

# Strategies Considered for Candidate SARS Coronavirus Vaccine Development

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- **Classical formalin inactivated whole virus vaccine**
  - Lytic virus grows to high titres on Vero
  - Inactivation with formalin
  - Sucrose gradient purification
  - Adjuvantation with alum
  
- **Attenuated recombinant vector**
  - (i) **MVA – S glycoprotein**
  - (ii) **dVV – S glycoprotein**
    - **S glycoprotein is responsible for both binding to receptor on host cells and for membrane fusion**
    - **Induces neutralising antibody**
    - **Elicits cell-mediated immunity**

# Strategies Considered for Candidate SARS Coronavirus Vaccine Development

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ORIGINAL ARTICLE

## A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

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However, a novel coronavirus was isolated from patients who met the case definition of SARS. Cytopathological features were noted microscopically in Vero E6 cells inoculated with a throat-swab specimen. Electron-microscopical examination of cultures revealed ultrastructural features characteristic of coronaviruses.

# Development of Inactivated Whole Virus SARS Coronavirus Candidate Vaccine (I)

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- Human corona virus SARS candidate grows to high titres in Vero (CDC)
- Baxter has developed a technology platform based on serum and protein free culture of Vero cells at large scale
- This technology has been optimised for a range of viral candidate vaccines
- High probability that Vero cell platform could be utilised for SARS coronavirus vaccine development

# Summary of Baxter's Serum- and Proteinfree Vero Cell Technology

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## Origin:

African Green Monkey (*Cercopithecus aethiops*) kidney cells ATCC CCL81 obtained from ATCC (American Type Culture Collection) at passage no. 124 in 1988.

## Cell Banks:

MCB (Master Cell Bank) passage no. 128  
(fully tested for absence of tumorigenicity, absence of adventitious agents and identity/genetic stability)

WCB (Working Cell Bank) passage no. 133

standard quality control tests:

- bacterial and mycotic sterility
- mycoplasmas
- extraneous agents

## Fermentation:

Stirred tank fermenter with microcarriers up to 1300 litres

## Host System:

Influenza viruses (*Orthomyxovirus*)  
Hepatitis A virus (*Picornavirus*)  
Ross River virus (*Alphavirus*)  
Japanese Encephalitis virus (*Flavivirus*)  
West Nile virus (*Flavivirus*)  
(Recombinant) Vaccinia virus (*Poxvirus*)  
Vaccinia (Smallpox Vaccine)

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0891-3668/97/\$03.00/0  
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# Fifteen years of experience with Vero-produced enhanced potency inactivated poliovirus vaccine

EMMANUEL VIDOR, MD, CARLTON MESCHIEVITZ, MD AND STANLEY PLOTKIN, MD

# Development of Inactivated Whole Virus SARS Candidate Vaccine (II)

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- Fully characterised Vero cell line – Master File reviewed by FDA for Smallpox (ACAM-2000) and Influenza IND's
- All preclinical development, process development, Q.C., manufacturing technologies are available to allow rapid development of such a candidate vaccine
- All validated systems in place for screening virus seeds for adventitious agents
- Large scale production capacity will be available at BSL-3

