Development of Vaxfectin®-adjuvanted DNA Vaccines

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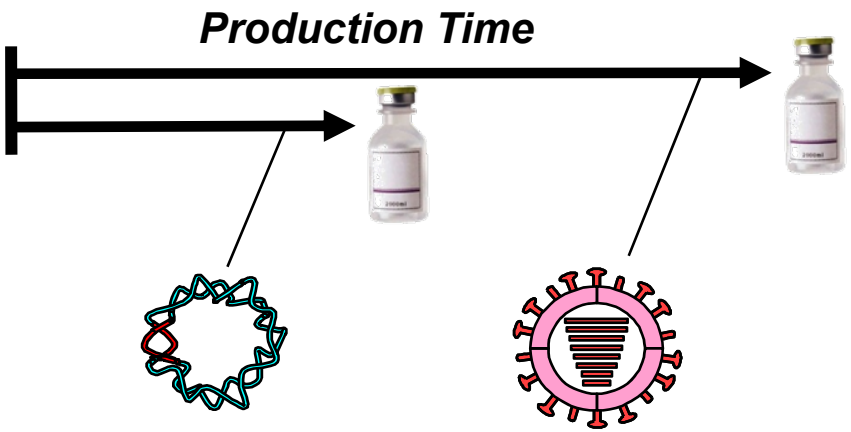
June 04, 2008 - ECI Vaccines - Portugal

Advantages of Plasmid DNA Vaccines



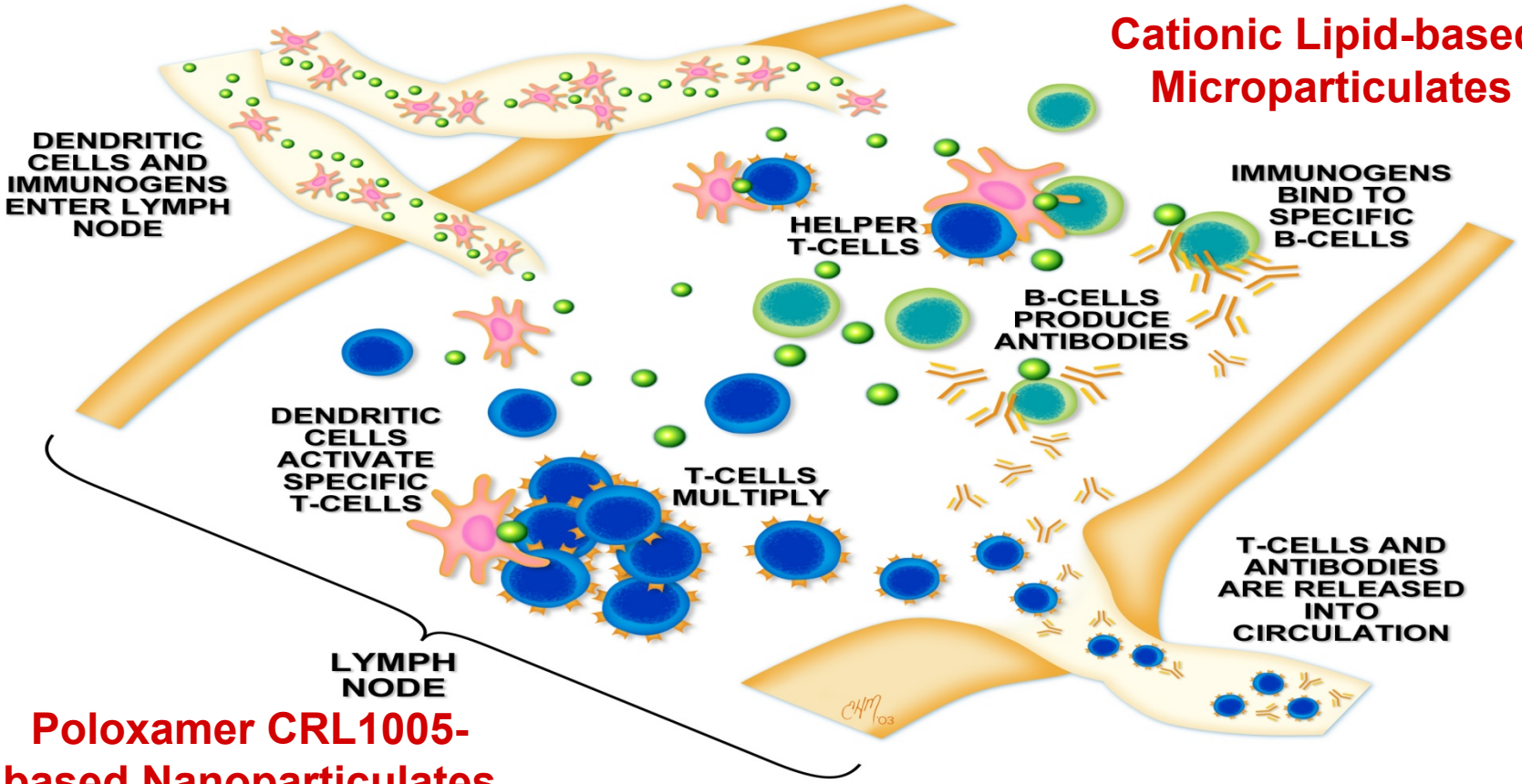
Fermentation-based Manufacturing

Egg-based Manufacturing

- Proven platform technology that induces humoral and cellular responses in animals
- 3 animal health vaccines approved
- Potential to prime strong memory responses
- Evidence of safety and immunogenicity in humans with and without adjuvants
- No infectious components
- Fermentation-based manufacturing
- Short manufacturing timeline
- Inherently stable

Gene Delivery Systems

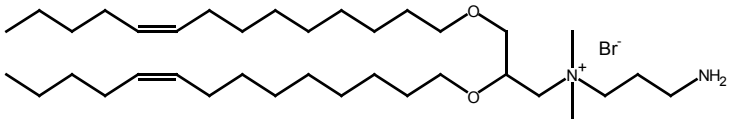
Improved Cellular and Humoral Response



Vaxfectin® Adjuvant

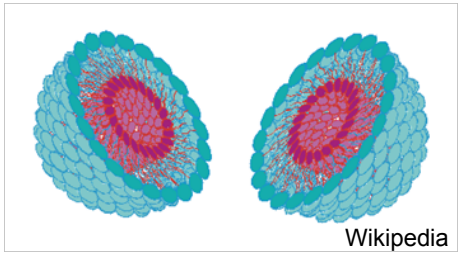
Cationic Lipid

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(cis-9-tetradeceneyloxy)-1-propanaminium bromide

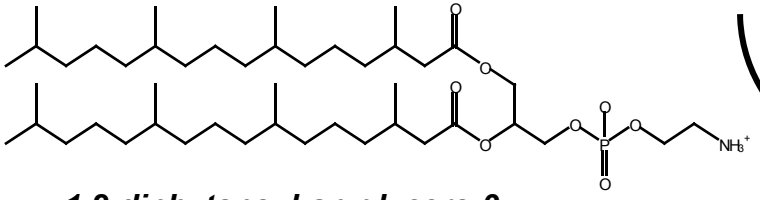


GAP-DMORIE

DPyPE



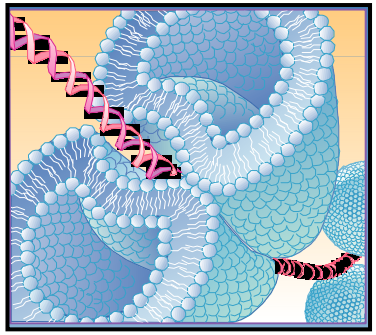
Cationic Liposomes



1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine

Co-Lipid

pDNA



pDNA/lipid Complex

Vaxfectin® Profile

- Two-lipid mixture
- Patented technology
- Dose sparing with DNA and protein-based vaccines
- Scaleable cGMP manufacturing
- Simple formulation

