ASFV Vaccine Overview in 2023 Dr. Douglas Gladue

### Live attenuated vaccines (LAV) are effective preventing homologous challenge Low virulence field isolate based vaccines

Comparative Study > Am J Vet Res. 1980 Nov;41(11):1867-9.

Western hemisphere isolates of African swine fever virus: asymptomatic carriers and resistance to challenge inoculation

C A Mebus, A H Dardiri



Virology Volume 198, Issue 1, January 1994, Pages 350-354



Short Communications

Passively Transferred African Swine Fever Virus Antibodies Protect Swine against Lethal Infection

D.V. Onisk, M.V. Borca, S. Kutish, E. Kramer, P. Irusta, D.L. Rock

A Vaccine for ASFV is not a new idea 1980: Asymptomatic carriers resistance to homologous challenge

1994: Passive transferred antibodies to protect against ASFV



# Global African Swine Fever Research Alliance

https://www.ars.usda.gov/GARA/





#### <sup>5th</sup> GARA Scientific Conference, Punta Cana, Dominican Republic May 2022

Webinar: ASFV vaccines May 2021 : <u>GARA Scientific Communications - YouTube</u> Webinar : ASFV Genomic Sequencing: <u>GARA Scientific Communications - YouTube</u>



## **ASFV Vaccine Gaps:**

- Protective immunity from viral proteins unknown
- Hard to make subunit vaccine
  - Any attempts have failed or give only a modest protection rate
  - Successful subunit vaccines have been to low-virulence strains
  - Required large doses commercially unviable
- Inactivation of ASFV does not offer protection even at high doses
  - Inactivated vaccine not possible
- Live Attenuated Vaccine only current option



## **GARA Scientific Conference Manila**



Update Vaccine Gaps and Concerns Specifically for ASIA

Live attenuated vaccines (LAV) are effective preventing homologous challenge

## Attenuated isolates were produced by:

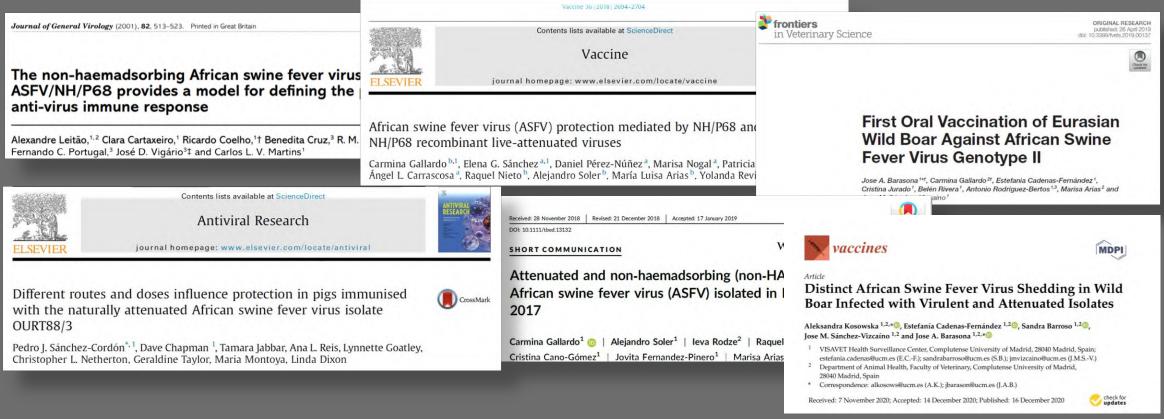
• Low virulence field isolates

• Viruses attenuated by tissue culture passages

• Viruses with genetically engineered deletions



### Live attenuated vaccines (LAV) are effective preventing homologous challenge Low virulence field isolate based vaccines



All Low virulent field isolates retain residual virulence Vaccines based on low virulent field isolates Need further attenuation / safety measures



