



Live, Attenuated Influenza Vaccine Manufactured in MDCK Cells

VRBPAC

September 25, 2008

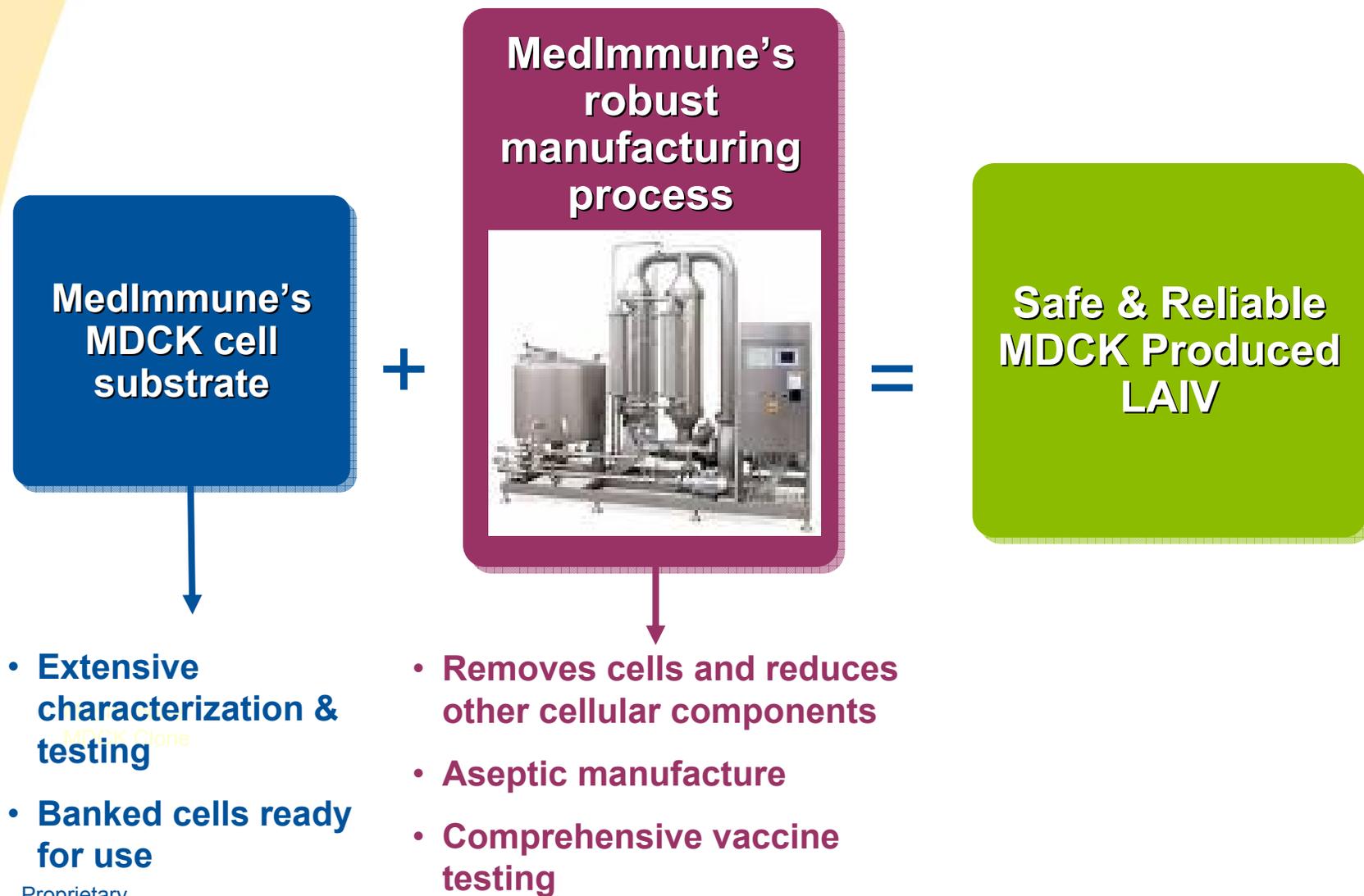
Silver Spring, Maryland

This project has been funded in part with \$169,462,231 in Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under Contract No. HHSO10020060010C.

To develop a safe, reliable vaccine technology to enhance the nation's supply of annual influenza vaccine and increase pandemic preparedness

- Describe the benefits of switching from egg production to cell-produced influenza vaccines
- Describe the safety of production of LAIV in MDCK cells
 - ◆ Characterization of the cell line
 - ◆ Manufacturing technologies for high quality vaccines
 - ◆ Defined risk assessments
- Enable VRBPAC to recommend moving forward with clinical development of a cell produced live, attenuated influenza vaccine (LAIV)

MDCK Cell Culture-Produced LAIV



Proprietary

■ Background

- ◆ MedImmune overview
- ◆ FluMist® (Live, Attenuated Influenza Vaccine)
- ◆ Egg and cell based production technologies

■ Producing a safe, reliable LAIV in cell culture

- ◆ Cell line selection
- ◆ Cell line testing results
- ◆ Manufacturing technology
- ◆ Product testing

■ Defined risk assessment of vaccine safety

■ Conclusions

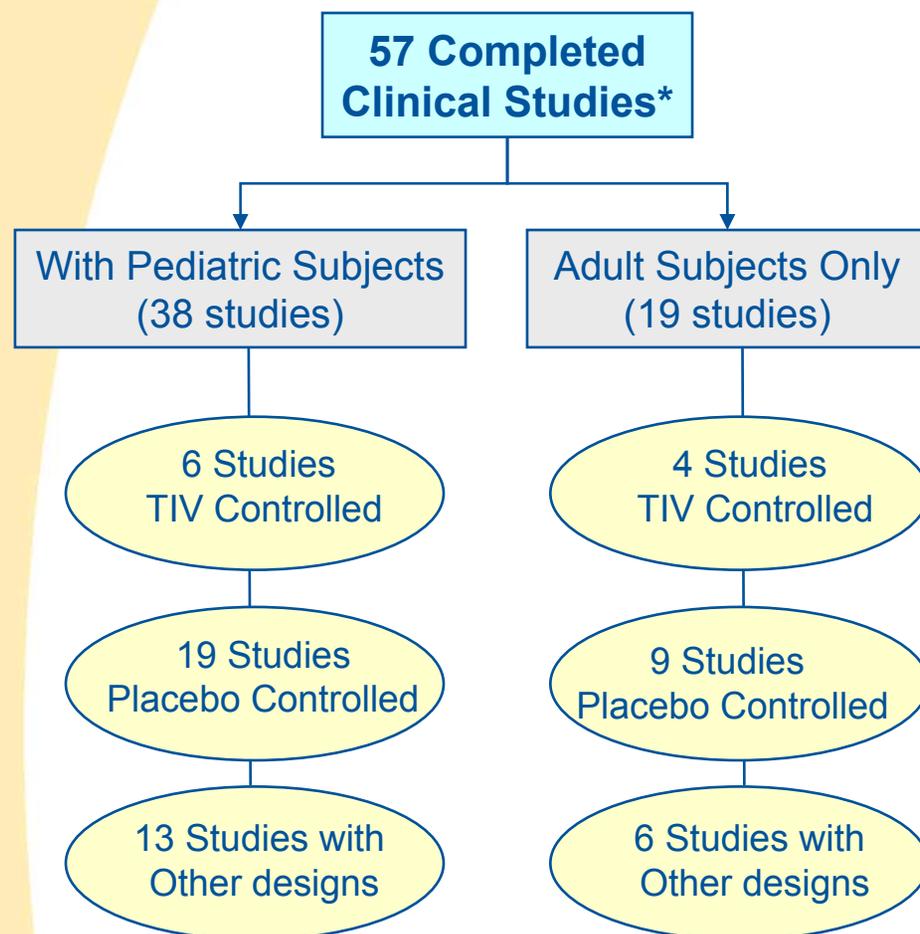
- Worldwide biologics unit for AstraZeneca
- Headquartered in Gaithersburg, MD
- Approximately 3,000 employees in the US, UK and the Netherlands
- FluMist[®] (LAIV) licensed in the US since 2003
 - ◆ Safety profile supported by nearly 11 million commercial doses distributed

