Immunogenicity and Efficacy of Live Attenuated Pandemic Influenza Vaccines: Preclinical Data

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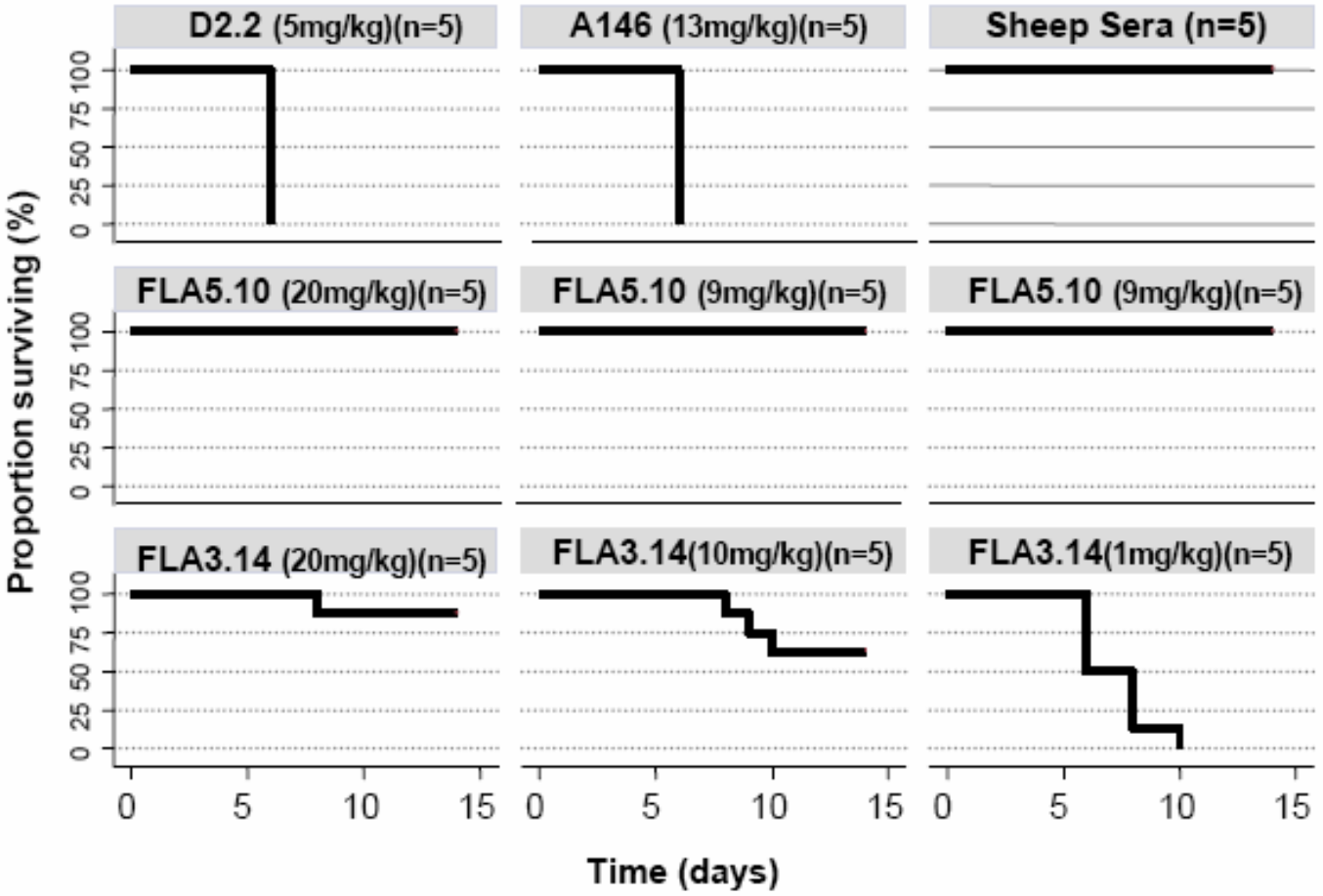
Efficacy of Immunoprophylaxis



