Development of Vaxfectin®-adjuvanted DNA Vaccines

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Advantages of Plasmid DNA Vaccines

- 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

Cationic Lipid-based Microparticulates

Poloxamer CRL1005-based Nanoparticulates

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Vaxfectin® Adjuvant

Cationic Lipid

\((\pm)-N-(3\text{-aminopropyl})-N,N\text{-dimethyl-2,3-bis(cis-9-tetradeceneyloxy)-1-propanaminium bromide}\)

GAP-DMORIE

DPyPE

Co-Lipid

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

Vaxfectin® Profile

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

pDNA/lipid Complex
## Vaxfectin® References
### Enhanced Responses

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Species</th>
<th>Immunogenicity</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 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 IFNy 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)

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

• ↓ serum anti-NP antibody titers in IL-6−/− mice (p = 0.02)
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 < 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)

Mouse & ferret H5N1 challenge studies

Gene and formulation screening studies

plasmid

cGMP vaccine manufacturing

Safety studies → IND → Human clinical trials

Human clinical trials
Mouse Challenge Model

Vaccination Regimen

Week 0

Week 3

Route: Intramuscular +/-Formulations

N ≥12/group

Challenge Regimen

Week 6

Week 6-8

Survival

Weight

LD₉₀

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 ($p < 0.032$)

![Graph showing survival rates and ELISPOT data](image-url)
Vaxfectin® provides significant survival benefit over DMRIE:DOPE at low doses.

Jimenez, Human Vaccines 2007
Cross-strain Protection

Influenza Challenge Model (H1N1)

**Survival: Vaxfectin®-formulated NP + M2**

- Vaxfectin®-formulated NP+M2 provides cross strain protection against H1N1 challenge
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 LD₅₀) at Day 42

- Inactivated HA vaccine
- H5
- H5 + NPav + M2av
- H5 + NPhu + M2hu
- NPav + M2av
- NPhu + M2hu
- pDNA backbone

p<0.001 by log-rank test
**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

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**Graph 1:**
- **X-axis:** Days After Infection
- **Y-axis:** Percent Survival
- **Legend:**
  - H5 + M2_{av} + NP_{av}
  - pDNA backbone

  *P value = 0.0009 by log rank test*

**Graph 2:**
- **X-axis:** Days After Infection
- **Y-axis:** Average Group Weight (kg)

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*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

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 LD50) at Day 42
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

No genomic integration
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)

![Graph 1](1x 100 µg gB pDNA w/ needle or B2000)

- Anti-gB GMT
- Days

![Graph 2](1x 500 µg gB pDNA in PBS IM ± EP)

- Anti-gB GMT
- Days
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® 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)
Vaxfectin® as a Th1 Adjuvant

**Anti-TIV IgG Isotypes**

**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.

<table>
<thead>
<tr>
<th>Isotype</th>
<th>GMT x fold compared to 0.1µgTIV</th>
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<tbody>
<tr>
<td>IgG1</td>
<td>17x</td>
</tr>
<tr>
<td>IgG2a</td>
<td>274x</td>
</tr>
<tr>
<td>IgG2b</td>
<td>208x</td>
</tr>
<tr>
<td>IgG3</td>
<td>294x</td>
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