# An Overview of Novel FMDV Vaccines and their Future use in Endemic Countries

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# PROPHYLACTIC VACCINATION

- FMD chemically-inactivated vaccines have been available for over 70 years.
- Today, approximately 2 billion doses per year of inactivated virus vaccine are sold worldwide.
- There is an increasing demand of FMD **vaccines tailored to the 7 FMD global pools needs** and to improve FMD control in endemic countries and the developing world where losses on animal productivity and trade have a direct impact on **food security**.

# CONCERNS WITH CURRENT INACTIVATED FMD VACCINES

- Require adaptation and growth of large volumes of wild type virus in cells
  - Risk of virus of release from manufacturing facilities
  - Ability to rapidly address new strains
- Some antigens lack stability (low potency/short shelf life)
- Onset of protection 7-14 days
- Short duration of immunity ≤6 months
- Difficult to differentiate vaccinated from infected animals (DIVA) due to presence of residual NS proteins. Added cost for antigen purification
- Vaccinated and exposed animals become carriers

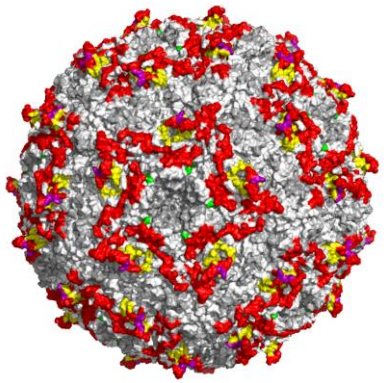
# Novel FMD Vaccine Strategies

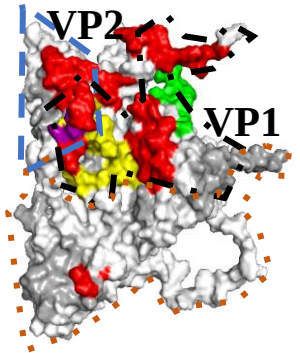
- Whole virus particle with built DIVA markers

- Empty Particles: Vectored Vaccines Adeno-FMD VLP
  Virus-like Particles (Baculovirus, Ecoli, mammalian cells)

- Synthetic Peptide. T and B cell peptide epitopes

Other strategies under investigation: modified live virus, DNA-encoding empty capsids, mRNA, alternative vectors for expression of VLP



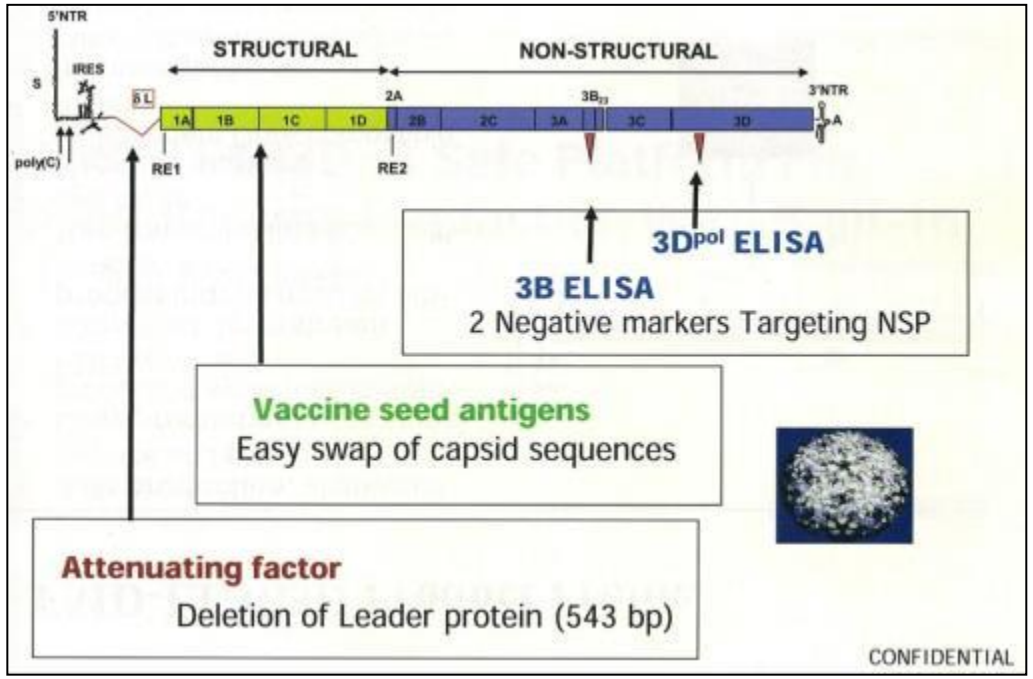


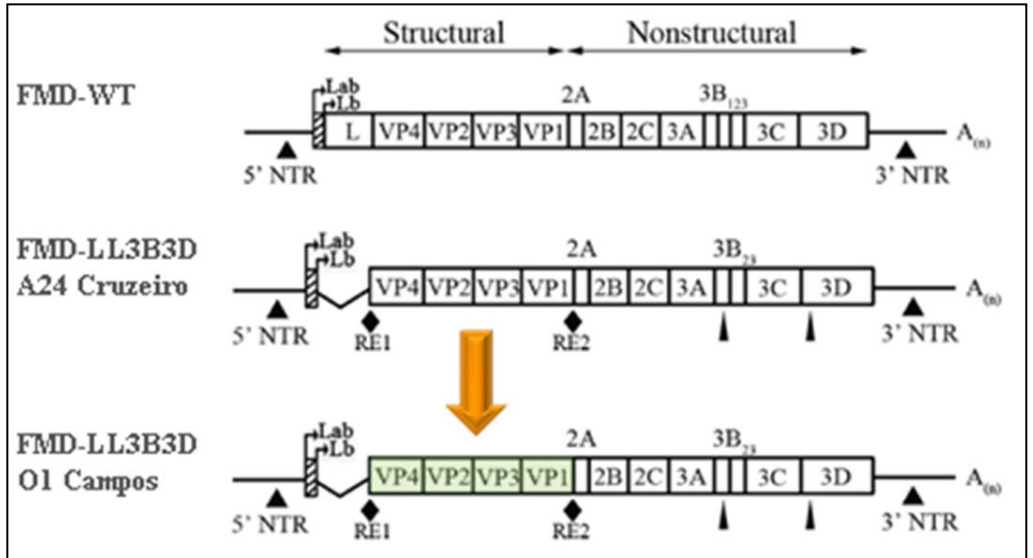
# FMD-LL3B3D
# Vaccine Platform

## A Safe Platform For FMD Vaccine Production With Built-In DIVA Markers

# FMD-LL3B3D VACCINE PLATFORM

- **Collaborative effort between the USDA-ARS and Zoetis**
- **Cassette construction**
  - Allows rapid insertion of capsid coding regions from emerging strains
- **Double BEI inactivated and formulated with a proprietary oil-based adjuvant**
- **Safe and easy production technology**
  - FMD-LL3B3D A24 Cruzeiro platform backbone lacks $L^{pro}$ region and one of three 3Bs
  - Attenuated in cattle and pig
  - Uses the same production systems as current inactivated FMDV vaccines
- **Non-transmissible (cattle & swine)**
- **Fully DIVA compatible**
  - Two independent and stable negative markers
  - Genetically altered key epitopes in 3B and 3D NSPs
- **High potency**
  - Potent immune responses to inserted capsid proteins of target strain
  - Proprietary adjuvant increases antibody and cellular immune responses