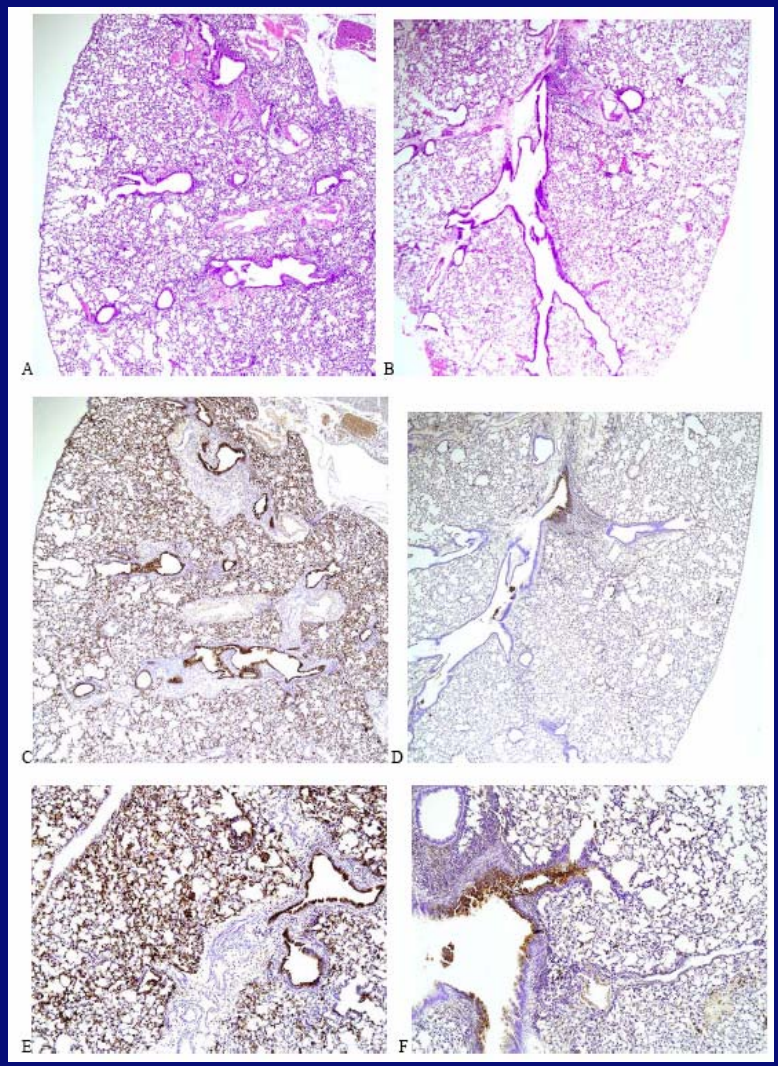
Histopathology in mice challenged with H5N1 virus following immunoprophylaxis

