Efficacy of a Trivalent Subunit Antigen Vaccine in Prevention of Genital Herpes
HSV Epidemiology

HSV-1
- 5 billion infected worldwide: 60% in high income countries, 90% in low income countries
- HSV-1 more common than HSV-2 as cause of 1st time genital herpes
- 200 million cases of genital herpes caused by HSV-1

HSV-2
- ½ billion infected worldwide: 11% seroprevalence 15-49 yr worldwide, 30% in Africa (higher in sub-Saharan Africa), 14% USA
- Ratio of infection female to male: 60:40
Rationale for an HSV-2 vaccine

- Some individuals get very ill
- Many worry about transmitting, including to newborns
- 3-4-fold higher risk of acquiring and transmitting HIV that is not reduced by acyclovir
- In settings with high HSV-2 prevalence, 25-50% of HIV infections are attributable to HSV-2

Concerns

- Animal models have not been used well to predict likelihood of success in humans
Latest HSV-2 subunit vaccine trial

GSK/NIH gD2 subunit vaccine

- **Design:** > 8000 HSV-1, -2 seronegative women, age 18-30 years
gD2 or hep A as control, 3 doses at 0, 1, 6 months
- **1° endpoint:** HSV-1 or HSV-2 genital herpes disease
- **Result:** Vaccine was efficacious against HSV-1 genital disease (57%) but not HSV-2
- **Comment:** Neutralizing antibody titers were low (peaked at 1:29) and did not persist

Belshe et al NEJM 2012
Why is developing an HSV-2 vaccine difficult?

- HSV-1 and -2 encode many proteins that inhibit innate and acquired immunity
- 2 immune evasion molecules are expressed on the virus envelop and at the surface of infected cells, making them potential targets for blocking antibodies
  
  gC - inhibits complement activation
  gE - inhibits antibody activities
Subunit antigen vaccine

Baculovirus subunit antigens - almost the entire ectodomains:

- gD2 – block entry
- gC2 – block immune evasion from complement
- gE2 – block immune evasion from antibody

Adjuvants:

- CpG – good B and T cell agonist
- Alum – good B cell agonist
Immunization with gC and gE generates antibodies that block C3b and IgG Fc binding.
gC2/gD2/gE2 as HSV-2 prophylactic vaccine

Goals:
Prevent acute disease
Prevent recurrent disease
Prevent risk of transmission to partner

The perfect result:
Acute disease: 0 days
Recurrent disease: 0 days
Genital shedding of HSV-2 virus: 0 days
gC2/gD2/gE2 as HSV-2 prophylactic vaccine

Goals:
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The perfect result:
Acute disease: 0 days
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Genital shedding of HSV-2 virus: 0 days

Our result:
Acute disease: 0.3% days
Recurrent disease: 1% days
Genital shedding of infectious virus during recurrent phase of infection: 0.2% of days
Prevention studies in guinea pigs

Immunizations: 85 animals

1. Mock (adjuvant alone) – 3 doses
2. gD2 with CpG/alum – 3 doses
3. gC2/gD2/gE2 antigen with CpG/alum adjuvants 3 doses
4. gC2/gD2/gE2 with CpG/alum + 4th dose with gD2 CpG/alum

Awasthi et al PLOS Path 2017
Survival and other disease events in guinea pigs

![Survival Graph]

- **Mock** n=24
- **gD2** n=25
- **Trivalent** n=27
- **Trivalent + gD2** n=9

*P*<0.001
**Guinea pigs: genital disease**

**Days acute genital lesions**

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock (n=24)</td>
<td>88/250 (35.2%)</td>
<td></td>
</tr>
<tr>
<td>gD2 (n=25)</td>
<td>9/350 (2.6%)</td>
<td></td>
</tr>
<tr>
<td>Trivalent (n=27)</td>
<td>1/378 (0.3%)</td>
<td>**</td>
</tr>
<tr>
<td>Trivalent + gD2 (n=9)</td>
<td>0/126 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

**P<0.001**

**Days recurrent genital lesions**

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock (n=8)</td>
<td>85/340 (25%)</td>
<td>***</td>
</tr>
<tr>
<td>gD2 (n=25)</td>
<td>77/1087 (7.1%)</td>
<td>***</td>
</tr>
<tr>
<td>Trivalent (n=27)</td>
<td>14/1158 (1.2%)</td>
<td>***</td>
</tr>
<tr>
<td>Trivalent + gD2 (n=9)</td>
<td>2/351 (0.6%)</td>
<td></td>
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</tbody>
</table>

**Vaccine efficacy based on days with lesions:**

- gD2 = 80%
- Trivalent = 97%
- Trivalent + gD2 = 99%
Vaginal shedding of replication competent virus

% days HSV-2 DNA shedding contains replication competent virus

- gD2
- Trivalent
- Trivalent + gD2

Immunogen

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Percent days</th>
</tr>
</thead>
<tbody>
<tr>
<td>gD2</td>
<td>7/25</td>
</tr>
<tr>
<td>Trivalent</td>
<td>1/28</td>
</tr>
<tr>
<td>Trivalent + gD2</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* denotes statistical significance
A trivalent vaccine that includes strategies to prevent HSV-2 immune evasion is a promising candidate for human trials.