Live attenuated vaccines (LAV) are effective preventing homologous challenge

## Attenuated isolates were produced by:

Low virulence field isolates

• Viruses attenuated by tissue culture passages

• Viruses with genetically engineered deletions



### Live attenuated vaccines (LAV) are effective preventing homologous challenge ASFV attenuated by cell culture passage



Journal of Virology

February 2015 Volume 89 Number 4

# The Progressive Adaptation of a Georgian Isolate of African Swine

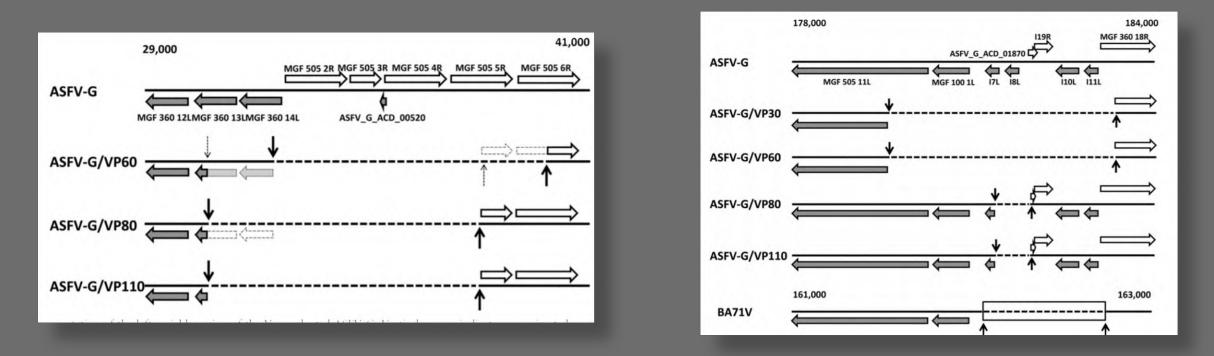
#### Fever Virus to Vero Cells Leads to a Gradual Attenuation of Virulence in Swine Corresponding to Major Modifications of the Viral Genome

#### Peter W. Krug,<sup>a</sup> Lauren G. Holinka,<sup>a</sup> Vivian O'Donnell,<sup>a,b</sup> Bo Reese,<sup>c</sup> Brenton Sanford,<sup>a</sup> Ignacio Fernandez-Sainz,<sup>a,b</sup> Douglas P. Gladue,<sup>a,b</sup> Jonathan Arzt,<sup>a</sup> Luis Rodriguez,<sup>a</sup> Guillermo R. Risatti,<sup>b</sup> Manuel V. Borca<sup>a</sup>

Agricultural Research Service, U.S. Department of Agriculture, Plum Island Animal Disease Center, Greenport, New York, USA<sup>a</sup>; Department of Pathobiology and Veterinary Science, CANHR, University of Connecticut, Storrs, Connecticut, USA<sup>b</sup>; Center for Applied Genetics and Technology, University of Connecticut, Storrs, Connecticut, USA<sup>c</sup>

		Mean (SD)				
	No. of survivors/total	Time to death (days)	Fever			
Dose and virus			No. of days to onset	Duration (days)	Max daily temp (°F)	
10 <sup>2</sup> HAD <sub>50</sub>						
ASFV-G	0/7	8.4 (0.56)	6.14 (1.57)	2.88 (0. 69)	107.1 (0.59)	
ASFV-G/VP30	3/5	11.5 (0.7)	8.8 (1.3)	3.4 (0.89)	105.42 (1.25)	
ASFV-G/VP60	5/5				103.72 (0.12)	
ASFV-G/VP80	5/5				103.18 (0.86)	
ASFV-G/VP110	5/5				102.7 (0.22)	
10 <sup>4</sup> HAD <sub>50</sub>						
ASFV-G	0/5	7.32 (1.03)	3.67 (0.52)	3.67 (0.82)	107.4 (0.52)	
ASFV-G/VP30	0/5	8 (0.71)	3.6 (0.55)	4.4 (1.14)	106.5 (0.14)	
ASFV-G/VP60	0/5	8.75 (0.5)	4 (0.0)	4.25 (0.5)	107.15 (0.34)	
ASFV-G/VP80	0/5	9.5 (0.5)	7 (0.25)	2.25 (0.5)	105.12 (1.06)	
ASFV-G/VP110	5/5				102.4 (0.17)	





Virus adaptation to grow in cell lines implies dramatic genomic changes that result in the inability for the attenuated virus to replicate in swine ( not vaccines)



