Cervical cancer immunotherapy: Induction of HPV specific CTLs in human volunteers after VGX-3100 immunization

Niranjan Sardesai
Inovio Pharmaceuticals

Follow this and additional works at: http://dc.engconfintl.org/vaccine_iv

Part of the Biomedical Engineering and Bioengineering Commons

Recommended Citation
Induction of HPV Specific CTLs in Humans After VGX-3100 Immunization

May 22, 2012
Albufeira, Portugal

Vaccine Technology IV
Best Vaccine Induced CD4+ and CD8+ T-Cells (Humans): HVTN 080 ICS Responses Against HIV Peptides

Ref: HVTN-080 Study; Kalams et al. 2011 HVTN Annual Meeting
### HIV Vaccines Comparison: Humans (HVTN Data)

**Inovio DNA-EP:** Better & faster CD8+ T cell responses than viral vectors and/or complex prime-boost regimens

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Details</th>
<th>Components</th>
<th>Immunization Schedule (Days)</th>
<th>% CD4 Response</th>
<th>% CD8 Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVTN 073-SAAVI</td>
<td>DNA‘, MVA-C</td>
<td>D, D, D, M, M</td>
<td>0, 28, 56, 112, 140</td>
<td>70</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>boost D, D, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HVTN 205-GeoVax</td>
<td>DNA‘, MVA-B</td>
<td>D, M, M, M</td>
<td>0, 56, 112, 140</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>HVTN 077-VRC</td>
<td>Ad35. Ad5</td>
<td>Ad35, Ad5</td>
<td>0, 28, 56, 168</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>DNA‘, Ad5</td>
<td>D, D, D, Ad5</td>
<td>0, 28, 56, 168</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>(Ad5 sero-) DNA’</td>
<td>D, D, D, Ad5</td>
<td>0, 28, 56, 168</td>
<td>23</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>(Ad5 sero+) DNA’</td>
<td>D, D, D, Ad5</td>
<td>0, 28, 56, 168</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>INOVIO’s DNA+EP</td>
<td>DNA/IL-12/EP</td>
<td>D, D, D</td>
<td>0, 28, 84</td>
<td>81</td>
<td>52</td>
</tr>
</tbody>
</table>

Source: McElrath - Compilation of HVTN study results

>90%
**HIV Therapy: PENNVAX™-B Phase I Study (HIV-001)**

- Open label Phase I clinical trial (therapy) - UPenn
- Four vaccinations over 4 months (0, 4, 8, 16 weeks)
- 12 vaccinated subjects:
  - PENNVAX™-B (1 mg each of EnvB, Gag, Pol) + IM EP
  - On HAART; Undetectable plasma viral load (< 75 copies/mL)
  - CD4 T lymphocyte counts > 400 cells/μL with nadir > 200 cells/μL

### T-cell Responses by IFN-γ ELISpot Assay

<table>
<thead>
<tr>
<th>Vaccine induced T-cell responses (2 SD above prevacc. ELISpot levels)</th>
<th>Vaccine + EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 1 Ag</td>
<td>75% (9/12)</td>
</tr>
<tr>
<td>2 or more Ag</td>
<td>50% (6/12)</td>
</tr>
</tbody>
</table>

**Responses predominantly CD8+ T Cells**

- T-cell immune responses superior to previously-tested HIV DNA vaccines in HIV+ population
Integrated Synthetic Vaccine Platform:

Revolutionizing Vaccines Through:

- Unparalleled safety profile
- Greater potency than viral vectors in primates and in humans (T-cells & Abs)
- Only platform to yield CD8+ effector T-cells in humans without anti-vector serology
- No vector induced responses - repeat boosts; multiple/combination vaccines; older/younger people
- Manufacturing advantages: Rapid, scalable, stable, stockable
Superior Vaccine Delivery Using Electroporation

1. **Vaccine Injection**
   - Syringe
   - DNA vaccine
   - Cells

2. **Electric fields applied**
   - Electrical pulses
   - Nucleus
   - DNA vaccine
   - Temporary openings