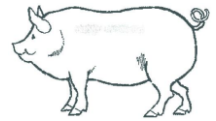


# SAFETY OF THE LIVE FMD LL3B3D VACCINE STRAINS - CATTLE AND SWINE

## FMD-LL3B3D platform viruses are highly attenuated in cattle or swine

| Construct | Inoculation Route | # Animals |
|---|---|---|
| FMD-LL3B3D-A24 Cruzeiro | Intralingual ($7x10^6$) | 2 |
| FMD-LL3B3D-A24 Cruzeiro | Aerosol ($1x10^6$ to $3x10^6$) | 3 |
| FMD-LL3B3D-A24 Cruzeiro | Aerosol and Contact / ($1x10^6$) | 9 |



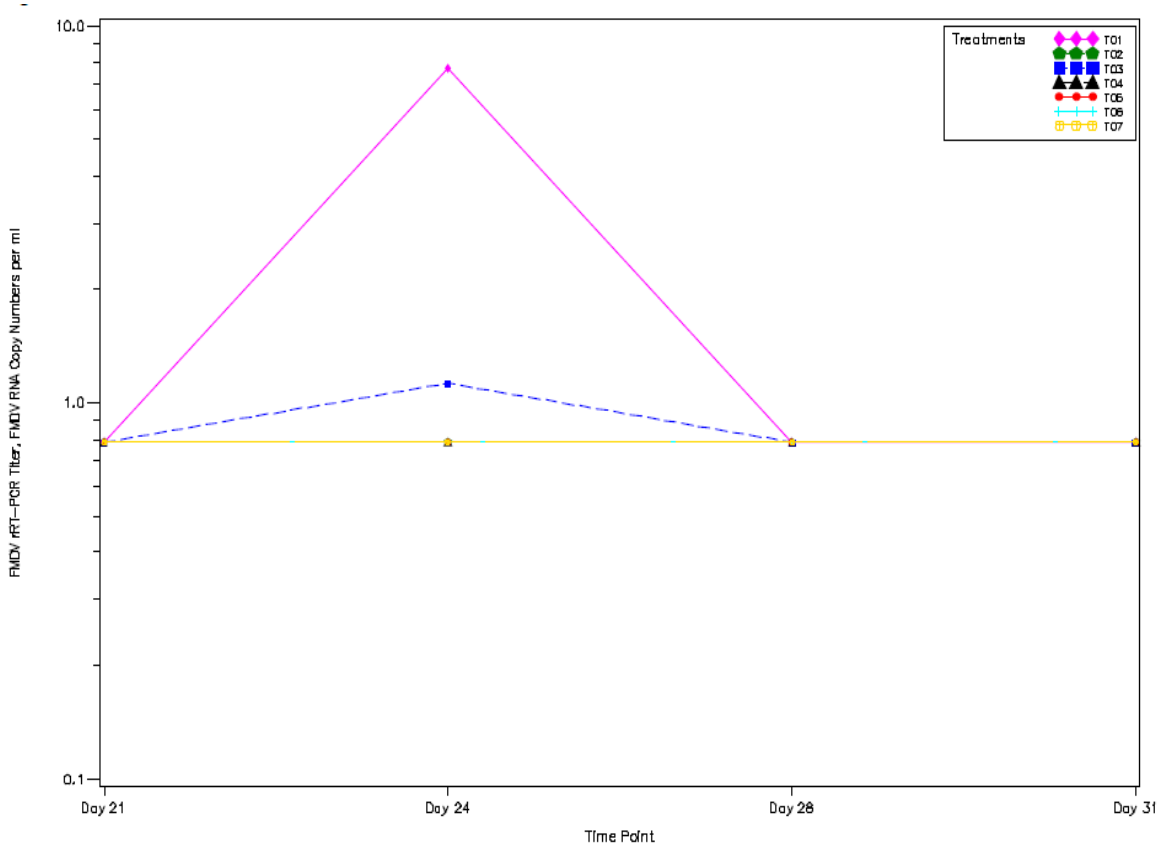
| Construct | Inoculation Route | # Animals |
|---|---|---|
| FMD-LL3B3D-A24 Cruzeiro | Heelbulb and Contact ($1x10^5$) | 4 |
| FMD-LL3B3D-Asia1 Shamir | Heelbulb and Contact ($1x10^6$) | 5 |
| FMD-LL3B3D-A Turkey 06 | Heelbulb and Contact ($1x10^6$) | 5 |
| FMD-LL3B3D-O1 Campos | Heelbulb and Contact ($1x10^6$) | 4 |
| FMD-LL3B3D-A Argentina 2001 | Heelbulb and Contact ($2x10^6$) | 4 |
| FMD-LL3B3D-C3 Indaial | Heelbulb and Contact ($2.8x10^6$) | 4 |

- Results
  - ✓ No clinical disease
  - ✓ No viral shedding
  - ✓ No fever spike
  - ✓ No contact transmission
  - ✓ Very limited if any immune response
- Safety study results supported the US Select Agent Exclusion

