Pros and cons of DNA vaccines against chronic viral infections and the advantages of combining different vaccination approaches

Maria Isagulians
Eukaryotic cell barriers to the viral life cycle and types of subversion mechanisms that are used by the virus

Generic Viral Life Cycle

*Initiation Phase*
- Attachment
- Cross plasma membrane
- Uncoating
- Cross nuclear membrane

*Replication Phase*
- DNA synthesis
- RNA synthesis
- Protein synthesis

*Release Phase*
- Assembly
- Maturation
- Exit from cell


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Common Viral Subversion Mechanisms

*Molecular mimicry*
- Cell surface receptors
- Immune evasion
- Cell survival

*Hijacking*
- Signal transduction pathways
- Transcription factors

*Oncogenes/Transformers*
- Cell cycle control
- Access to the nucleus

Examples of the viruses causing chronic infections:

- HIV-1
- HPV family
- HSV-1/HSV-2
- CMV
- HCV

Examples of the approaches to vaccine design
<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live, attenuated vaccine</td>
<td>Measles, mumps, rubella, polio (Sabin vaccine), yellow fever</td>
</tr>
<tr>
<td>Inactivated or “killed” vaccine</td>
<td>Cholera, flu, hepatitis A, Japanese encephalitis, plague, polio (Salk vaccine), rabies</td>
</tr>
<tr>
<td>Toxoid vaccine</td>
<td>Diphtheria, tetanus</td>
</tr>
<tr>
<td>Subunit vaccines</td>
<td>Hepatitis B, pertussis, pneumonia caused by <em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>Conjugate vaccines</td>
<td><em>Haemophilus Influenza</em> type B, pneumonia caused by <em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>DNA vaccines</td>
<td>In clinical testing</td>
</tr>
<tr>
<td>Recombinant vector vaccines</td>
<td>In clinical testing</td>
</tr>
</tbody>
</table>
Needles versus No needles

- Oral vaccines: polio, typhoid, cholera, rotovirus, nasal influenza
- Cutaneous vaccination: liquid-jet injection deliver antigen id, sc or im
  (Advantage-Langerhans cells in skin-requires lower dose of antigen. Disadvantage-blood transfer)
- Particle bombardment of the skin - epidermal powder administration
- Topical application to skin using adjuvants and or permeabilizing agents
Genetic vaccines – new technique, new hope

• Genetic vaccines are based on DNA or RNA
• Encodes the vaccine antigen
• Stimulates both B and T cell responses
• Induces an immune response resembling that during a viral infection
The cells of the body act as vaccine factories

DNA

transcription

mRNA

protein

Presentation of protein fragments on MHC class I molecules

Secreted protein

Muscle/skin/lymphoid cell

G. Karlsson-Hedestam, SMI 2006
DNA vaccines

**PROS**

- **Efficacy**
  - Immunity similar to a modified live

- **Stability**
  - Highly stable over long periods of time

**CONS**

- **Efficacy**
  - Protective antigen must be known

- **Prime/boost may require different technology**

- **Safety**
  - Limited data on in field conditions

  - Unknown risk associated with incorporation into genome – food producing animals
Efficacy: stepwise changes make a difference

Felber BK, Velantin A et al, 2014
Manipulations with encoded antigens allowing retargeting of processing and presentation
Targeting of drug resistant HIV-1 reverse transcriptase (RT) to the lysosomal degradation pathway

Isaguliants M & Starodubova E, 2009
Potentiating immune response against drug resistant HIV-1 RT after its targeting to the lysosomal degradation pathway

Specific IL-2 production

Specific IgG response
Efficacy: stepwise changes make a difference

Felber BK, Velantin A et al, 2014

Combination of DNA Vaccine with Molecular Adjuvants to Increase Immunogenicity (i.e., Plasmids expressing IL-12, IL-15, IL-2, GM-CSF)

Delivery Sites:
- Intramuscular
- Skin
- Intranasal
- Oral
- Intestinal
- Vaginal

Delivery Methods:
- Needle/syringe of naked DNA
- In vivo electroporation of naked DNA
- DNA formulated in liposomes
- Needle-free injections using gene gun, biojector
- Nanoparticles
- Skin patches
Ways of gene delivery

29G needles OR Microneedles OR Biojector (delivery by gas pressure)

