Case Study: Recombinant virus-like particle vaccines: Recombivax-HB® and Gardasil®

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Vaccine Basic Research,
Merck Research Laboratories
VLPs as immunogens

- Commonly more immunogenic than subunits
- Stimulate both humoral and cellular arms of the immune system
- Spatial structure of display enhance production of neutralizing antibodies
- Possible to present multiple proteins
- Size-suitable for uptake by dendritic cells (DCs): MHC II, DC maturation, stimulation of the innate immune response
# HBV and HPV VLP vaccines

<table>
<thead>
<tr>
<th>Virus</th>
<th>Particle Composition</th>
<th>Type / Expression system</th>
<th>Size</th>
<th>Licensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>HBsAg</td>
<td>Native SVP (plasma)</td>
<td>~22nm</td>
<td>1981</td>
</tr>
<tr>
<td></td>
<td>HBsAg</td>
<td>rec. VLP (yeast)</td>
<td>~22nm</td>
<td>1986</td>
</tr>
<tr>
<td>HPV</td>
<td>L1, Major Capsid protein</td>
<td>rec. VLP (yeast)</td>
<td>~55-57nm</td>
<td>2006</td>
</tr>
</tbody>
</table>

To consider when developing a vaccine

- Knowledge of the pathogen, disease, host’s immune response
- Vaccine production and delivery techniques
- Expectations of vaccine – required immune response
- Clinical endpoints
- Vaccine components
- Safety
HBV

- 42 nm infectious virion – Dane particle
- 22 nm non-infectious subviral particle
  – Composed of HBsAg and lipid
- Association Ab to HBsAg and immunity to infection
- 10 mIU/mL protects against acute infection
- Correlate of protection

Venters C Expert Rev Vaccines 2004 3:119-129,
Zhoa Q Human Vaccines 2006 2:174-180
Plasma HBsAg

- Human chronic carrier plasma to serve as immunizing antigen
- Required: Purity and Inactivation
- 3 Steps
  - Pepsin digestion
  - 8M Urea Disassoc/Reassociation
  - Formaldehyde treatment
HBV

- rHBsAg ~25 kDa protein 226 aa
- 2 forms: +/- glycosylation, at Asn-146
- Self assembles into VLP of ~100 rHBsAg with 30-50% lipid (lipoprotein particles)
- Intra- and intermolecular disulfide bonds stabilize particle, maintain antigenic and immunogenic properties of the VLP

Zhoa Q Human Vaccines 2006 2:174-180
Envelope VLPs

- Assembled from envelope proteins that bud from the usual cellular compartments
- ER, plasma membrane etc
- Viral lipoprotein envelope contain cellular lipids and in some case may contain host cell proteins as well
Plasma vs Recombinant HBsAg VLPs

• Self assemble
• Rec. more homogeneous array of particles
• UV adsorption pattern same
• Reducing gel
  – Rec. major band 23kDa = non-glycosylated
  – Plasma 23 and 27 kDa -/+ glycosylation
• Reactivity in RIA (AUSRIA II, Abbott)
• Comparability: Efficacy and immunogenicity established

To consider when developing a vaccine

- Knowledge of the pathogen, disease, host’s immune response
- Vaccine production and delivery techniques
- Expectations of vaccine – required immune response
- Clinical endpoints
- Vaccine components
- Safety
HPV

• Non-enveloped, naked virus
• Infects human epithelial tissues in a tissue tropic and species specific manner
HPV

- > 100 HPV types
- ~55-57 nm infectious virion
- Viral capsid composed of L1, major capsid protein and L2, minor capsid protein
- L1 protein ~500 aa self associates into pentamers
- 72 pentamers self associate into VLP
- Neutralizing antibodies can be elicited by VLPs
- Minimum Ab correlate of protection not yet established
HPV L1 Protein Spontaneously Assembles into Virus-Like Particles (VLPs)

Bioengineered L1 Proteins (5) → L1 Pentamer → Self-Assembled Virus-Like Particle
Passive transfer confers Protection