### Live attenuated vaccines (LAV) are effective preventing homologous challenge ASFV attenuated by cell culture passage

MDPI

### 👹 viruses

#### Article

A Cell-Adapted Live-Attenuated Vaccine Candidate Protects Pigs against the Homologous Strain VNUA-ASFV-05L1, a Representative Strain of the Contemporary Pandemic African Swine Fever Virus

Quang Lam Truong <sup>1,\*,†</sup>, Lihua Wang <sup>2,†</sup><sup>(0)</sup>, Tuan Anh Nguyen <sup>1</sup>, Hoa Thi Nguyen <sup>1</sup>, Son Danh Tran <sup>1</sup>, Anh Thi Vu <sup>1</sup>, Anh Dao Le <sup>1</sup>, Van Giap Nguyen <sup>3</sup><sup>(0)</sup>, Phuong Thi Hoang <sup>1</sup>, Yen Thi Nguyen <sup>1</sup>, Thi Luyen Le <sup>1</sup>, Thang Nguyen Van <sup>1</sup>, Thi My Le Huynh <sup>3</sup>, Huong Thi Lan Lai <sup>1</sup><sup>(0)</sup>, Rachel Madera <sup>2</sup>, Yuzhen Li <sup>2</sup>, Jishu Shi <sup>2,\*</sup><sup>(0)</sup> and Lan Thi Nguyen <sup>1,\*</sup>



### MDPI

#### Article

African Swine Fever Vaccine Candidate ASFV-G-∆I177L Produced in the Swine Macrophage-Derived Cell Line IPKM Remains Genetically Stable and Protective against Homologous Virulent Challenge

Manuel V. Borca <sup>1,2,\*</sup>, Ayushi Rai <sup>1,3</sup>, Nallely Espinoza <sup>1,2</sup>, Elizabeth Ramirez-Medina <sup>1,2</sup>, Edward Spinard <sup>1,2</sup>, Lauro Velazquez-Salinas <sup>1,2</sup>, Alyssa Valladares <sup>1,3</sup>, Ediane Silva <sup>2</sup>, Leeanna Burton <sup>2</sup>, Amanda Meyers <sup>1,3</sup>, Cyril G. Gay <sup>4</sup> and Douglas P. Gladue <sup>1,2,\*</sup>

IPKM is first cell line to not induce changes in ASFV Good for existing vaccine canidates Live attenuated vaccines (LAV) are effective preventing homologous challenge

## Attenuated isolates were produced by:

## Low virulence field isolates

Viruses attenuated by tissue culture passages

## • Viruses with genetically engineered deletions

#### 🐲 viruses

Revieu

**Recombinant ASF Live Attenuated Virus Strains as Experimental Vaccine Candidates** 

Douglas P. Gladue \* @ and Manuel V. Borca \*



## African swine fever virus genome

	Contents lists available at ScienceDirect	
- AL	Virus Research	RESEARCH
ELSEVIER	journal homepage: www.elsevier.com/locate/virusres	

