Development of Vaxfectin®-formulated HSV-2 Plasmid DNA Vaccines for Prophylactic and Therapeutic Applications

Sean M. Sullivan, PhD
Executive Director, Pharmaceutical Sciences and Process Development

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Herpes Simplex Virus Type 2 (HSV-2)

- Herpes virus family (dsDNA enveloped virus)
- Leading cause of genital herpes worldwide (STD)
- 1 in 6 infected in U.S. (40-60MM)
- 1 in 6 infected worldwide (>500M)
- Latent infection (nerves/ganglia)
  - 20% symptomatic patients with recurrences
- No licensed vaccine, only antiviral treatment
- HSV-2 costs in the U.S. estimated >$1B
- Unmet medical need
  - Prevention of infection (prophylactic vaccine)
  - Prevention of recurrence of lesions and transmission (therapeutic vaccine)
Epidemiology and Pathogenesis of Mucocutaneous HSV Infection

- **Initial infection:** Initial infection with retrograde transport of HSV to sensory nerve ganglia.
- **Transmission:** Mucosal viral shedding leads to sexual or perinatal transmission.
- **Latency:** Latency maintained by immune surveillance; immune control of virus also present at the mucosa.
- **Reactivation:** Reactivation from latency with mucosal shedding and lesions; virus travels anterograde to skin or mucosae.
HSV-2 pDNA-based Therapeutic Vaccine

- Selection of antigens
  - Envelope glycoprotein gD and tegument proteins VP11/12(UL46), VP13/14(UL47)
  - Based on human immunological response in HSV-2 infected subjects and preclinical animal models
  - Consensus protein sequences obtained by sequencing HSV-2 clinical isolates

- Antigen expression plasmids
  - Expression cassette optimized for maximal gene expression
  - Gene sequences codon-optimized for maximal protein translation

- Adjuvant
  - Vaxfectin® cationic lipid-based adjuvant
  - Extensive preclinical database and successful Phase 1 clinical trials showing favorable safety profile, and humoral and cell-mediated immune responses
HSV-2 Antigen Selection
Rationale for Antigen Targets

UL46 and UL47
- Abundant proteins (>1,000 copies) in virion
  - CD8+ targets prior to TAP shutdown by ICP47
  - DCs can cross present tegument proteins to CD8+ T cells
- Prevalent recognition by IFNγ-CD8+ human T cells
- CD4+ and CD8+ epitopes identified
- Skin-homing (CLA+) T cells found in lesions
- CD8+ T cells found at dermal-epidermal junction near nerves

Glycoprotein D (US6)
- Therapeutic POC in humans (Straus, Lancet 1994)
- Therapeutic POC in guinea pigs (Vical/UTMB)
- Prevalent recognition by IFNγ-CD4+ and CD8+ human T cells
- CD4+ and CD8+ epitopes identified
Mice received 100 μg of plasmid encoding UL46, UL47, or gD at 0, 2, 4 weeks IFN-γ ELISPOT assay performed 2-3 weeks later
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/lipid Complex

Vaxfectin® Profile
- Two-lipid mixture
- Forms microparticles
- Increases immune responses and protection in animal models
- Dose sparing
- Scaleable cGMP manufacturing
- Simple formulation
- Patented technology
H5 DNA Vaccine Phase 1 Trials

- Safety & immunogenicity endpoints
- Double-blind placebo-controlled
- 103 healthy subjects 18-45 years of age
- IM injections on Days 0 and 21
- All DNA vaccines formulated with Vaxfectin®

<table>
<thead>
<tr>
<th>Needle</th>
<th>N=56</th>
<th>Needle-free</th>
<th>N=47</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg Trivalent (0.03 mg H5 DNA)</td>
<td>6</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>0.5 mg Trivalent (0.17 mg H5 DNA)</td>
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<td>0.5 mg H5 Monovalent</td>
<td>6</td>
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<tr>
<td>1 mg Trivalent (0.3 mg H5 DNA)</td>
<td>15</td>
<td>1 mg H5 Monovalent</td>
<td>15</td>
</tr>
<tr>
<td>1 mg Monovalent</td>
<td>15</td>
<td>1 mg Trivalent (0.3 mg H5 DNA)</td>
<td>15</td>
</tr>
<tr>
<td>PBS</td>
<td>14</td>
<td>PBS</td>
<td>11</td>
</tr>
</tbody>
</table>
Antibody and T-cell Responses to Influenza H5 DNA Vaccine

HI antibody titers ≥40 in 47%-67%
- In range of protein vaccines

H5 IFN-γ T cells in 75%-100%

Smith et al., Vaccine 2010
Mouse Challenge Model

Vaccination
- Route: Intramuscular +/- Vaxfectin® (N = 10/group)