Irrelevant MAb D2.2

MAb D2.2

MAb D2.2

X100



H5 MAb FLA5.10

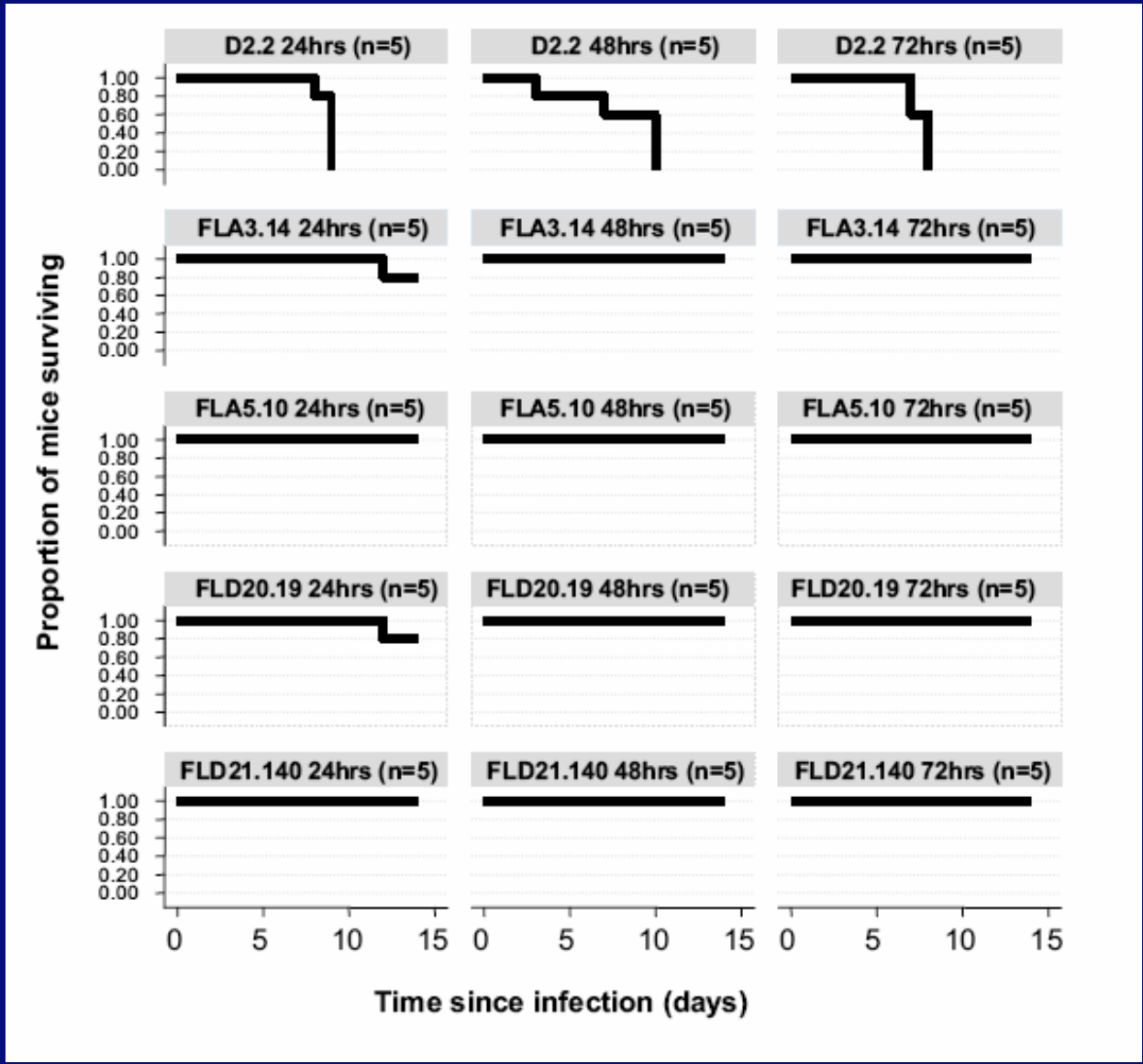
H5 MAb FLA5.10

H5 Mab

FLA5.10

X100

Efficacy of H5 Mabs administered 24, 48 and 72 h after infection with $5LD_{50}$ VN/04



Attenuation

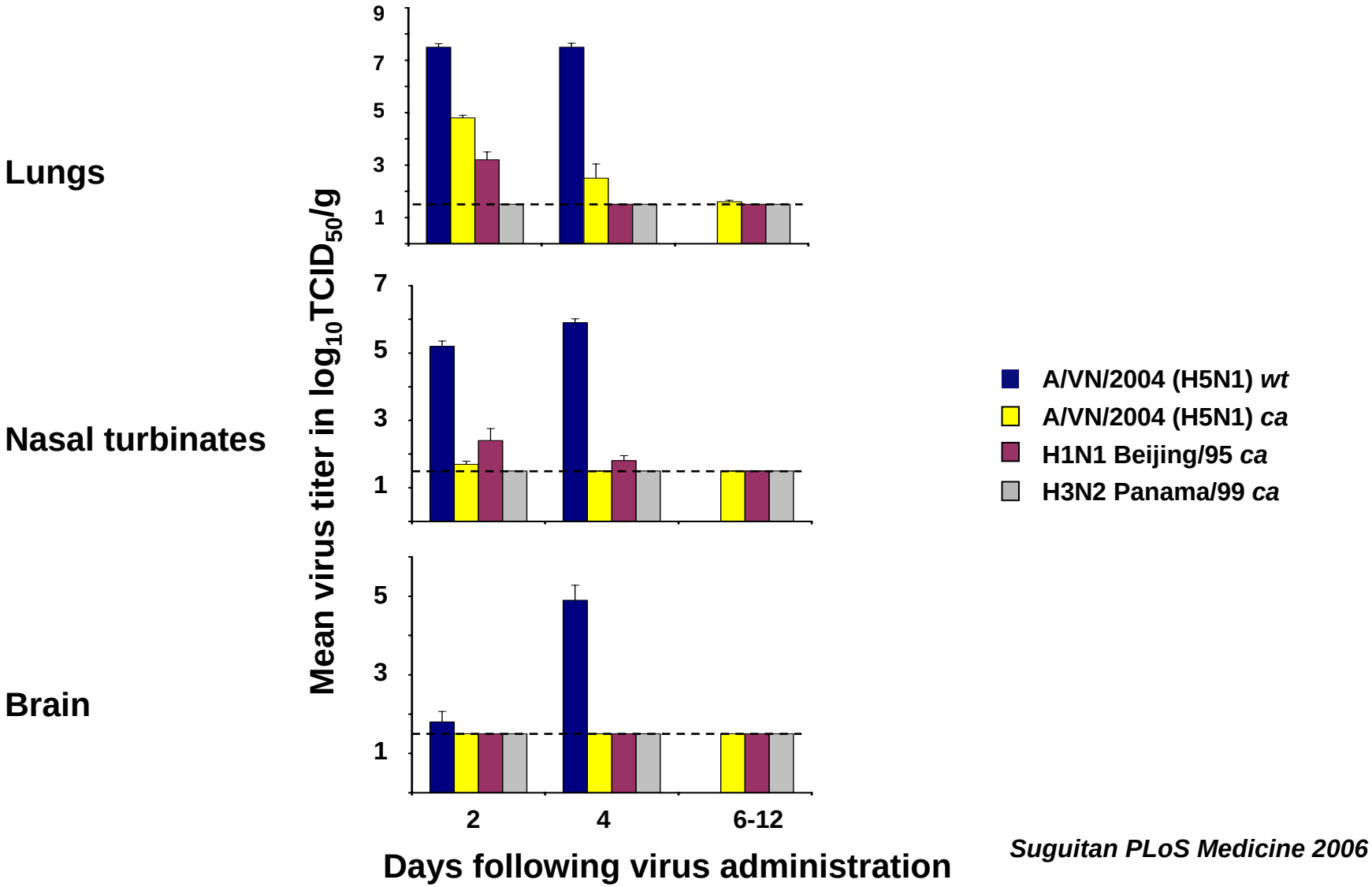
- In chickens:
  - if HA derived from HPAI
- In mice:
  - lethality (if relevant)
  - virus replication in respiratory tract
- In ferrets:
  - virus replication in the respiratory tract

The H5N1 ca Reassortant Viruses are not Highly Pathogenic for Chickens

Virus	# inoculated	# died	Mean time to death
1997, 2003 and 2004 H5N1 wt	8	8	1-2 days
1997 H5N1 ca	8	0	-
2003 H5N1 ca	8	0	-
2004 H5N1 ca	8	0	-

4-week-old SPF White Plymouth Rock chickens were inoculated intravenously with a 1:10 dilution of stock virus ($10^{8-8.75}$/ml) and observed for 10 days.

