Clinical Development of Formulated Therapeutic and Prophylactic DNA-based Vaccines

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Human Cytomegalovirus (CMV)

- Beta herpesvirus (HHV-5)
- Infects 50-85% of US adults by age 40
- CMV not eliminated after 1° infection
  - Remains in latent state within granulocytes, macrophages, and dendritic cell precursors
- Asymptomatic in immunocompetent host
- High risk groups
  - Hematopoietic stem cell transplant patients
  - Solid organ transplant patients
  - AIDS patients and other immunocompromised patients
  - Fetuses of mothers infected during pregnancy
- Current therapies
  - Antiviral drugs (e.g., Ganciclovir, Valganciclovir)
  - Cytogam®: Pooled human immune globulin
CMV DNA Vaccines

- **TransVax™ CMV therapeutic vaccine**
  - Phase 2 clinical data in 2010 in CMV-seropositive HCT recipients
  - Potential breakthrough in a niche market with the potential to expand to SOT

- **CyMVeclin™ CMV prophylactic vaccine**
  - Potential for clinical efficacy data in CMV-seronegative women of childbearing potential
  - Addresses one of the last large ID markets for vaccines
TransVax™ CMV Vaccine

Product Description

- Single vial formulation
  - Two plasmids: gB + pp65 (5 mg total DNA in 1 mL dose)
  - CRL1005 poloxamer (7.5 mg/mL) + Benzalkonium chloride (BAK; 0.11 mg/mL)
  - Stable at -30°C for >3 years

- Completed Phase 1 trial
  - Successfully dose-escalated up to 5 mg DNA dose by IM injection (*J Infect Dis* 2008)
  - Induced T-cell and antibody responses
  - Noninfectious

- Orphan drug status for HCT and SOT in the U.S.
CMV in Hematopoietic Cell Transplant
Hypotheses and Assumptions

- Reactivation in ~60% of CMV+ recipients within 100 days after transplant; if untreated can cause pneumonitis, hepatitis, gastroenteritis, retinitis, leukopenia, encephalitis
  - Meyers et al., 1986; Junghanss et al., 2002, 2003
- DNA vaccination should increase CMV-specific T-cell and antibody responses
  - Selinsky et al., 2005; Hartikka et al., 2008; Wloch et al., 2008
- Increased cellular immune responses should reduce viremia (incidence, onset, duration)
  - Cwynarski et al., 2001; Aubert et al., 2001, Hakki et al., 2003
- Reduced viremia should decrease antiviral use and CMV disease
  - Emery et al., 2000
TransVax™ CB01-202 Phase 2 HCT Trial

- Randomized, double-blind, placebo-controlled
  - 18-65yo CMV+ HCT recipients with leukemia or lymphoma
  - 6/6 or 5/6 HLA allele match
  - Stratified by site, donor CMV status, HLA match
  - Randomized 1:1 for active vs. placebo
- Multicenter (16 enrolling U.S. sites)
- Completed enrollment 4Q08
  - 1 year follow-up after transplant
TransVax™ Phase 2 Trial
Recipient-only Immunization Regimen

Hematopoietic Cell Transplant (HCT Study)

HCT Donor Related or Unrelated

HCT Recipient (Immunosuppressed by full/partial myeloablation)

CMV Seropositive Recipient (CMV - Recipients Excluded)

HCT Day -5 to -3
Day 21 to 42
Day 84 (+ 7)
Day 196 (+ 14)
Viral Load and Antiviral Therapy Use

- Viral load monitored by both local and central lab assays
  - Weekly (wk 3-13), Biweekly (wk 14-28), Monthly (wk 29-52)
- Central Mayo lab used standardized assay for comparing viral loads from all sites
  - Roche LightCycler PCR using polymerase primers
- Different local lab assays and treatment algorithms at each site used to determine dose and duration of antiviral therapy
  - PCR or antigenemia assays
- Four antivirals used for induction and maintenance Rx
  - Ganciclovir, Valganciclovir, Foscarnet or Cidofovir
Interim Analysis Endpoints