3. **Cellular vaccine uptake**
   - Cell membrane

4. **Antibodies and T-cells clear antigens**
   - Lymph node
   - Antigen-presenting cell
   - Antibodies
   - Killer T cells

5. **Immune system responds**
   - Lymph node

6. **Cell produces coded antigen**
Better Delivery = Improved Immune Responses

Display of Green Fluorescence Protein (GFP) gene expression after electroporation delivery into rabbit muscle


1000x enhancement in expression

> 10-100x enhancement in immunogenicity
DNA/EP by the Numbers

- Number of DNA Trials: > 3,600
- Number of study participants: Est. > 50,000+; > 150,000 Imm.; decades of follow up
- Number of DNA + EP Trials: 32
- Number of DNA + EP study participants: >1,600 (enrolled/scheduled)
- Number of EP vaccinations: > 4,800+ (estimated)
- Inovio Figures (enrolled); # Vaccinations:
  - IM EP: 300+; 1000+
  - ID EP: 120+; 280+


Keyword DNA (2/2010): 2612 studies worldwide 1466 US; 530 EU

2/2012:
3631 studies worldwide
1938 US; 719 EU
HPV & HPV Associated Cancers

- **USA**: Annual incidence of HPV associated cancers
  - > 20,000 in women; > 11,000 in men

- **Cervical Cancer** > 99% HPV associated (> 70% are 16/18)
  - Merck (Gardasil) & GSK (Cervarix): >$1.5 B in annual sales
  - Worldwide: Annually
    - ~500,000 new cases cervical cancer - 50% fatal
    - 2nd largest cancer killer of women > 250k deaths
  - USA: Annually
    - 3M ASCUS (atypical cells) with HPV
    - 250K CIN 2/3 (stage prior to cancer) with HPV16/18
    - 13,000 cervical cancer; 5,000 deaths
  - $6 B spent: cervical cancer diagnosis and treatment
  - Surgery/ablation invasive + feared impact on childbirth
  - Need for less-invasive, more readily distributable treatment of cervical dysplasia and cancer

- **Head and Neck (HNSCC)** ~ 23.5% - 35.6% associated with HPV
  - HPV 16 (68-86%); HPV 18 (1-8%)
  - USA Annually: >40,000 new cases; >7,800 deaths
  - 5 year survival: 26.5% - 81.8% (median 59.1%)
VGX-3100 targets E6 + E7 oncogenes which transform HPV-infected cells into precancerous & cancerous cells.

- HPV Types 16 and 18

* Deletions or mutations important for p53 binding and degradation.

△ Mutations in Rb binding site
## High Concentration, High Purity Formulations

<table>
<thead>
<tr>
<th>Test</th>
<th>pGX3001</th>
<th>pGX3002</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleic Acid Concentration</strong></td>
<td>6.0 mg/mL</td>
<td>6.0 mg/mL</td>
</tr>
<tr>
<td>Purity (A260/280)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Host-Cell RNA</td>
<td>≤ 0.1%</td>
<td>1%</td>
</tr>
<tr>
<td>Host-Cell Protein</td>
<td>≤ 0.1%</td>
<td>≤ 0.1%</td>
</tr>
<tr>
<td>Host-Cell DNA</td>
<td>≤ 0.001%</td>
<td>≤ 0.001%</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>≤ 0.1 EU/mg</td>
<td>≤ 0.1 EU/mg</td>
</tr>
<tr>
<td>Microbial Limits</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleic Acid Concentration</strong></td>
<td>9.2 mg/mL</td>
<td>8.1 mg/mL</td>
<td>8.5 mg/mL</td>
</tr>
<tr>
<td>Purity (A260/280)</td>
<td>2.0</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Host-Cell RNA</td>
<td>≤ 0.06%</td>
<td>≤ 0.08%</td>
<td>≤ 0.07%</td>
</tr>
<tr>
<td>Host-Cell Protein</td>
<td>≤ 0.03%</td>
<td>≤ 0.04%</td>
<td>≤ 0.04%</td>
</tr>
<tr>
<td>Host-Cell DNA</td>
<td>≤ 0.001%</td>
<td>≤ 0.001%</td>
<td>≤ 0.001%</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>1.1 EU/mg</td>
<td>≤ 1.2 EU/mg</td>
<td>≤ 1.9 EU/mg</td>
</tr>
<tr>
<td>Microbial Limits</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Now routinely achieve upwards of 10+ mg/mL to support multi-plasmid formulations
Vaccination with pConE6E7 delays/prevents tumor growth

