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-aminopropyl)-N,N-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
## Vaxfectin® References
### Enhanced Responses

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<td>Hartikka, <em>Vaccine</em> 19:1911; 2001</td>
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<td>NP and various other antigens</td>
<td>Mice</td>
<td>↑ Ab titers 3-10X</td>
<td>Reyes, <em>Vaccine</em> 19: 3778; 2001</td>
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<td>D'Souza, <em>Inf Imm</em> 70: 3681; 2002</td>
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<td>Influenza NP or hGH</td>
<td>Rats</td>
<td>↑ NP Ab titers and CTLs</td>
<td>Sankar, <em>Oral Dis</em> 8: 275; 2002</td>
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<td>JEV prM/E</td>
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<td>↑ Neut Ab titers 8X</td>
<td>Nukuzuma, <em>Vir Imm</em> 16: 183; 2003</td>
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<td>Locher, <em>DNA Cell Biol</em> 23: 107; 2004</td>
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<td>HIV-2 Env tat nef gag/pro</td>
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<td>Plasmodium yoelli CSP</td>
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<td>Sedegah, <em>Vaccine</em> 24: 1921; 2006</td>
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<td>Margalith, <em>Gen Vac Ther</em> 4: 1; 2006</td>
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<td>Romano, <em>Vaccine</em> 24: 3353; 2006</td>
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<td>Hermanson, <em>PNAS</em> 101: 13601; 2004</td>
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<td>↑ Ab titers at 5 months vs rPA in Alhydrogel</td>
<td>Hahn, <em>Vaccine</em> 24: 4795; 2006</td>
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<td>Influenza HA, NP, M2</td>
<td>Mice</td>
<td>↑ # of IFNγ T cells vs PBS-formulation Protection against H5N1 challenge</td>
<td>Jimenez, <em>Human Vaccines</em>; 2007</td>
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<td></td>
<td>Mice/Ferrets</td>
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<td>Lalor et al., <em>JID</em>; 2008</td>
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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

plasmid

Mouse & ferret H5N1 challenge studies

cGMP vaccine manufacturing

Safety studies → IND

Human clinical trials
Mouse Challenge Model

Vaccination Regimen

Route: Intramuscular +/- Formulations

N ≥ 12/group

Week 0

Week 3

Challenge Regimen

Week 6

Week 6-8 Survival Weight

LD$_{90}$

Mouse-adapted A/HK/8/68 (H3N2) challenge

or

Mouse-adapted A/PR/8/34 (H1N1) challenge
Vaxfectin® and DMRIE:DOPE provide significant survival benefit over Poloxamer CRL1005 ($p < 0.032$)
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®

**Survival: Vaxfectin® vs. PBS at low doses**

- 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
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

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.

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

p<0.001 by log-rank test

P=0.14 by log-rank test
**DNA-based Pandemic Influenza Vaccine**

**Ferret H5N1 Challenge Study**

**HI Titers**

- [Day 0]
- [Day 14]
- [Day 35]
- [Day 63]

**Vaccine**

- Empty Plasmid
- NP+M2
- H5+NP+M2
- H5+NP+M2 (1X)

**Nasal Wash Virus Titers**

- Day 3
- Day 5
- Day 7

**Average Nasal Wash Titers (log10 EID50/mL)**

- Empty Plasmid
- NP+M2
- H5+NP+M2
- H5+NP+M2 (1 dose) [Day 21 injection]

**p-values**

- p = 0.044
- p = 0.041
- p = 0.004
- p = 0.021

*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) - 2$^{nd}$ 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 showing anti-gB GMT over days for 1x 100 µg gB pDNA w/ needle or B2000](image1)

![Graph showing anti-gB GMT over days for 1x 500 µg gB pDNA in PBS IM ± EP](image2)
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)

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

**Day 42 H1 HI**
- TIV alone
- + 300µg VAX
- + 900µg VAX

**Day 42 H3 HI**
- TIV alone
- + 300µg VAX
- + 900µg VAX
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>IgG Type</th>
<th>GMT x fold compared to 0.1µgTIV</th>
<th>GMT x fold 0.1µg TIV+ 900µg Vaxfectin®</th>
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<tr>
<td>IgG1</td>
<td>17x</td>
<td>17x</td>
</tr>
<tr>
<td>IgG2a</td>
<td>274x</td>
<td>274x</td>
</tr>
<tr>
<td>IgG2b</td>
<td>208x</td>
<td>208x</td>
</tr>
<tr>
<td>IgG3</td>
<td>294x</td>
<td>294x</td>
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**Pie Chart**

- **GMT x 10^3**
  - 0.1 µg TIV
  - 0.1 µg TIV + 900 µg Vaxfectin®

- **GMT x 10^6**
  - IgG1: 1.1
  - IgG2a: 2.7
  - IgG2b: 4.5
  - IgG3: 0.1
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