LAIV is an Important Component of Influenza Prevention and Pandemic Preparedness

- Established efficacy against seasonal influenza
 - ◆ Cross protection against mismatched strains*
- Strong immune responses seen after a single dose in immuno-naive populations
- Considerable manufacturing efficiency
 - ◆ Live vaccine produced in either eggs or cells requires lower manufacturing capacity than inactivated vaccine
- Innovative intranasal delivery

* FluMist PI; June 2008

FluMist Shown to be Highly Efficacious in Both Adults and Children



FluMist efficacy demonstrated:

- In both adults and children
- Across multiple influenza seasons
- Through trials conducted worldwide

* Company sponsored studies. Sponsors included Aviron, Wyeth and MedImmune

Benefits of Cell-Based Production



	PRODUCTION SUBSTRATE	
	Eggs (SPF)	Cell Culture
Exposure of flock to environmental agents	Low risk, high impact	NA
Preproduction characterization	- Limited - Inherent contamination	- Extensive - Sterile
Manufacturing procedures	Need to control contamination *	Controlled
Egg allergies limit use	Yes	No

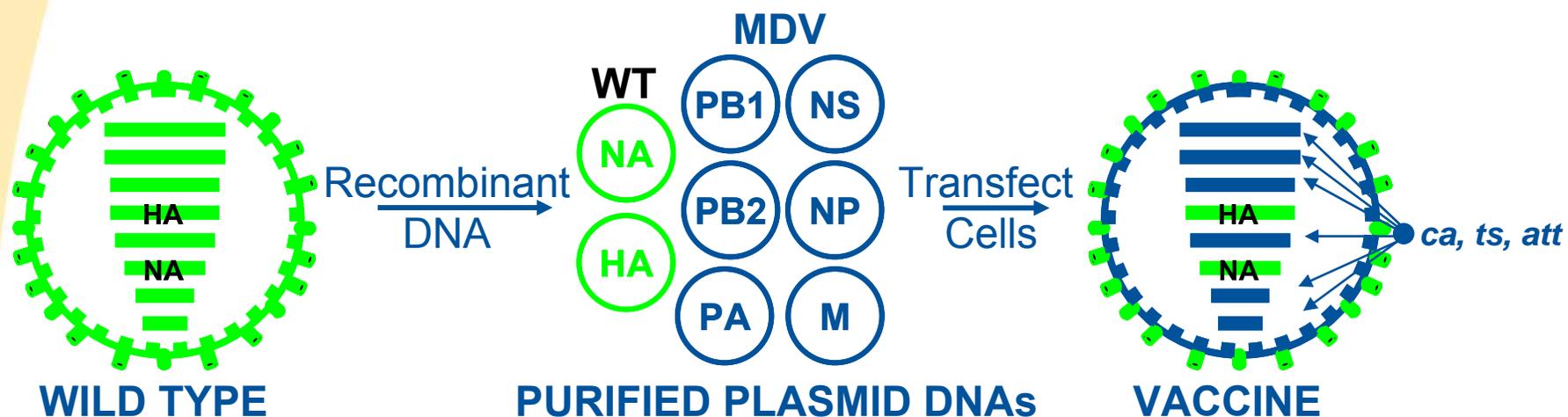
* Contributed to significant inactivated vaccine shortages in 2004/05 season

Cell Culture Production is More Scalable Than Eggs

- Larger quantities of bulk doses are produced more rapidly than in eggs
 - ◆ To produce 150 million bulk doses of vaccine
 - > 12 weeks in eggs
 - > 4 weeks with 2 (2,500L) bioreactors
- Increasing scale is faster than eggs
 - ◆ 12 months needed to increase size of chicken flock & egg production
 - ◆ Scale is limited by availability of number of bioreactors

Plasmid Rescue Eliminates AVA Risks

- LAIV vaccines are 6:2 reassortants
- The internal genes of cell and egg produced vaccines are genetically identical



- Plasmid rescue of 6:2 vaccine strains is part of the current egg produced FluMist® product

Plasmid rescue eliminates the risk from any potential contaminants in the wild type (human) isolate

Vaccines Produced Using Egg and Cell Substrates Are Comparable

- Vaccine traits are encoded in the sequence of the vaccine strains
 - ◆ Cell and egg produced strains are genetically identical

- The *ca* and *ts* characteristics are retained that make the vaccine safe

Analytical Test	Comparability between egg and cell produced vaccine
Complete Genomic Sequence	✓
Phenotypic Analysis (<i>ca</i> and <i>ts</i>)	✓
Host Cell Susceptibility	✓
Virus Protein Expression	✓
Virus Morphology and Size	✓
Replication and Attenuation in Ferrets	✓
Immunogenicity and Efficacy in Ferrets	✓
Safety profile in Animal Models	✓

Cell Culture Production of LAIV is a Significant Advance for Public Health

- Cell culture produced LAIV is an important component of influenza prevention and pandemic preparedness
 - ◆ Increases reliability of supply of influenza vaccines
 - ◆ Accelerates speed and quantity of vaccine supply
 - ◆ Retains all the advantages of LAIV

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Considerations for Selecting a Safe Cell Line

- Readily characterized to assure product safety
 - ◆ No evidence of inherent oncogenic agents
- Supports replication of different influenza serotypes and strains
- Consistent cell growth and high virus productivity at large scale production
- Grows in serum-free media

MDCK Cells Outperformed All Others

- Of the 13 cell substrates assessed, only MDCK cells had all the requisite characteristics for manufacturing LAIV.
 - ◆ MRC-5, WI-38; human diploid cells used for other vaccines
 - ◆ 293, CHO, FRhL-2, MDCK, NIH 3T3, Vero and other mammalian continuous cell lines
 - ◆ CEF, CEK, DF-1 and other avian cell lines

MDCK Cell Substrate History

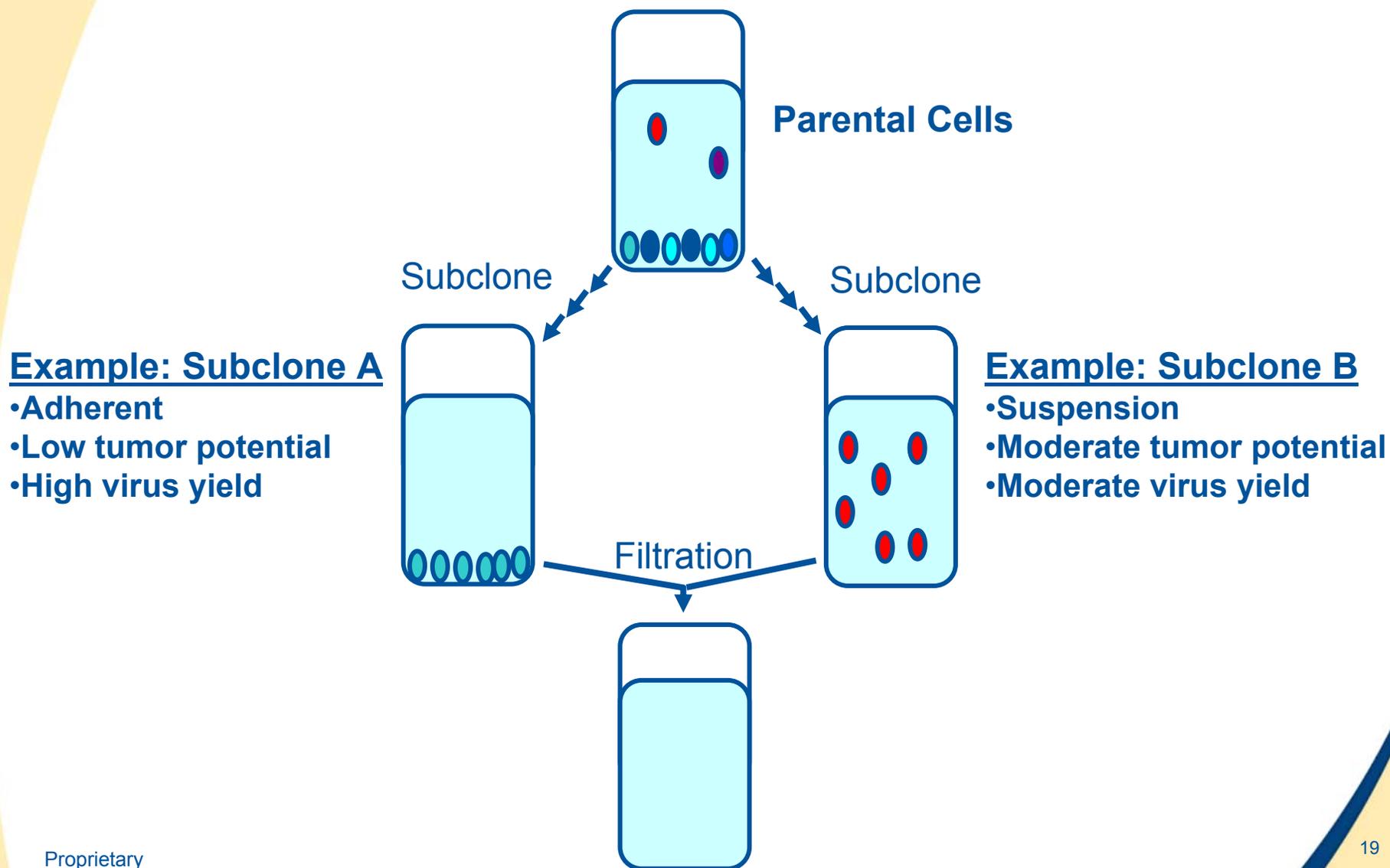
- 1958 - Madin-Darby canine kidney (MDCK) cell line was derived from the kidney of a normal cocker spaniel
- 1964 - Deposited at American Type Culture Collection (ATCC CCL-34)
- 2001 - MedImmune obtained cells from ATCC CCL-34 for preparation of pre-Master Cell Bank