USDA zoetis

# FMD-LL3B3D A24 CRUZEIRO VACCINE IN ADJUVANT – PD50 STUDY with proprietary adjuvant

## A: Viremia




## B: Clinical Signs and Persistence

| | | Clinical Signs | | | | Log10 RNA Copies (Probang) | | | | Virus Isolation (Probang) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day of Study | | | | | | | | | | | |
| Group | Animal | 20 | 23 | 27 | 30 | 38 | 42 | 49 | 52 | 38 | 42 | 49 | 52 |
| PBS | R14-84 | 0 | 2 | 4 | 4 | 4.29 | 4.72 | 0 | 3.83 | Positive | Positive | Positive | Positive |
| | R14-85 | 0 | 4 | 4 | 4 | 4.26 | 6.01 | 5.14 | 4.7 | Positive | Positive | Positive | Positive |
| | R14-86 | 0 | 1 | 4 | 4 | 0 | 3.62 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-87 | 0 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| Full Dose DSP1 | R14-72 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-73 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-74 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-75 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| 1/4th Dose DSP1 | R14-76 | 0 | No | No | No | 4.98 | 4.68 | 0 | 0 | Positive | Positive | Negative | Positive |
| | R14-77 | 0 | 1 | 1 | 1 | 5.52 | 3.43 | 0 | 0 | Positive | Positive | Negative | Negative |
| | R14-78 | 0 | No | No | No | 0 | 4.35 | 0 | 5.3 | Positive | Positive | Positive | Positive |
| | R14-79 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| 1/16th Dose DSP1 | R14-80 | 0 | No | No | No | 0 | 0 | 4.88 | 4.59 | Positive | Negative | Positive | Positive |
| | R14-81 | 0 | No | No | No | 5.08 | 4.01 | 3.98 | 4.65 | Positive | Positive | Positive | Positive |
| | R14-82 | 0 | No | No | No | 0 | 4.47 | 6.12 | 4.32 | Positive | Positive | Positive | Positive |
| | R14-83 | 0 | No | No | No | 0 | 0 | 0 | 0 | Positive | Positive | Positive | Positive |
| Full Dose DSP2 | R14-60 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-61 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-62 | 0 | No | No | No | 4.75 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-63 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| 1/4th Dose DSP2 | R14-64 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-65 | 0 | No | No | No | 4.1 | 4.11 | 0 | 3.39 | Positive | Positive | Positive | Positive |
| | R14-66 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-67 | 0 | No | No | No | 4.14 | 5.08 | 5.18 | 4.82 | Positive | Positive | Positive | Positive |
| 1/16th Dose DSP2 | R14-68 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-69 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-70 | 0 | No | No | No | 0 | 0 | 0 | 0 | Negative | Negative | Negative | Negative |
| | R14-71 | 0 | No | No | No | 5.34 | 5.46 | 4.49 | 3.7 | Positive | Positive | Positive | Positive |

Total abnormal vesicle score code

| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|

# EFFICACY OF THE FMD-LL3B3D A24 CRUZEIRO IN CATTLE

## FMD-LL3B3D A24 Cruzeiro vaccine prevented FMD lesions at 1/16$^{th}$ dose

- Vaccines formulated with inactivated FMD-LL3B3D A24 Cruzeiro and a proprietary Zoetis oil-based adjuvant
- Clinical outcomes
  - Higher serum antibody titers compared to conventional vaccine
  - More robust CMI response compared to conventional vaccine
  - Complete prevention of FMD lesions
    - Even with 1/16 dose (0.5 μg antigen)
  - Full dose (8 μg) vaccine prevented persistent infection (DSP # 1 and # 2)
  - Prevention of fever
  - Prevention of viremia (DSP # 2)
  - Significant reduction in shedding (data not shown)

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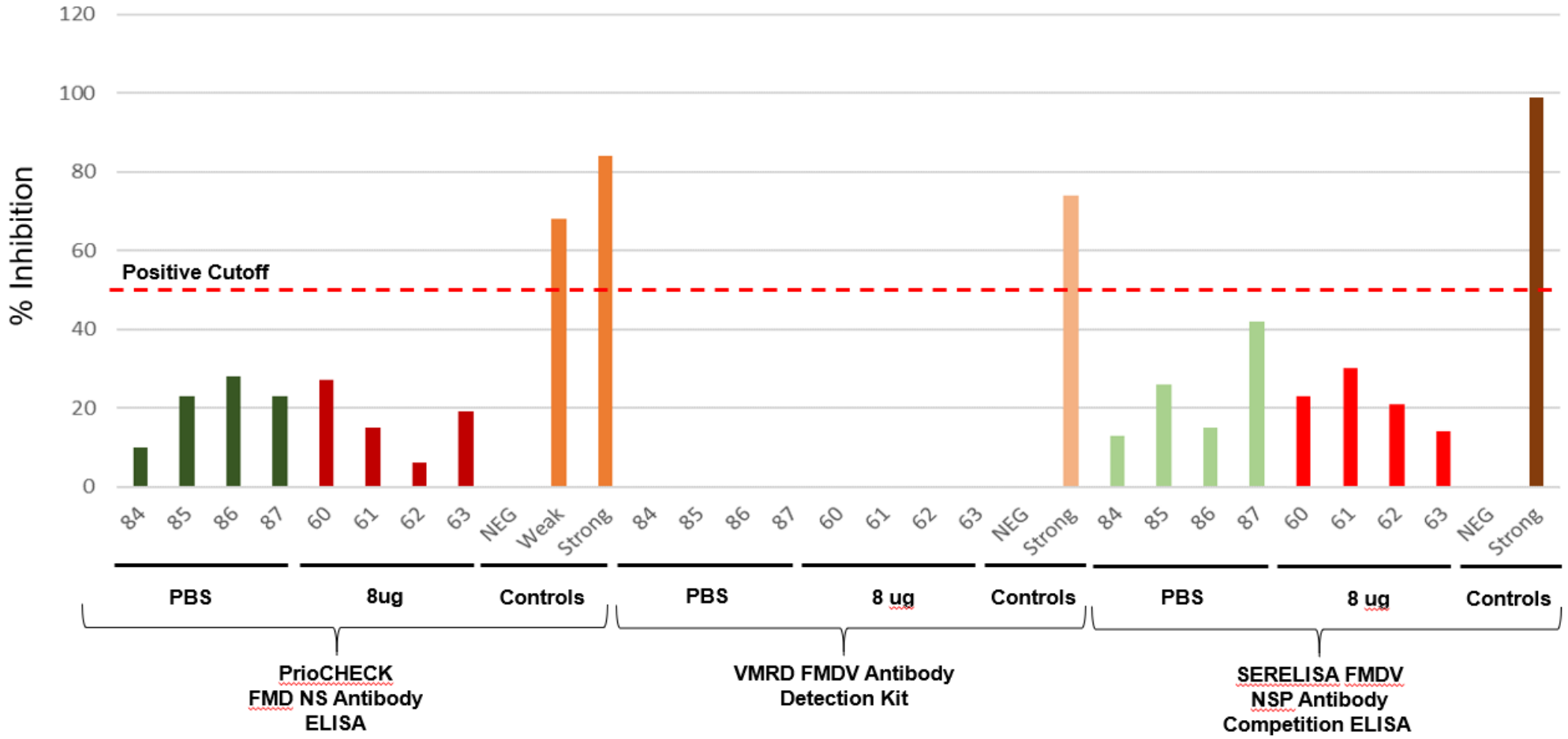
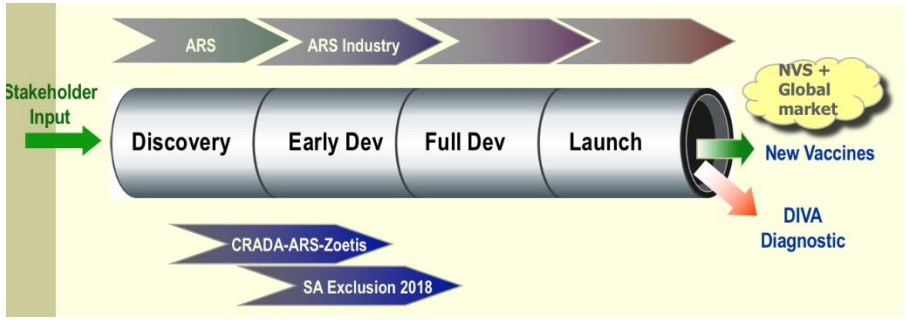
# DIVA COMPATIBILITY WITH CURRENT COMMERCIAL ASSAYS - (PrioCHECK SERELISA AND VMRD)