# Results of Influenza Seed Virus Bank PCR Testing

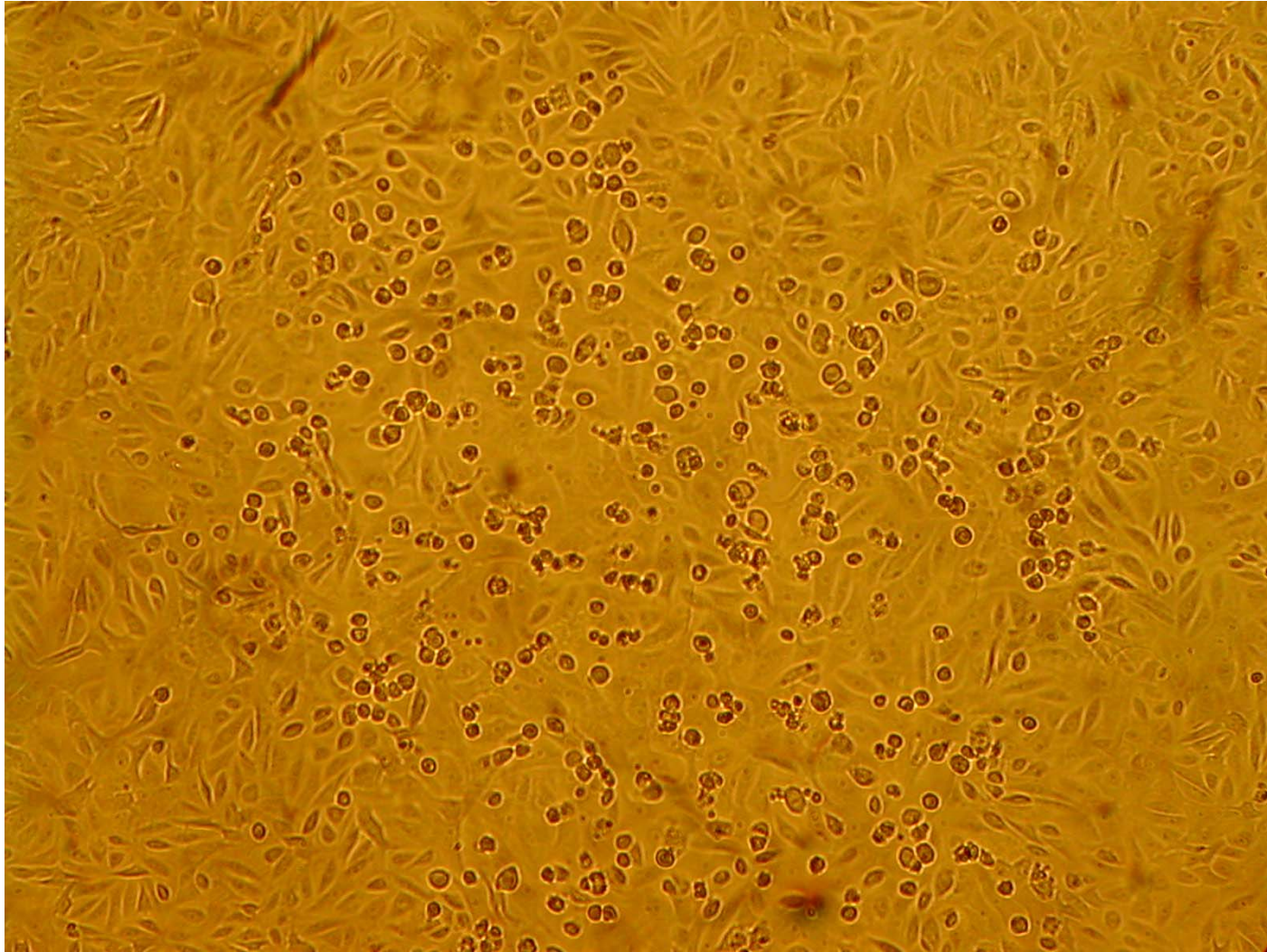
Target Virus(es)	Seed virus bank	
	1999/2000 A/Beijing/262/95 (A/H1N1) A/Sydney/5/97 (A/H3N2) B/Yamanashi/166/98 (B)	2001/2002 A/New Caledonia/20/99 (A/H1N1) A/Panama/2007/99 (A/H3N2) B/Johannesburg/5/99 (B)
Human Cytomegalovirus (CMV)	negative	negative
Human herpes virus (HHV 6 and 7)	negative	negative
Epstein-Barr virus (EBV)	negative	negative
Human enteroviruses (Poliovirus types 1, 2, 3; Coxsackievirus A13, A20-22, B4; Enterovirus 70, 71; Echovirus 2-9, 11, 12, 30)	negative	negative
Influenza type C	negative	negative
Measles (wild type and defective)	negative	negative
Adenovirus (types 1-3 and 5-7)	negative	negative
Herpes Simplex virus (HSV 1 and 2)	negative	negative
Corona virus (229E and 0C43)	negative	negative
Human respiratory syncytial virus (RSV A and B)	negative	negative
Human parainfluenza virus (HPI 1, 2 and 3)	negative	negative
Human rhinovirus	negative	negative
Varicella-zoster virus (VZV)	negative	negative
Rubellavirus	negative	negative