MDCK Cells Contain Different Subpopulations

- Subclones with differing biochemical properties can be isolated
- Tumorigenicity from different sources are variable

	Minimum number of cells needed to form tumors in nude mice	Notes
Stiles, et al (1976)	$>10^6$	No tumors detected
Percheson, et al (1999)	$>10^7$	No tumors detected
Solvay (VRBPAC 2005)	$>10^7$	ATCC parent cells
Solvay (VRBPAC 2005)	10^5	Solvay cell bank
Novartis (VRBPAC 2005)	10^1	Suspension cells

Biological Cloning Isolates Subclones with Specific Properties



Steps to Isolate a Uniform MDCK Cell Line with Low Tumorigenicity

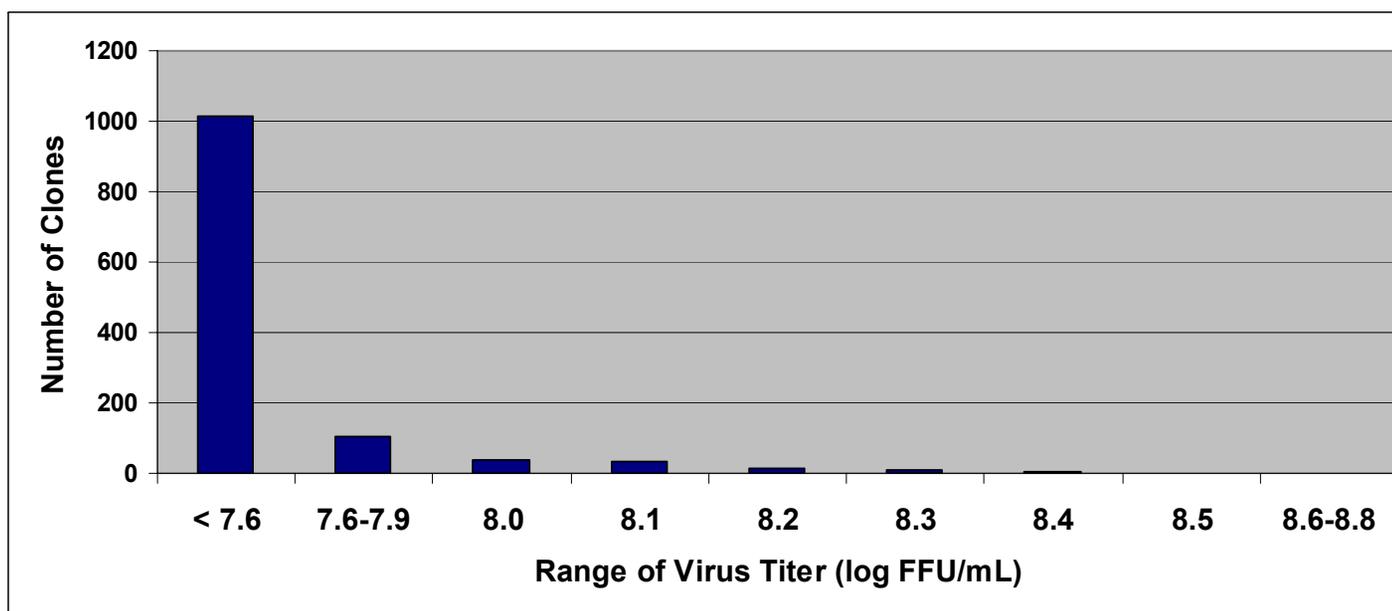
- Tumorigenicity of MedImmune's MDCK cell line controlled by focusing on 3 key areas
 - ◆ Clonal isolation of a cell line
 - ◆ Contact inhibited growth
 - ◆ Use of a robust serum-free growth media

Quality Built into the Cell Bank From Multiple Aspects

- Obtain low passage MDCK cells from ATCC
- Biologically clone the cells by limiting dilution
 - ◆ Establish a uniform population from a single genetic parent
 - ◆ Evaluate and choose a clone which supports vaccine strain replication
 - ◆ Enable tracking of exposure of cells to animal derived products
- Transfer cells to serum-free media
 - ◆ Elimination of exposure to adventitious agents from animal derived products
- Produce cell banks in compliance with cGMP

Adherent Subclone Chosen with High Productivity

- A limited number of cell clones supported higher levels of virus productivity



Productivity stable over 25 passages

- One clone was subsequently expanded in serum free media
- Master and working cell banks produced under cGMP in serum-free media

Assessing Potential Risks of Using MDCK Cell Lines Through Testing

Potential risks

- Adventitious agents
- Tumorigenicity
 - ◆ Evaluating whether intact cells can establish a tumor
- Oncogenicity
 - ◆ Evaluating whether cellular components can induce tumors

Assessing Potential risks

- in vitro and in vivo testing for specific and general agents
- Evaluate tumorigenicity in nude mice
- Evaluate oncogenicity of MDCK DNA and cell lysate in multiple rodent species

Based on CBER's approach for continuous cell lines

Extensive Testing Did Not Detect Adventitious Agents in MedImmune's MDCK Cells

- General tests (broad detection assays)
 - ◆ Sterility, mycoplasma, mycobacterium
 - ◆ in vivo safety
 - > Newborn mice, adult mice, guinea pigs and embryonated eggs
 - ◆ in vitro safety
 - > MRC-5, Vero, MDCK, MDBK, HeLa, BHK-21 & RK-13