African swine fever virus evasion of host defences

#### L.K. Dixon\*, M. Islam, R. Nash, A.L. Reis

A Gene Deleted	B Function	C Isolate	D Growth in cells	E Virulence in pigs	F Ref.
ONA repair pathway	, genome integrity and nucle	otide metabolism			(1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.
Q174L	DNA repair	BA71V (a)	Required for efficient	ND	(Redrejo-Rod riguez et al., 2013)
	polymerase X		macrophage growth		
E296R	AP endonuclease	BA71 V (a)	Required for macrophage growth	ND	(Redrejo-Rodriguez et al., 2006)
2165R	dUTPase	BA71 V (a)	Required for macrophage growth	ND	(Oliveros et al., 1999)
A240L	Thymidine kinase	Malawi (v) Haiti (v)	Required for macrophage growth	Attenuated Partial protection	(Sanford et al., 2016)
ype I Interferon Re	sponse				
MGF360	Type I Interferon	Benin 97/1(v)	No effect	Attenuated	(Reis et al., 2016)
MGF 505/530	Inhibitors			Good protection	
MGF360	Type I	Pr4 (v)	No effect	Attenuated	(Zsak et al., 2001)
MGF 505/530	Interferon Inhibitors			Good protection	
MGF360	Type I	Georgia (v)	No effect	Attenuated	(O'Donnell et al. (2015b))
MGF 505/530	Interferon Inhibitors			Good protection	
DP96R(UK)	IFN inhibitor	Malawi (v)	No effect	Attenuated Induced protection	(Zsak et al., 1998)
EP402R/CD2v	Binding to red blood cells	BA71 (v)	No effect	Attenuated Good protection	(Monteagudo et al., 2017)
EP402R/CD2v/8-DR	Binding to red blood cells	Malawi LIL20/ 1(v)	No effect	Delay in clinical signs	(Borca et al., 1998)
B119L(9GL)	morphogenesis	Malawi (v)	Reduced replication	Attenuated Induced protection	(Lewis et al., 2000)
B119L(9GL)	morphogenesis	Georgia (v)	Reduced replication	At low doses attenuated and Induced protection	(O'Donnell et al. (2015c))
DP96R(UK) B119L (9GL)		Georgia (v)	Reduced replication	Attenuated Induced protection	(O'Donnell et al. (2017))
MGF 360 MGF 505/530 9 G L	IFN inhibitor morphogenesis	Georgia (v)	Reduced replication	Attenuated No protection	(O'Donnell et al. (2016))
1224 1/4CL	IAP apoptosis inhibitor	Malawi (v)	No effect	No reduction in virulence	(Neilan et al., 1997a; Reis et al., 2017)
0P71 L/NL		Malawi (v) Pr4 (v)			(Afonso et al., 1998a)
0P71 L/NL		E70 (v)		Attenuated Induced protection	(Afonso et al., 1998a)
831.	IL-1beta binding protein	Georgia (v)	No effect	No reduction in virulence	(Borca et al., 2018)
0P148R		Benin 97/1 (v)	No effect	Attenuated Induced protection	(Reis et al., 2017)
P153R	C-type lectin	Malawi (v)	No effect	No reduction in virulence	(Neilan et al., 1999)
2381.	Inhibitor of inflammatory responses	Malawi (v)	No effect	No reduction in virulence	(Neilan et al., 1997b)
111	Transmembrane	Malawi (v)	No effect	No reduction in virulence	(Kleibocker et al., 1998)

### In 2019

- Only 16 different genes have been deleted from any ASFV strain.
- 7 genes associated with virulence
  - 9GL, NL, UK, MGF, DP148R, TK, CD2
- 5 genes attenuated field isolates
- 9GL, NL, UK, MGF, DP148R

### Single gene deletions in ASFV that resulted in no phenotype

Gene	Isolate	Reference
A224L (4CL)	Malawi	[61]
A859L	Georgia	[62]
C962R	Georgia	[63]
CD2	Georgia	[30]
E165R	Ba71V	[64]
E296R	Ba71V	[65]
H240R	HLJ/2018	[66]
I8L	Georgia	[67]
KP117R (p22)	Georgia	[68]
L11L	Malawi	[69]
L83L	Georgia	[70]
MGF360 13L-14L	Georgia	[71]
MGF100-1R	GZ201801	[72]
MGF110-1L	Georgia	[73]
MGF360-16R	Georgia	[74]
MGF-360-1L	Georgia	[75]
Q174L	Ba71V	[76]
X69R	Georgia	[77]

Gladue & Borca. Viruses. 2022 Apr 23;14(5):878

Many genes can be deleted from ASFV without affecting

- Growth in cell cultures
- Virulence in swine
- Often indistinguishable from WT ASFV

# African swine fever virus virulence determinants

7 genes historically associated with virulence introduced into ASFV-G
9GL, NL, UK, MGF, DP148R, TK, CD2

Gene Deleted	<b>Fully Attenuated</b>	Protection
NL-S	No	-
UK	No	-
TK	Yes	No
9GL	Low doses, higher doses lethal	Yes
DP148R	No	
CD2	No	-
	Gladue & Borca. Viruses. 20	22 Apr 23;14(5)

• Clear that new determinants of virulence were needed to attenuate ASFV-G



# **Genetic Deletions that Attenuate ASFV-Georgia**

 Table 5. Genetic deletions in ASFV-Georgia viruses resulting in experimental vaccines.