Challenge
- Week 6: Intramuscular +/− Vaxfectin® (N = 10/group)
  - HSV-2 strain 186
  - 50 x LD50 (1.5 x 10^4 PFU)

Ganglia Dissection
- Week 18

Week 0
- Vaccination
- Challenge
- Ganglia Dissection

Week 2
- Vaccination
- Challenge
- Ganglia Dissection

Week 4
- Vaccination
- Challenge
- Ganglia Dissection

Week 6
- Vaccination
- Challenge
- Ganglia Dissection

Week 6-8
- Vaccination
- Challenge
- Ganglia Dissection

Week 18
- Vaccination
- Challenge
- Ganglia Dissection

Serum for antibody by ELISA

Daily vaginal swab for HSV-2 qPCR

HSV-2 qPCR
Impact of HSV-2 Vaccine on Antibody Titers, Primary Infection and Latency

Anti-gD Antibody Titers
- PBS-S vs Vax-S: p = 0.034
- PBS-FL vs Vax-FL: p = 0.026

Vaginal HSV-2 DNA Copy Number
- Day 5 vaginal qPCR copies
  - PBS-S vs Vax-S: *p = 0.024
  - PBS-FL vs Vax-FL: *p = 0.019
- Day 90 DRG qPCR copies
  - PBS-FL vs Vax-FL: p = 0.007

Week 8 gD ELISA GMT t-test
- PBS-S vs Vax-S
- PBS-FL vs Vax-FL

Day 5 vaginal qPCR copies
Wilcoxon rank sum test

Day 90 DRG qPCR copies
Wilcoxon rank sum test
Summary of Murine Studies

- Vaxfectin® increased immunogenicity of gD pDNA
  - 5-6 fold increase in antibody titers compared to pDNA alone
- Vaxfectin®-formulated plasmid DNA decreased vaginal HSV-2 copy number following viral challenge
  - Vaxfectin®-formulated pDNA expressing full length or secreted gD reduced vaginal HSV-2 copy number compared to gD pDNA alone
  - pDNA expressing full length gD resulted in lower vaginal HSV-2 copy number than pDNA expressing secreted gD
- Vaxfectin®-formulated gD pDNA reduced viral latency
  - 60% of mice had undetectable HSV-2 viral genomes in Vaxfectin®/gD pDNA treatment group
Guinea Pig Prophylactic Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Vaccine</th>
<th>pDNA dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>15</td>
<td>PBS</td>
<td>NA</td>
</tr>
<tr>
<td>gD +Vaxfectin®</td>
<td>15</td>
<td>FL-gD pDNA Backbone</td>
<td>300 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>300 µg</td>
</tr>
<tr>
<td>gD/UL46/UL47 +Vaxfectin®</td>
<td>15</td>
<td>FL-gD UL46/UL47</td>
<td>300 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150 µg/150 µg</td>
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</table>

**Primary Infection**

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>21</th>
<th>42</th>
<th>63</th>
<th>78</th>
<th>126</th>
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</thead>
<tbody>
<tr>
<td>DNA Vaccinations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-2 Strain MS (10^6 PFU)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Lesion Recurrence**

Days 112-126 Assayed for Viral Shedding
## HSV-2 Neutralizing Antibody Titers

<table>
<thead>
<tr>
<th>Group</th>
<th>Neutralizing Ab titer</th>
<th>Post 2\textsuperscript{nd} immunization</th>
<th>Post 3\textsuperscript{rd} immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>&lt;1:10</td>
<td>&lt;1:10</td>
<td></td>
</tr>
<tr>
<td>gD</td>
<td>1:2560</td>
<td>1:10240</td>
<td></td>
</tr>
<tr>
<td>gD/UL46/UL47</td>
<td>1:1280</td>
<td>1:5120</td>
<td></td>
</tr>
</tbody>
</table>
### HSV-2 Primary and Recurrent Disease

<table>
<thead>
<tr>
<th>Group</th>
<th>Primary Disease</th>
<th>Recurrent Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence</td>
<td>Severity&lt;sup&gt;a&lt;/sup&gt;, mean ± SD</td>
</tr>
<tr>
<td>Naïve</td>
<td>15/15</td>
<td>9.3 ± 4.7</td>
</tr>
<tr>
<td>gD</td>
<td>0/15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>gD/UL46/UL47</td>
<td>0/15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Severity defined as cumulative daily lesion score
<sup>b</sup> Frequency defined as recurrent lesion days between days 15-63 post inoculation
<sup>c</sup> p<0.001 compared to naïve
Guinea pigs were vaccinated three times three weeks apart and infected with 10^6 pfus of HSV-2 MS strain 3 weeks after last vaccination. Vaginal swabs were taken and assayed for viral genomes (GE) by qPCR on days 1-5 postinfection.
## HSV-2 Shedding in Recurrent Disease

