice of immunogens and virulence-associated genes
Protection mechanisms
Cross-protection
Cell lines and DIVA for LAVs
In vitro testing and Animal models

Last but not least..

- A relatively small number of government and academic laboratories with top tier ASF vaccine expertise.

- Global funding levels for ASF vaccine research and development have been historical relatively low and intermittent.

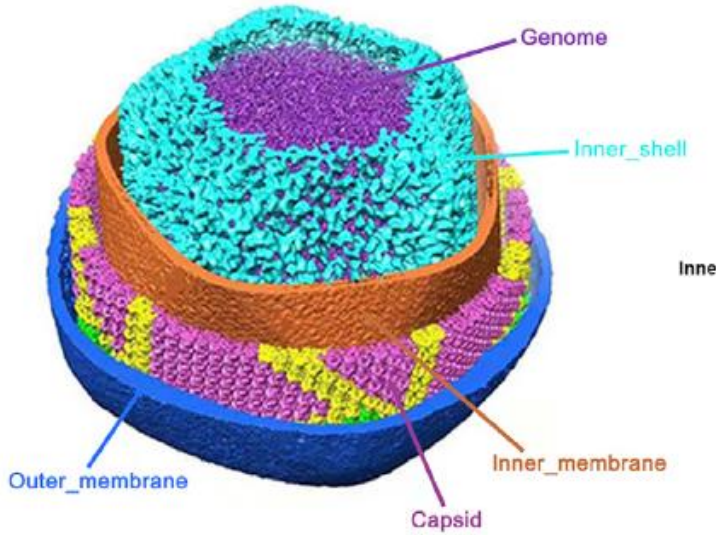
Research is a key!

ASF R**&D** in Thailand?