Dermavax, Cellectis CUY21EDIT, BEX
OLD CONS

- Very efficient in (small) animal models, cheap, easy to produce, handle and store;
- Safe in small animals, larger species, humans

NEW CONS

- Low immunogenicity in larger species;
- Difficult to enhance the efficacy in large species;
- Multiple clinical trials with little success.
Methods for gene delivery

- Virus vector
- Bacterial vector
- Plasmid DNA
The recombinant Modified Vaccinia virus Ankara (MVA)

**The plasmids**

- gp160 env B
- gp160 env A
- gp160 env C
- rev B
- RTmut B
- p37 gag B
- p37 gag A
- p17 Gag B
- p24 Gag A

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The recombinant Modified Vaccinia virus Ankara

**HIVIS:**

Example of therapeutic HIV vaccine
Prime Boost Strategy

Prime

Boost

Immune Response
Heterologous Prime-Boost

- Same antigen encoded by the plasmid and presented by the heterologous boost;
- Focuses the immune system on the vaccine antigen

Plasmid DNA

Same antigen in another form: recombinant virus vaccine; protein; peptides etc

G. Karlsson-Hedestam
25μg/plasmid injected intradermally using the Biojector. Plasmid injected as 2 entities (env/rev and gag/RT)

1μg rGM-CSF injected at the site of injection of envelope-encoding DNA

Animals boosted with $10^7$ pfu i.m. rMVA

Wahren B et al, 2007
## MVA and Ad5 as Vectors in HVTN Trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVTN 055</td>
<td>Therion MVA or MVA+FPV</td>
</tr>
<tr>
<td>HVTN 065</td>
<td>GeoVax MVA or DNA+MVA</td>
</tr>
<tr>
<td>HVTN 050</td>
<td>Merck Ad5 gag</td>
</tr>
<tr>
<td>HVTN 502/503</td>
<td>Merck Ad5 gag/pol/nef</td>
</tr>
<tr>
<td>HVTN 054</td>
<td>VRC Ad5 env/gag/pol</td>
</tr>
<tr>
<td>HVTN 057</td>
<td>VRC DNA + Ad5 env/gag/pol</td>
</tr>
<tr>
<td>HVTN 069</td>
<td>VRC DNA + Ad5 env/gag/pol</td>
</tr>
<tr>
<td>HVTN 204</td>
<td>VRC DNA + Ad5 env/gag/pol</td>
</tr>
</tbody>
</table>
Thai HIV Vaccine Study

- Community-based study
  - Not specifically high-risk
  - Moderate risk more likely predominated
- 31.2% Efficacy to prevent infection
  - Two-tailed p value 0.039 with 95% CI 1.1%-52.1%
  - 51/8197 infected vaccinees
  - 74/8198 infected placebo

Rerks-Ngarm S, Pitisuttithum P et al, 2009
## From HIVIS to TaMoVac

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HIVIS01/02/05 Stockholm</td>
<td>40 phase 1</td>
<td>3xDNA</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; MVA</td>
<td>Published</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; MVA</td>
<td></td>
<td></td>
<td></td>
<td>Published</td>
</tr>
<tr>
<td>HIVIS03/06 Dar es Salaam</td>
<td>40 (+20) phase 1/2</td>
<td>3xDNA + 1&lt;sup&gt;st&lt;/sup&gt; MVA</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; MVA</td>
<td>Published</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; MVA</td>
<td>Analysis ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaMoVac I (Tz) Dar + Mbeya</td>
<td>108 (+12) phase 2</td>
<td>3xDNA</td>
<td>2xMVA</td>
<td>rgp140/ GLA</td>
<td></td>
<td></td>
<td></td>
<td>I: In press II: Manuscript</td>
<td></td>
</tr>
<tr>
<td>TaMoVac I (Moz) Maputo</td>
<td>20 (+4) phase 1</td>
<td>3xDNA</td>
<td>2xMVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Analysis ongoing</td>
<td></td>
</tr>
<tr>
<td>HIVIS07 Stockholm</td>
<td>22 (+5) phase 1</td>
<td>3xDNA +/- EP</td>
<td>2xMVA</td>
<td>+/- rgp140</td>
<td></td>
<td></td>
<td></td>
<td>Manuscript submitted</td>
<td></td>
</tr>
<tr>
<td>TaMoVac II Dar+ Mbeya+ Maputo</td>
<td>180 +(18) phase 2</td>
<td>3xDNA +/- EP</td>
<td></td>
<td>Addition of rgp140/GLA to MVA boost</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MVA: MVA-CMDR  DNA: 7 plasmid DNA multigene/multisubtype vaccine  EP: Electroporation
Thai HIV Vaccine Trial: Surprising findings