- C



# FMD-LL3B3D Vaccine Platform Commercialization Pathway




## Proof of Concept

- Patent US8765141
- Live virus safety studies
- Preliminary vaccine efficacy studies

## Select Agent exclusion

- Production of full-length constructs
- Produce vaccine candidates for different pools
- Second DIVA Test Developed (VMRD)

## Full Development

- Establishment of vaccine development facility
- Pre-MS and MS Production
- Development of additional full-length constructs
- Process optimization and scale-up

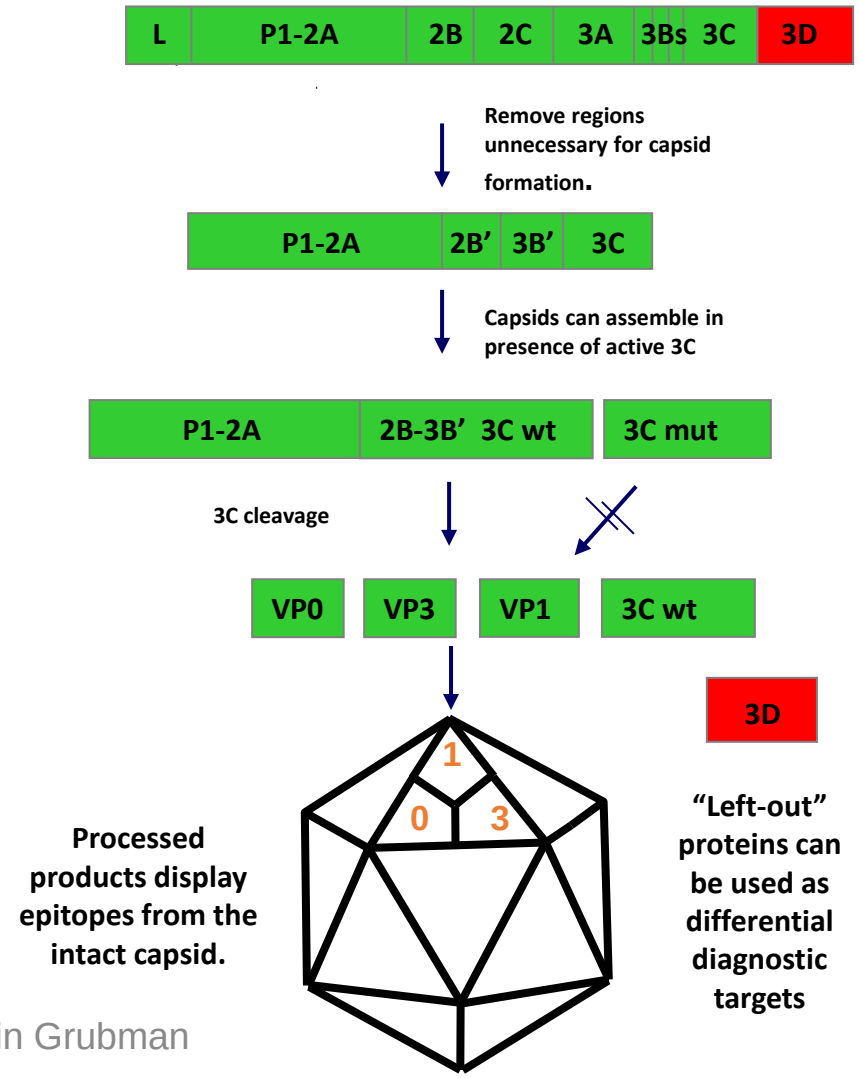
USDA zoetis

# Viral Vector FMD VLP Vaccine platform.

**Recombinant replication-defective Adenovirus for in vivo expression of FMD VLPs with DIVA capability**

# Human Ad5-based FMD vaccine: in vivo delivery of VLPs

- Contains all protective epitopes present on current inactivated virus vaccine but lacks infectious viral nucleic acid and non-structural protein (NSP)
- This vaccine can be produced without the need of high biosecurity conditions, and is compatible with DIVA testing
- Can be safely produced in the United States with commercial partner






*Mayr et al, 1999, 2001*

# Brief History of Adt.A24 USDA CVB Conditional License Program (DHS S&T PIADC –Industry Partner)

**2005. DHS successfully completes 1st POC efficacy study in direct and contact challenge models @ 7 days post-vaccination using industry produced discovery research vaccine**

**2006. Vaccine dose titration to establish MID50. Benchmarked Adt.A24 against conventional inactivated vaccine from vaccine manufacturer. Demonstration that vaccine platform can be used for different serotypes**

**2007-09. Regulatory development plan w/ industry partner and USDA CVB initiated. 20-liter process development. Master Cell Stock and Master Seed Virus produced; suitable cattle adjuvant identified. First FMD vaccine (safety) study on U.S. mainland completed**

**2010-11. Pivotal efficacy completed including minimum protective dose. Pre-licensing serials made by manufacturer. Field safety study in 500 cattle (dairy and beef)**