The 2004 H5N1 *ca* vaccine candidate is attenuated for mice and does not spread to the brain



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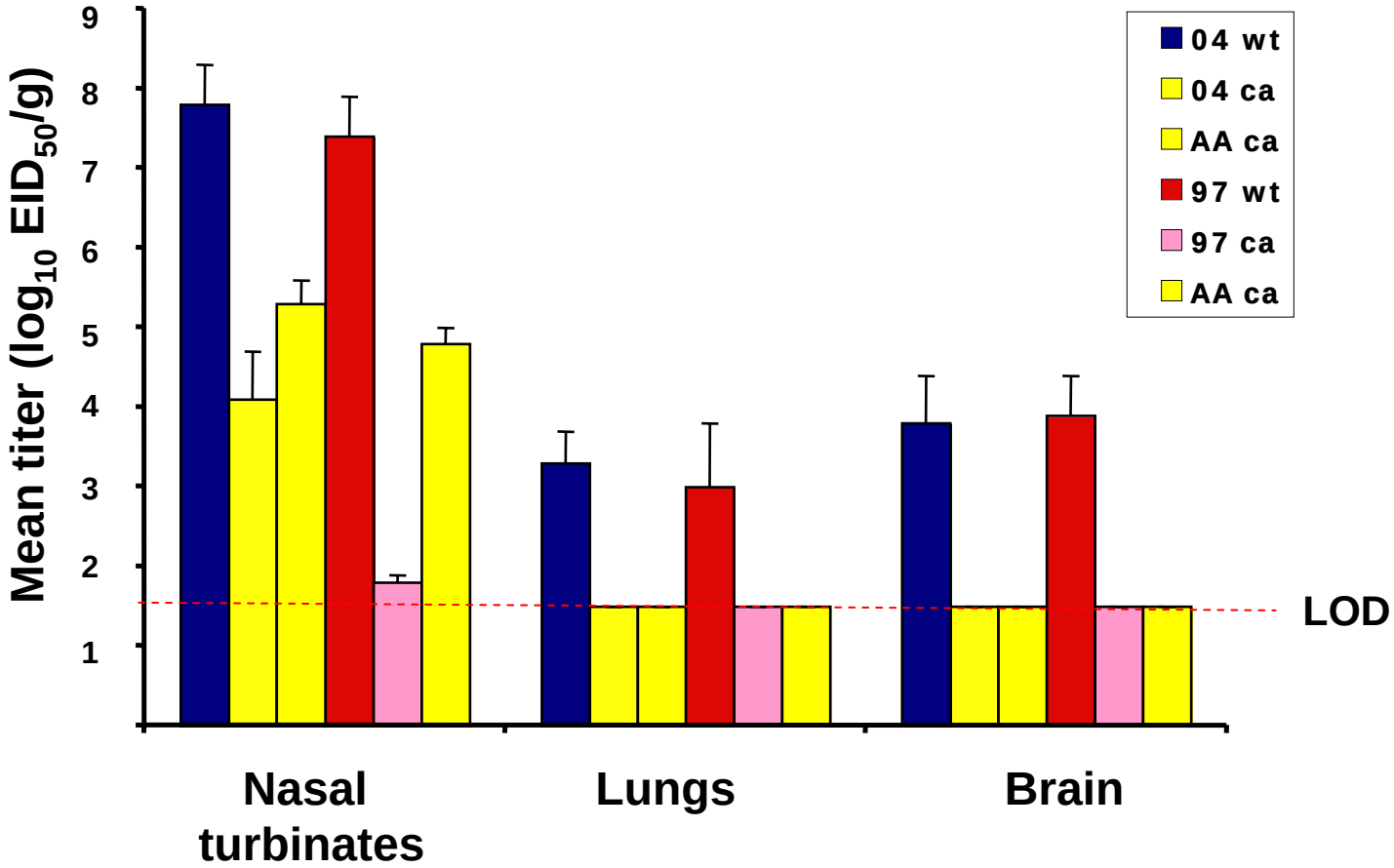
The 2004 and 1997 H5N1 ca viruses are attenuated in ferrets



10^7 $TCID_{50}$ inoculated i.n., tissues harvested on day 3 post-infection

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Immunogenicity

- In mice and in ferrets
- Following one dose or two doses
- Tested against homologous and heterologous viruses

A single dose of H5N1 *ca* vaccine fails to elicit serum neutralizing Abs in mice