# Baxters Vaccine Production Plants in Bohumil, Czech Republic and Krems, Austria



**Production Capacity:**  
**Approx. 42 million doses of  
trivalent influenza vaccine**

# SARS Plaque (d4)



# Titer of Qualified SARS Coronavirus in Vero Cells

MOI	Titer ( $\log_{10}$ TCID <sub>50</sub> /ml)	
	Standard Vero (+ FBS)	Serumfree Vero
0.0001	6.5	7.3
0.00001	6.6	7.4
0.000001	6.5	7.4

Titer Qualified Seed Virus (CDC): 6.4  $\log_{10}$  TCID<sub>50</sub>

# Recombinant Vaccinia Strategy

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- Safety considerations for development and manufacturing
  - BSL-3 required for SARS coronavirus
  - CoV is transmitted via aerosol, with fatality rate of 4-5%
  - Recombinant Vaccinia-SARS would be BSL-2
- Efficacy
  - Live viral vector induces both cellular and humoral immune responses
  - Spike protein, a very large transmembrane glycoprotein, is correctly folded and transmitted to immune system by vaccinia vectors
  - viral glycoprotein – vaccinia recombinants have been demonstrated to induce protective immune responses in a variety of models
  - virus nucleocapsid may also be a target for such a strategy

# Vaccinia – Coronavirus Recombinants

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Murine coronavirus MHV-JHM:

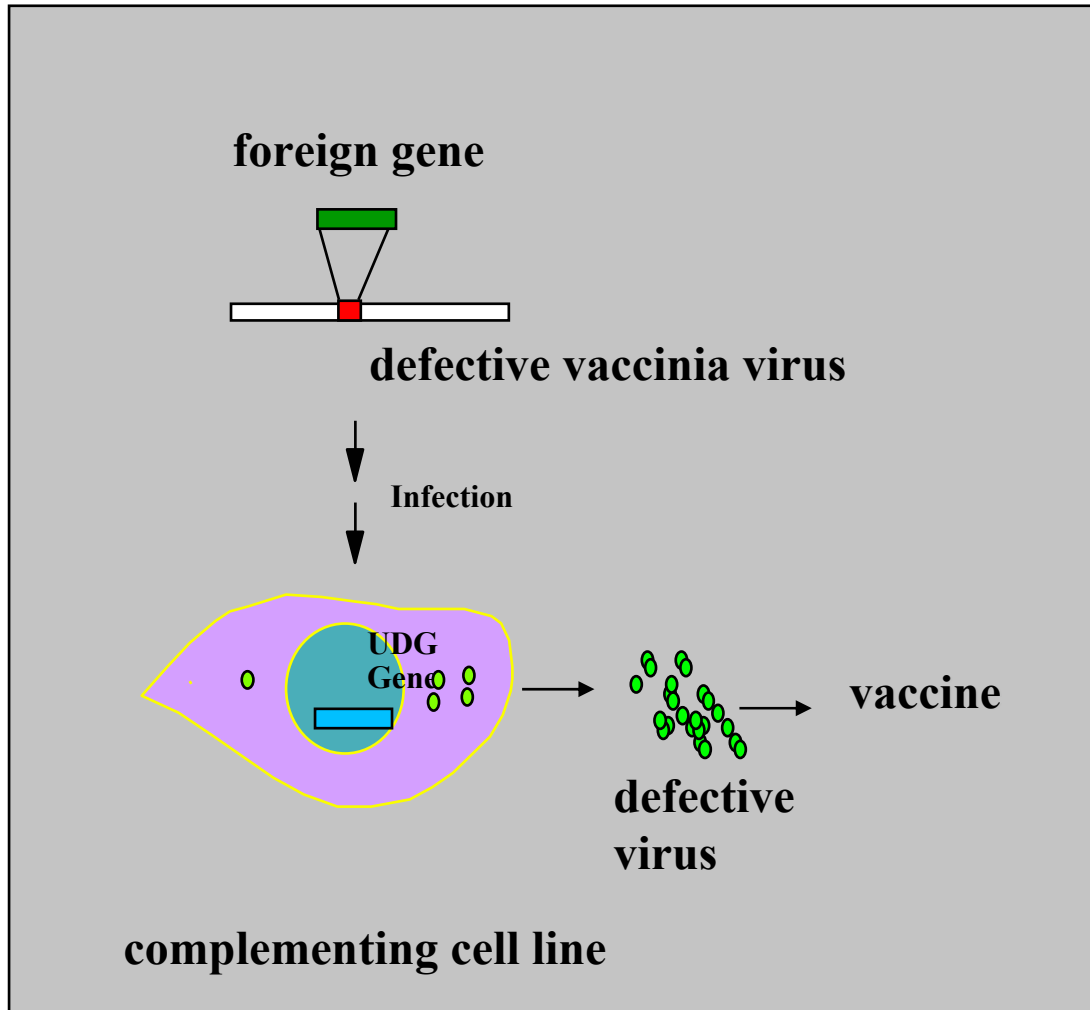
**Flory, E., A. Stuhler, H. Wege and S. Siddell (1993).**

"Recombinant vaccinia viruses which express MHV-JHM proteins: protective immune response and the influence of vaccination on coronavirus-induced encephalomyelitis." Adv Exp Med Biol **342**: 401-6.

**A strong protection against acute encephalomyelitis (AE) was mediated in Lewis rats** which were immunized by VV-Sfus+ and challenged with an otherwise lethal dose of MHV-JHM before the induction of S-specific IgG antibodies.

# The defective Vaccinia Virus (dVV) Technology

(Holzer et al., 1999. J. Virol. 73:4536-42)



- a complementing cell line substitutes an essential vaccinia gene
- cell line permits the growth of a defective virus
- the defective virus infects cells (abortive infection) and induces immunity
- dVV does not replicate in the host

# dVV - SARS

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- dVV vectors are highly protective in a Tick-borne encephalitis virus challenge model

Holzer, G. W., G. Remp, G. Antoine, M. Pfleiderer, O. M. Enzersberger, W. Emsenhuber, T. Hammerle, F. Gruber, C. Urban, F. G. Falkner, and F. Dorner. 1999.

**Highly efficient induction of protective immunity by a vaccinia virus vector defective in late gene expression.**

J. Virol. **73**:4536-4542.

# dVV - SARS

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## Advantages of SARS-dvv compared to SARS-MVA

- Production cell line will be a complementing Vero cell line
- Fully characterised cell bank
- Upscaling to > 1000 Liter scale possible
- No dependence on SPF eggs
- No retrovirus issues (adventitious viruses, CEC)

# Summary

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- **Baxter is very well positioned for the rapid development of a candidate SARS vaccine**
  - (i) **Technology platforms are available to allow development of both inactivated and live viral vaccines**
  - (ii) **We have substantial experience in developing a Vero cell-derived inactivated vaccine against a respiratory disease i.e. flu**
  - (iii) **We have 20 years experience in developing recombinant vaccinia vaccines**
  - (iv) **We have substantial manufacturing capacity at BSL-3**