**2012. Conditional licensed issued (A24 serotype)**

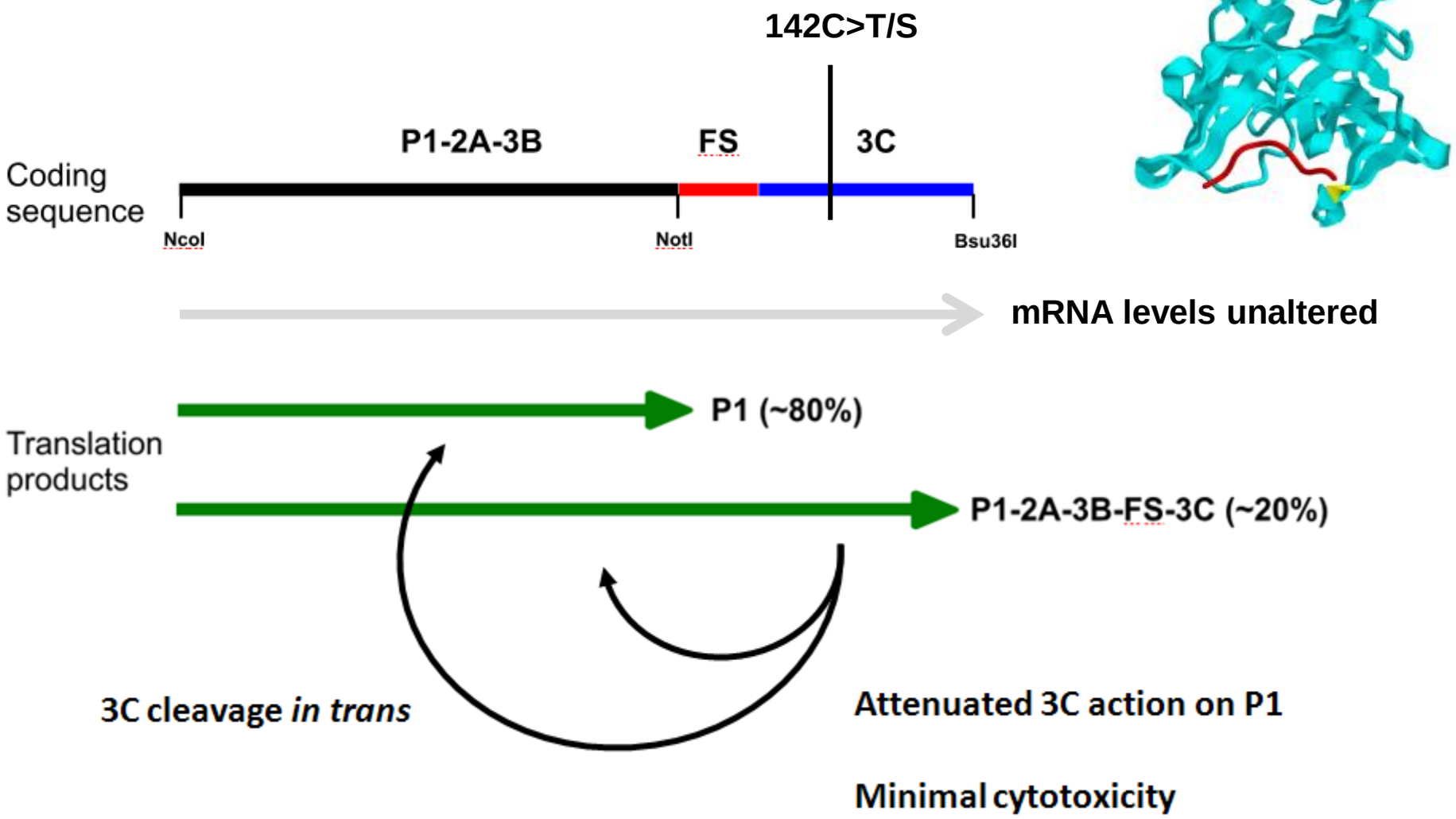
**2013-2018. Commercial scale-up 200 liter studies w/ commercial partner; conditional license renewed. (10) AdtFMD Master Seed Virus stocks produced and tested in efficacy studies**

**2020. Adt.A24 conditional licensed renewed (Huvepharma current license holder)**

# FMD VLP
# Vaccine Platform
# (baculovirus)

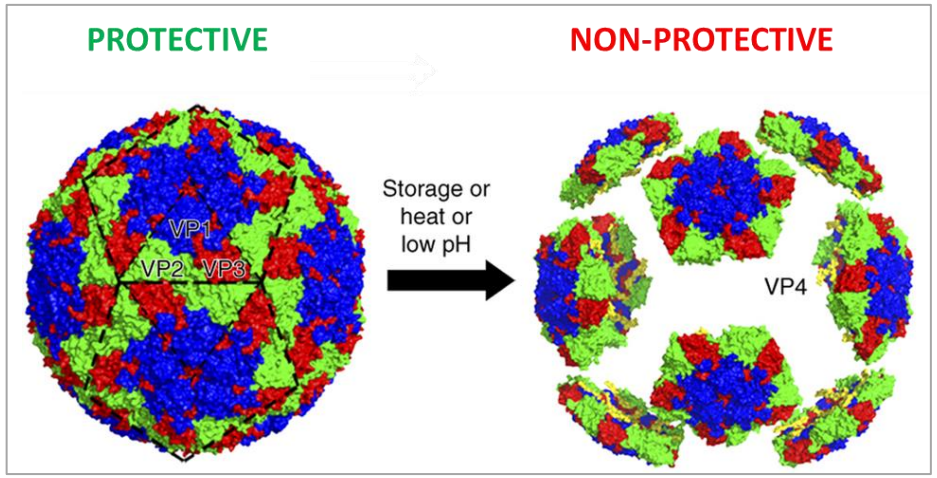
**Stabilized empty FMDV capsids produced *in vitro***

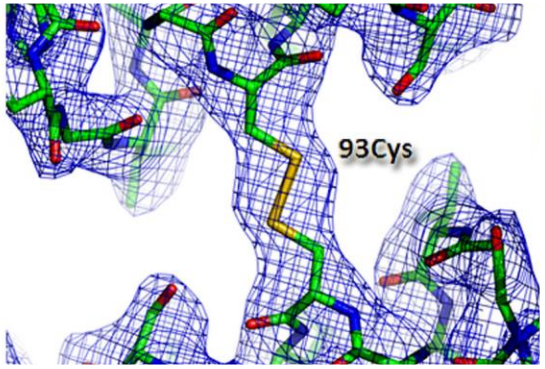
# Translation strategy – selectively reduce 3C translation and enzymatic activity



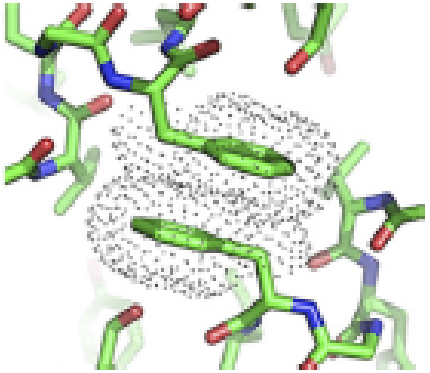
# Stabilisation of VLPs using multiple methods

(not all tolerated in live viruses)