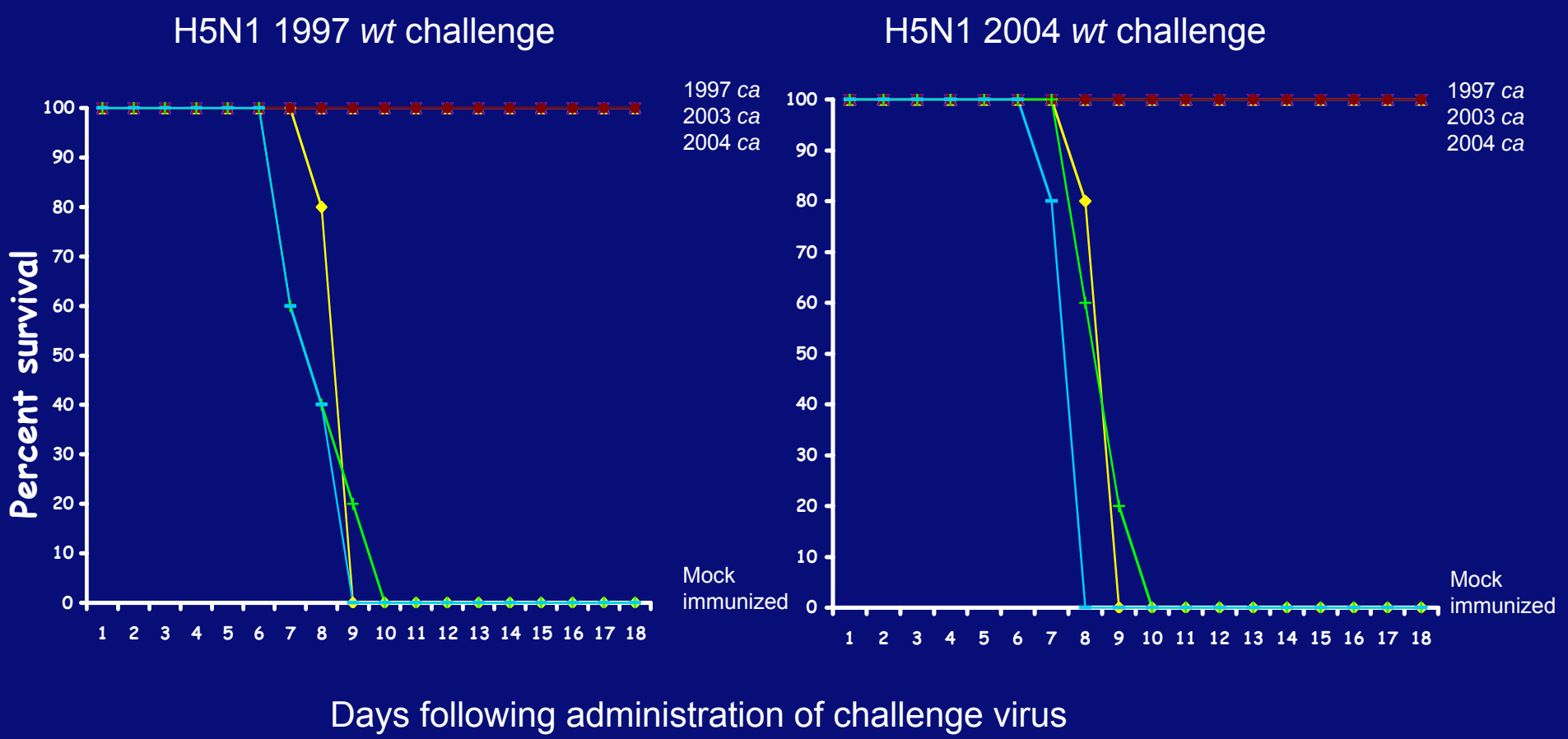
Immunizing virus	Doses	Geometric mean serum neutralizing Ab titers against indicated virus		
		1997 *wt*	2003 *wt*	2004 *wt*
A/VN/2004 *ca*	1	10	10	10
A/HK/2003 *ca*	1	10	37	10

Sera were collected before (prebleed) and 28 days following each dose of vaccine; an undetectable titer is assigned a value of 10

Efficacy

- In mice and in ferrets
- Following one dose or two doses
- Tested against homologous and heterologous viruses
- Efficacy against lethal challenge (if relevant)
- Protection from pulmonary replication or systemic spread of challenge virus

A Single Dose of H5N1 *ca* Vaccine Protects Mice from Lethal Challenge with 50, 500 or 5000 LD_{50} of Homologous and Heterologous Wild-type H5N1 Viruses