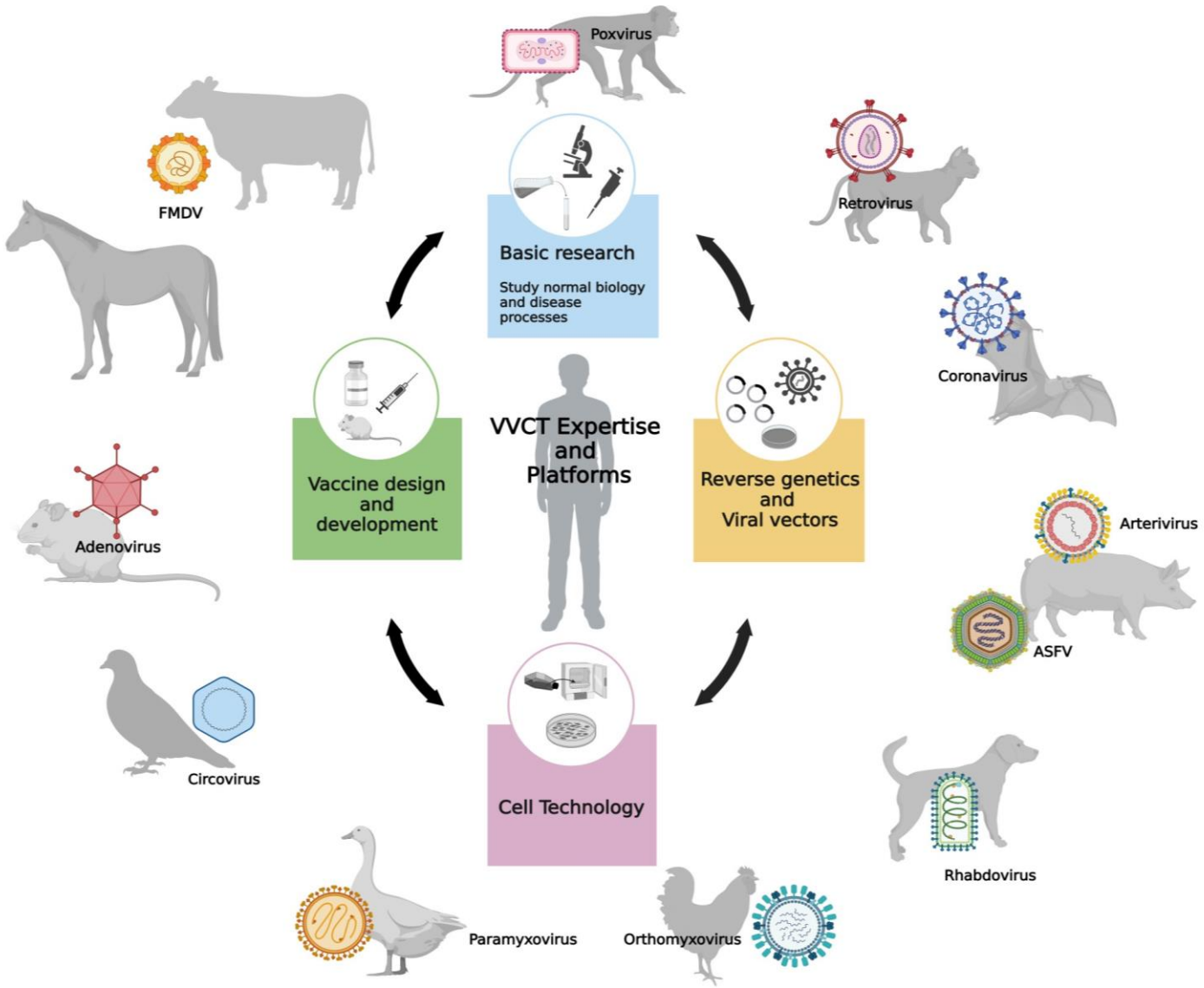
AVCT
VIROLOGY & CELL TECHNOLOGY LAB

Knowledge
&
Experience
&
Creativity

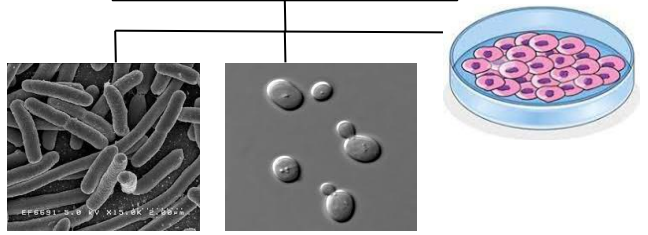
VS



AVCT
VIROLOGY & CELL TECHNOLOGY LAB

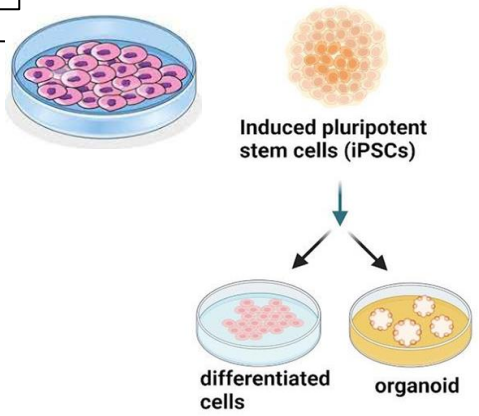


Recombinant protein production platforms

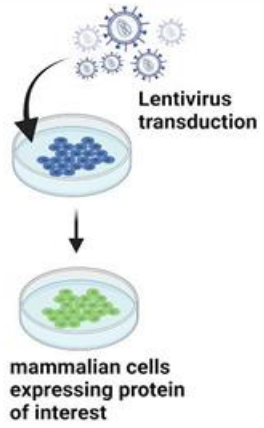