Vaxfectin® References

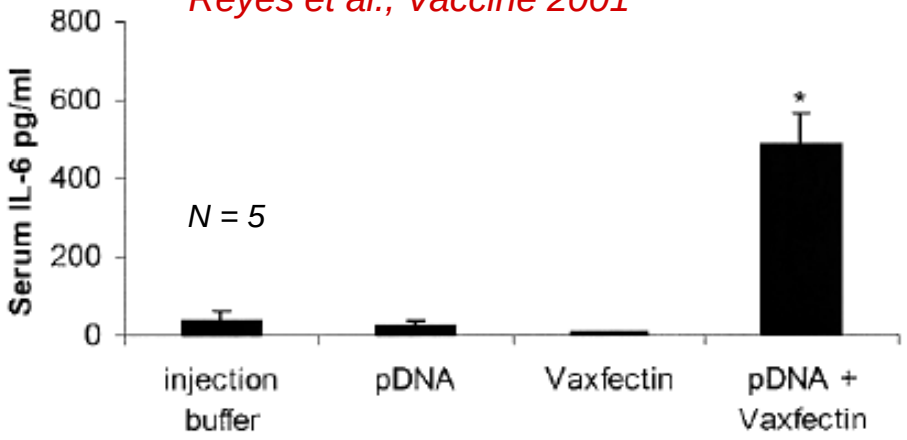
Enhanced Responses

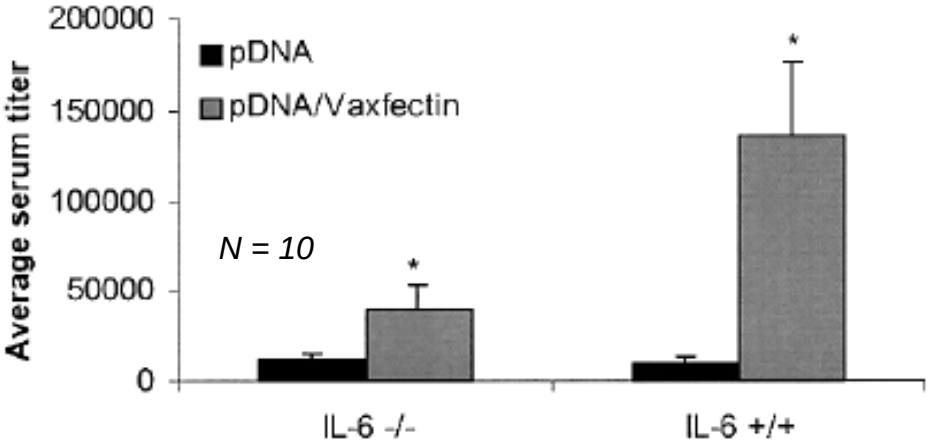
Antigen	Species	Immunogenicity	Reference
Influenza NP	Mice Rabbits	↑ NP Ab titers 20X ↑ NP Ab titers 50X	Hartikka, *Vaccine* 19:1911; 2001
NP and various other antigens	Mice	↑ Ab titers 3-10X	Reyes, *Vaccine* 19: 3778; 2001
Tuberculosis Ag85	Mice	↑ Ag85 Ab titers 3-10X	D'Souza, *Inf Imm* 70: 3681; 2002
Influenza NP or hGH	Rats	↑ NP Ab titers and CTLs	Sankar, *Oral Dis* 8: 275; 2002
JEV prM/E	Mice	↑ Neut Ab titers 8X	Nukuzuma, *Vir Imm* 16: 183; 2003
HIV-2 Env	Mice	↑ Serum and mucosal Ab	Locher, *DNA Cell Biol* 23: 107; 2004
HIV-2 Env tat nef gag/pro	Baboons	Partial protection from HIV-2	Locher, *Vaccine* 22: 2261; 2004
Plasmodium yoelli CSP	Mice	↑ Ab titers, ELISPOT responses, protection	Sedegah, *Vaccine* 24: 1921; 2006
Rabies G protein	Mice Rabbits	Protective neutralizing titers sustained 120 days	Margalith, *Gen Vacc Ther* 4: 1; 2006
Tuberculosis Ag85	Mice	↑ Cytokines but not survival against MTB	Romano, *Vaccine* 24: 3353; 2006
Anthrax PA83	Rabbits	↑ Neut Ab and protection	Hermanson, *PNAS* 101: 13601; 2004
Anthrax PA83	Sheep	↑ Ab titers at 5 months vs rPA in Alhydrogel	Hahn, *Vaccine* 24: 4795; 2006
Influenza HA, NP, M2	Mice Mice/Ferrets	↑ # of IFNγ T cells vs PBS-formulation Protection against H5N1 challenge	Jimenez, *Human Vaccines*; 2007 Lalor et al., *JID*; 2008

Vaxfectin®-enhanced Antibody Responses

IL-6 Dependency

Reyes et al., Vaccine 2001



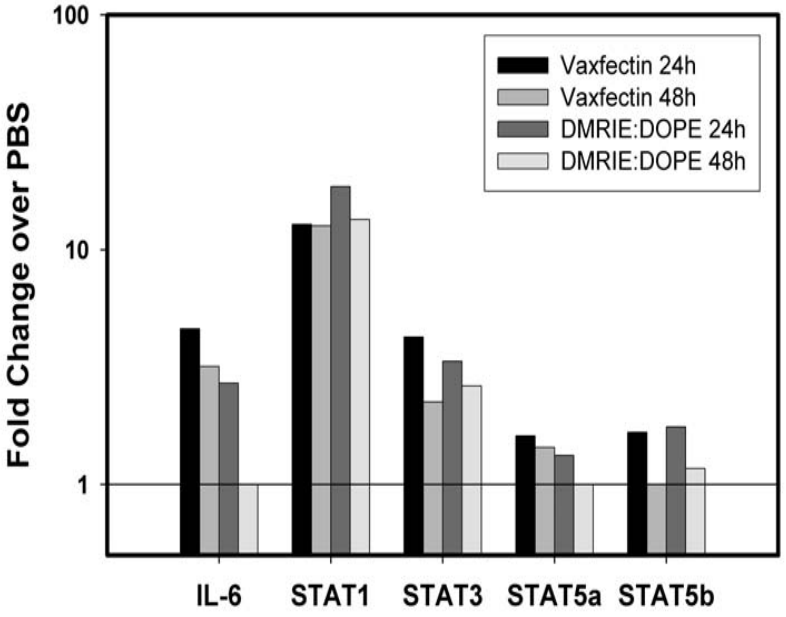


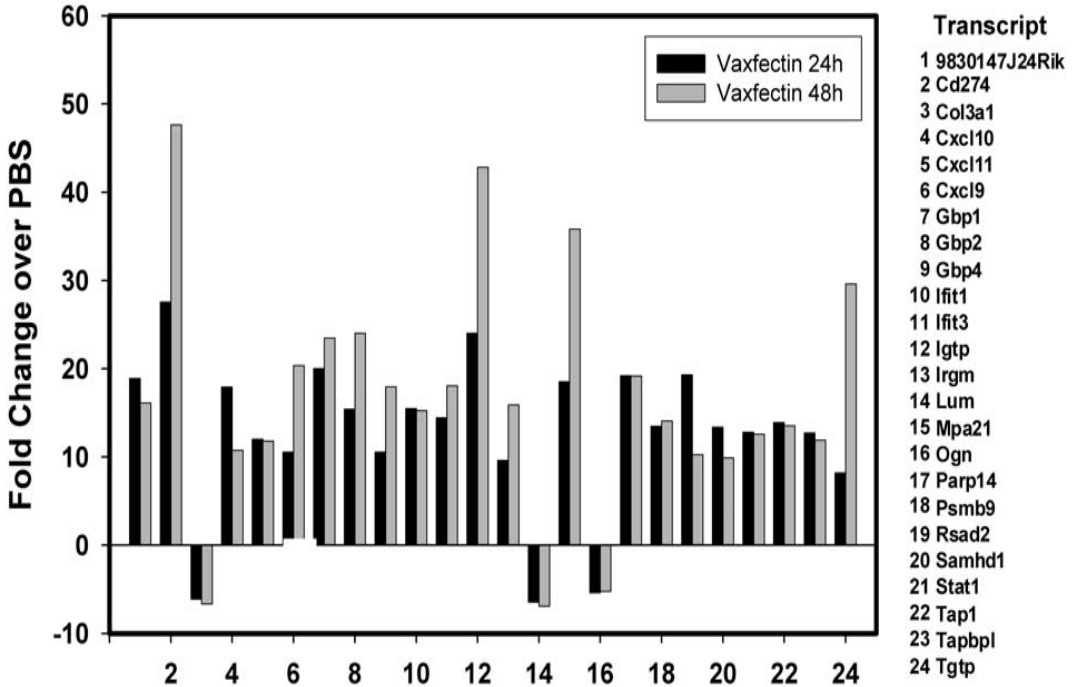
- ↑ serum IL-6 at 4 hrs ($p < 0.05$)
- ↓ serum anti-NP antibody titers in IL-$6^{-/-}$ mice ($p = 0.02$)

IM injections in C57BL/6 mice (5 μg NP pDNA in each rectus femoris) at 0 and 3 weeks; IgG titers (mean ± SEM) at 6 weeks

Vaxfectin® Mechanism of Action

Induction of Immunostimulatory Gene Expression





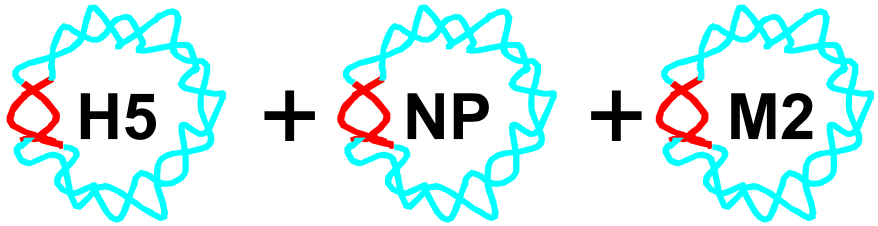
Analysis of transcript levels (Affymetrix DNA Chip: 39,000 transcript represented) in mouse muscles (N =3) at 24 and 48h after IM injection of cationic lipid (Vaxfectin® or DMRIE:DOPE)-formulated VR6365 pDNA (Fold-increase over injected VR6365/PBS muscles with $p \leq 0.05$)

Pandemic Influenza DNA Vaccine Strategy

Goal: Develop a vaccine that is efficacious for current perceived threats that will also be beneficial in the event of a mismatched pandemic virus

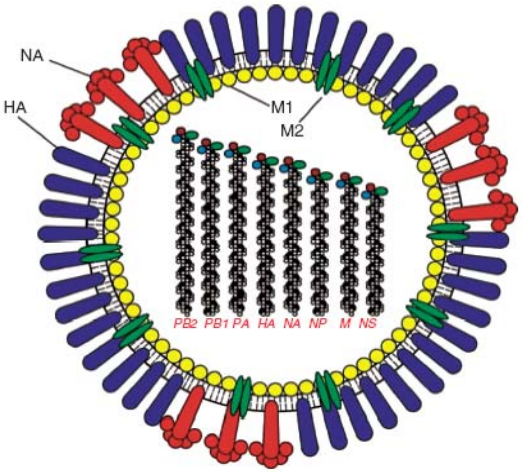
- Select HA component for current H5 threat
- Select highly conserved proteins to enable cross protection
- Create consensus sequences (>85% identical)
- Test to determine lead constructs and formulation
- Evaluate preclinical efficacy
- Evaluate preclinical safety
- Evaluate in the clinic

Pandemic Influenza DNA Vaccine



Product profile

- H5 HA (A/Vietnam/1203/04)
- Conserved NP + M2 for cross-protection
- Vaxfectin® adjuvant
- 1 or 2 IM (possibly ID) injections
- 1 mg dose or less
- Needle or Biojector® 2000

