Human Cytomegalovirus Vaccines

by Stanley A. Plotkin
I am a paid consultant to almost all of the CMV vaccine projects discussed here and thus have multiple conflicts of interest, which cancel each other.
Why a CMV Vaccine?

- To prevent congenital infection in infants of seronegative women, and if possible seropositive women

- To prevent CMV infection in transplant recipients
  - Seronegative solid organ transplant recipients at high risk of primary infection
  - Seropositive bone marrow transplant patients at high risk of reactivation
<table>
<thead>
<tr>
<th>Year(s)</th>
<th>Vaccine Type and Collaborators</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974-79</td>
<td>Attenuated AD-169 (Elek + Stern with Merck)</td>
</tr>
<tr>
<td>1975-79</td>
<td>Attenuated Towne (Plotkin with GSK)</td>
</tr>
<tr>
<td>1980-85</td>
<td>Attenuated Towne (Plotkin with Merck)</td>
</tr>
<tr>
<td>1985-90</td>
<td>Towne (Plotkin with NIAID)</td>
</tr>
<tr>
<td>1991-2001</td>
<td>gB (Chiron)</td>
</tr>
<tr>
<td>1995</td>
<td>Canarypox vector (Sanofi)</td>
</tr>
<tr>
<td>1996</td>
<td>Towne-Toledo Recombinants (MedImmune)</td>
</tr>
<tr>
<td>1997</td>
<td>Peptides (City of Hope)</td>
</tr>
<tr>
<td>2000</td>
<td>IOM Report on Vaccine Priorities</td>
</tr>
<tr>
<td>2001</td>
<td>gB (Sanofi)</td>
</tr>
<tr>
<td>2005</td>
<td>DNA plasmids (Vical)</td>
</tr>
</tbody>
</table>
Responses to Towne Vaccine

- Local reaction
- ACIF Ab
- NT Ab-Towne
- NT Ab-Toledo
- HCMV-LPR
Outcome of Exposure to Transplanted Kidney from a CMV-Seropositive Donor (D+) in Renal Transplant Recipients
Cadaveric (CAD) Renal Allograft Actuarial Survival for R-D+ Group

![Graph showing actuarial survival rates for VACCINE/CAD (n=11) and PLACEBO/CAD (n=15) groups over 60 months.](image-url)
Cytomegalovirus

non-structural protein: IE1

pp65, pp150

gB

gH/gl/
UL128-131

Copyright 1994 - '97 Marko Reschke
Chief inducer of antibodies that prevent entry into fibroblasts

Developed by Chiron together with MF/59 adjuvant

Well tolerated in hundreds of vaccinees

Immunogenic in adults and toddlers in 3 dose regimen: 0, 1, 6 mo

Now developed by Sanofi Pasteur and GSK
Kaplan-Meier Curve of CMV Infections Related to Vaccination with gB

B) After 2 Doses Per Protocol Population

Probability of CMV Infection (%)

Study Month

Log Rank Test
p-value = 0.0824

Bernstein, D., Et al, doi: 10.1016/j.vaccine.2015.11.056
Sanofi Pasteur gB/MF59 in Kidney or Liver Transplant Patients

Proportion of days that patients in the three subgroups at risk of CMV infection

Viremia

Antiviral Use

Antibody and Memory B-Cell Responses to GSK 15 mcgx 3 gb/AS01

**Anti-gB IgG antibody**

**Anti-gB IgG avidity**

**Anti-CMV neutralising antibody**

**gB-specific memory B-cells**

Natural infection level of immunity is defined by testing sera from 39 healthy subjects with the same assays.
Translation to Enveloped Viruses has been Challenging

• Nature
  o Enveloped virions share three key features
  o Glycoprotein antigens do not natively dictate particle structure

• Viral Mimic
  o VBI eVLPs mimic key elements of enveloped viruses
  o Glycoprotein antigens find a “native like” home in lipid bilayer
  o T-cell antigens can be fused in-frame with protein capsid core

Neutralizing Antibody Titers in Rabbits Equivalent to or Exceeding Natural Infection

The endpoint neutralization titer of individual rabbit sera (n=5/group) collected 28 days after a 2nd immunization are shown against CMV infection of fibroblast and epithelial cells. The neutralizing activity of CMV+ human sera were also evaluated.
Cytomegalovirus

non-structural protein: IE1

pp65, pp150

gB

gH/gL/
UL128-131
CTL Induction by Canarypox-pp65

- ▲ Seronegative vaccinated
- □ Originally seropositive vaccinees
- ○ Seronegative placebo-inoculated

% pp65-specific lysis vs. Time after initial immunisation (month)

Viral (Astellas) CMV DNA Vaccine

- Bivalent – DNA for gB and pp65
- Poloxamer adjuvant (nanoparticle)
- After 5 mg dose x3 or 4 in Seropositive
- Bone Marrow Transplant recipients
  - ↓ viral load
  - ↓ antiviral therapy
% Subjects with ≥500 CMV Copies/ml

* p-value from a log-rank test with stratification by site; Plotted circles represent censored data; Viral load determined by a central lab PCR assay
Global study initiated June 2013

500 CMV seropositive allogeneic HCT recipients

1:1 randomized, double blind, placebo-controlled

5 doses: 0, 1, 2, 3, 6 months

Primary endpoint: Composite of overall mortality and CMV end organ disease

~ 80 sites in U.S., Canada, EU, Australia, Japan, Korea, Taiwan

Enrollment completion expected 3Q16
Effect of City of Hope pp65 Peptide Vaccine on CMV Reactivation

Figure 2: Kaplan-Meier estimates of relapse-free survival
Patients were followed up to May 31, 2015. HCT = haemopoietic cell transplantation. HR = hazard ratio.