Gene Deleted	Dose Tested with Full Attenuation	Homologous Protection	Challenge Route and Dose	Reference
9GL	$10^2, 10^3$	Yes	IM 10 <sup>2</sup>	[35]
9GI, UK	$10^2, 10^4, 10^6$	Yes	IM 10 <sup>3</sup>	[48]
A137	$10^4, 10^7$	Yes	IM 10 <sup>2</sup>	[46]
CD2, UK	$10^{4}$	Yes	IM 10 <sup>2</sup>	[49]
DI 177L/DLVR *	$10^2, 10^4, 10^6$	Yes	IM 10 <sup>2</sup>	[39]
I177L	$10^2, 10^4, 10^6$	Yes	IM 10 <sup>2</sup>	[36-38]
I1226R	10 <sup>2</sup>	Yes	$IM 10^4$	[45]
Multiple MGF *	$10^2, 10^4$	Yes	IM 10 <sup>3</sup>	[25,51]

- No standard for testing ASFV genetic deletions for attenuation, different doses for attenuation, different challenge doses/ route of challenge
- Hard to compare studies from different laboratories

# Genetic Deletions that Attenuate ASFV-Georgia

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I177L	$10^2, 10^4, 10^6$	Yes	IM 10 <sup>2</sup>	[36-38]
I1226R	10 <sup>2</sup>	Yes	$\mathrm{IM}10^4$	[45]
Multiple MGF *	$10^2, 10^4$	Yes	IM 10 <sup>3</sup>	[25,51]

\* multiple MGF consists of a deletion of 6 MGF genes.

Gladue & Borca. Viruses. 2022 Apr 23;14(5):878



NAVETCO Vaccine is based on the ASFV-G- $\Delta$ I177L Co-Developed with the USDA

#### Safe and Effective Vaccine for ASFV

www.navetco.com.vn

# African swine fever virus virulence determinants

Table 3. Determinants of virulence that attenuate ASFV-Georgia-derived viruses.

Gene Deleted	Fully Attenuated	Homologous Protection	Reference	
9GL	Low doses, higher doses lethal	Yes	[35]	
9Gl, UK	Yes	Yes	[48]	
A137	Yes, only low doses tested	Yes	[46]	
CD2, UK	Yes	Yes	[49]	
E184L	No	Surviving animals	[47]	
I177L	Yes	Yes	[36-38]	
I226R	Yes	Yes	[45]	
I267L	No	-	[43,44]	
L7L-L11L *	No	Surviving animals	[50]	
MGF-110-9L	Partial at low doses	-	[40]	
MGF360-9L	Partial at low doses	-	[42]	
MGF-505-7R	Yes, only low doses tested	-	[41]	
Multiple MGF #	Yes	Yes	[25,51]	
QP509L/QP383R	Yes	No	[52]	

\* L7L-L11L consists of genes L7L, L8L, L9R, L10L, L11L; <sup>#</sup> multiple MGF consists of a deletion of 6 MGF genes.

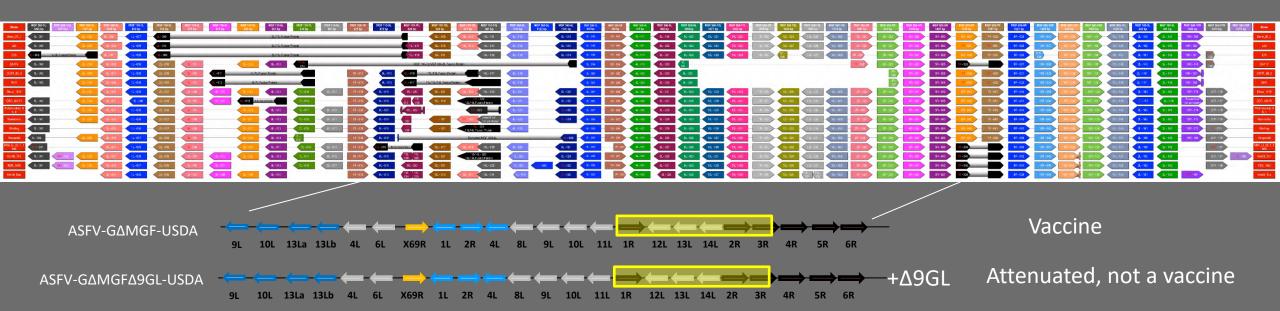
Gladue & Borca. Viruses. 2022 Apr 23;14(5):878

- Five Family Members: 100, 110, 300, 360, 505
- Varying number and combinations of MGF genes in Sequenced ASFV genomes.