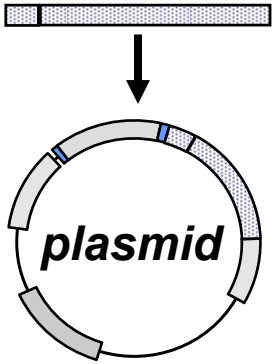


Comprehensive immune responses

- Antibodies against HA and M2
- T cells against all encoded antigens
- One vaccine against any pandemic strain

Nonclinical Development Pathway

Influenza genes
(HA, M2, NP)

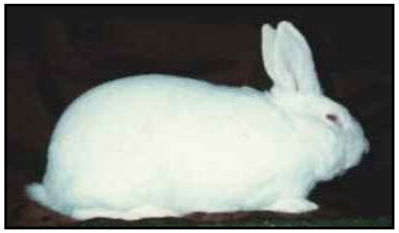


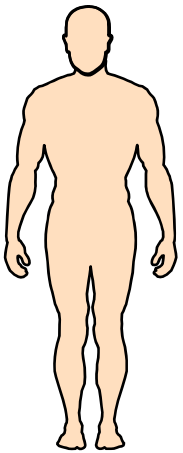
Gene and formulation screening studies

Mouse & ferret H5N1 challenge studies

cGMP vaccine manufacturing

Safety studies → **IND** →





Human clinical trials

Mouse Challenge Model

Vaccination Regimen

Challenge Regimen

3 week assessment

Week 0 **Week 3** **Week 6** **Week 6-8**

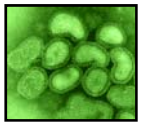
Survival

Weight

Route*:* Intramuscular
+/-Formulations

N ≥12/group

LD_{90}

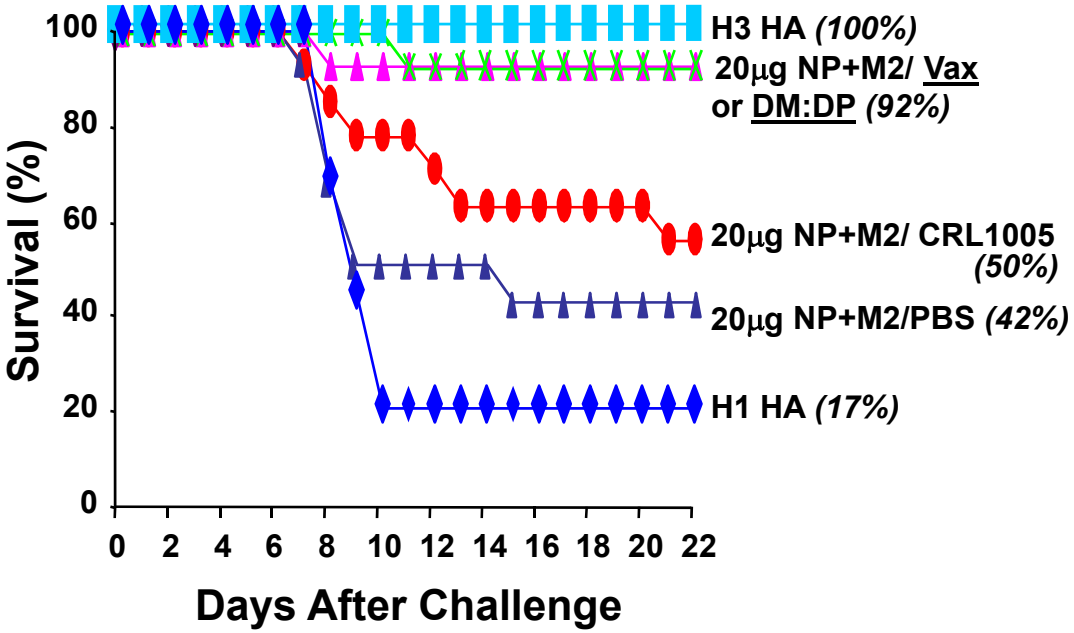


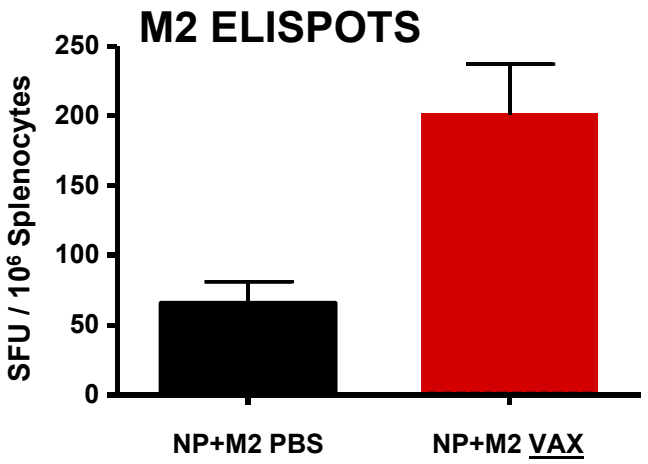
Mouse-adapted A/HK/8/68 (H3N2) challenge
or
Mouse-adapted A/PR/8/34 (H1N1) challenge

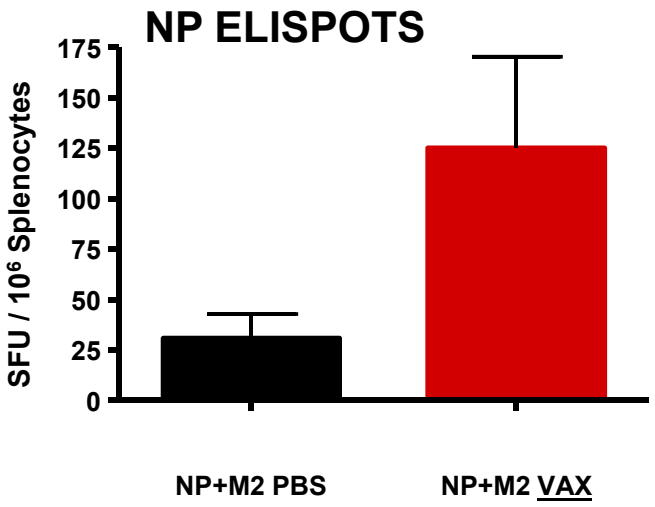
Formulation Screening

Influenza Challenge Model (H3N2)

Cationic Lipids vs. Poloxamer vs. PBS

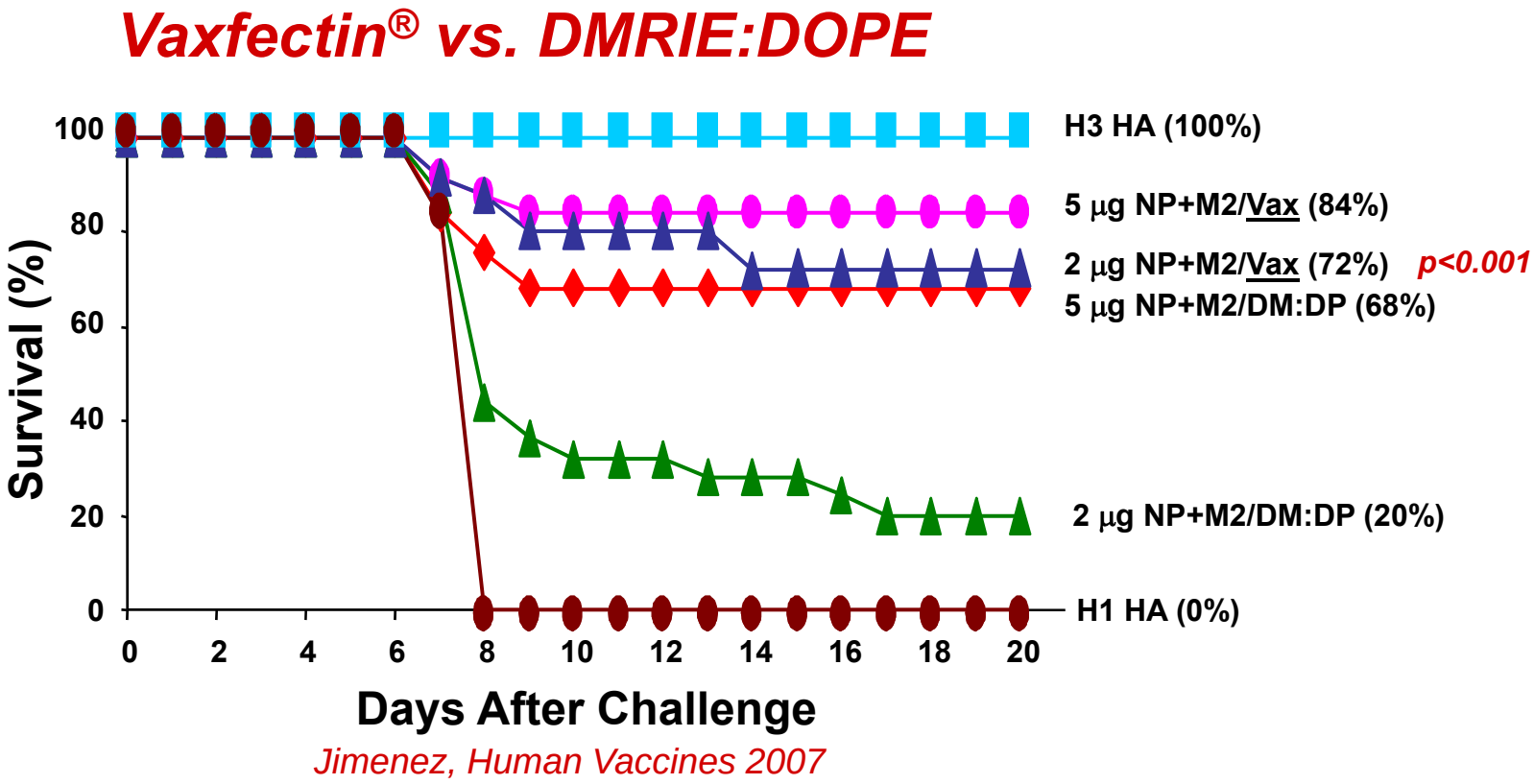






- Vaxfectin® and DMRIE:DOPE provide significant survival benefit over Poloxamer CRL1005 *($p \leq 0.032$)*