Additional Testing Did Not Detect Adventitious Agents in MedImmune's MDCK Cells

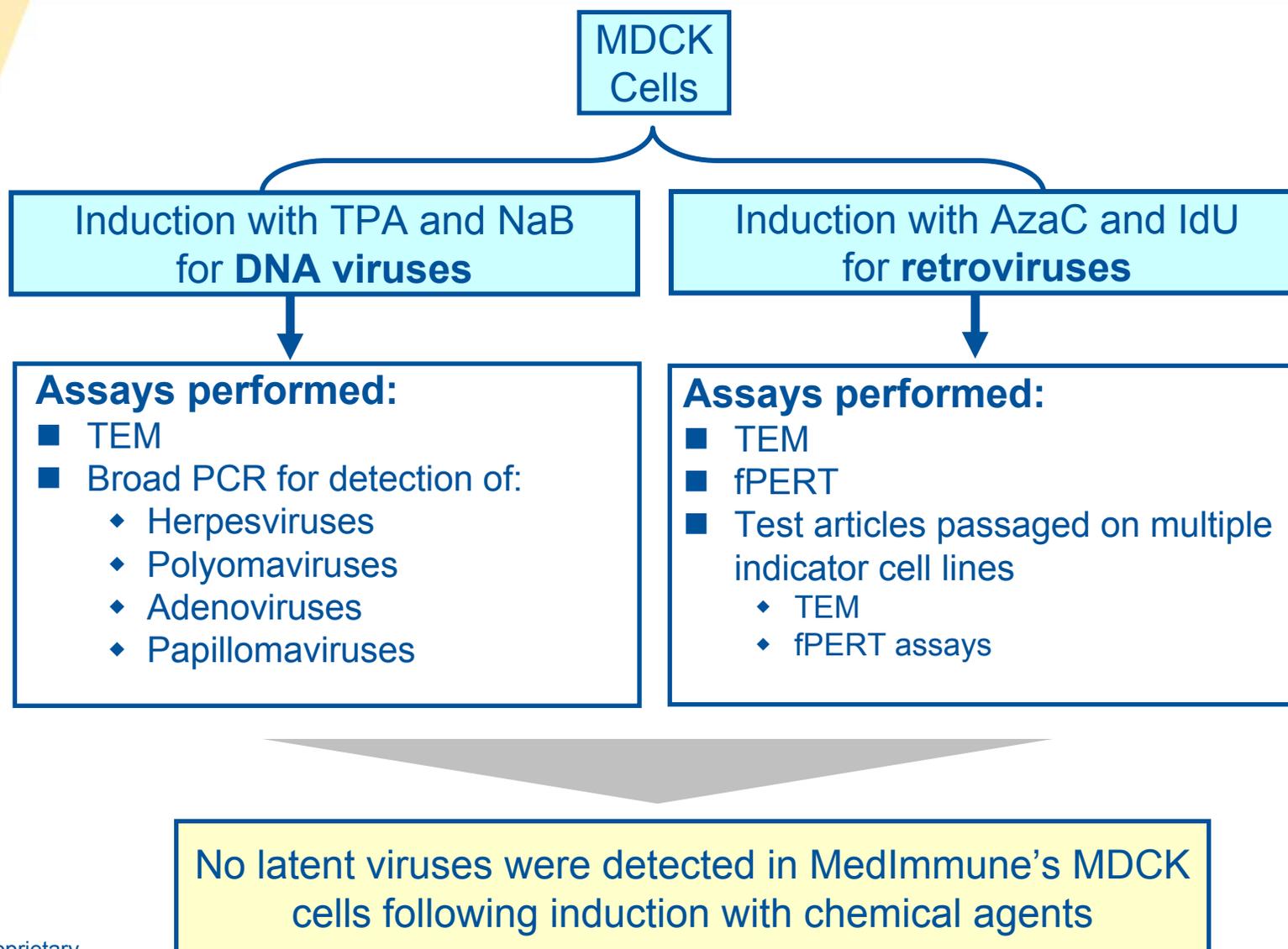
■ Specific tests (targeted agents)

- ◆ Over 30 PCR and other tests
- ◆ Human, simian, canine, rodent, equine, and porcine agents

■ Induction studies

- ◆ MDCK cells induced with chemical agents
- ◆ Cells evaluated for the presence of latent RNA and DNA viruses

Induction Studies Look for Latent Viruses in the Cell Line



MedImmune's MDCK Cells Contain No Detectable Adventitious Agents

- Multiple testing strategies employed
- Conclusion
 - ◆ No evidence of adventitious agents detected in MedImmune's MDCK cells

Extensive Tumorigenicity & Oncogenicity Tests of MedImmune's MDCK Cells

■ Studies - overview

- ◆ MDCK EOP cells (approximately 3 passages beyond manufacture of vaccine)
- ◆ Approximately 40 animals per group
- ◆ Observed for 6 months
- ◆ Conducted in compliance with GLP requirements

■ Tumorigenic potential of intact MDCK cells

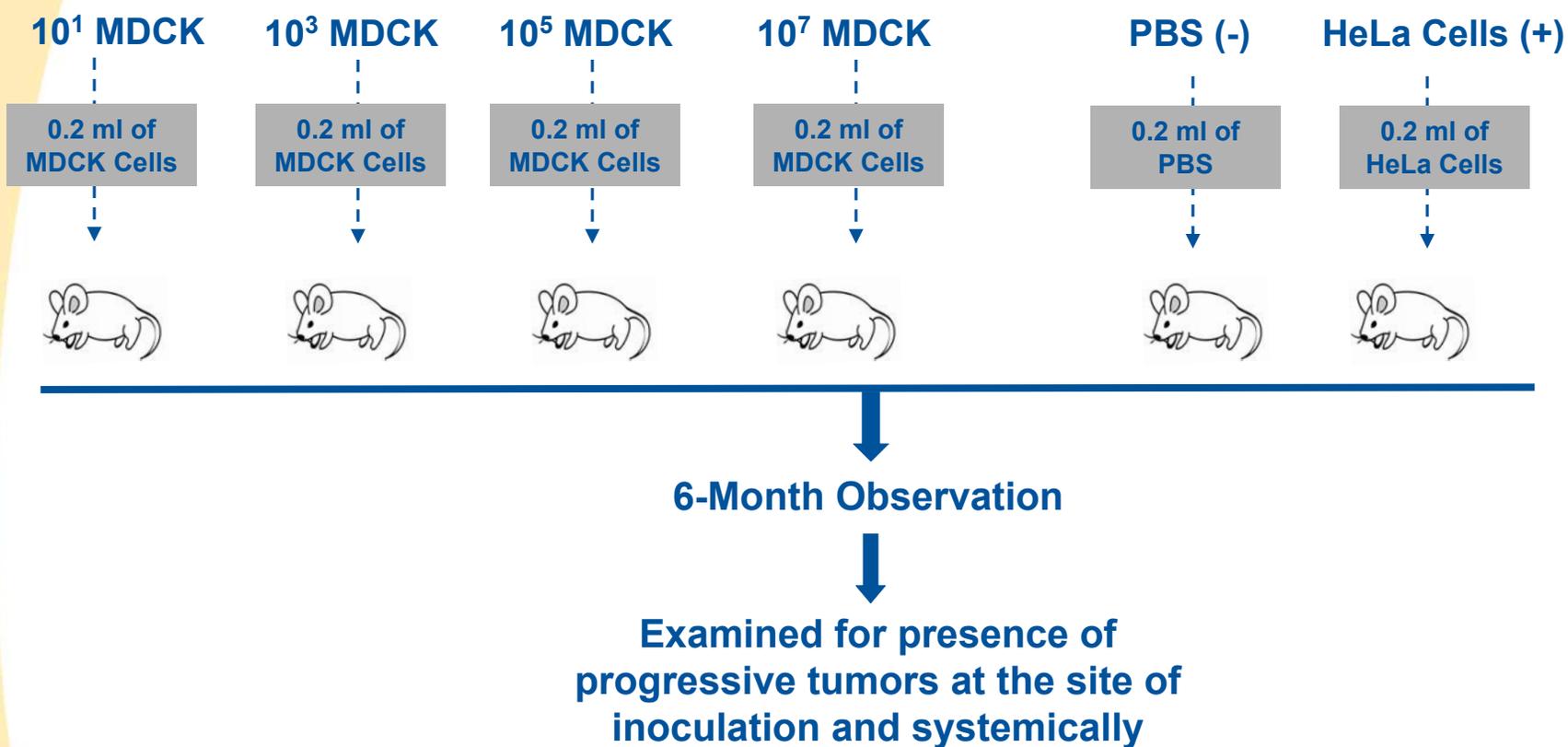
- ◆ Adult athymic nude mice
- ◆ Newborn athymic nude mice

■ Oncogenic potential of MDCK cell lysate and cell DNA

- ◆ Newborn rodents (athymic nude mice, rats & hamsters)