#### Image : Home - Viral Bioinformatics Research Centre (4virology.net





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

Vivlan O'Donnell,<sup>a,b</sup> Lauren G. Holinka,<sup>a</sup> Douglas P. Gladue,<sup>a,b</sup> Brenton Sanford,<sup>a</sup> Peter W. Krug,<sup>a</sup> Xiqiang Lu,<sup>c</sup> Jonathan Arzt,<sup>a</sup> Bo Reese,<sup>d</sup> Consuelo Carrillo,<sup>a</sup> Guillermo R. Risatti,<sup>b</sup> Manuel V. Borca<sup>a</sup>

Agricultural Research Service\* and APHIS," USDA, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, Sector Disease, Center, G

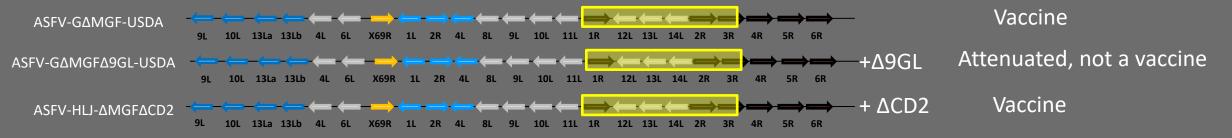


#### ASFV-G-∆MGF

- Deletion of 6 MGF genes
- Protective at single low dose
- No clinical symptoms at higher doses one day of mild fever

#### $ASFV-G-\Delta MGF\Delta 9GL$

- Deletion of 6 MGF genes and 9GL
- Full Attenuation
- No replication in swine
- Addition on 9GL to MGF : Not a Vaccine



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#### D Springer Link

Research Paper | Published: 01 March 2020

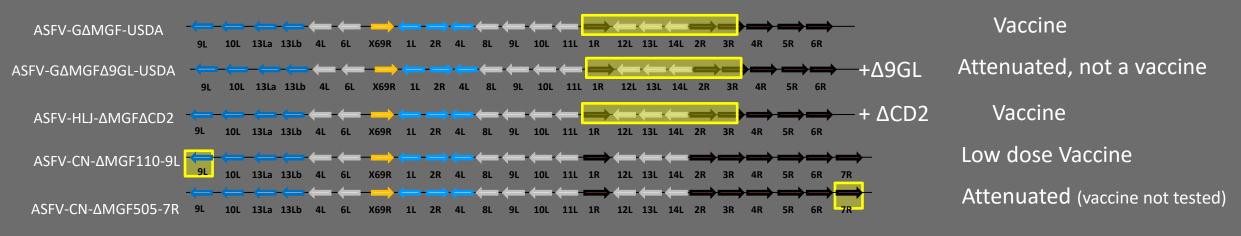
A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs

<u>Weiye Chen, Dongming Zhao, Xijun He, Renqiang Liu, Zilong Wang, Xianfeng Zhang, Fang Li, Dan Shan,</u> <u>Hefeng Chen, Jiwen Zhang, Lulu Wang, Zhiyuan Wen, Xijun Wang, Yuntao Guan, Jinxiong Liu & Zhigao Bu</u>

Science China Life Sciences (2020) Cite this article 193 Accesses 29 Altmetric Metrics

#### HLJ/18-7GD –( $\Delta$ MGF $\Delta$ CD2)

- Using the ASFV-G-∆MGF-USDA
- Add deletion to CD2
- CD2 by itself is not attenuated (confirming Borca et.al)
- HLJ/18-7GD is protective
- Reversion to virulence: 5 passages in pigs
- Tested in pregnant sows
- Being tested in field trials in China