Effect of Cationic Lipid Formulations



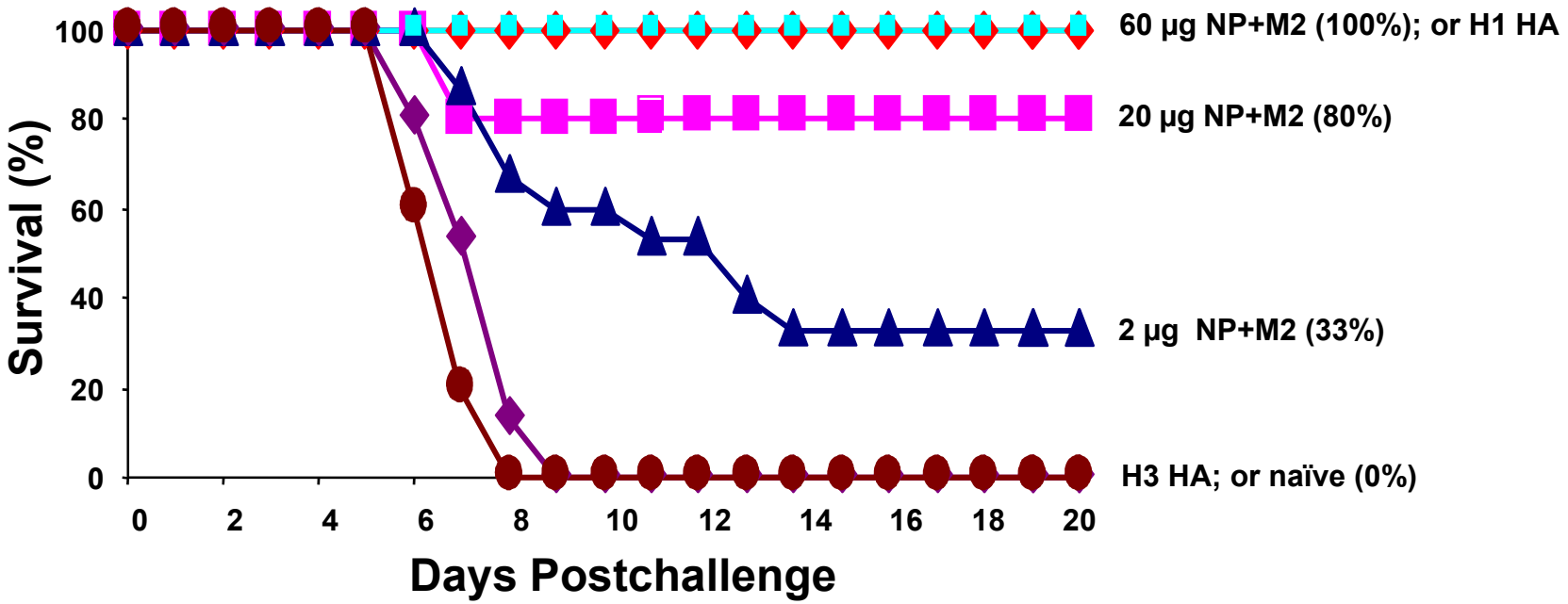
Jimenez, Human Vaccines 2007

- Vaxfectin® provides significant survival benefit over DMRIE:DOPE at low doses

Cross-strain Protection

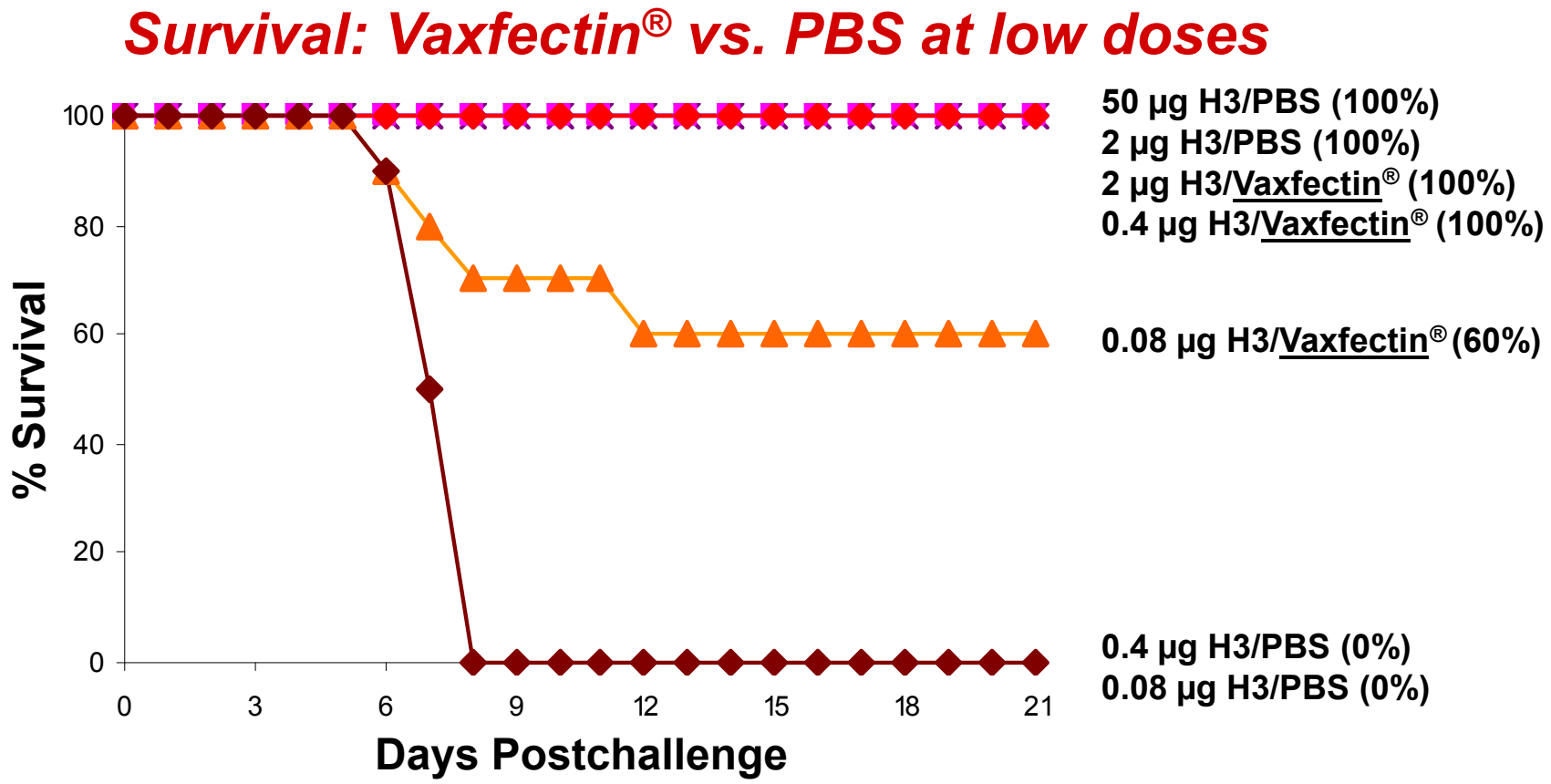
Influenza Challenge Model (H1N1)

Survival: Vaxfectin®-formulated NP + M2



- Vaxfectin®-formulated NP+M2 provides cross strain protection against H1N1 challenge