ELISA

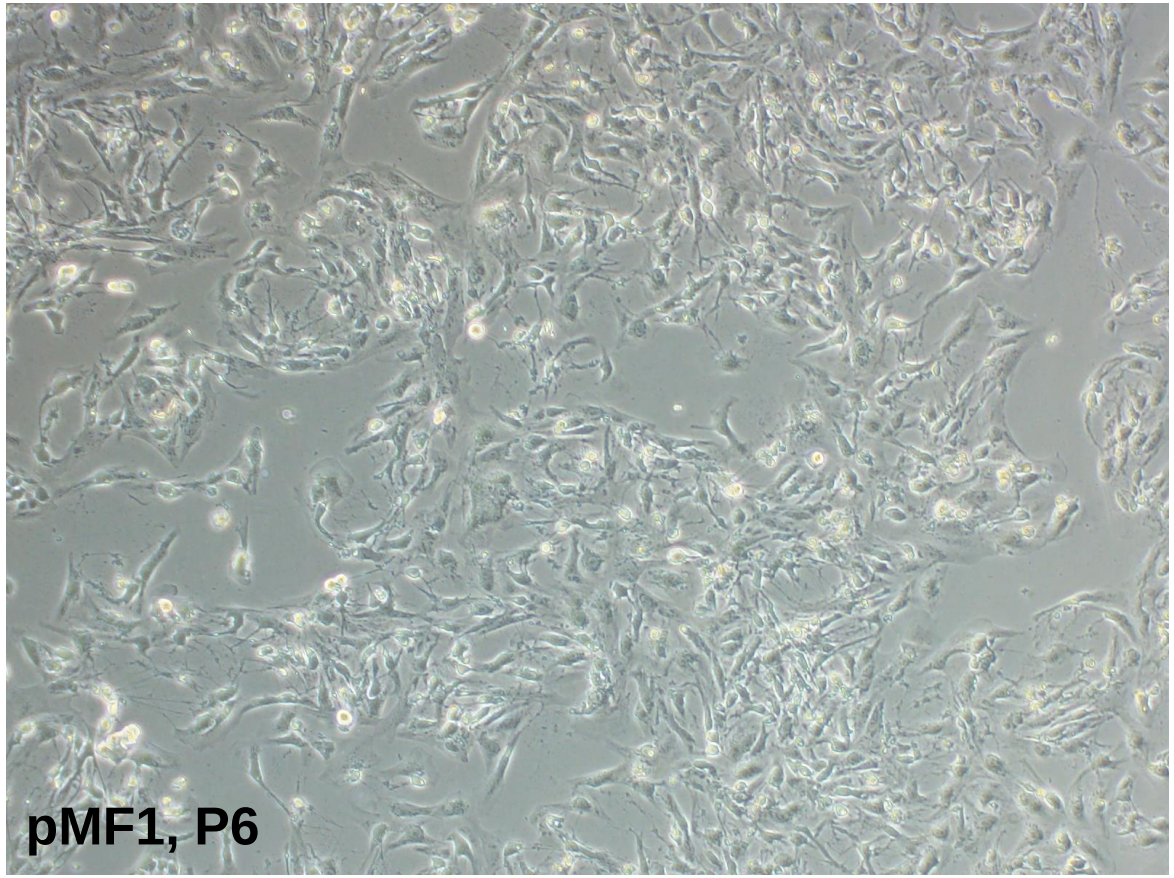
Stem cell technology for disease models

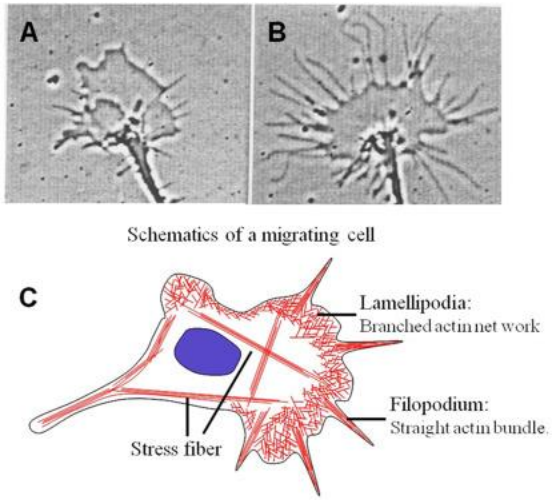


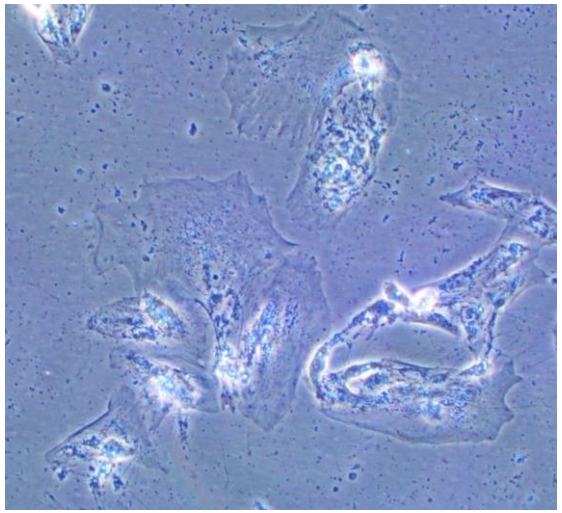
Genetic modification of cell lines

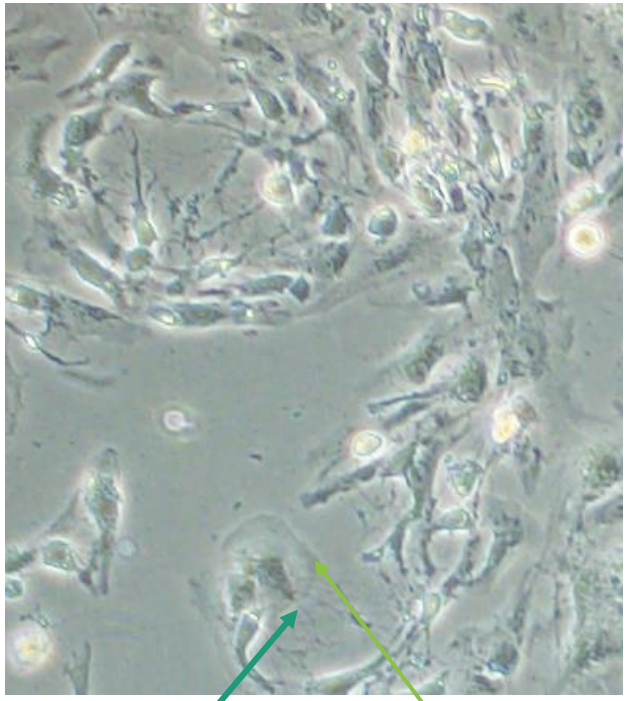


Features of porcine macrophage (pMF) cells