- Protection correlated with Ab against V2 region
- Early, high protective immune response
  - First 12 months post-vaccination cumulative vaccine efficacy was est. 60.5% (95% CI 22–80)
  - Efficacy declined quickly

Hence: Maybe additional boost or other ↑immune response can ↑efficacy.
# Common HPV Types Associated With Benign and Malignant Disease

| High-Risk | Types 16, 18, 31, 33, and 45 | Low-grade cervical changes  
High-grade cervical changes  
Cervical cancer  
Anogenital and other cancers |
|-----------|-----------------------------|------------------------------------------------------------------|
| Low-Risk  | Types 6 and 11              | Benign low-grade cervical changes  
Condylomata acuminata (Genital warts) |

Cox JT, 1995; Munoz et al., 2003.
<table>
<thead>
<tr>
<th>ORF</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>L1 protein, major capsid protein</td>
</tr>
<tr>
<td>L2</td>
<td>L2 protein, minor capsid protein</td>
</tr>
<tr>
<td>E1</td>
<td>Initiation of viral DNA replication</td>
</tr>
<tr>
<td>E2</td>
<td>Transcriptional regulatory protein with an auxiliary role in viral DNA replication</td>
</tr>
<tr>
<td>E3</td>
<td>No known function</td>
</tr>
<tr>
<td>E4</td>
<td>Late protein; disrupts cytokeratins</td>
</tr>
<tr>
<td>E5</td>
<td>Membrane-transforming protein; interacts with growth factor receptors</td>
</tr>
<tr>
<td>E6</td>
<td>Transforming protein of HPVs; targets degradation of p53</td>
</tr>
<tr>
<td>E7</td>
<td>Transforming protein of HPVs; binds to the retinoblastoma protein</td>
</tr>
<tr>
<td>E8</td>
<td>No known function</td>
</tr>
</tbody>
</table>

ORF, open reading frame; HPV, human papillomavirus.

(From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Table 66-1)
Vaccination aims:
Prevention of HPV infection: generation of humoral mucosal immunity

Vaccination approaches:
Delivery of: L1 or L1/L2 virus–like particles (VLPs).
Delivery of vaccinia vector expressing E6/E7
Delivery of chimeric VLPs (with E6/E7 peptides or L1/L2-E7 fusions)
Anti-tumor DNA vaccines based on the expression of HPV16 E6/E7 oncoproteins

C57Bl/6 mice immunized with gD/E6/E7 DNA and challenged with neoplastic TC-1 cells expressing HPV16 E6 & E7

Lasaro MO et al, 2005
Vaccines are based on virus-like particles (VLPs) derived from the non-infectious L1 outer capsid proteins (Fife, Wheeler et al. 2004). The vaccines are non-infectious.

Gardasil®, Merck. HPV 6, 11, 16, 18

Cervarix™, GlaxoSmithKline HPV 16, 18

Three FDA-approved vaccines—Gardasil®, Gardasil-9®, and Cervarix®—prevent HPV infection and therefore guard against the major cause of cervical and ano-genital cancers and potentially head and neck cancer.

Gardasil® protects against the HPV types 16, 18, 6, and 11.

Gardasil-9® is approved for the prevention of cervical, vulvar, vaginal, and anal cancers caused by HPV types 16, 18, 31, 33, 45, 52, and 58, and for the prevention of genital warts caused by HPV types 6 or 11.

Men between the ages of 9 and 26 may receive Gardasil-9® to protect their future partners and to protect themselves against anal cancer and potentially head and neck cancer, as well as genital warts.