Dose-sparing Effect of Vaxfectin®

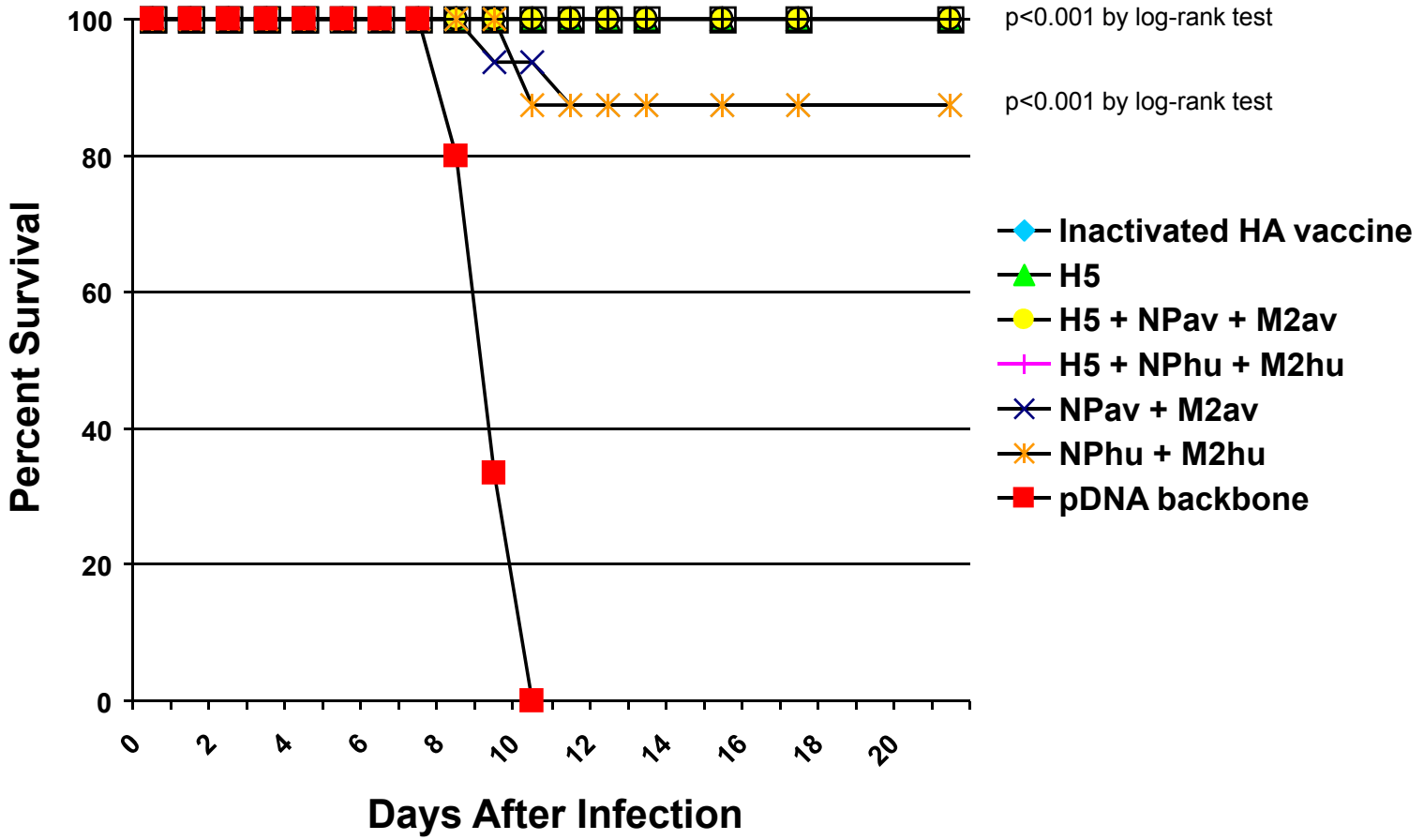


- H3 HA formulated with Vaxfectin® protects against H3N2 challenge at a single 80 nanogram dose *(p=0.0042)*

DNA-based Pandemic Influenza Vaccine

Mouse H5N1 Challenge Study - Survival

Collaboration with Dr. Richard Webby at St Jude Children's Research Hospital



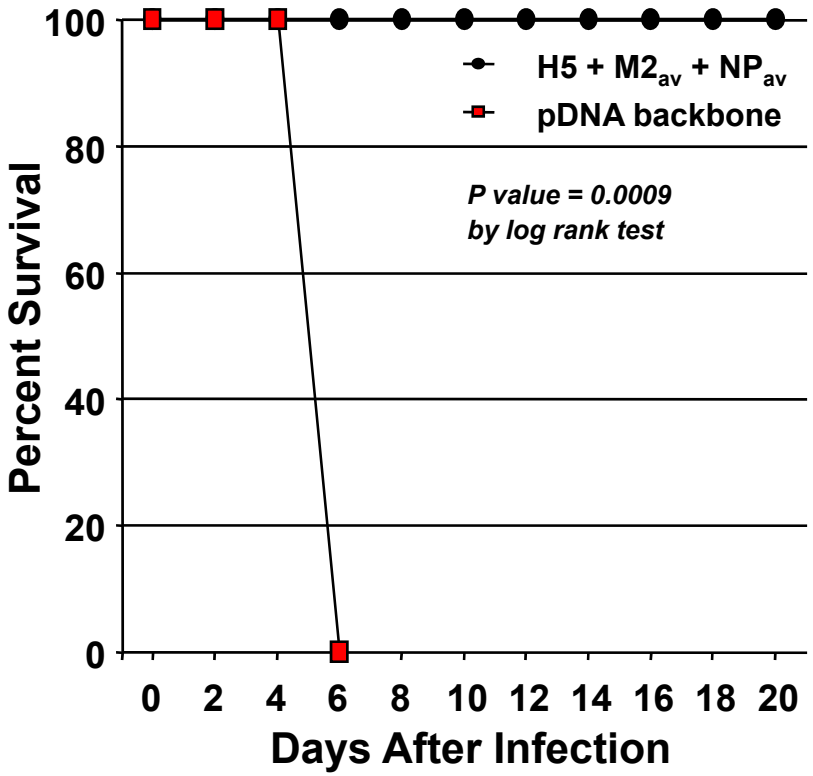
BALB/c mice (n = 16 / group) vaccinated at Days 0, 21 with 33 µg each Vaxfectin®-formulated pDNA or inactivated H5N1 vaccine (15 µg HA); A/Vietnam/1203/04 challenge (100 x LD_{50}) at Day 42

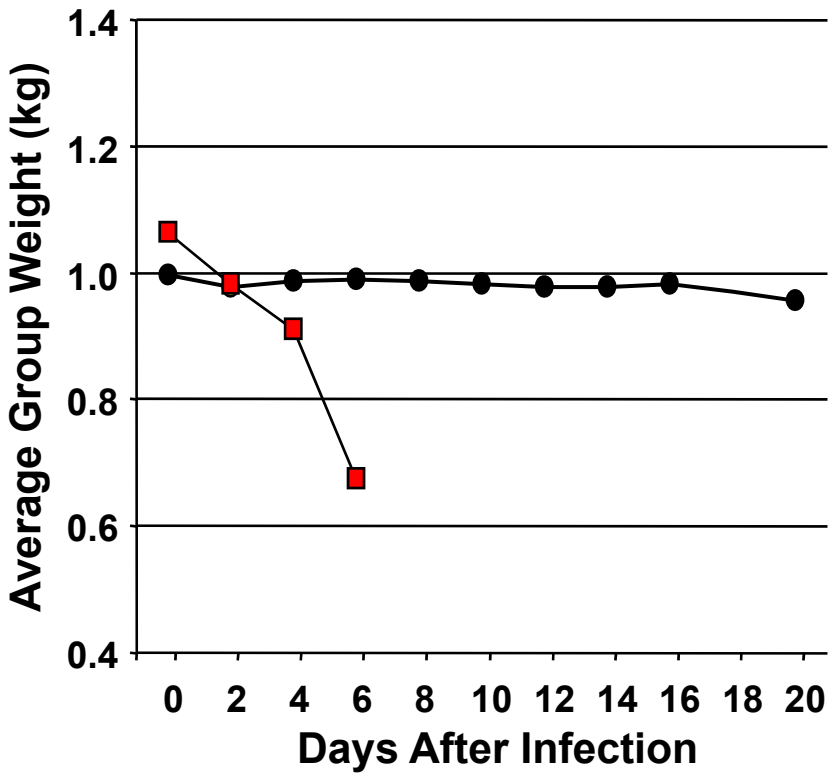
DNA-based Pandemic Influenza Vaccine

Ferret H5N1 Challenge - Survival, Weight Loss

Lalor et al., JID 2008

Collaboration with Dr. Richard Webby
St. Jude Children's Research Hospital



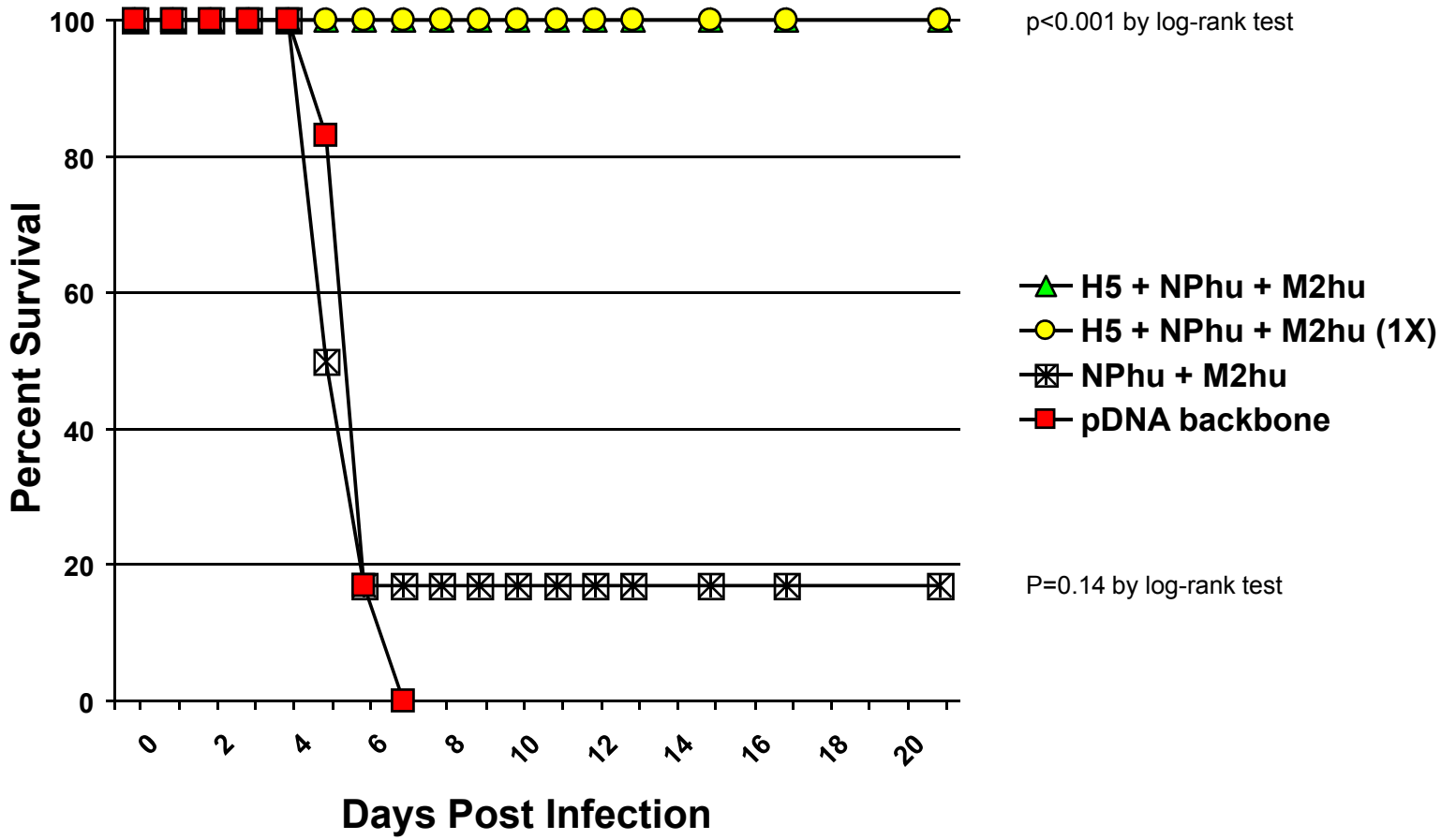


Fitch ferrets (n = 6 / group) serologically H5N1 flu-free vaccinated at Days 0 and 21 with 1.0 mg total Vaxfectin®-formulated pDNA; A/Vietnam/1203/04 challenge (100 x LD_{50}) at Day 42