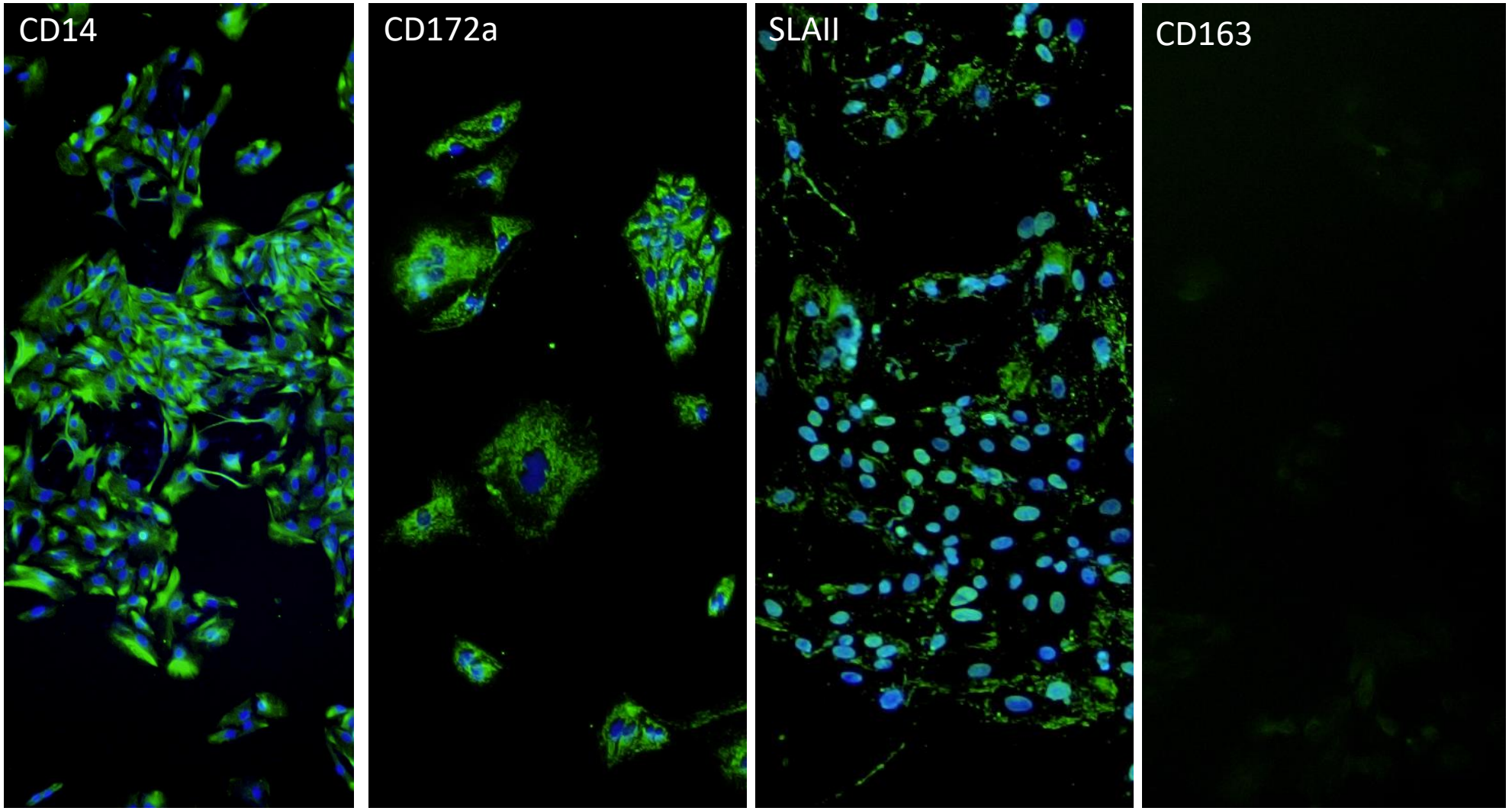




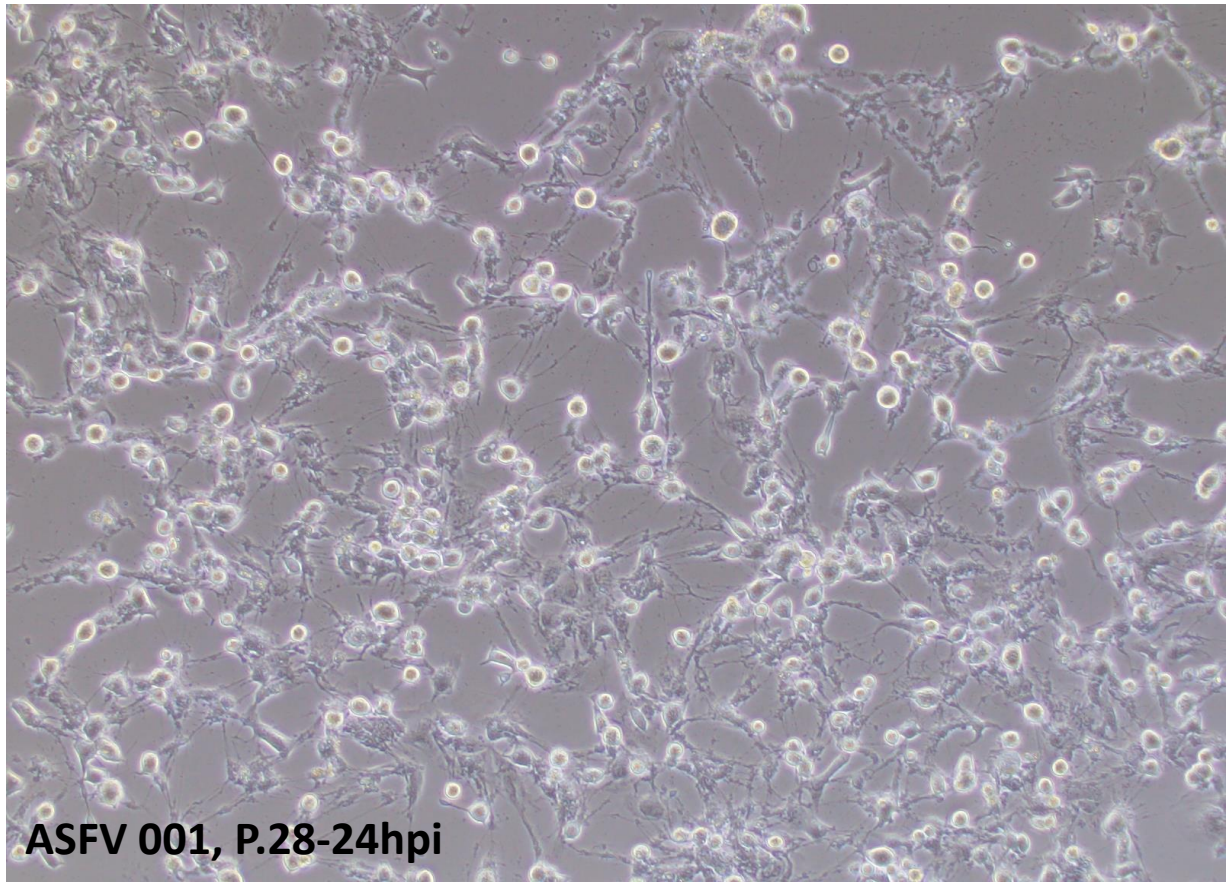


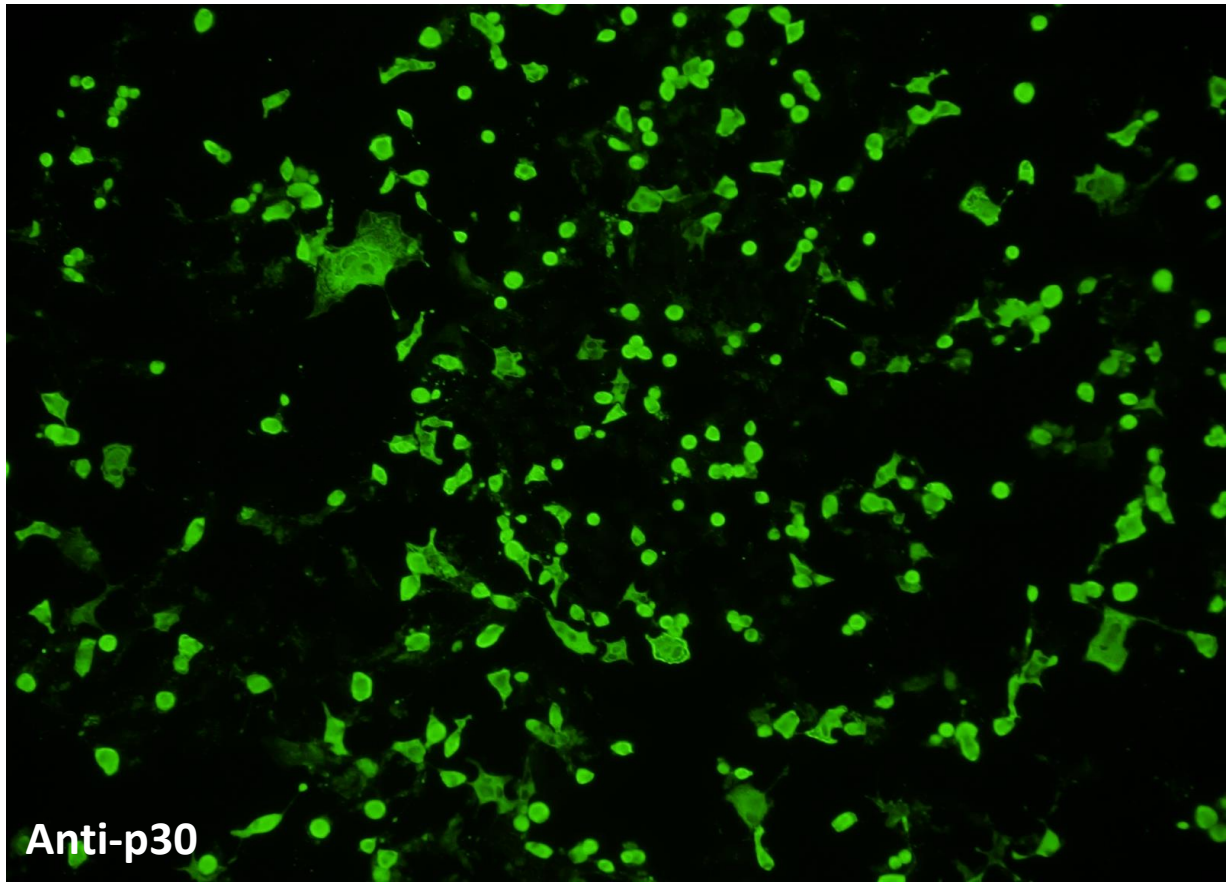
filipodia and lamellipodia

Features of porcine macrophage (pMF) cells



ASFV infection in pMF





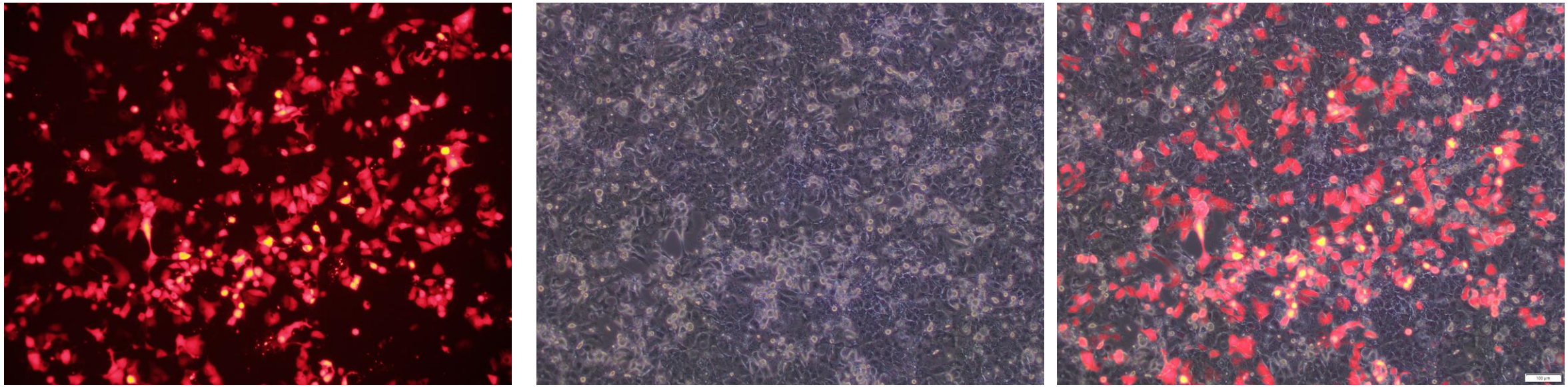
Applications

Support virus replication

- Virus isolation
- Wild-type ASFV propagation (to avoid virus adaptation)
- Virus-host interaction
  - How the virus interacts with macrophages as to identify the protective mechanisms and how the virus evades them.
  - Identify viral receptors and ligands (identification, how virus interacts).
  - Identify viral attachment proteins.

A macrophage based - ADCC assay

Recombinant ASFV001 expressing mCherry generated by homologous recombination technique



LAVs

Virus-host interaction

Inhibition assay
(Antivirals, Immune response)

Development of attenuated ASFV that can be classified as BSL2 agent.

Acknowledgment

ห้องปฏิบัติการไวรัสวิทยาและเซลล์เทคโนโลยี
ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ (BIOTEC)
สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ สวทช.

50

สวทช NSTDA

NAC2023
18th NSTDA Annual Conference
การประชุมวิชาการประจำปี สวทช. ครั้งที่ ๑๘

Thank you

28-31
มีนาคม 2566