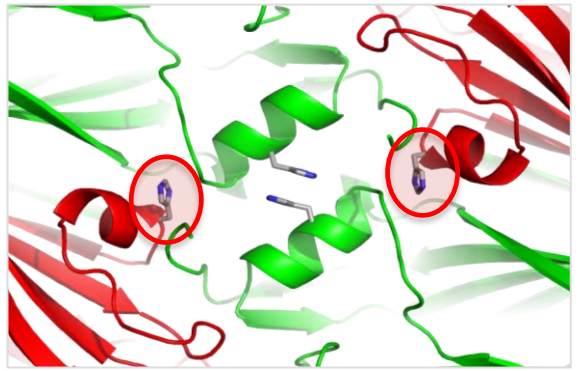



Incorporating Disulphide Bonds



Hydrophobic Stacking



Improving pH Stability

UNIVERSITY OF OXFORD

THE Pirbright INSTITUTE

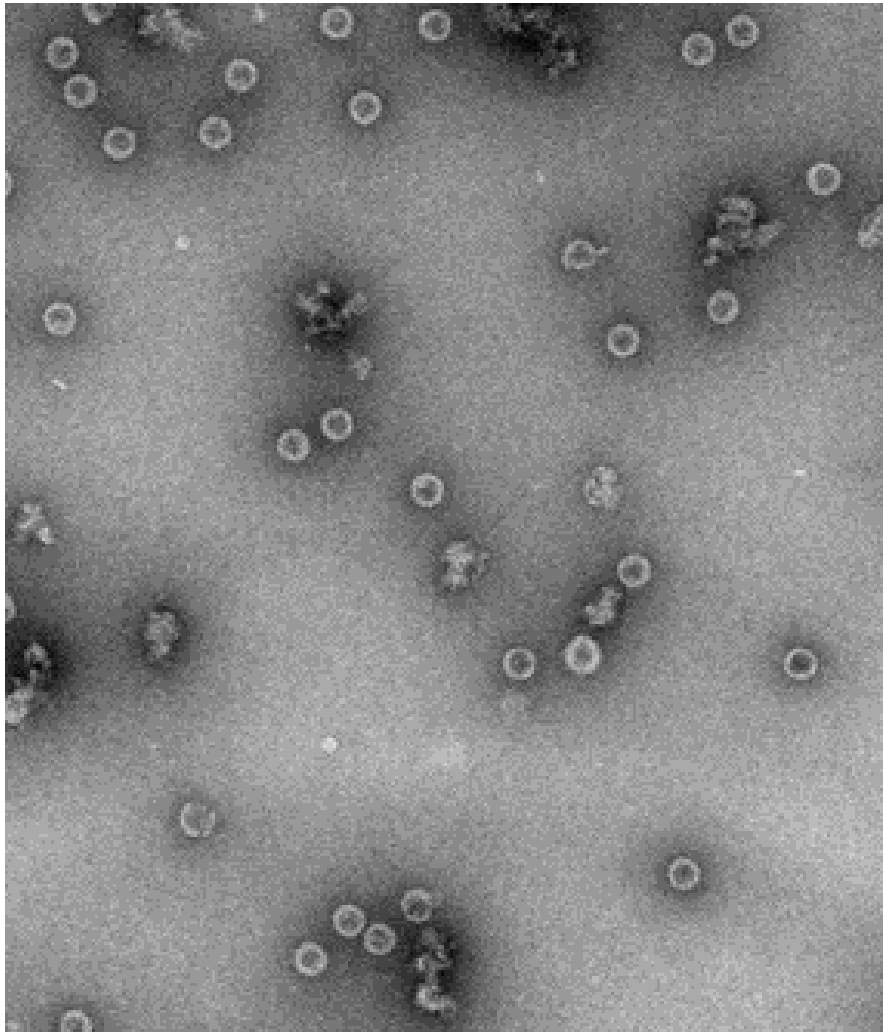
University of Reading

MSD Animal Health

diamond

# Recombinant FMD vaccines – virus like particles

- Stabilized VLPs now available for multiple strains of most serotypes
- Made in insect cells using baculovirus, an industry standard
- They are entirely safe as no live virus is ever used
- Low containment manufacturing
- Yields optimized significantly; VLPs are commercially viable
- Vaccines are of high potency (≥6$PD_{50}$); same vaccination schedule as current vaccines



# Benefits of stabilised VLPs

**Improved storage characteristics:** vaccine ready for deployment, less reliant on cold chain

**Safe production:** no live FMD virus, enhanced production capacity

**Quick response to new FMDV variants:** no need to isolate virus and adapt to tissue culture; sequence → gene synthesis → expression

**No non-structural proteins:** companion DIVA diagnostic tests, greater certainty of discriminating between vaccinated and infected animals

**Similar to or better than current FMD vaccines:** same (cross-protective) immune response in animals, but VLP vaccines are more stable and potentially more affordable

# Pathway to a commercial product

- GMP manufacturing processes have been developed
- Virus-like particles are included in EMA/CVMP/IWP/105506/2007 Rev. 1 ‘Multi-strain dossiers’
- Ph.Eur Monograph 0063 (FMD vaccines) is applicable
- VLPs are not yet included in OIE Terrestrial Manual & OIE Terrestrial Code

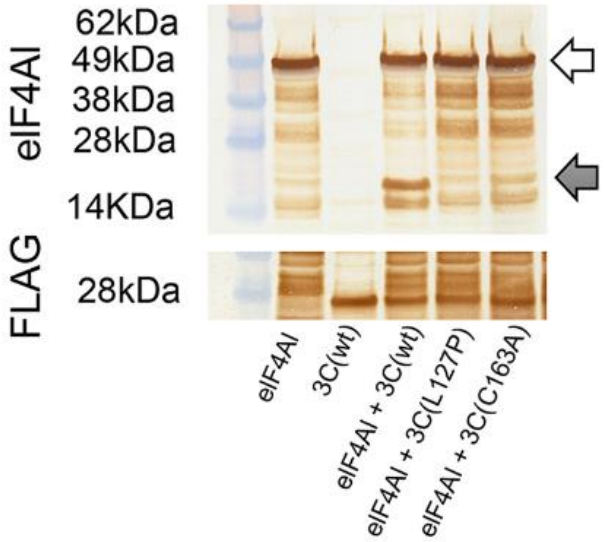
# Funding support

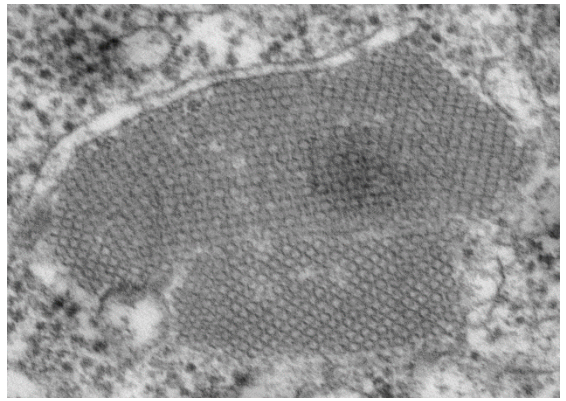
defra
Department for Environment Food and Rural Affairs
wellcome
UK Research and Innovation
University of Oxford
The Pirbright Institute
University of Reading
MSD Animal Health
diamond