Proof of Concept – Humoral Immunity, CRPV Infection Model

Challenge with CRPV

Warty lesions appear
Immune response
Serum Ab produced
Lesions regress

Collect sera

Passive transfer
IP Ab transfer

Protection

Immune Response to HPV Vaccines: A Proposed Mechanism

- VLPs in vaccine
- Antigen presenting cell
- Helper T cell
- Naïve or memory B cell
- Plasma cell
- Macrophage eliminates antibody-coated virus
- Neutralizing antibody prevents HPV infection
- Cervical epithelium
- Antibodies bind to virus
- Cytokines
- Anti-HPV neutralizing antibodies
- Antibodies bind to virus
Clone L1 gene for the capsid protein of HPV types 6, 11, 16 and 18

**Key expression features:**
- tightly regulated promoter
- common host strain
- common vector

Intracellular expression in *S. Cerevisiae* – baker’s yeast

Cell lysis and purification

VLPs

HPV virion

Plasmid

On - galactose
Off - glucose

Leader    ATG               HPV L1                              Stop
Enhancer promoter
Fermentation of *S. cerevisiae*

- Common host and plasmid with tightly controlled *GAL1* promoter
- Defined media fermentation for process consistency
- Required both process development and host strain genetic modifications (extensive screening of host/plasmid combinations)
- Established an “universal” platform for all types
- Achieved desired cell growth and VLP expression
Fermentation of *S. cerevisiae*

- 3 phase process using two carbon sources
HPV VLP Manufacturing Process

- **Fermentation/Harvest**
  - Produce in recombinant yeast

- **Cell Thaw/Disruption**
  - Release intracellular HPV VLPs

- **Nuclease Treatment**
  - Digest DNA/RNA, facilitate clearance

- **Microfiltration**
  - Clarify yeast lysate

- **Capture Chromatography**
  - Remove majority yeast impurities

- **Polishing Chromatography**
  - Reduce nuclei acid and HCP

- **Ultrafiltration**
  - Remove residual impurities and buffer exchange

- **Sterile Filtration**

- **Adjuvant Adsorption**

Jansen et al., (Merck and Co.) US Patent 5,888,516 1999
Volkin et al., (Merck and Co.) US Patent 6,245,568 2001
Cook et al Prot. Exp. Purif. 1999
Use of Size Exclusion Chromatography to Monitor VLP Aggregation

Crude lysate sample

Fermentation Harvest Time, VLP Aggregation, and Purification Performance
Disulfide Cross-linking and VLP Assembly

- Disulfide bonds are involved in inter-capsomeric contacts
- Bonds are important for VLP stability

Model:
SV40, Polyomavirus
(Stehle et al. 1996. Structure, 4, 165-182)
At the capsomere interfaces:
- Carboxylates are neutralized where [Ca\(^{2+}\)] high
- Capsomeres held together by hydrophobic forces and disulfide bonds
- Inside cell [Ca\(^{2+}\)] low
- Disulfide bonds reduced
- Carboxylates negatively charged
  strong charge repulsion allows for viral disassembly
Improved biophysical properties benefited the performance of the purification process & helped ensure process consistency.

Composite TEM images of HPV VLPs

Generated through a collaboration between Merck and Scripts Inst.
VLP Product

• To monitor the product… an *in vitro* relative potency assay (IVRP) was developed utilizing type HPV L1 VLP conformational and type specific antibodies capable of detecting neutralizing epitopes on the VLPs

• IVRP results correlated with traditional mouse potency results
VLP antigenicity

*In Vitro Relative Potency Assay*

**Sandwich enzyme immunoassay**
(with reference standard and 4-parameter fit)

Detection mAb used:
- HPV16: H16.V5
- HPV18: H18.R5

- VLP capturing mAb
- HPV neutralizing mAb
- HRP Conjugated goat anti-mouse IgG<br>2β antibody
GARDASIL®
Merck’s Quadrivalent Recombinant Vaccine

Quadrivalent HPV (Types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine
VLPs manufactured in Saccharomyces cerevisiae
Amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant 225 μg per dose
0.5 mL injection volume
3 doses within 6 months
VLP-16 Crystal Structure

A. Side view
B. Tilted side view
C. Interior

S.C. Harrison et al. Molecular Cell, 2000
HPV Virus-Like Particle (VLP)

VLP has many regions available for antibody binding. Not all sites induce protection from Infection.
A = HPV 11  
B = HPV 35  
C = HPV 16  
D = HPV 18
Potential issues VLP platforms

- Size restrictions on intended antigen
- Glycosylation
- Host cell proteins associated with lipid envelope
- Purity
- Conformation
- Anti-carrier (VLP platform) immunity due to universal vaccination