**Next steps**

Find a sponsor for phase I/II human trials

Use guinea pig model to determine immune correlates of protection to improve predictive power of animal studies for human trials
Antibody correlates of protection against gD2

Neutralizing antibody titers

Reciprocal neutralization titer

Animal number
Antibody correlates of protection against gD2

Neutralizing antibody titers

Genital disease in gD2 immunized g. pigs
Neutralizing antibody response correlates with protection from HSV-2 disease

Threshold neutralizing antibody titer: $\geq 1:320$ correlates with strong protection
Carterra high throughput biosensor imager (left) and sensor chip with printed MAb (right).

Measuring epitope-specific antibody responses

Print 40 MAbs

FLOW gD

FLOW αgD MAb #1

competes

Regenerate

Repeat for each MAb
Group MAb into communities and sub communities
Assign functions to MAb by in vitro assays

Blue: Blocks gD interaction with gH/gL
Green: Blocks gD interaction with gH/gL
Yellow: Blocks HVEM binding
Red: Blocks nectin-1 binding
Pink: Blocks nectin-1 & HVEM binding
Brown: Blocks cell-to-cell spread
Passive transfer in mice to assess whether MAb to crucial epitopes protect

<table>
<thead>
<tr>
<th>Community</th>
<th>MAb prototype</th>
<th>Function blocked</th>
<th>Survival (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink</td>
<td>DL11</td>
<td>Entry via nectin-1 &amp; HVEM</td>
<td>100%</td>
</tr>
<tr>
<td>Blue</td>
<td>MC5</td>
<td>gD interaction with gH/gL</td>
<td>80%</td>
</tr>
<tr>
<td>Yellow</td>
<td>1D3</td>
<td>Entry via HVEM</td>
<td>80%</td>
</tr>
<tr>
<td>Green</td>
<td>MC2</td>
<td>gD interaction with gH/gL</td>
<td>60%</td>
</tr>
<tr>
<td>Brown</td>
<td>DL6</td>
<td>Cell-to-cell spread</td>
<td>60%</td>
</tr>
<tr>
<td>Red</td>
<td>MC23</td>
<td>Entry via nectin-1</td>
<td>40%</td>
</tr>
<tr>
<td>None</td>
<td>MC16</td>
<td>Binds non-crucial gD2 epitope</td>
<td>0%</td>
</tr>
<tr>
<td>None</td>
<td>Nonimmune IgG</td>
<td>None</td>
<td>0%</td>
</tr>
</tbody>
</table>

All MAb that block crucial gD functions \textit{in vitro} protected \textit{in vivo}, but some are more protective than others.
Biosensor-based guinea pig immune IgG competition assay

Print MAbs

Mix gD2 + g. pig IgG#1

Flow gD2 + g. pig IgG#1

Repeat for each g. pig IgG

Competes

Regenerate
Define epitope-specific antibody responses in immunized guinea pig IgG

Most animals produce antibodies to epitopes recognized by pink and blue but not yellow communities.
Define epitope-specific antibody responses in immunized guinea pig IgG

- **Green:** gD - gH/gL
  - 60% protection in mice

- **Brown:** Cell - cell spread
  - 60% protection in mice

- **Red:** nectin-1
  - 40% protection in mice

Few animals produce antibodies to **green** and **brown** communities
Comparison of epitope-specific responses in gD2 immunized guinea pigs and humans

Table. gD2 immunization of humans

<table>
<thead>
<tr>
<th>MAb</th>
<th>Community</th>
<th>Function of gD2 epitope</th>
<th>% protection in mice</th>
<th>Humans(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL11 or 77S</td>
<td>Pink</td>
<td>Binds to nectin-1 &amp; HVEM receptors</td>
<td>100%</td>
<td>29/29 (100%)</td>
</tr>
<tr>
<td>MC5</td>
<td>Blue</td>
<td>Interacts with gH/gL</td>
<td>80%</td>
<td>28/29 (97%)</td>
</tr>
<tr>
<td>1D3</td>
<td>Yellow</td>
<td>Binds to HVEM receptor</td>
<td>80%</td>
<td>0/29 (0%)</td>
</tr>
<tr>
<td>MC2</td>
<td>Green</td>
<td>Interacts with gH/gL</td>
<td>60%</td>
<td>28/29 (97%)</td>
</tr>
<tr>
<td>DL6</td>
<td>Brown</td>
<td>Promotes cell-to-cell spread</td>
<td>60%</td>
<td>0/29 (0%)</td>
</tr>
<tr>
<td>MC23</td>
<td>Red</td>
<td>Binds to nectin-1 receptor</td>
<td>40%</td>
<td>28/29 (97%)</td>
</tr>
</tbody>
</table>

\(^1\) Whitbeck et al. J Virol 2014

Future goal: Improve antibody responses to yellow and brown communities, which were also weak immunogens in guinea pigs
Correlation between epitopes blocked and genital lesions

gD2-immunized guinea pigs

Lesion days vs. # epitopes blocked

$r = -0.71$

$p < 0.0005$
Conclusions

Protection correlates strongly with the number of crucial gD2 epitopes blocked.

gD2 immunization in humans failed to produce antibodies to some epitopes that are highly protective.

Future directions

Develop strategies to produce antibodies to all crucial gD2 epitopes.

Perform similar studies with gC2 and gE2.

Defining epitope-specific immune correlates of protection will improve the accuracy of animal models in predicting outcomes of human trials.
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