0

Check for updates

### African Swine Fever Virus MGF-110-9L-deficient Mutant Has Attenuated Virulence in Pigs

Dan Li<sup>1</sup> · Yinguang Liu<sup>1</sup> · Xiaolan Qi<sup>1</sup> · Yuan Wen<sup>1</sup> · Pan Li<sup>1</sup> · Zhao Ma<sup>1</sup> · Yongjie Liu<sup>1</sup> · Haixue Zheng<sup>1</sup> · Zhijie Liu<sup>1</sup>

Received: 18 July 2020 / Accepted: 17 December 2020 © Wuhan Institute of Virology, CAS 2021

A Immunology

**RESEARCH ARTICLE** 

African Swine Fever Virus MGF-505-7R Negatively Regulates cGAS– STING-Mediated Signaling Pathway

This information is current as of April 27, 2021. Dan Li, Wenping Yang, Lulu Li, Pan Li, Zhao Ma, Jing Zhang, Xiaolan Qi, Jingjing Ren, Yi Ru, Qingli Niu, Zhijie Liu, Xiangtao Liu and Haixue Zheng

#### ASFV-CN/GS/2018-ΔMGF110-9L

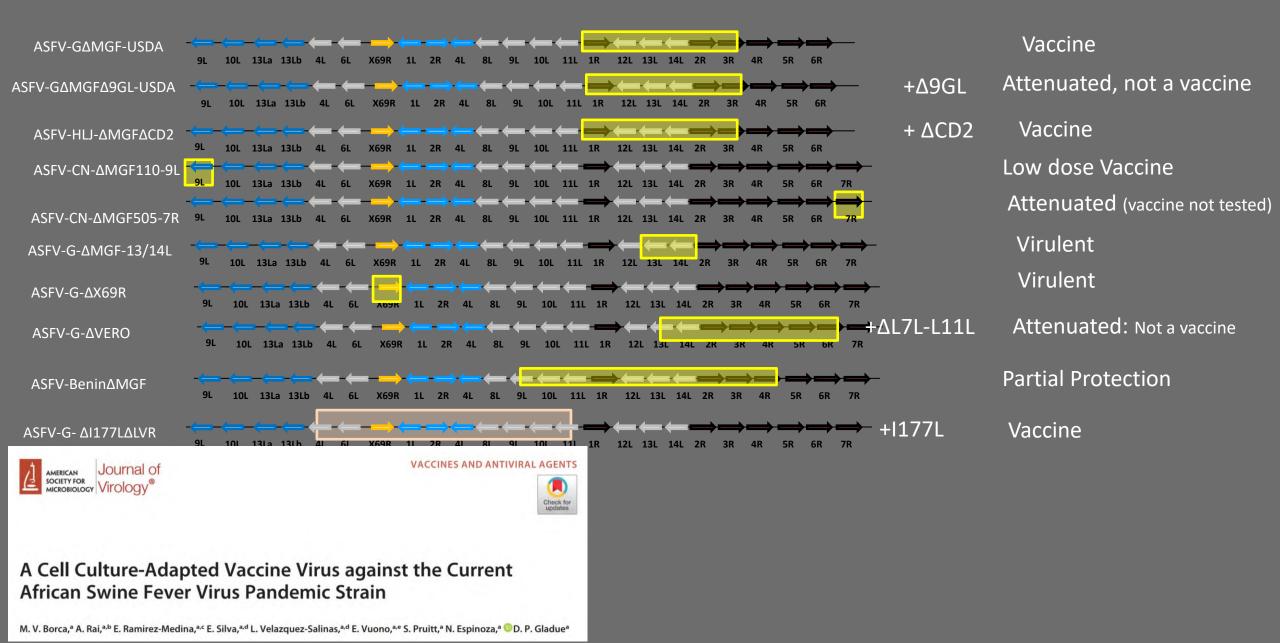
- Attenuated in swine at very low doses (HAD10)
- Challenged animals survived
- Vaccine detected in nasal swabs (possible shedding)
- Higher doses not tested