DNA-based Pandemic Influenza Vaccine

Ferret H5N1 Challenge Study - Survival

Collaboration with Dr. Richard Webby at St Jude Children's Research Hospital

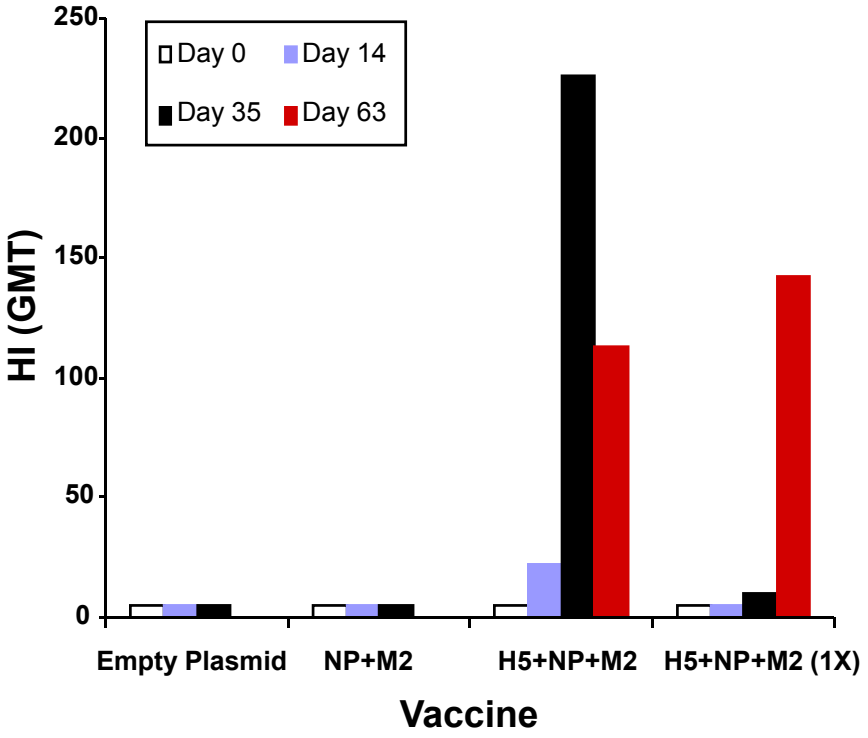


Fitch ferrets (n = 6 / group) serologically H5N1 flu-free vaccinated at Days 0 and 21 or Day 21 only (1X) with 0.3 mg each Vaxfectin®-formulated pDNA; A/Vietnam/1203/04 challenge (100 x LD_{50}) at Day 42

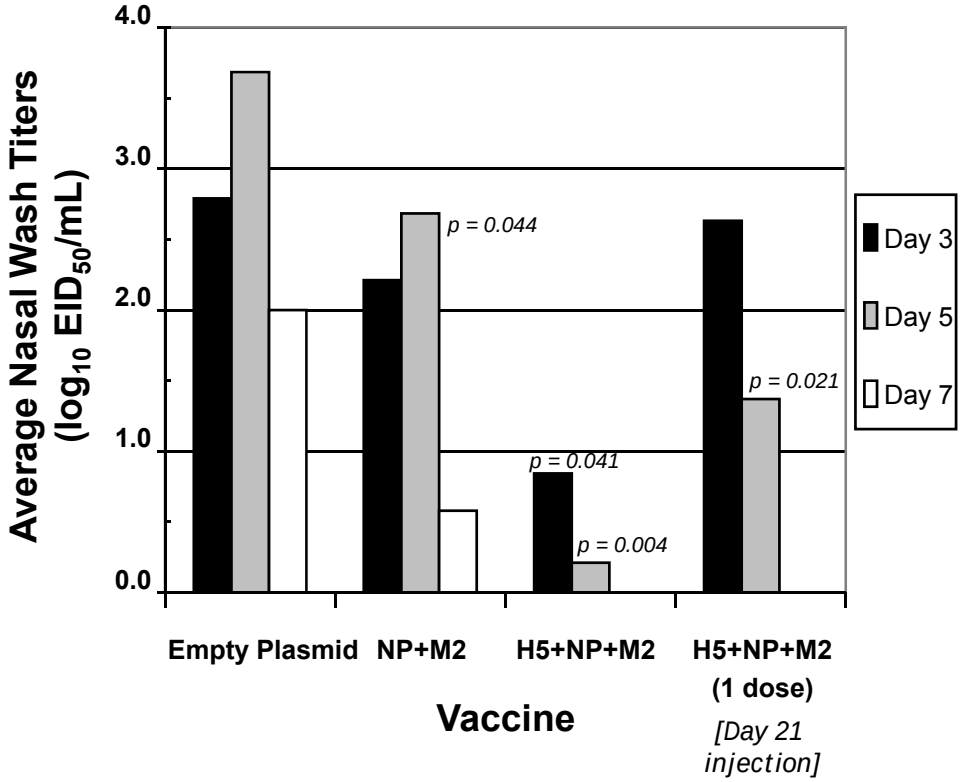
DNA-based Pandemic Influenza Vaccine

Ferret H5N1 Challenge Study

HI Titers



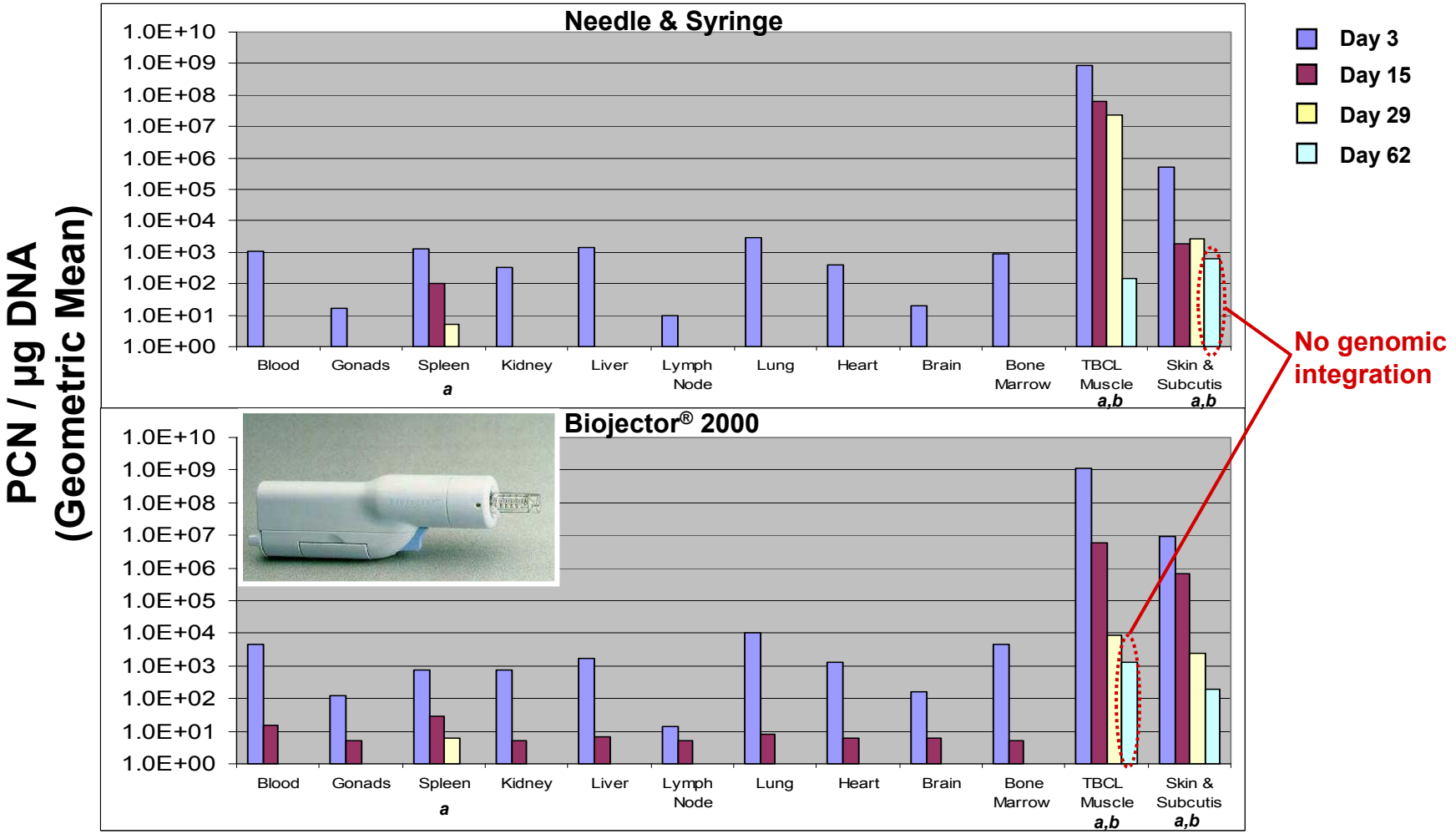
Nasal Wash Virus Titers



HI titers in vaccinated ferrets prior to vaccination (Day 0), on Days 14 and 35, and 3 weeks after infection with A/Vietnam/1203/04 (Day 63); virus titers in the upper respiratory tract of vaccinated and control ferrets measured from nasal washes collected on Days 3, 5, and 7 after infection