MDCK Cell Substrate Characterization – Tumorigenicity Testing

Methods: Adult & Newborn Nude Mouse Models



MedImmune's MDCK Cells Do Not Form Tumors in Nude Mice

Study	Test Sample	Number of animals injected	Tumors at site of Injection (SOI)	Tumors at other locations
Tumorigenicity (Adult Mouse)	Negative Control (DPBS)	33	0	2 ^a
	Positive control (10 ⁷ HeLa cells)	41	37	0
	MDCK cells (10 ¹ , 10 ³ , 10 ⁵ , 10 ⁷ ; 44/grp)	176	0	1 ^b
Tumorigenicity (Newborn Mouse)	Negative Control (DPBS)	43	0	0
	Positive control (10 ⁷ HeLa cells)	44	44	0
	MDCK cells (10 ¹ , 10 ³ , 10 ⁵ , 10 ⁷ ; ~44/grp)	171	0	0

^a Lymphoma & a bronchiolo-alveolar adenoma: spontaneous murine tumors

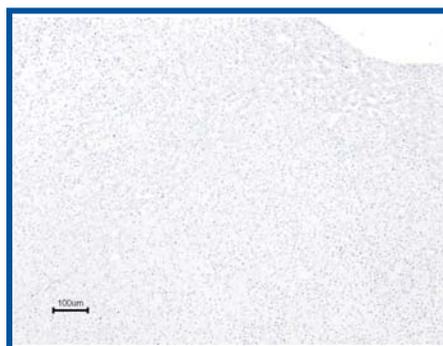
^b Histiocytic sarcoma (10⁵ MDCK group): spontaneous murine tumor confirmed by antibody staining and SINE PCR

No MDCK tumors detected

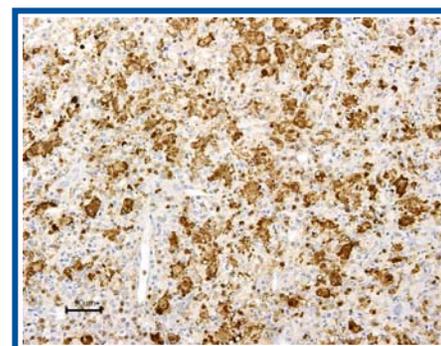
Immunohistochemistry Confirmed Murine Origin of Tumor

Histiocytic Sarcoma, Liver
(Tumor study, 10^5 group)

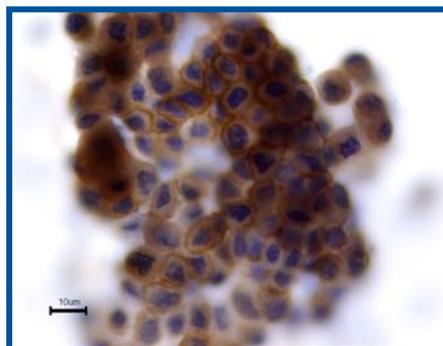
α Canine Ezrin



α Murine Galectin-3



Control MDCK cell pellet



SINE PCR Confirmed Murine Origin of Tumor

- Canine-specific short interspersed nuclear elements (*Can-SINE*)*
 - ◆ 130 - 150 bp
 - ◆ Present approximately every 5 - 8.3 kb in the canine genome
 - ◆ Constitute about 1.8 - 3% of the genome

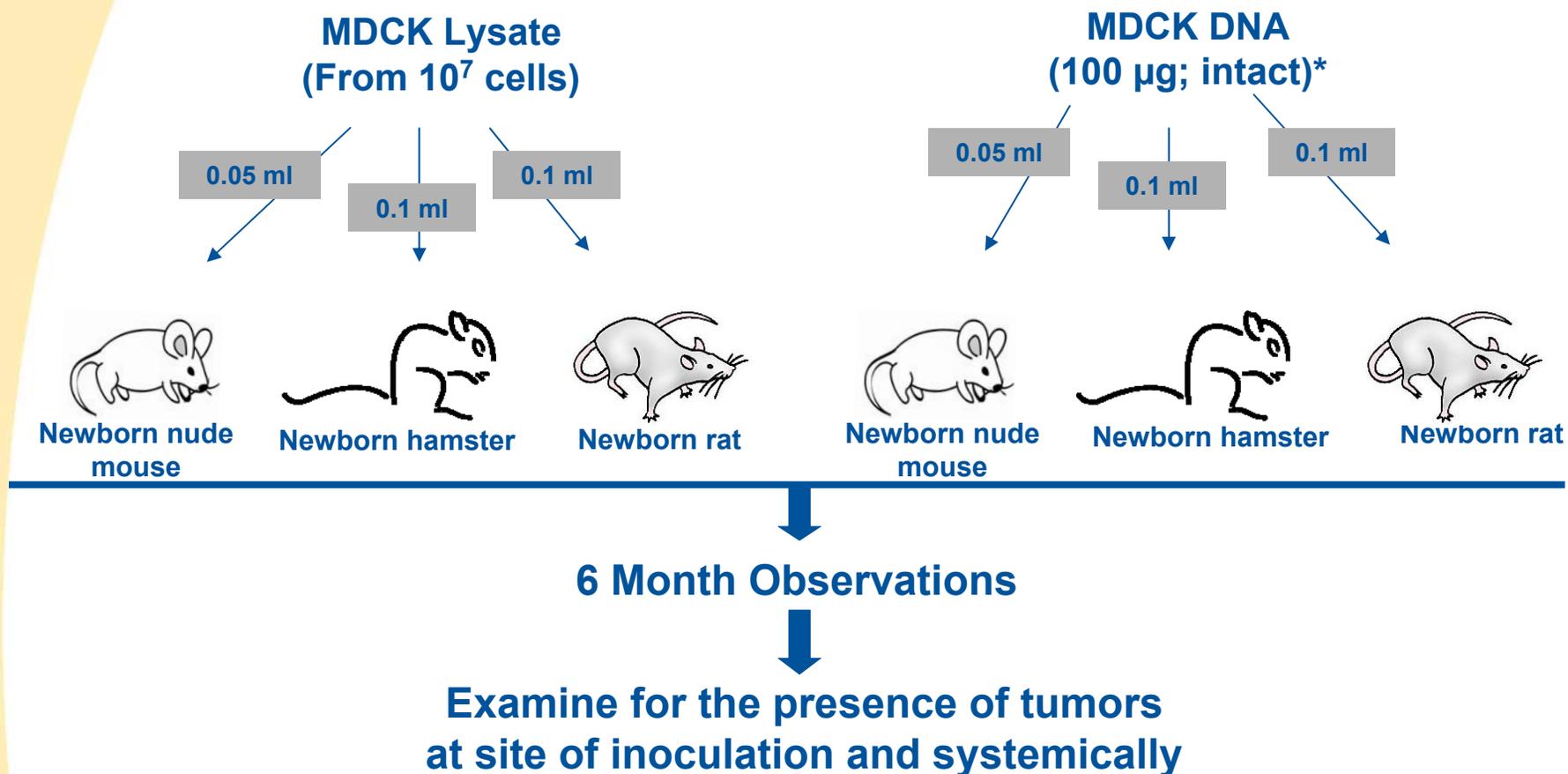
*Das M et al. (1998) *Mamm Genome* **9**, 64–69

- Observation of histiocytic sarcoma

	Canine	Rodent
SINE PCR	—	+

Spontaneous Murine Tumor—Not MDCK Derived

MDCK Cell Substrate Characterization – Oncogenicity Testing



MedImmune's MDCK Cells Do Not Contain Oncogenic Components

Groups	MDCK Lysate (10 ⁷ cell equiv)			MDCK DNA (100 µg)			Tumor incidence
	Mice	Hamster	Rat	Mice	Hamster	Rat	
Non Injected (n=25)	1 ^a	0	0	0	0	0	1/150 (0.6%)
PBS (n=45)	0	0	0	0 ^b	0	2 ^c	2/269 (0.7%)
Test (n=45)	0	0	1 ^d	1 ^a	1 ^e	0	3/270 (1.1%)

^a Bronchiolo-alveolar adenoma in the lung; spontaneous tumor / no canine DNA by SINE; confirmed rodent origin