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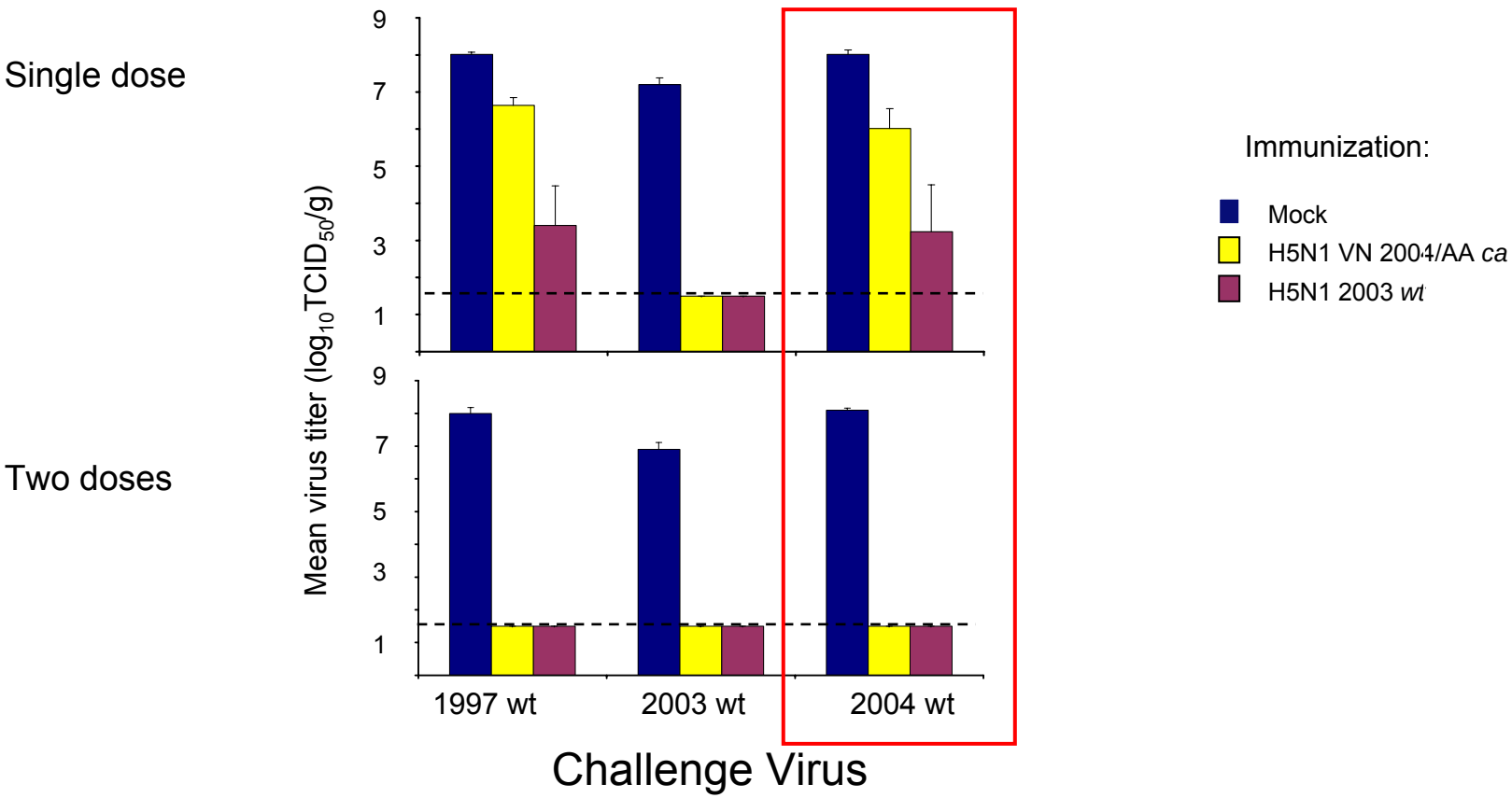
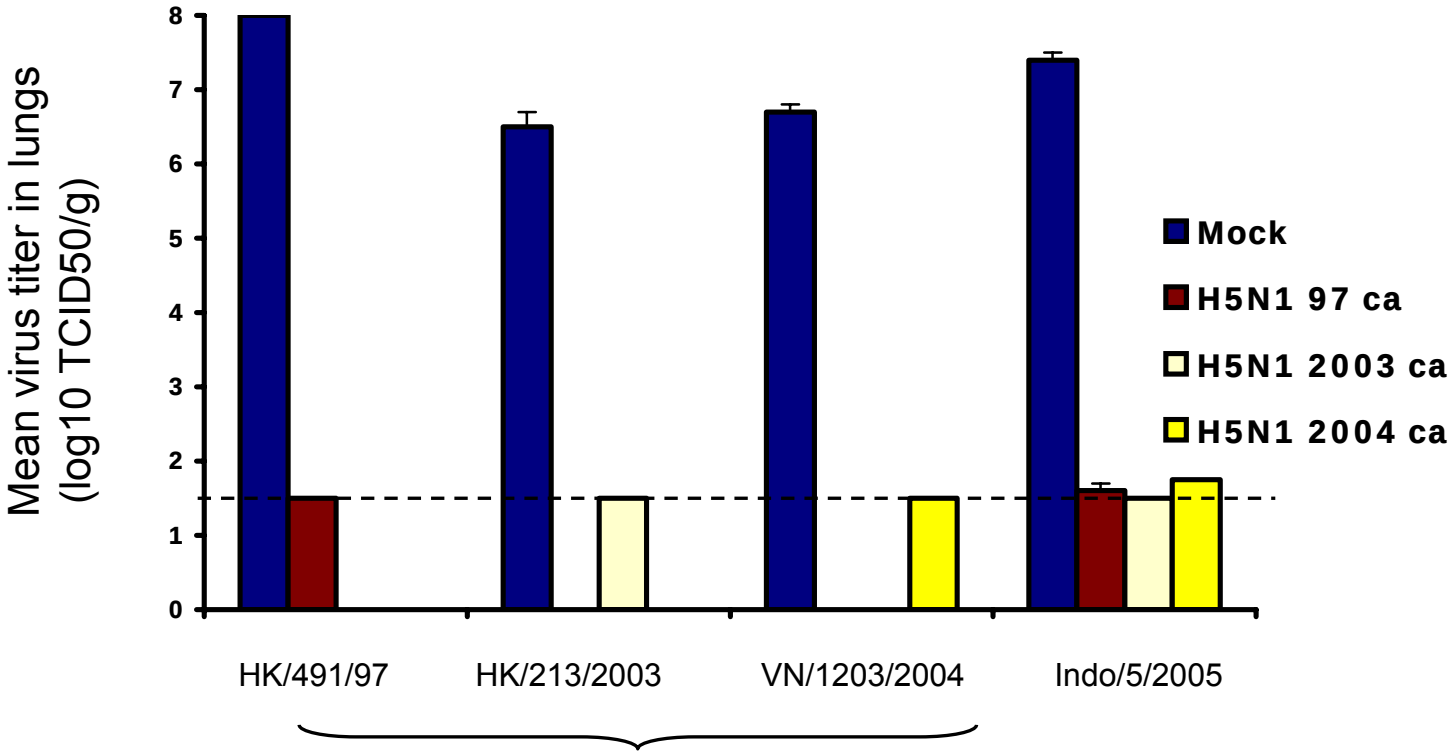
Complete Protection from Pulmonary Replication of *wt* H5N1 Challenge Viruses is Conferred by 2 doses of the 2004 H5N1 *ca* Vaccine