Vaxfectin®-formulated Influenza Vaccine

GLP Tissue Distribution in Rabbits



Single bilateral i.m. injection of 0.5 mg DNA/0.5 mL/muscle [~28X human dose (mg/kg)]; 5 rabbits/sex/timepoint; PBS as negative control; qPCR of extracted DNA; only tissues analyzed at Day 29 [a] and day 61 [b]

Vaxfectin®-formulated Influenza Vaccine
Phase 1 Clinical Trials

- Double-blind placebo controlled study
- Dose escalation 0.1 to 1 mg total DNA
  - Vaxfectin®-formulated H5 + NP + M2 or H5 alone
  - Vaccinations IM on Days 0 and 21
  - Needle or Biojector® 2000
- 103 normal healthy adults (18-45 yrs)
  - 3 clinical sites: SNBL (Maryland), Stony Brook (New York), Accelovance (San Diego)
- Safety, tolerability, immunogenicity
  - Antibodies measured by HI and neutralizing assays
  - T-cell responses measured by ELISPOT assays

Pandemic Influenza DNA Vaccine
Summary

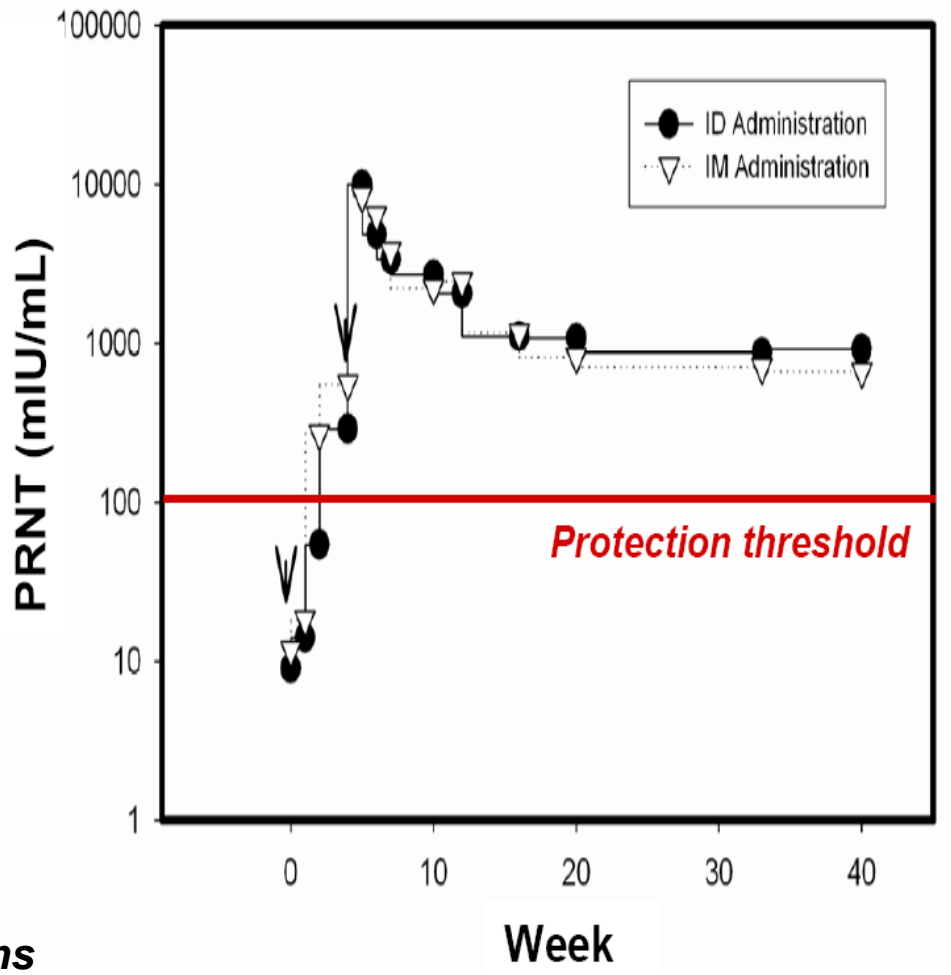
- Mouse studies at Vical
  - In the absence of HA, NP + M2 provides the best cross strain protection in mice
  - Vaxfectin® adjuvant provides dose-sparing advantage
- Mouse and ferret H5N1 studies at SJCRH
  - 100% protection in mice and ferrets with H5 + NP + M2
  - 1 dose vaccine protects ferrets against Vietnam H5N1 strain
  - High-level protection in mice with NP + M2 vaccine
- GLP safety studies
  - Rabbit repeat dose study demonstrates safety
  - Clearance over time with no evidence of integration
- Two ongoing clinical trials

Vaxfectin®-formulated DNA Vaccine
Protection of Macaques against Measles

- Juvenile rhesus macaques (2 yrs)
- H + F DNA on Days 0, 28
  - 0.5 mg ID (n= 5)
  - 1 mg IM (n = 5)
- Challenge at Week 55 with 10^4 $TCID_{50}$ of Bilthoven strain intratracheally
- No clinical signs of measles and no viremia in vaccinated animals
- Rash and viremia in controls

- Infant macaques (6-8 wks) - 2nd study
  - Challenge at 1 year
  - No clinical signs or viremia

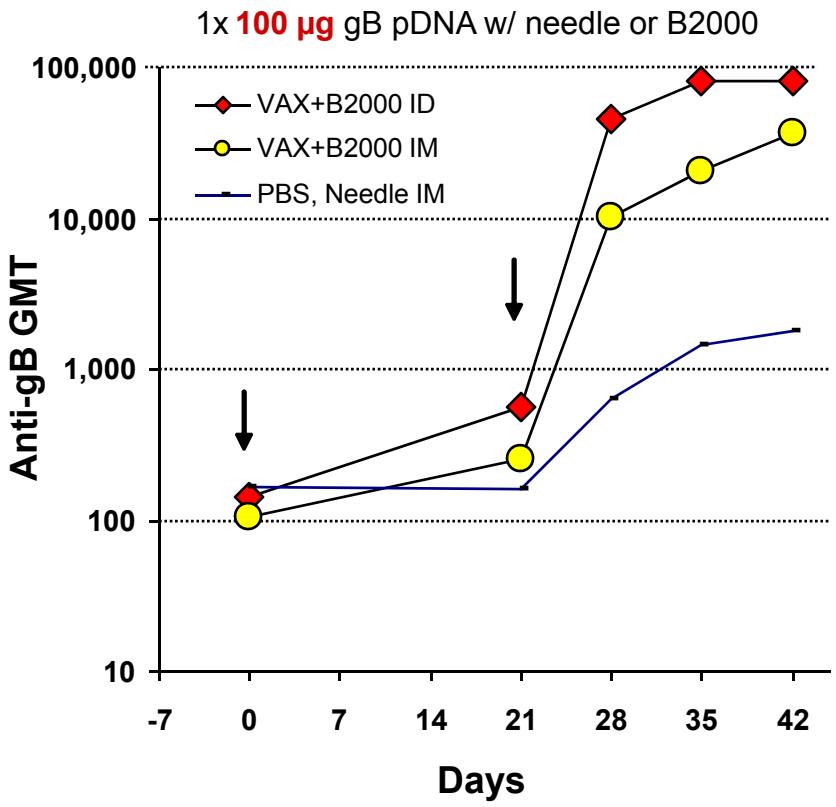
Pan et al., Clinical and Vaccine Immunology (2008)

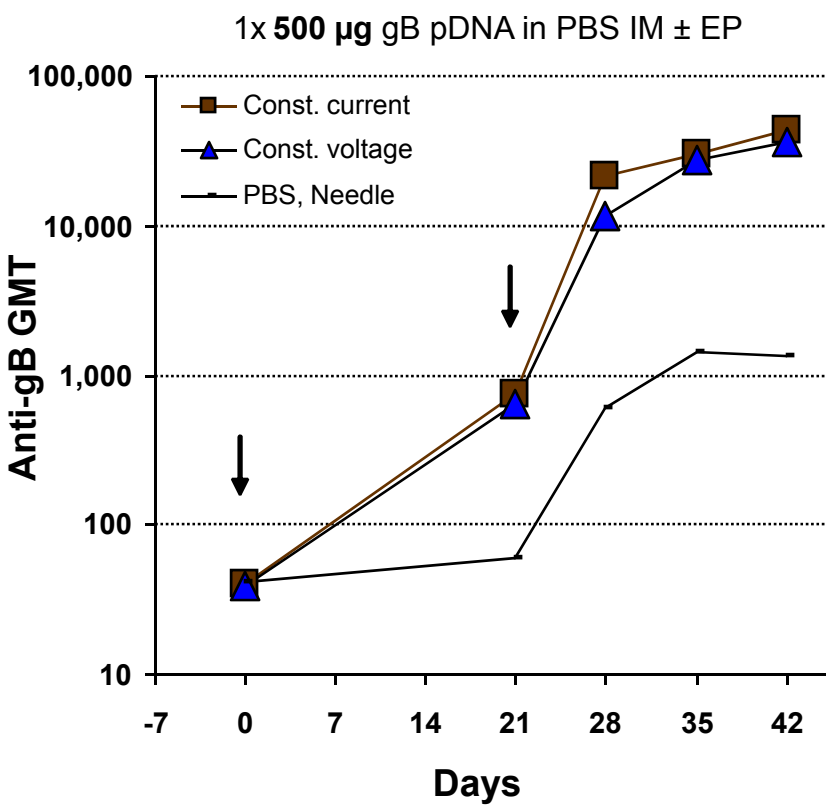


Collaboration with Dr. D. Griffin, Johns Hopkins

Needle-free Injection of Vaxfectin®-formulated DNA vs Electroporation

- IM or ID delivery of Vaxfectin®-formulated pDNA (hCMV gB) vaccine delivered with needle-free device resulted in anti-gB titers similar to those obtained with EP-assisted delivery (historical study)