^b Only 44 animals were available for post-weaning randomization in this group

^c Hepatocellular adenoma & skin hemangiosarcoma; spontaneous tumors

^d Hind leg carcinoma; no canine DNA by SINE; confirmed rodent origin

^e Nephroblastoma; no canine DNA by SINE; confirmed rodent origin

No oncogenicity detected

No Oncogenicity Detected in MedImmune's MDCK Cells

- All tumors were of rodent origin; no canine DNA detected
- Tumors observed in these studies were spontaneous and observed in other studies in these species
- Balanced frequency between negative control groups and test article groups

Summary – Extensive Data Demonstrates Safety of MedImmune’s MDCK Cells

■ Adventitious Agent Testing

- ◆ No evidence of adventitious agents by comprehensive testing regimen

■ Tumorigenicity

- ◆ No evidence of local or systemic tumorigenicity (up to 10^7 cells)

■ Oncogenicity

- ◆ No evidence of local or systemic oncogenicity caused by MDCK cellular components (cell DNA or cell lysate)

Addressing Potential Risks of Using MDCK Cell Lines Through Manufacturing

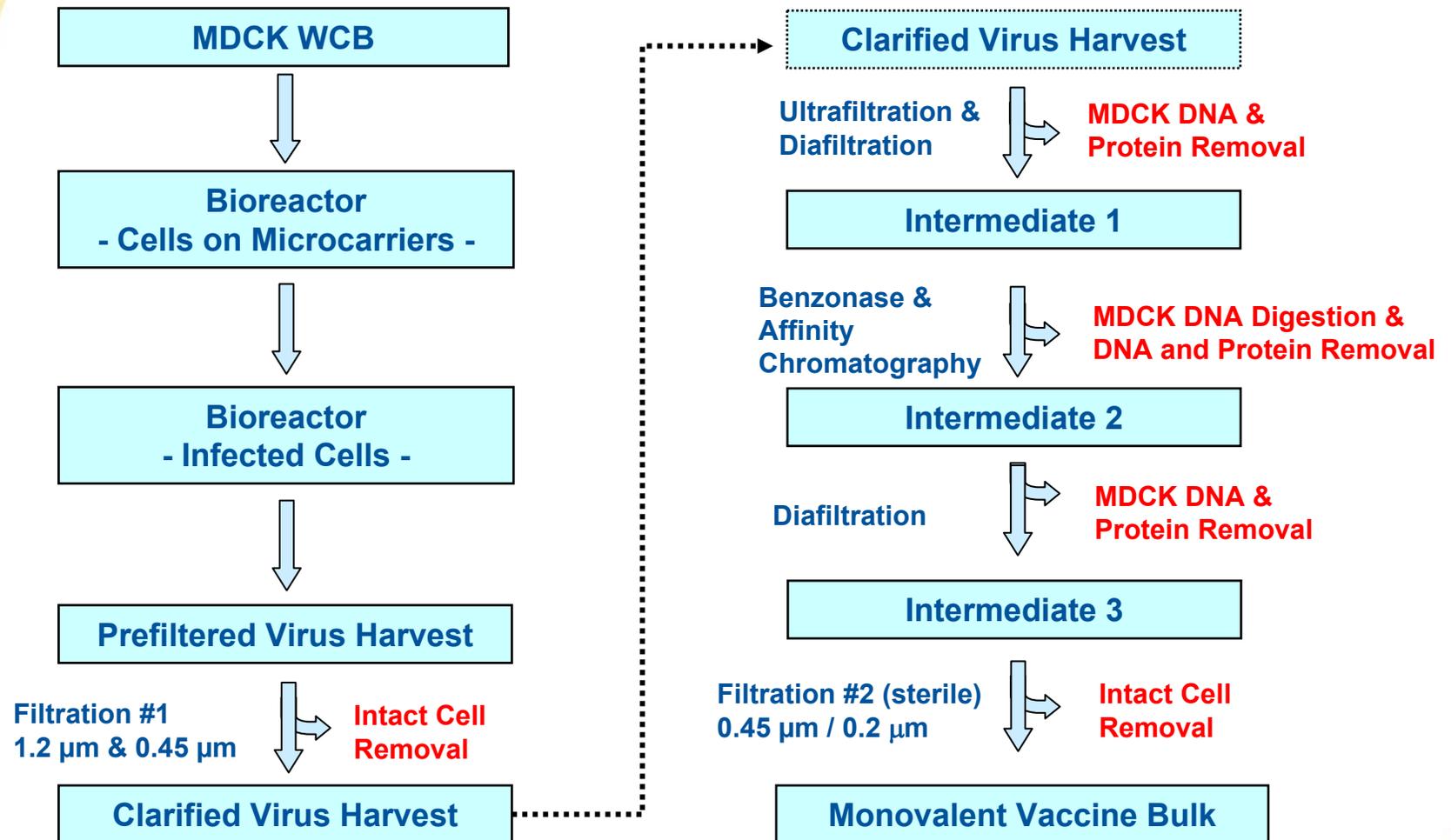
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Addressing Potential risks

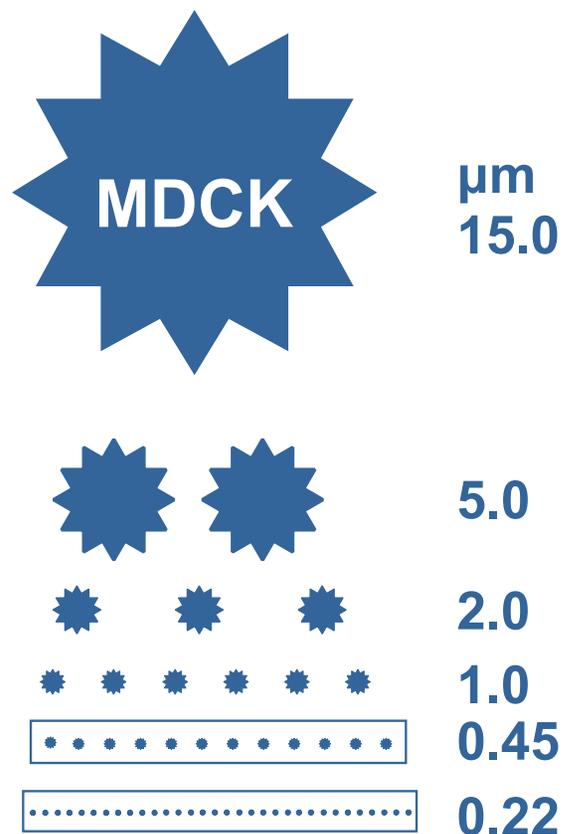
- Control through plasmid rescue and manufacturing processes
- Removal of all cells through multiple filtration steps
- Reduce quantity and size of residual MDCK DNA and quantity of MDCK proteins

Manufacturing Process Removes MDCK Components at Multiple Steps



Filtration Removes Intact Cells

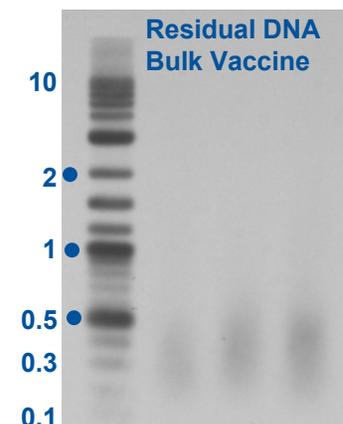
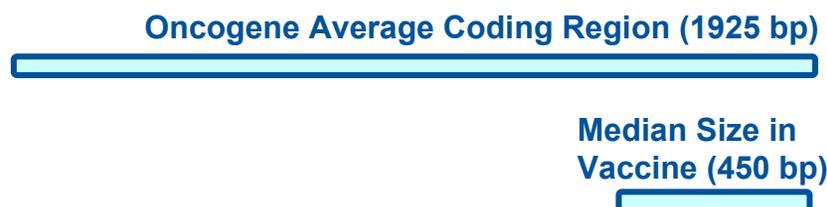
- Removal of intact cells occurs at multiple steps
- Process capable of removing at least 10^{21} cells
 - ◆ This represents 100 billion times more cells than in a typical bioreactor
 - ◆ Laboratory studies demonstrate capabilities of the filters
- Multiple filtration steps ensure safety of the process