# FMD VLP
# Vaccine Platform
# E.coli and Mammalian expression system.

# 3C(L127P) mutation

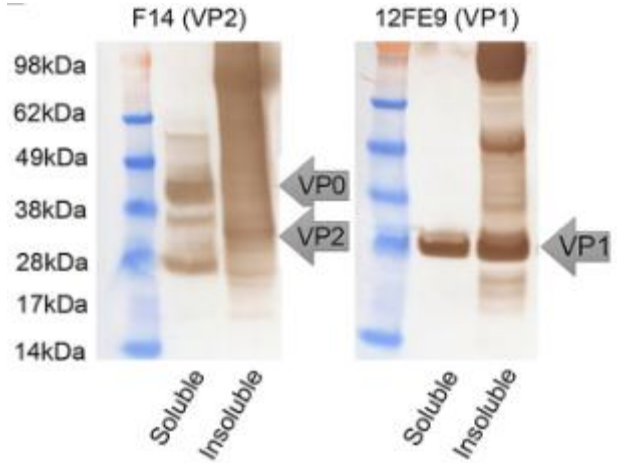
- Expression of wild type 3C has a negative effect on host cells
  - Causes issues in transgene expression, vaccine production, and genome stability
- L127P mutation dramatically reduces processing of host proteins
  - In particular eIF4AI
  - This enhances transgene expression
- L127P allows processing of FMDV P1 and enhanced production of FMDV virus like particles (VLPs) in all tested platforms
  - Pan-platform technology
    - Baculovirus, Ad5, bacteria, mammalian cell culture
- L127P made previously non-viable vaccine platforms viable

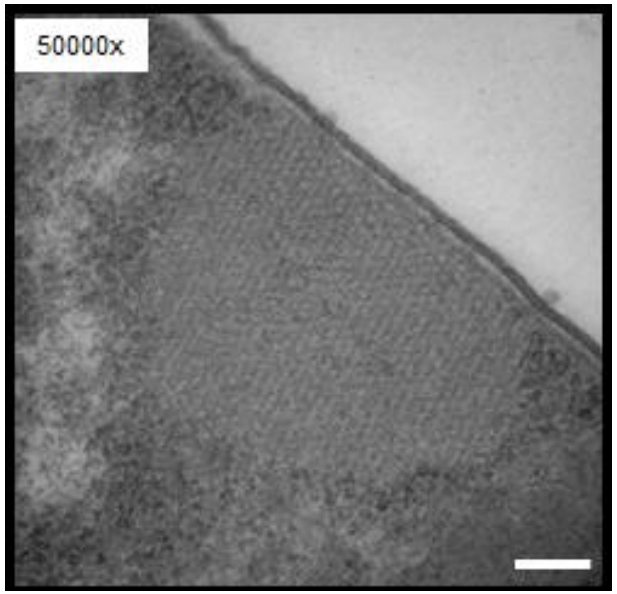


# Bacterial platform

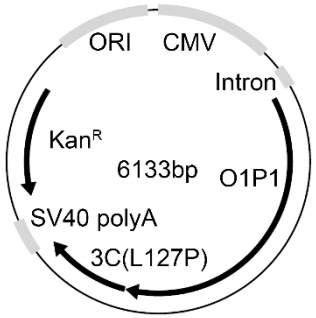
- Expresses P1 polypeptide and 3C protease in E. coli from plasmids
- Processed VPs and VLP arrays found by western blot and EM
- DHS developed an extraction protocol that left antigen in soluble fraction
- Soluble fraction administered to cattle and swine in non-challenge studies
  - VNTs generated in both groups
- Plasmid system is readily adaptable
- Does not need biocontainment facilities for production
- Issues with endotoxins need to be overcome before platform can be fully realized

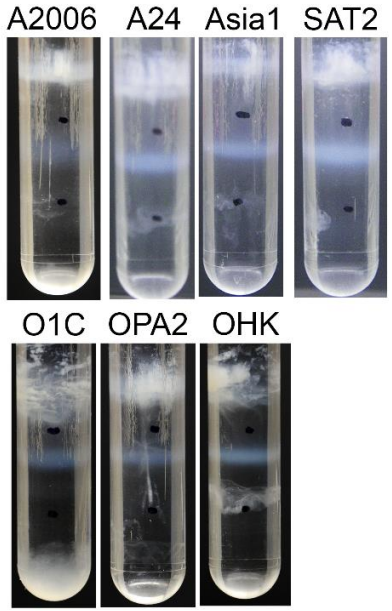


# MamVLP platform is highly adaptable

- DHS has invested in transiently transfected mammalian cell culture as a rapid response FMDV vaccine platform
  - Highly adaptable - plug and play system
  - Only need to change the P1
    - Compatible with USDA Vet Service Memorandum 800.213 for platform licensure
- Can stockpile DNA as master seed and then rapidly scale up production
  - Plasmids are cheap and easy to store for long periods of time
- Easy to adapt to new and emerging strains
  - Direct gene synthesis into plasmids
- No need for high containment manufacturing facilities
- 100% protection in swine after two doses against Serotype O
- System compatible with all tested serotypes and strains
- Advancing platform with industry partners



# FMD Peptide
# Vaccine platform

**Dendrimeric B and T cell peptides epitopes**

# A Dendrimeric Peptide Construction to prevent Foot-and-Mouth Disease (FMD)


Francisco Sobrino

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Consejo Superior de Investigaciones Científicas

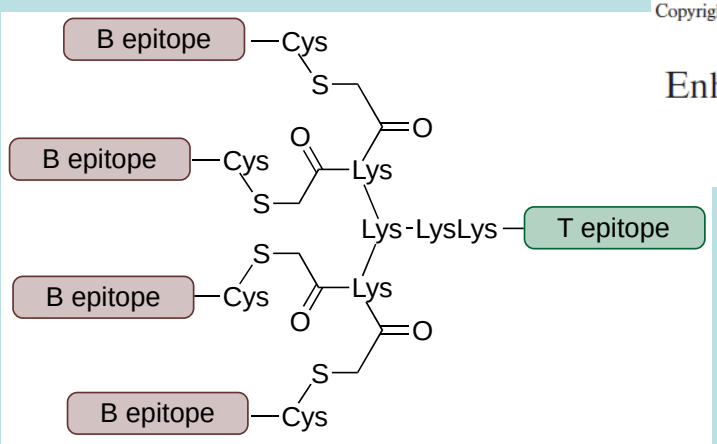
Esther Blanco

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

David Andreu

upf. Universitat Pompeu Fabra Barcelona

# •2008: 1st prototype ($B_4T$)




JOURNAL OF VIROLOGY, July 2008, p. 7223–7230 Vol. 82, No. 14
0022-538X/08/$08.00+0 doi:10.1128/JVI.00401-08