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th>Frequency&lt;sup&gt;a&lt;/sup&gt;, mean ± SD</th>
<th>Quantity&lt;sup&gt;b&lt;/sup&gt;, log&lt;sub&gt;10&lt;/sub&gt; GE/mL (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>9/11</td>
<td>2.8 ± 1.4</td>
<td>4.0 ± 0.9</td>
</tr>
<tr>
<td>gD</td>
<td>6/15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5 ± 1.0</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>gD/UL46/UL47</td>
<td>6/15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8 ± 1.3</td>
<td>3.6 ± 1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Frequency is number of days of vial shedding between days calculated using only animals experiencing shedding last 14 days of the study (days 112-126)

<sup>b</sup> Quantity is amount of virus shed per event

<sup>c</sup> P=0.05 compared to naïve
### HSV-2 DRG Load

<table>
<thead>
<tr>
<th>Group</th>
<th>Dorsal Root Ganglia virus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Virus load, $\log_{10}$ GE/mL (mean ± SD)</td>
</tr>
<tr>
<td>Naïve</td>
<td>10/10</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>gD</td>
<td>5/15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>gD/UL46/UL47</td>
<td>1/15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Incidence is defined as the number of animals in which HSV-2 DNA was detectable from ganglia/number tested

<sup>b</sup> $p<0.001$ compared to naïve

<sup>c</sup> $p<0.0001$ compared to naïve
Conclusions from Guinea Pig Prophylactic Study

- gD monovalent and gD/UL46/UL47 trivalent produced approximately equivalent neutralizing antibody titers
- Monovalent and trivalent vaccines reduced viral replication by >2 logs
- Monovalent and trivalent vaccines reduced latent infection with the trivalent vaccine showing greater reduction in viral latency
## Guinea Pig Therapeutic Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Vaccine</th>
<th>pDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>gD/UL46/UL47 + Vaxfectin®</td>
<td>14</td>
<td>FL-gD UL46/UL47</td>
<td>300 µg</td>
</tr>
<tr>
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<td>Naïve</td>
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<td>NA</td>
</tr>
</tbody>
</table>

### Primary Infection
- HSV-2 Strain MS (10⁶ PFU)

### Lesion Recurrence
- DNA Vaccinations
- Days 42-63 Assayed for Viral Shedding
Reduction in Frequency of Recurrent Lesions in Vaccinated Guinea Pigs

60 guinea pigs infected with $10^6$ pfu HSV-2 strain MS on day 0; 15 days after primary infections resolved, guinea pigs randomized into treatment groups.

Guinea pigs monitored daily for recurrent lesions and score; vaginal swabs taken last 14 days and assayed for HSV-2 DNA by PCR.
### Reduction of Viral Shedding in Vaccinated Guinea Pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals Shedding Virus</th>
<th>Days of Virus Shedding&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Virus Shed (Log&lt;sub&gt;10&lt;/sub&gt; HSV-2 Genomes)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>gD/UL46/47</td>
<td>9/14 (64%)</td>
<td>1.07 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.45 ± 0.20</td>
</tr>
<tr>
<td>Naive</td>
<td>12/14 (86%)</td>
<td>2.29 ± 0.41</td>
<td>2.91 ± 0.19</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean (± SE) number of shedding days/animal over the last 14 day period

<sup>b</sup> Mean (± SE) HSV-2 genome copies

<sup>c</sup> p < 0.05 compared to control by t-test
Conclusions from Guinea Pig Therapeutic Studies

- gD/UL46/UL47 trivalent vaccine significantly reduced lesion recurrence compared to naïve control
- Trivalent vaccine reduced viral shedding
- UL46/UL47 bivalent vaccine did not significantly reduce lesion recurrence compared to naïve control showing the importance of including gD pDNA in the vaccine
Clinical Development

- FDA Pre-IND meeting
- cGMP manufacture of each plasmid DNA
- Manufacture single vial drug products
- Rabbit repeat dose toxicology study (shown to be well tolerated)
- Rabbit biodistribution study (shown to have acceptable clearance rate)
- Submit IND
HSV-2 Therapeutic Vaccine
Phase 1/2 Trial Design Overview

- Placebo-controlled trial in ~150 HSV-2+ adults
  - History of symptomatic genital herpes lesions
- Regimen
  - 2 months pre-vaccination shedding data
  - 3 vaccinations at monthly intervals
  - 2 months post-vaccination shedding data
- Primary endpoints
  - Safety and tolerability in HSV-2+ healthy subjects
  - Comparison of HSV-2 shedding rate in each subject before and after
- Planned initiation late 2013