Multiple Steps Reduce the Quantity of MDCK DNA and Protein

■ Reduction of MDCK Quantity and Size

- ◆ Multiple steps combine to remove >90% of MDCK DNA
- ◆ One dose contains less than 1 ng of MDCK DNA
 - > WHO recommends a 10 ng limit for parenteral products produced from continuous cell line substrates
- ◆ Median size is reduced to 450 bp
- ◆ 90% DNA below 1 kb

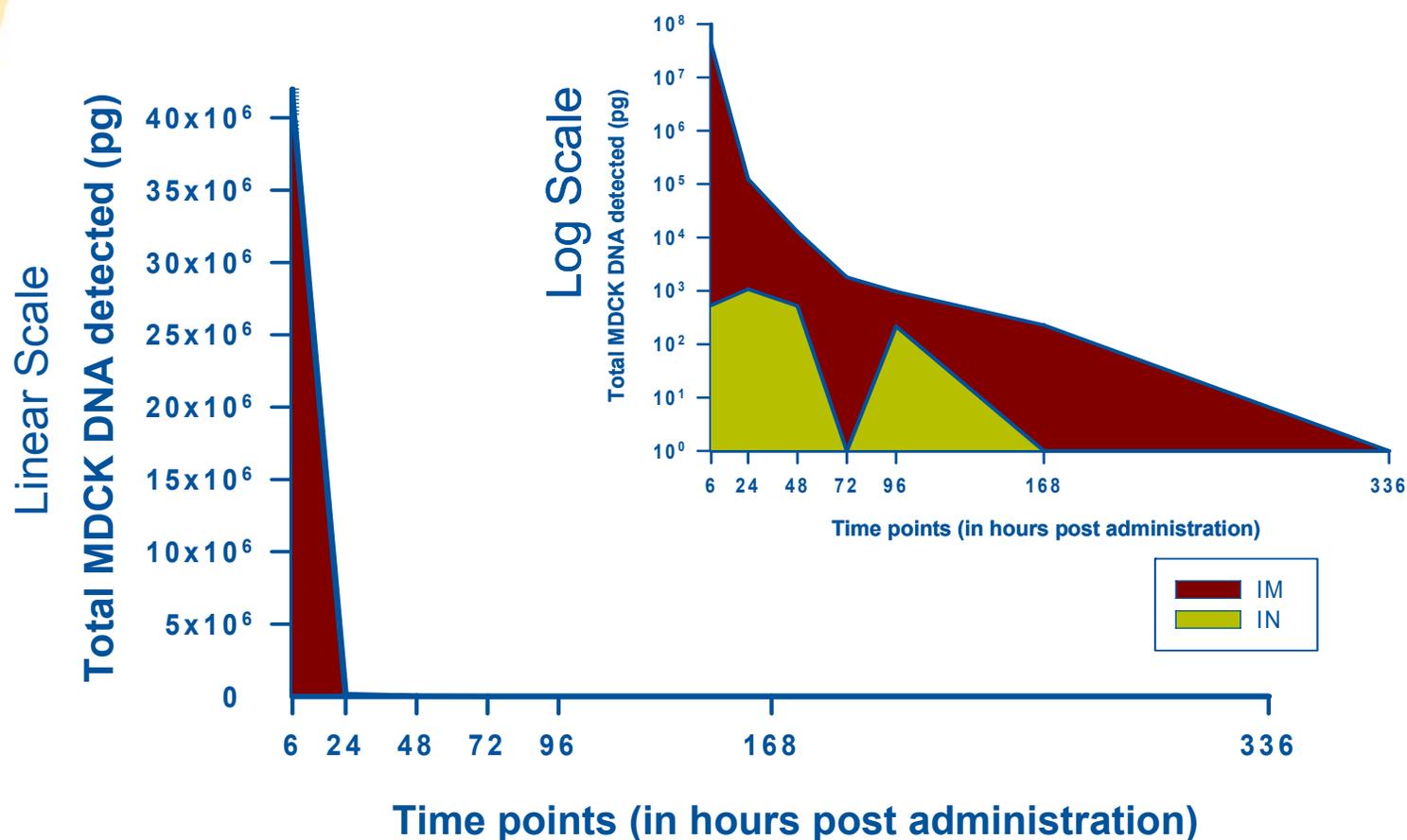


■ Reduction of host cell protein

- ◆ Removes >90% of MDCK protein
- ◆ One dose contains approximately 0.5 µg of MDCK protein

Route of Administration May Add to Safety of Vaccine - MDCK DNA Clearance Study

- Rats were given equivalent amount (100 µg) of sheared MDCK DNA
- Tissues measured for residual DNA at various time points
- Route of vaccine administration (intranasal) provides an additional safety barrier



The Bulk Vaccine is Extensively Tested to Ensure Safety and Purity

Pre-filtration

- Mycoplasma
 - ◆ Microbiological Culture
 - ◆ Cell Culture
- in vitro Adventitious Agents
 - ◆ Neutralize influenza
 - ◆ Multiple indicator cell lines
- in vivo Adventitious Agents
 - ◆ Neutralize influenza
 - ◆ Multiple species of sensitive host systems
- Potency
- Bioburden

Post-filtration

- Sterility
- Potency
- Genotype
- Phenotype
- Attenuation
- Endotoxin
- pH
- Color and Appearance
- Residual Host Cell DNA
- Residual Host Cell Protein
- Residual Benzoylase
- Osmolality

Manufacturing Process Ensures Production of Safe Vaccines

- Control of all materials
 - ◆ Minimal exposure to animal derived components
 - ◆ Highly characterized MDCK cell banks
 - ◆ Highly characterized vaccine seeds

- Production equipment and environment
 - ◆ Closed systems; isolated from the environment

- Multiple purification steps ensure safety of the product
 - ◆ Removes all intact cells
 - ◆ Reduces the quantity and size of DNA
 - ◆ Reduces host proteins
 - ◆ Sterile filtration

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■ Conclusions

Applying a Defined Risk Assessment Approach

- A defined risk assessment was employed based on CBER guidelines (and in line with other manufacturers) addressing theoretical concerns associated with:
 - ◆ Intact cells
 - ◆ Oncogenicity
 - ◆ Infectivity

- Reinforces product safety assurance

(Lewis AM Jr, Krause P, Peden K. A defined-risks approach to the regulatory assessment of the use of neoplastic cells as substrates for viral vaccine manufacture. Dev Biol (Basel). 2001;106:513-35.)

■ Observations

- ◆ Modern processes remove intact cells from the product
 - > Filtration removes at least 21 log cells

■ Calculation of safety margin

- ◆ Theoretical cells in one dose (no removal or lysis of cells): 5.6 log
Clearance factor – cell number = safety margin
21.4 log – 5.6 log = 15.8 log margin of safety

■ Conclusion

- ◆ Risk of one dose containing an intact MDCK cell is 1.6×10^{-16}
- ◆ Only 1 out of 6.3 quadrillion (6.3×10^{15}) doses may contain a single intact MDCK cell
 - > This value represents the risk that one person would receive a cell-containing dose if the entire world population were immunized every 50 minutes for 100 years
- ◆ MedImmune's MDCK cells demonstrate low tumorigenic potential

Risk Assessment Due to Residual DNA Oncogenicity Overview

- Oncogenicity assessment – quantitative modeling uses conservative assumptions
 - ◆ Need to assume an active oncogene in genomic DNA
 - > Animal data demonstrated no oncogenicity in MDCK DNA
 - ◆ Quantitation extrapolated from conservative assumptions
 - > 1 ng of oncogenic plasmid DNA elicits tumors in nude mice
 - ◆ Determine how much genomic DNA would be needed to deliver the same oncogene dose as 1 ng of the plasmids

- Safety factor calculation – what does it tell us
 - ◆ Based on a conservative amount of MDCK DNA in one dose (1 ng)
 - > How many doses of vaccine would equal the oncogene dose in the mouse experiments?
 - > What is the oncogenicity risk in one single dose?
 - ◆ Assess quantitative impact of DNA digestion