- Safety
- Cellular response (pp65 and gB)
- Antibody response (gB)
- **Viral load** (≥ 500 copies/mL lower limit of detection by PCR)
  - Occurrence of CMV infection (*any occurrence of ≥ 500 copies/mL*)
  - Duration of viremia (*% of days on study with ≥ 500 copies/mL*)
  - Viral load Area Under the Curve (*total copy numbers for time on study*)
  - Peak viral load (*highest copy number by PCR during study*)
# Demographic and Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>TransVax™ (N=40)</th>
<th>Placebo (N=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (52%)</td>
<td>12 (35%)</td>
</tr>
<tr>
<td>Male</td>
<td>19 (48%)</td>
<td>22 (65%)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>37 (93%)</td>
<td>31 (91%)</td>
</tr>
<tr>
<td>African American</td>
<td>3 (7%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (years)</td>
<td>49.8</td>
<td>49.1</td>
</tr>
<tr>
<td><strong>Donor CMV status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20 (50%)</td>
<td>19 (56%)</td>
</tr>
<tr>
<td>Negative</td>
<td>20 (50%)</td>
<td>15 (44%)</td>
</tr>
<tr>
<td><strong>HLA Allele Matching</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6</td>
<td>37 (93%)</td>
<td>32 (94%)</td>
</tr>
<tr>
<td>5/6</td>
<td>3 (7%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td><strong>Relatedness to Donor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related</td>
<td>22 (55%)</td>
<td>15 (44%)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>18 (45%)</td>
<td>19 (56%)</td>
</tr>
<tr>
<td><strong>Conditioning Regimen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloablative</td>
<td>29 (73%)</td>
<td>23 (68%)</td>
</tr>
<tr>
<td>Nonmyeloablative</td>
<td>11 (27%)</td>
<td>11 (32%)</td>
</tr>
</tbody>
</table>

*Groups were stratified by site, donor CMV status, and HLA match*
Preliminary Safety

- **Data Safety Monitoring Board**
  - DSMB reported ‘No safety concerns detected’ for first 20 patients through Day 56
  - Continued review of SAEs and safety reports for remainder of study

- **SAEs**
  - One report of angioedema after second dose, possibly related to study drug or metaclopramide
  - No other study discontinuations due to related AEs
  - No difference between groups in SAEs
### Clinical Observations Up to 4 Months

<table>
<thead>
<tr>
<th></th>
<th>TransVax™ (N=40)</th>
<th>Placebo (N=34)</th>
<th>TransVax™ vs Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of injections</td>
<td>2.6</td>
<td>2.6</td>
<td>-</td>
</tr>
<tr>
<td>Initiation of CMV antiviral Rx</td>
<td>19 (48%)</td>
<td>18 (53%)</td>
<td>- 9%</td>
</tr>
<tr>
<td>Recurrent use of CMV antiviral Rx</td>
<td>4 (10%)</td>
<td>7 (21%)</td>
<td>- 52%</td>
</tr>
<tr>
<td>CMV disease</td>
<td>3 (7%)</td>
<td>3 (9%)</td>
<td>-</td>
</tr>
<tr>
<td>Death</td>
<td>3 (7%)</td>
<td>3 (9%)</td>
<td>-</td>
</tr>
</tbody>
</table>
T-cell Responses to pp65
Up to 1 Year

Median pp65 IFN-γ ELISPOT Response

<table>
<thead>
<tr>
<th>Days</th>
<th>Vaccine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>215</td>
<td></td>
<td></td>
</tr>
<tr>
<td>290</td>
<td></td>
<td></td>
</tr>
<tr>
<td>365</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Wilcoxon rank sum test

N=20-33 for Placebo
N=26-33 for Vaccine

SFU/10^6 PBMC
T-cell Responses to gB
Up to 1 Year

Median gB IFN-γ ELISPOT Response

Vaccine
Placebo

N=20-33 for Placebo
N=26-33 for Vaccine
Antibody Responses to gB
Up to 1 Year

Geometric Mean gB Antibody (90% CI)

N=20-34 for Placebo
N=26-38 for Vaccine
Wilcoxon rank-sum test
# Viral Load Endpoints
## Up to 4 Months

<table>
<thead>
<tr>
<th></th>
<th>TransVax™ (N=40)</th>
<th>Placebo (N=34)</th>
<th>% Reduction vs Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Occurrence of CMV infection</strong> (subjects reaching ≥ 500 copies/mL)</td>
<td>12 (30%)</td>
<td>17 (50%)</td>
<td>-40%</td>
</tr>
<tr>
<td><strong>Recurrence of CMV infection</strong> (subjects with 2 or more episodes of ≥ 500 copies/mL)</td>
<td>3 (7%)</td>
<td>7 (21%)</td>
<td>-67%</td>
</tr>
<tr>
<td><strong>Duration of viremia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (days)</td>
<td>7.8</td>
<td>11.9</td>
<td>-35%</td>
</tr>
<tr>
<td>Mean (percentage)</td>
<td>7.1</td>
<td>10.5</td>
<td>-32%</td>
</tr>
<tr>
<td><strong>AUC viral load (total copies)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>779</td>
<td>1021</td>
<td>-24%</td>
</tr>
<tr>
<td>Range</td>
<td>83-14,474</td>
<td>198-14,417</td>
<td></td>
</tr>
<tr>
<td><strong>Peak viral load (copies/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2575</td>
<td>8537</td>
<td>-70%</td>
</tr>
<tr>
<td>Median</td>
<td>ND</td>
<td>1125</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>ND-29,500</td>
<td>ND-145,000</td>
<td></td>
</tr>
</tbody>
</table>

*ND: not detectable

Based on PCR results from central lab (Mayo Clinical Trial Services)*
Time to Initial Viral Reactivation