Viraemia, immunogenicity, and survival outcomes of cytomegalovirus chimeric epitope vaccine supplemented with PF03512676 (CMV PepVax) in allogeneic haemopoietic stem-cell transplantation: randomised phase 1b trial.

Nakamura R1, La Rosa C2, Longmate P1, Drake J1, Staple C1, Zhou Q2, Larriga MG2, O’Donnell M1, Cai L1, Facal L1, Sailhotra A1, Snyder DS1, Aldoss I1, Forman SJ1, Miller JS1, Zaia JA1, Diamond DJ1
Figure 4: Kaplan-Meier estimates of CMV reactivation.

Patients were followed up for at least 6 months after HCT. HCT = haemopoietic cell transplantation. HR = hazard ratio.
Cytomegalovirus

non-structural protein: IE1

pp65, pp150

gB

gH/gL/UL128-131
Antibody Response to HCMV: Rationale for gH/gL/UL128 UL130/UL131 as a candidate Vaccine
Speed of Response to Pentamer Correlates with CMV Fetal Transmission

Lilleri et al, PLOS One 2013
The appearance of rhCMV-neutralizing antibodies is delayed in acutely rhCMV-infected, CD4+ T-cell–depleted females.

Kristy M. Bialas et al. PNAS 2015;112:13645-13650
Importance of Pentamer in Protection Against Intrauterine Infection

- Antibodies block infection of cytotrophoblasts
- Rapid production of antibodies reduces transmission
- Magnitude and kinetics of CD4+ T cells reduces transmission

CMV Vaccine Concept is Based on Replication Defective Virus

- inclusion of pentameric complex
- T-cells that may contribute to protective immunity
- UL51 and IE1/2 are fused to ddFKBP, which renders the CMV proteins unstable and therefore prevents replication, whereas the addition of Shld-1 stabilizes the ddFKBP and therefore permits replication.

Vaccine production (with Shld-1)  Vaccination (no Shld-1)
# Live CMV Vaccines in Development

<table>
<thead>
<tr>
<th>Attenuated strain (Towne)</th>
<th>Med Coll VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinants with wild virus (Towne-Toledo)</td>
<td>Medimmune</td>
</tr>
<tr>
<td>Replication-defective virus</td>
<td>Merck</td>
</tr>
<tr>
<td>Alphavirus Replicon Vectored: Pox, adeno, LCMV, VSV</td>
<td>Novartis, Sanofi Pasteur, City of Hope, Queensland Inst., Paxvax, Hookipa, Yale</td>
</tr>
</tbody>
</table>
MVA Vectored Vaccine

- Don Diamond and associates of City of Hope
- Designed for transplant patients
- Contains peptides from pp65 and IE1 proteins
- With tetanus helper epitope and a CpG adjuvant
- Stimulates CD4+ and CD8+ T cells in normal volunteers
- Now being tested in stem cell transplant patients (randomized phase 2)
## Non-living CMV Vaccines in Development

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Company(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant gB glycoprotein with adjuvant (2)</td>
<td>Sanofi Pasteur, GSK</td>
</tr>
<tr>
<td>DNA plasmids</td>
<td>Vical, Inovio</td>
</tr>
<tr>
<td>Self-replicating RNA</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>Peptides</td>
<td>City of Hope</td>
</tr>
<tr>
<td>Dense bodies</td>
<td>Vaccine Project Management (Germany)</td>
</tr>
<tr>
<td>Virus-like particles</td>
<td>Variations Bio, Redbiotech</td>
</tr>
<tr>
<td>Soluble Pentamers</td>
<td>Humabs</td>
</tr>
</tbody>
</table>
pp65 T cell responses do not prevent reinfection but reduce viral dissemination during primary infection.

Reinfection is mediated by the action of US2-11, which inhibits HLA-mediated host responses.
likely protective immune correlates

- Neutralizing antibodies against:
  - Fibroblast entry mediated by gB
  - Epithelial cell entry mediated by pentameric gH/gL/UL128-131 complex
  - *but not mutually exclusive*

- CD4+ T cells providing help
  - mediated by Tfh cells for B cells
- CD8+ T cells providing CTL:
  - mediated principally by pp65 tegument protein and IE1 non-structural protein
Chief Unanswered Questions About Prevention of CMV

- Importance of cellular immune response in maternal-fetal transmission?
- Can maternal-fetal infection in seropositive women be prevented by boosting antibody or CMI to US 2-11 proteins?
- Can protective immune responses be prolonged over age of child-bearing?
How to Demonstrate Efficacy of a CMV Vaccine

- Artificial challenge with low passage virus
- Prevent infection of women whose children are in day care
- Prevent infection of children entered in day care
- Prevent disease or infection in solid organ and Hematogenous transplant recipients
- Cohort study in pre-pregnant women to prevent later fetal infection
- Prevention of fetal disease
Probable First Targets for CMV Vaccination

- Girls 11-13 yrs. of age  
  (association with HPV, TdAcP, MCV4)

- Seronegative women of child-bearing age

- All infants, to reduce viral circulation

- Solid organ transplant recipients

- Hematogenous stem cell transplant recipients
Vaccination in Solid Organ Transplantation (Recipients)

Possible Endpoint:

Viral Load

Use of Antivirals

Graft rejection
In 2016

- There is ample need for a CMV vaccine
- There is proof of concept for a CMV vaccine
- There are many experimental candidates
- The important antigens, depending on the clinical population, are:
  - gB
  - Pentamer complex
  - pp65
- The path to licensure has been defined by FDA