Vaccine dose: 10^6 TCID$_{50}$ per dose 2004 H5N1 *ca*; Challenge virus dose: 10^5 TCID$_{50}$ of *wt* virus

2 Doses of H5N1 *ca* Vaccines Provide Complete Protection from Pulmonary Replication of Homologous and Heterologous *wt* H5N1 Viruses



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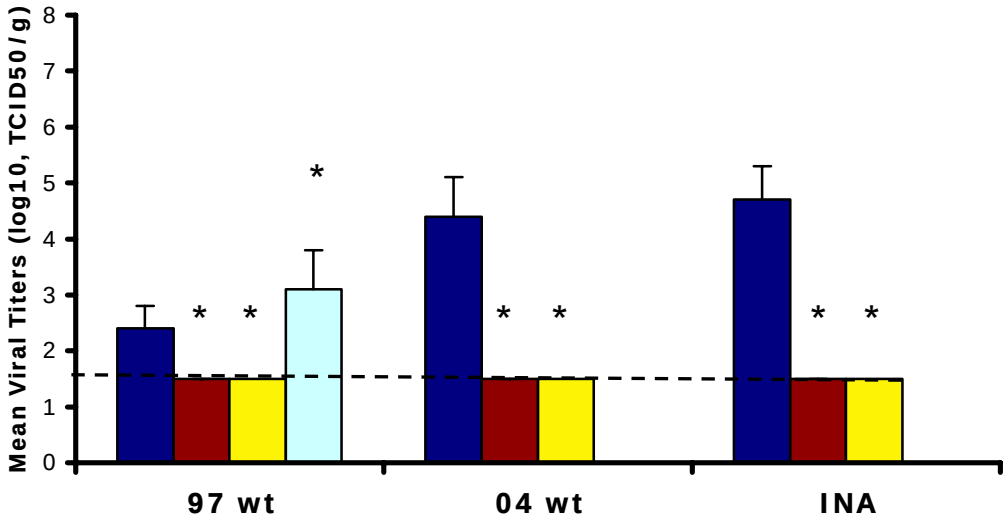
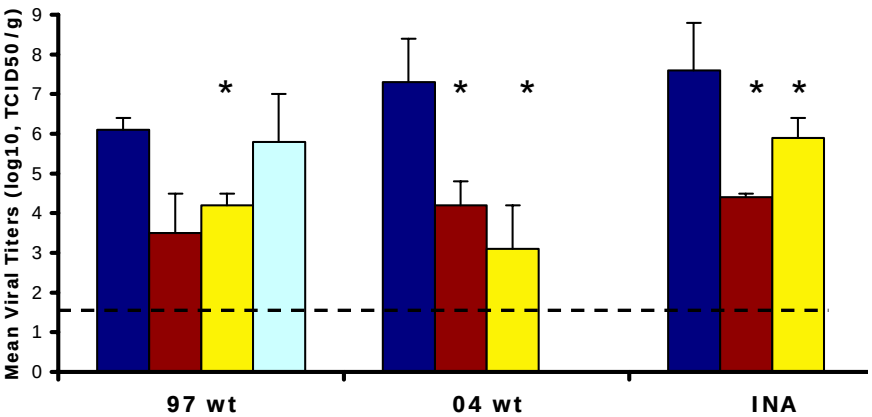
Efficacy following 2 Doses of H5N1 *ca* Vaccines in Ferrets