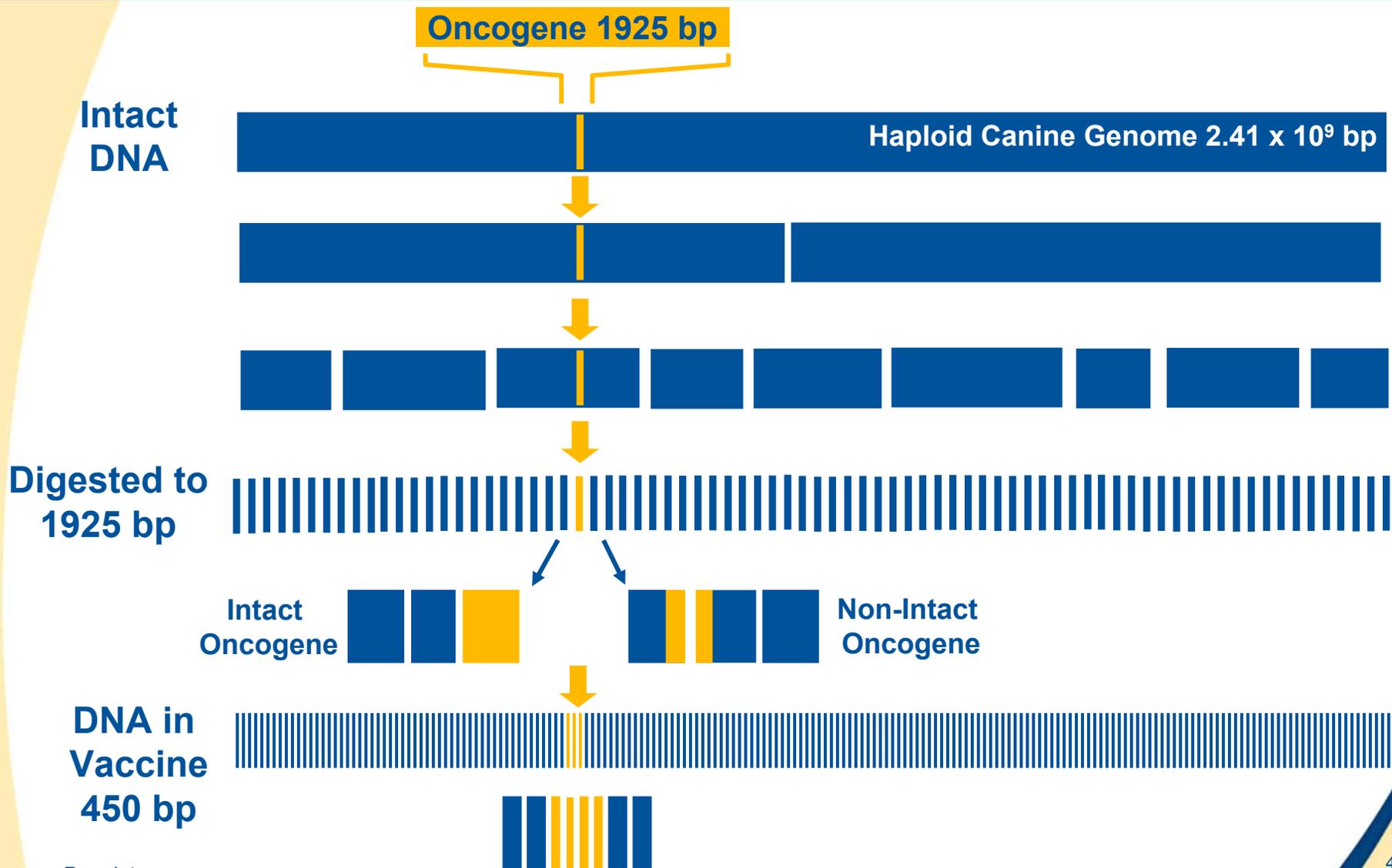
Risk Assessment Evaluation of Oncogenicity of DNA

- *If* cellular DNA contained an active oncogene it would take over 1 million doses to deliver the oncogenic dose used in the mouse studies
 - ◆ It would take over 5 billion doses assuming 25 µg of plasmid was required*

*Sheng L, Cai F, Zhu Y, Pal A, Athanasiou M, Orrison B et. al. Oncogenicity of DNA in vivo: Tumor induction with expression plasmids for activated H-ras and c-myc. *Biologicals*. 2008;36(3):184-97.

Benzonase Digestion Genomic DNA

Oncogene Sequence Intact or Non-Intact



Digestion of DNA Adds Significant Assurance of Safety

- Calculate the safety factor including DNA digestion
 - ◆ Model worst case scenario 1 ng of MDCK DNA digested to 1925 bp in length
 - ◆ Approximately 1 in every 2000 oncogene fragments is intact

- Final Safety Factor for oncogenicity of residual MDCK DNA
 - ◆ The risk of an oncogenic event in one dose is 4.2×10^{-10}
 - ◆ The safety factor is 2.4×10^9
 - > It would take over 2 billion doses of vaccine to deliver the dose administered to one mouse (equals over 400,000 L of vaccine)

Risk Assessment of Potential Infectivity in Residual DNA

- Infectivity assessment – quantitative modeling uses conservative assumptions
 - ◆ Need to assume an active provirus in genomic DNA
 - > No evidence of infectious proviruses in MDCK DNA
 - ◆ Quantitation extrapolated from in vitro studies*
 - > 150 ng of HIV-1 cDNA in a plasmid results in infectious virus
 - > Degrading the DNA to a mean of 650 bp abolished infectivity
 - ◆ Determine how much genomic DNA would be needed to deliver the same provirus dose as 150 ng of the plasmid

*Peden K, Sheng L, Pal A, Lewis A. Biological activity of residual cell substrate DNA. Dev Biol (Basel) 2006;123:45-56; discussion 55-73.

- Safety factor calculation – what does it tell us
 - ◆ Based on a conservative amount of MDCK DNA in one dose (1 ng)
 - > How many doses of vaccine would equal the provirus dose in the in vitro experiments
 - OR –
 - > What is the risk of infectivity from a provirus in genomic DNA in one single dose
 - ◆ Assess quantitative impact of DNA digestion

Risk Assessment Outcome of Infectivity of DNA

- Risk of a provirus in one dose is 1.3×10^{-12}
- Safety factor is 7.2×10^{11}
 - ◆ Extrapolation of in vitro data – no infectivity would be detected in at least 700 billion doses of vaccine

Addressing Potential Risks of Using MDCK Cell Lines

Testing

- **Adventitious agents**
 - ◆ General and specific tests on MDCK Cells
 - ◆ Tests of vaccine bulk

- **Tumorigenicity**
 - ◆ Adult nude mice
 - ◆ Newborn nude mice

- **Oncogenicity**
 - ◆ MDCK genomic DNA
 - ◆ MDCK cell lysate
 - ◆ Multiple species

Manufacturing

- **Adventitious agents**
 - ◆ Plasmid rescued seeds
 - ◆ Closed process
 - ◆ Multiple filtration steps

- **Tumorigenicity**
 - ◆ Multiple filtration steps remove intact cells

- **Oncogenicity**
 - ◆ Reduce DNA size and quantity
 - ◆ Reduce protein quantity

Risk Assessment

- **Adventitious agents**
 - ◆ Not applicable

- **Tumorigenicity**
 - ◆ 6.3×10^{15} times excess cell clearance

- **Oncogenicity**
 - ◆ Safety factor for DNA oncogenicity: 2.4×10^9
 - ◆ Safety factor for provirus infectivity: 7.2×10^{11}

MDCK Cell Culture-Produced LAIV

MedImmune's
MDCK cell
substrate

- No detectable adventitious agents
- Low tumorigenicity
- No detectable oncogenicity

+

MedImmune's
robust
manufacturing
process



- Acellular vaccine
- Reduction of DNA quantity & size
- Reduction of host protein
- Routine vaccine Testing

=

Safe & Reliable
MDCK Produced
LAIV

Cell Culture Produced LAIV is Safe and Fills a Need for Influenza Vaccine

- Safety of our products is MedImmune's primary focus
- Scientifically sound advance in influenza vaccine production
- Cell culture production increases the supply and reliability of vaccine
 - ◆ Seasonal flu impact
 - ◆ Pandemic preparedness



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