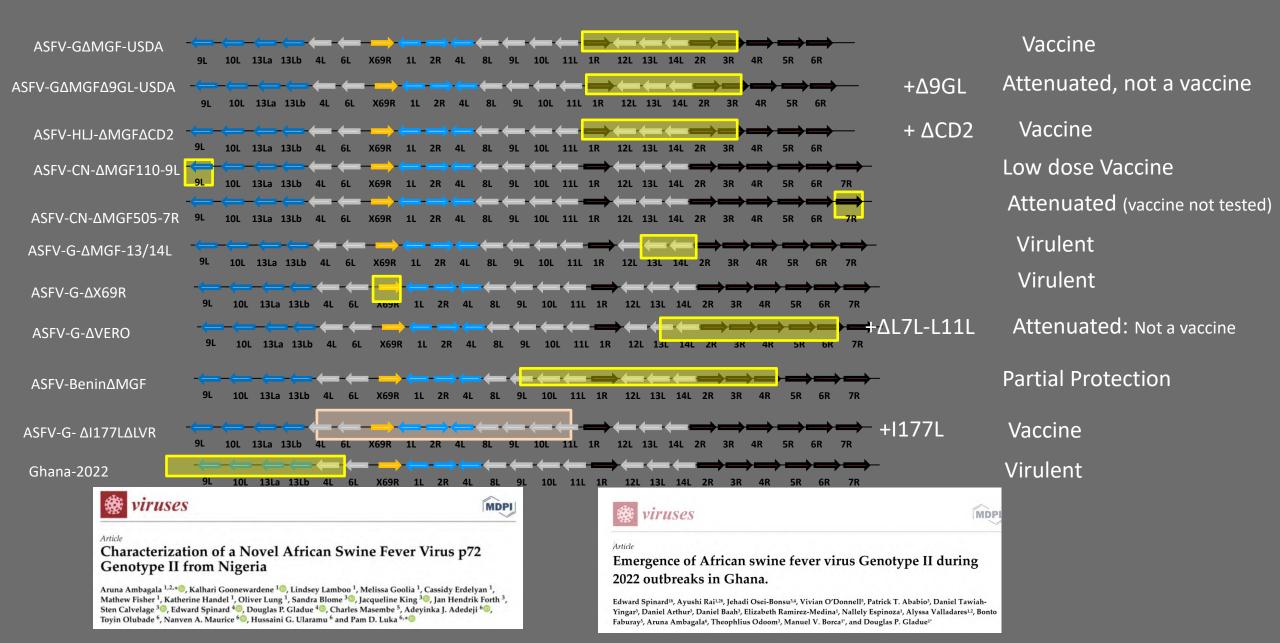
#### ASFV-CN/GS/2018-ΔMGF505-7R

- Attenuated in swine at very low doses (HAD10)
- Higher doses or vaccine efficacy not tested









- Not all ΔMGF deleted vaccines are the same
- Specific MGF deletions are Vaccines
- Additional deletions or genomic changes can severely effect vaccine efficacy
  - Different ΔMGF strains can go undetected if only analyzed by PCR



#### AVAC ASF LIVE VACCINE AN EFFECTIVE SOLUTION FOR PREVENTION OF AFRICAN SWINE FEVER



#### Manila, Philippines 2023



Nguyen Van Diep et al

AVAC vaccine is based on the USDA ASFV-G- $\Delta$ MGF vaccine platform

### Safe and Effective Vaccine for ASFV

## World-Wide Standard for ASFV Vaccine?

- Currently there is no standard for ASFV vaccine
- First vaccines are required for standards to be developed

## ASFV-G- $\Delta$ I177L is the first commercial produced vaccine for ASFV



ASFV-G- $\Delta$ MGF is the second commercial produced vaccine for ASFV





## World-Wide Standard for ASFV Vaccine?

- Currently there is no standard for ASFV vaccine
- First vaccines are required for standards to be developed

## USDA / WOAH: ASFV Vaccine International Standards

Recommendations for International Standard Guidelines for ASF First Generation Live Attenuated Virus Vaccines 2023 GARA Gap Analysis December 05 -07, 2023 Manila, Philippines

Cyril Gay and David Brake

USDA ARS/WOAH SPONSORED, CONTRACTED (CRDF GLOBAL) 2022 -2023 PROJECT TO BIOQUEST ASSOCIATES, LLC.