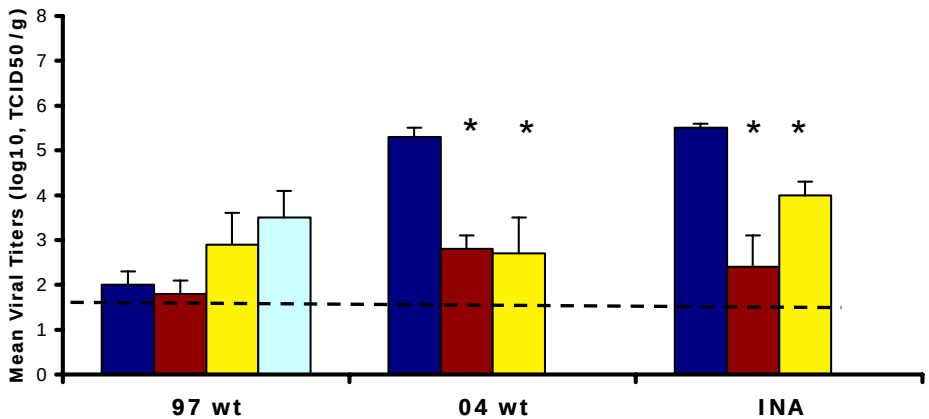
LUNGS



NT



BRAIN



Challenge Virus

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What have we learned?

- The kinetics of the neutralizing Ab response to the H5 viruses in mice are slow.
- The magnitude of the neutralizing Ab response to the H5 viruses in mice and ferrets is poor.
- When vaccines elicit a robust neutralizing Ab response in mice and ferrets, the Abs are cross-reactive and vaccines are cross protective.
- The changes in the H5 HA do not appear to be driven by positive selection in humans
- A vaccine that elicits sufficiently robust nt Ab to be cross-reactive will likely provide cross-protection against genetically and antigenically distinguishable H5N1 viruses.
- The poor infectivity of H5N1 *ca* virus in humans was not predicted from the preclinical data; the H5N1 *ca* viruses replicated efficiently in embryonated eggs, MDCK cells, mice and ferrets.
- The poor infectivity of the live attenuated vaccines may correlate with the lack of efficient person to person transmission of the *wt* H5N1 virus.

Live Attenuated Pandemic Influenza Vaccines

Subtype (# evaluated)	ts ca att	Immunogenicity		Efficacy	
		Mice	Ferrets	Mice	Ferrets
H5 (3)	√	√	√	√	√
H6 (3)	√	√	√	√	√
H7 (1)	√	√	√	√	√
H9 (1)	√	√	√	√	√

Summary

- Candidate live attenuated vaccines have been generated against 4 avian influenza virus subtypes- they are attenuated in chickens, mice, ferrets
- Immunogenicity: Two doses are required to observe consistent serum antibody responses (mice & ferrets) against H5N1 viruses; one dose was immunogenic for H7N3 and H9N2 viruses in mice and ferrets.
- Protection (challenge) studies
  - Mice and ferret models have been used to evaluate efficacy against challenge with homologous and heterologous wt viruses from the same subtype
  - Mice
    - A single dose protects from lethal challenge in mice (H5N1, H7N3)
    - When one dose of vaccine elicits a neutralizing Ab response, it protects from pulmonary replication and in other cases two doses are required (H7N3, H9N2 vs. H5N1)
  - Ferrets
    - A single dose prevents pulmonary replication
    - Two doses do not abolish replication of the *wt* H5N1 virus in the upper respiratory tract.

Goals of the Pandemic Influenza Vaccine Program

- Generate and evaluate a library of vaccines against viruses of each subtype (H2, H4-16) to protect humans against pandemic influenza
- Proceed to clinical trials to evaluate safety, infectivity and immunogenicity in healthy adults
- Bank sera from vaccinated volunteers to test against avian viruses that emerge in humans
- Determine the significance of antigenic differences among avian influenza viruses in humans

Program: CRADA with MedImmune Vaccines, collaboration with CIR, Johns Hopkins Univ.

Approach: Live attenuated vaccines

Evaluation: In inpatients, during the summer

Acknowledgements

Laura, Amorsolo, Josie, Tomy, Catherine, Celia, and Kim (and Mike)

Jack

LID: Brian Murphy
SEPRL, USDA: David Swayne and Joan Beck
MedImmune: George Kemble, Hong Jin, Bin Lu, Greg Duke, Zhaoti Wang, Melissa Dixon
CIR, JHU: Ruth Karron, Kawsar Talaat, Karen Callahan, Bhavin Thumar
Comparative Medicine Branch, NIAID, NIH

MAbs: Antonio Lanzavecchia, IRB, Switzerland, Cameron Simmons, Oxford Unit, Vietnam, Jerrold Ward, CMB, NIAID