Cervarix is FDA approved for use in preventing the two strains of HPV that cause most cervical cancers, HPV 16 and 18.

http://www.cancerresearch.org/cancer-immunotherapy/impacting-all-cancers/cervical-cancer#sthash.qbSQJ5H1.dpuf
First HPV DNA vaccines clinical trials

DNA vaccine encoding a signal sequence linked to E7 with abolished Rb binding site (E7detox) and **fused to heat shock protein 70 (Sig/E7detox/Hsp70)** - Phase I trials on HPV-16 positive patients with high-grade CIN lesions 2/3. Homologous DNA-prime-boost vaccination regimen of three vaccinations per patient, at three dose levels, 500, 1,000, and 3,000 ug. Regression in 3/9 pts (Dr Trimble, Johns Hopkins University).

DNA vaccine encoding **calreticulin (CRT) fused to E7detox** using a PowderMed/ Pfizer proprietary gene gun device - Phase I in HPV-16 positive patients with stage 1B1 cervical cancer (Dr Alvarez, University of Alabama at Birmingham)

*Hung CF et al, DNA vaccines for cervical cancer, 2007*
Therapeutic DNA-based HPV Vaccines

A phase II clinical trial of TVGV-1 vaccine for patients with HPV-induced cervical pre-cancer (NCT02576561).

A phase I/II trial of VGX-3100, a vaccine that targets HPV types 16 and 18, and INO-9012, a DNA construct that induces human interleukin 12 (IL-12), are being tested in patients with cervical cancer (NCT02172911).

ADXS11-001, a vaccine against the E7 protein, which is made by HPV, is in phase I/II trials in patients with anal cancer (NCT01671488).

There are two phase I clinical trials testing pNGVL4a/E7 (Detox)/HSP70 DNA vaccine in patients with HPV16+ cervical intraepithelial neoplasia. The first one will determine the best dose (NCT00988559) and the second one will be a combination with imiquimod, an innate immune activator (NCT00788164).

http://www.cancerresearch.org/cancer-immunotherapy/impacting-all-cancers/cervical-cancer#sthash.qbSQJ5H1.dpuf
Reasons for failure of herpes candidate vaccines

- Poorly controlled studies
- Insufficient dose
- Insufficient immunogenicity (Ab)
- No induction of CTL
- No induction of mucosal antibodies
- In case of recurrent disease, difficult to eradicate reservoir
Attempts to create a vaccine against Herpes simplex

- Auto-inoculation
- Live, deletion mutants (replication limited)
- Pox virus and adenovirus vectors for glycoproteins
- Inactivated whole virus
- Inactivated infected cell extracts
- Subunit glycoproteins
- Disabled Infectious Single Cycle (DISC)
- DNA plasmids
- Peptides

HSV-1 glycoproteins

gB, gC, gD, gE, gG, gH, gI
# DNA-immunization

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Model</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>gB/Sindbis</td>
<td>HSV-1, mouse</td>
<td>Hariharen 1998</td>
</tr>
<tr>
<td>gB</td>
<td>HSV-1, mouse</td>
<td>Manicken 1995</td>
</tr>
<tr>
<td>gD/bupivacaine</td>
<td>HSV-2, mouse.g.pig</td>
<td>Bernstein 1999</td>
</tr>
<tr>
<td>tgB + gD 1996/1997</td>
<td>HSV-2, mouse/g.pig</td>
<td>McClements</td>
</tr>
<tr>
<td>gD + IL12</td>
<td>HSV-2, mouse</td>
<td>Sin 1999</td>
</tr>
<tr>
<td>gD</td>
<td>HSV-1, mouse</td>
<td>Ghiasi 1995</td>
</tr>
<tr>
<td>gd/tgD</td>
<td>BHV-1, cattle</td>
<td>Van Drunnen 1998</td>
</tr>
<tr>
<td>ICP27</td>
<td>HSV-1, mouse</td>
<td>Manicken 1995</td>
</tr>
</tbody>
</table>
Benefits of host immune response to CMV

- Maternal immunity ameliorates effects of intrauterine infection on fetus
- Premature neonates with maternal antibody are protected from postnatal infection
- Pre-transplant immunity protects organ allograft recipients from severe disease
- Passively administered antibody protects organ allograft recipients
- Protective efficacy demonstrated in murine and guinea pig models of (homologous strain) CMV infection using attenuated strains
- Experience with Towne vaccine in humans
Experimental CMV vaccines