*Up to 4 Months*

**Figure:** Time to Initial $\geq 500$ copies/mL, Through 4 Months
Per Protocol Subjects with Recipient Immunization Only

Note: Plotted circles represent censored data.
DNA vaccination was safe and immunogenic in the HCT population
  - Apart from an allergic response in one subject, no evidence of unexpected safety concerns in this population

TransVax™ improved cellular responses to pp65 and gB vs. placebo

TransVax™ enhanced the level of gB antibodies, once the immune system recovered, and these were sustained at one year

A 4th dose of TransVax™ was safe and augmented the response

Viral load endpoints at 4 months favored TransVax™ vs. placebo
  - Decreased occurrence of CMV infection (detectable viremia by Mayo Clinic PCR)
  - Decreased recurrence of CMV infection
  - Delayed time to initial detectable viremia
  - Decreased duration of viremia
  - Decreased AUC & peak viral load
Congenital CMV Infection

Unmet Medical Need

- Most common intrauterine infection in the U.S.
  - 30%-40% risk if primary infection occurs during pregnancy
  - 1% of newborns are infected
- Results in death or severe disability in >8,000 infants per year
  - Leading infectious cause of sensorineural hearing loss and mental retardation
  - Every hour another child becomes permanently disabled from congenital CMV Infection
- 400 infants die each year
- Major priority for vaccine as sanctioned by Institute of Medicine
- No standard of care therapy
- No licensed vaccine or late-stage vaccines in development
CyMVectin™ CMV Vaccine

**Product Concept**

Vaxfectin®-formulated gB +/- pp65 pDNA vaccine

- Antibodies to gB are the predominant correlate of protection for CMV infection
  - gB protein vaccine reduced maternal infection (*Pass, NEJM 2009*)
- pp65 alone can reduce maternal viral load in guinea pig model
- Strong gB antibody responses induced in animal models
- Phase 1 trials of Vaxfectin®-formulated DNA vaccine completed (H5N1 influenza)
  - Well-tolerated safety profile
  - Antibody and T-cell responses achieved with only 2 doses
    - H5 considered to be poor-to-modest immunogen
  - 3 IM injections of Vaxfectin®-formulated CMV DNA vaccine to be tested with regimen similar to Gardasil®
Vaxfectin® Adjuvant

Cationic Lipid

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

GAP-DMORIE

DPyPE

Cationic Liposomes

Co-Lipid

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

Vaxfectin® Profile

• Two-lipid mixture
• Forms microparticles
• Increases immune responses and protection in animal models
• Dose sparing
• Scaleable cGMP manufacturing
• Simple formulation
• Patented technology

pDNA/lipid Complex
NZW rabbits (n=6 per group) injected with 100 µg gB DNA (PBS or Vaxfectin® formulation) by single IM (vastus lateralis) or ID injection with needle or Biojector®2000 on Days 0 and 21
Immune Responses to Vaxfectin®-formulated DNA vs Electroporation

Immunizations
- IM or ID delivery of Vaxfectin®-formulated pDNA CMV vaccine resulted in anti-gB titers similar to those obtained with EP-assisted delivery (historical study).

- 1x 100 µg gB pDNA w/ needle or B2000
- 1x 500 µg gB pDNA in PBS IM ± EP

- Const. current
- Const. voltage
- PBS, Needle
Biodistribution of Vaxfectin®-formulated DNA Vaccine in Rabbits

**Needle & Syringe**

**Biojector® 2000**

Bilateral IM injection of 0.5 mg DNA/muscle (~28X human dose (mg/kg)); 5 rabbits/sex/timepoint; tissues analyzed at Day 29\(^a\) and Day 61\(^b\).
Summary of Phase 1 Vaxfectin®-formulated DNA Vaccine (H5N1)

Safety
- All vaccine doses were well tolerated
  - No discontinuations due to vaccination/adverse events
  - Adverse events typically resolved in a few days
- No vaccine-related clinical or laboratory serious adverse events
- Most common reactions: Injection site pain, headache, malaise, myalgia

Immune responses
Monovalent cohorts:
- HI antibody response rates from 47%-67%
  - Peak titers by day 56, sustained titers to day 182 in 33%-50%
- HI antibody responses in the reported range of protein-based vaccines
- H5 T-cell responses in 75%-100% sustained for at least 6 months
CyMVectin™

Proof-of-Concept Clinical Trial

- IND allowed by the FDA
- A Phase 1 trial in CMV seronegative women with a child in day care center
  - CMV infection rate in women of childbearing age > 20% if child in day care center
  - Immunization schedule: 0, 1 and 6 months
- Endpoints
  - Immunogenicity
  - Safety
  - Occurrence rate of, and time to CMV infection
- Clinical “proof of concept” with approximately 80 subjects
  - Randomized 1:1 for active vs. placebo
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University at Stony Brook