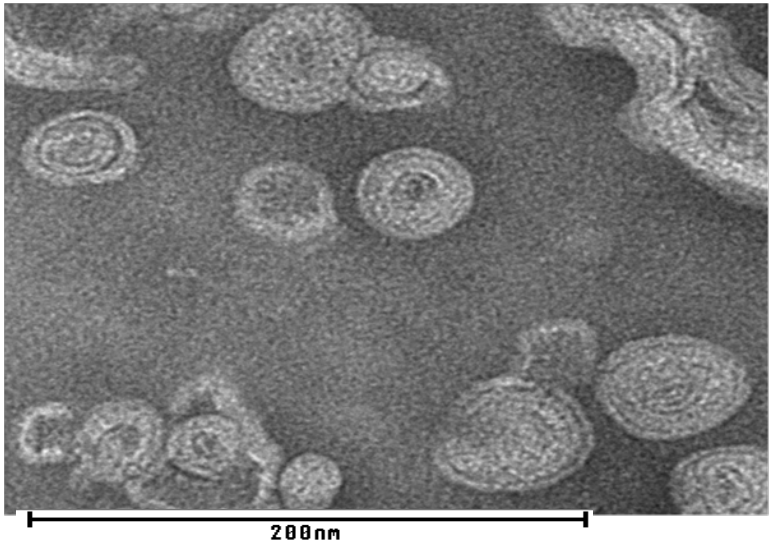


Vaxfectin® as an Adjuvant for Protein-based Vaccines

Fluzone®

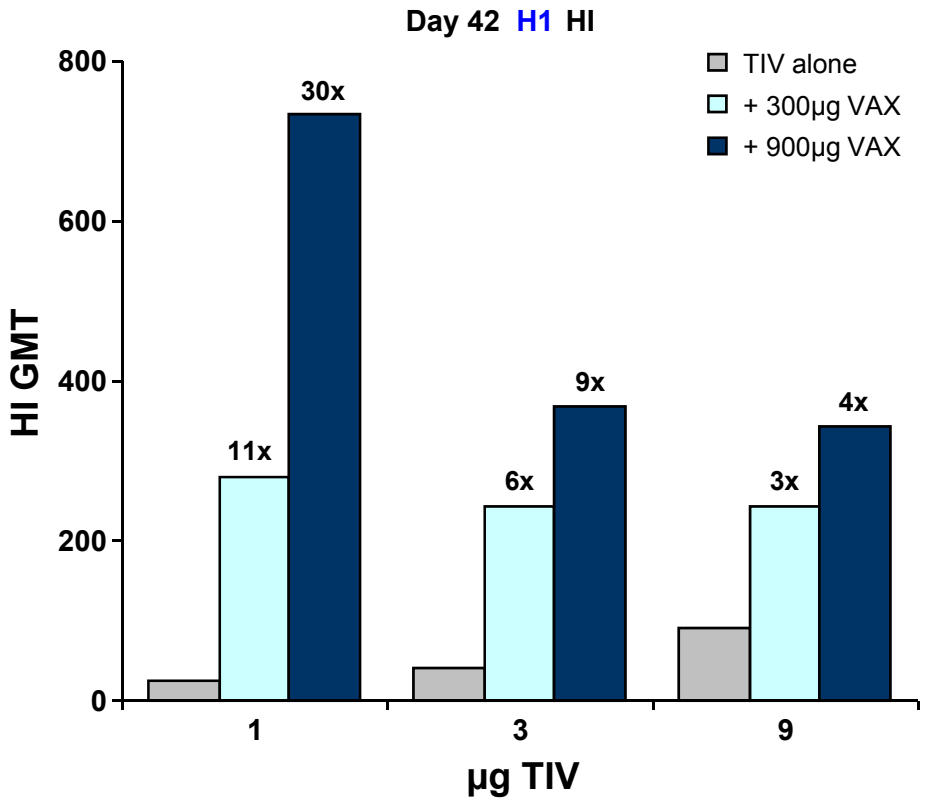
- Split inactivated trivalent inactivated flu vaccine (TIV; from sanofi pasteur)
- 2006-07 formulation contains 15 µg of each HA (A/New Caledonia/20/99 [H1N1], A/Wisconsin/67/2005 [H3N2], and B/Malaysia/2506/2004) per 0.5 mL

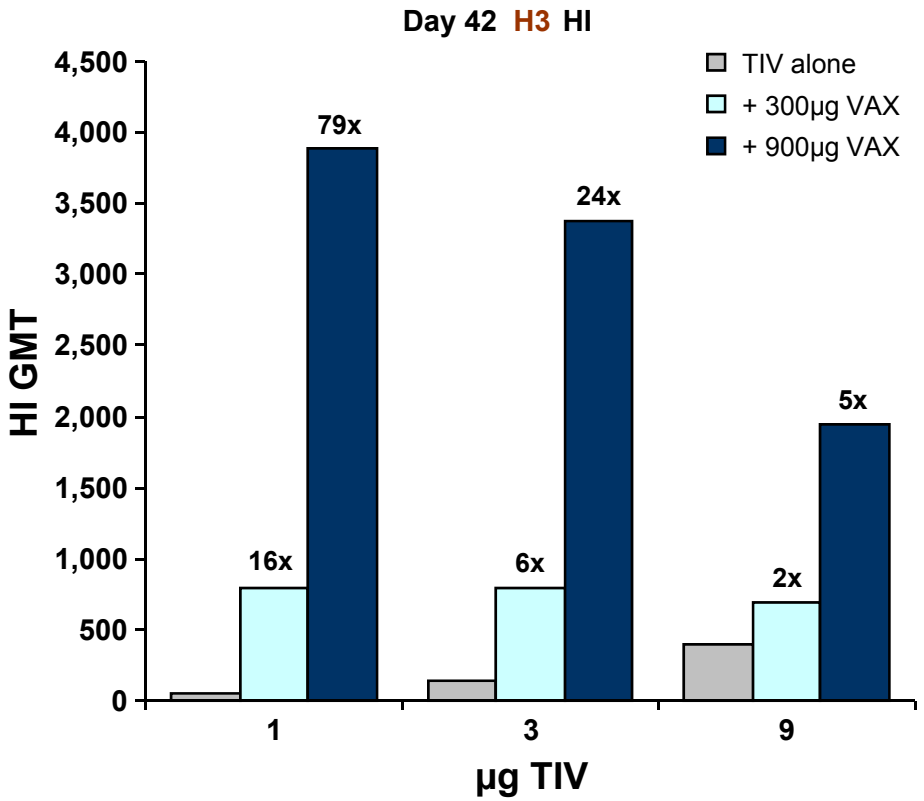
+ Vaxfectin®



Vaxfectin® as Dose-sparing Adjuvant
Protein-based Influenza Vaccine

- Vaxfectin® at any dose of TIV (Fluzone®) significantly ($p < 0.001$) increased HI titers in mice compared to TIV alone
- Dose-sparing for protein vaccines (≥10x)

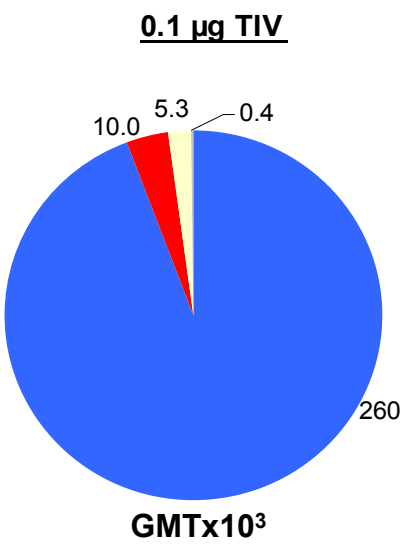


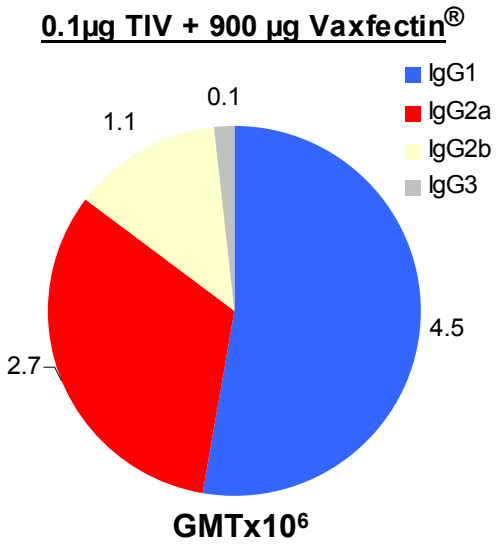


BALB/c mice (n = 10 / group) injected IM with Vaxfectin®-formulated protein on Days 0 and 21

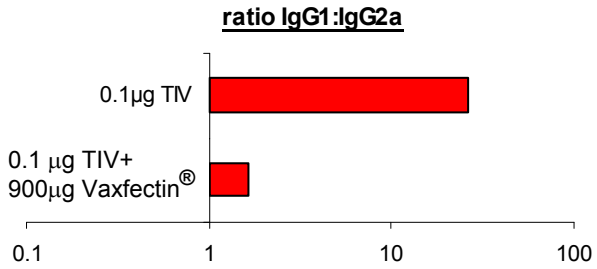
Vaxfectin® as a Th1 Adjuvant

Anti-TIV IgG Isotypes





	GMT x fold compared to 0.1µgTIV
	0.1µg TIV+ 900µg Vaxfectin®
IgG1	17x
IgG2a	274x
IgG2b	208x
IgG3	294x



Conclusions:

- With 0.1 µg total TIV dose, IgG1 is the dominant isotype
- Vaxfectin® (900 µg) increased IgG2/3 titers more than IgG1 titers, resulting in a more balanced isotype distribution

Acknowledgements

- Vical's Product Discovery-Development Teams and Departments
- SJCRH and Johns Hopkins Researchers
- Clinical investigators

Grants

SBIR # 1R43AI065016-01

Biodefense # 1UC1AI067161-01

DARPA # W911NF-05-1-0545