- Live attenuated Towne strain
- Recombinant of Towne with genes from virulent virus
- Subunit gB glycoprotein
- Subunit gH glycoprotein
- DNA plasmids
- gB in adenovirus vector
- Multiple genes in canarypox vector
- Prime boost with canarypox/subunit gB
<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Status</th>
<th>Characteristics and results</th>
</tr>
</thead>
</table>
| gB/MF59 adjuvant                             | Phase II study completed   | Acceptable safety for further studies  
Evaluating in HCMV-seronegative women within 1 year after they had given birth  
Vaccine efficacy of 50% on the basis of infection rates per 100 person years | [20] |
| gB/pp65/IE1 alphavirus replicon trivalent vaccine | Phase I study completed   | Favorable safety profile  
Evaluating in healthy, nonpregnant adults  
Elicits humoral and cellular immune responses  
Based on replication-deficient alphavirus technology | [39] |
| gB/pp65 bivalent DNA vaccine                 | Ongoing Phase II study     | Well tolerated with no serious adverse events in a Phase I study  
HCMV-seropositive or -seronegative healthy adults in Phase I and HCT recipients in Phase II studies  
Higher frequencies of HCMV-specific pp65 and gB T cells compared with placebo | [42,47] |
| Towne ± rhIL-12 ± priming by DNA vaccine encoding pp65, IE1 and gB | Phase I studies completed | Favorable safety profile; no evidence for viral latency or viral shedding in recipients  
Evaluating in HCMV-seronegative healthy adults  
Augmentation of immunogenicity by inclusion of rhIL-12 or DNA vaccine in Phase I studies  
Sung H & Schleiss MR, 2010 | [52,53] |

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3595507/
### Table 66-1. Comparative Features of Hepatitis Viruses

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hepatitis A</th>
<th>Hepatitis B</th>
<th>Hepatitis C</th>
<th>Hepatitis D</th>
<th>Hepatitis E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name</td>
<td>“Infectious”</td>
<td>“Serum”</td>
<td>“Non-A, non-B-post-transfusion”</td>
<td>“Delta agent”</td>
<td>“Enteric non-A, non-B”</td>
</tr>
<tr>
<td>Virus structure</td>
<td>Picornavirus; capsid, RNA</td>
<td>Hepadnavirus; envelope, DNA</td>
<td>Flavivirus; envelope, RNA</td>
<td>Viroidlike; envelope, circular RNA</td>
<td>Norovirus; capsid, RNA</td>
</tr>
<tr>
<td>Transmission</td>
<td>Fecal-oral</td>
<td>Parenteral, sexual</td>
<td>Parenteral, sexual</td>
<td>Parenteral, sexual</td>
<td>Fecal-oral</td>
</tr>
<tr>
<td>Onset</td>
<td>Abrupt</td>
<td>Insidious</td>
<td>Insidious</td>
<td>Abrupt</td>
<td>Abrupt</td>
</tr>
<tr>
<td>Incubation period (days)</td>
<td>15–50</td>
<td>45–160</td>
<td>14–180</td>
<td>15–64</td>
<td>15–50</td>
</tr>
<tr>
<td>Severity</td>
<td>Mild</td>
<td>Occasionally severe</td>
<td>Usually subclinical; 70% chronicity</td>
<td>Coinfection with HBV occasionally severe; superinfection with HBV often severe</td>
<td>Normal patients, mild; pregnant women, severe</td>
</tr>
<tr>
<td>Mortality pregnant</td>
<td>&lt;0.5%</td>
<td>1%–2%</td>
<td>~4%</td>
<td>High to very high</td>
<td>Normal patients, 1%–2%; women, 20%</td>
</tr>
<tr>
<td>Chronicity/ carrier state</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Other disease associations</td>
<td>None</td>
<td>Primary hepatocellular carcinoma, cirrhosis</td>
<td>Primary hepatocellular carcinoma, cirrhosis</td>
<td>Cirrhosis, fulminant hepatitis</td>
<td>None</td>
</tr>
<tr>
<td>Laboratory diagnosis</td>
<td>Symptoms and anti-HAV IgM</td>
<td>Symptoms and serum levels of HBsAg, HBeAg, and anti-HBc IgM</td>
<td>Symptoms and anti-HAV IgM</td>
<td>Anti-HDV ELISA</td>
<td>—</td>
</tr>
</tbody>
</table>

ELISA, Enzyme-linked immunosorbent assay; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBc IgM, hepatitis B core IgM
THANK YOU FOR YOUR ATTENTION!!!