Enhanced Mucosal Immunoglobulin A Response and Solid Protection against Foot-and-Mouth Disease Virus Challenge Induced by a Novel Dendrimeric Peptide▽†

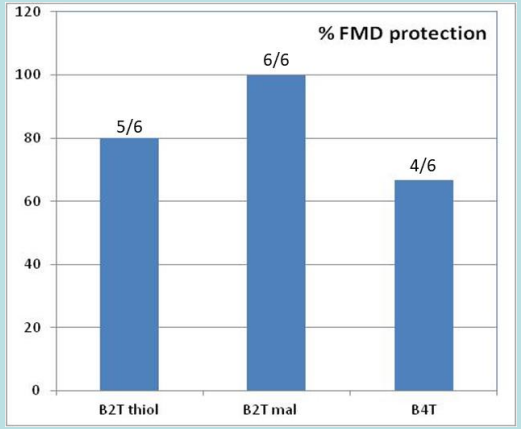
- •B epitope from antigenic site A, VP1, C-serotype
- •T epitope from non-structural protein 3A (21-35)
- •100% protection of pigs (n=4)
- •Control contact animals, housed with vaccinated ones, uninfected for 10 days after viral challenge
- •Strong IgA mucosal response, comparable to conventional vaccine

- •**$B_4T$ inconvenients:**
- •Ligation reaction sluggish, end product non fully homogeneous
- •Purification of ligated end product time-consuming, low yields

## •2008-12: 2nd generation ($B_2T$) vaccines

*« smaller is better »*

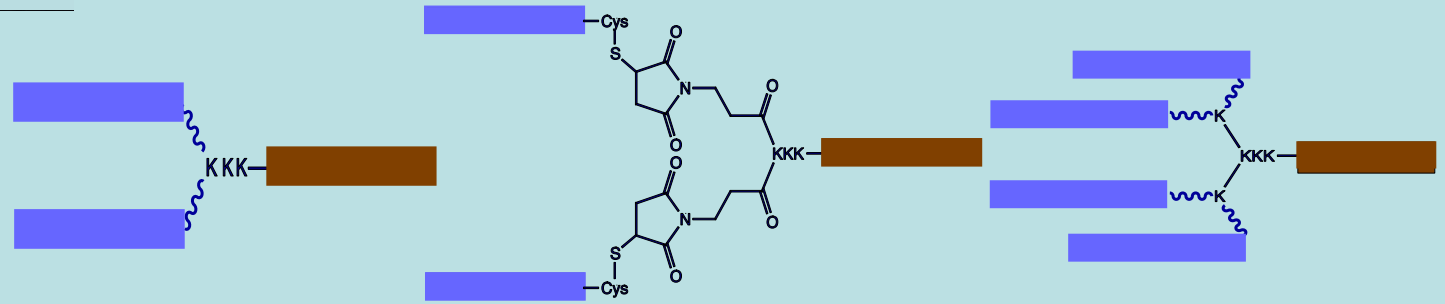
**Protection conferred by type O tetra - and bivalent dendrimers in pigs**




**n = 6 pigs**

***2 mg peptide/ 2 doses***
***Montanide ISA 50V2***
***(commercial oil adjuvant)***

*Blanco et al. Antiviral Res. (2016)*



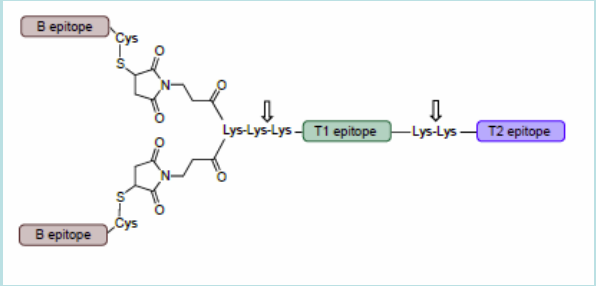

•Same concept than 1st generation $B_4T$ but simpler design and improved chemistry make it considerably more attractive, particularly maleimide-built constructions. Conjugation of the two moieties is essentially quantitative, minimal subsequent purification required.

•A homogeneous, well characterized product (HPLC, MS) that could be registered as a **pharmaceutical.**

• Feasible Industrial scale-up.

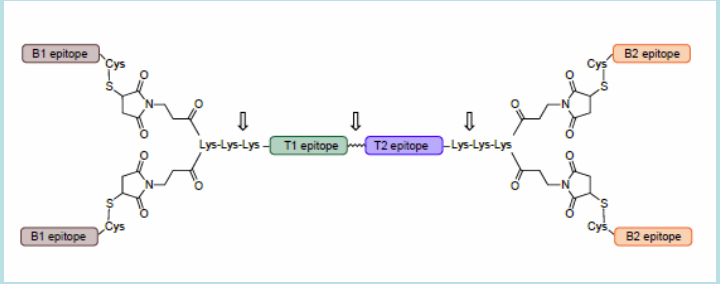
# $B_2T$ dendrimers can afford solid and long-lasting FMD protection in swine: current work with this modular approach

- Optimizing conjugation chemistry and inclusion of new T cell epitopes taking advantage of the versatility of the dendrimeric modular approach. Protection after a single peptide dose.




- Cañas-Arranz et al., 2020. **Vaccines** 8,19.(2020).
- Cañas-Arranz, et al., **Transbound. Emerg. Dis.** 67:1614-1622 (2020).
- Cañas-Arranz, et al., **Frontiers Vet. Sci**. 7:498. (2020).

- Multimerization strategies improve immunogenicity




- Defaus et al.,**Vaccines**. 22;8 (2020)
- Forner et al. **J. Org. Chem.** 85(3):1626-1634 (2020)

- Inclusion of different B cell peptide sequences in single dendrimers/combination of different dendrimeric molecules (heterotypic vaccines)

**Intellectual Property**

- Peptide vaccines for the prevention of foot-and-mouth disease. PCT. Application EP20382406.5. Date of presentation: 14/05/2020.

**Contacts with Companies**

-VIRBAC (2013-2015)
-Biogénesis Bagó (2015-2017)
-ACM Biolabs (2020-2021)