Prophylactic model - mice

Groups
1. pVAX
2. pConE6E7
3. pE7

Therapeutic model - mice

Days after tumor implantation

Note: TC1 Challenge model - Lung Epit. Cell line with HPV-16 - E6, E7
HPV-001/002: Phase 1 Study - Safety & Immunogenicity

- Combination of HPV DNA vaccine delivered IM using Cellectra® EP device - HPV 16, 18 (E6 + E7)
- Indication: Treatment of HPV types 16 and/or 18 associated CIN2/3

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Number of Patient</th>
<th>Dose (mg)</th>
<th>Plasmids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>0.3 X 2</td>
<td>Plasmids</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1 X 2</td>
<td>Plasmids</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>3 X 2</td>
<td>Plasmids</td>
</tr>
</tbody>
</table>

Patients with a history of CIN 2/3 previously treated by LEEP procedure were vaccinated 3x at 0, 1, 3 mo.
Serum and PBMC samples collected at multiple time points before and post immunization to evaluate immune responses.
HPV: Therapeutic Vaccines

HPV Clinical Reports - Previous Studies:
- MVA – based HPV E6/E7 vaccines from Transgene: Phase Ila (n = 21) completed; Phase IIb (n = 200) near completion
- Ad5, SFV (Semliki Forest virus)-based HPV E6/E7 vaccines
- Protein/Peptide-based vaccines – Recent encouraging data in VIN (Malief et al)
- Listeria-based vaccines
- DNA-based vaccines (TC Wu) - Use gene gun techniques to introduce HPV DNA vaccines directly into APCs; However...
- **Low immune responses observed** - cultured ELISpots needed to see cellular responses
  - no antibody responses induced

IM injection: **5/15** patients showed E7-specific responses, **2/15** patients showed E6-specific responses, the T cell responses required culture to observe. Vaccination did not induce Abs

**Improving Immune potency important**

HPV001: Best Vaccine Induced IFN-γ ELISpot Responses to HPV Peptides and HPV-Specific CTL Activity (GrzB Assay) (Humans)

**IFN-γ ELISpot Responses:**
- 5/6 positive
- All 4 antigens represented
- CD4+ & CD8+ T-cells
- Durable & boostable > 2y

**3 mg/plasmid**

- HPV16 E7
- HPV18 E7
- HPV16 E6
- HPV18 E6

**HPV-specific killing phenotype:**
- 6/6 positive (5–35%)

**Effectors = Activated CD8+/ HLA-DR+ /CD38+/ perf+/grz+ after pep. stim.**
Whole PBMCs are effectors. Killing Magnitude scales with the number of CTLs.
No association between Tregs (PBMC CD4/CD25hi/FoxP3+) present pre-vac. and response rate in ELISpot, CTL ICS or Quantitative Killing
VGX-3100/EP Phase 1 Study Summary

- **IM VGX-3100 + CELLECTRA™ EP Safety**
  - No safety concerns to date, well tolerated
  - No discontinuations or related SAEs or Gr 3 or 4 AEs
  - Mean VAS 6.2/10 decreases to 1.4/10 within 10 mins

- **Antibodies against all 4 antigens with high titers in 15/18 (83%) and Western Blot confirmation in all persist to 9 mos**

- **Antigen-specific cellular responses to HPV16,18 E6, E7**
  - 13/18 (72%) POS by IFN-γ ELISpot (>50 SFU/10^6 PBMC)
  - Increase w/ dose up to > 2500 SFU/10^6 PBMC for 1 Ag, > 5,670 SFU/10^6 PBMC for all 4 antigens
  - 5 subjects responded to all 4 antigens
  - Responses persist to 9 months after primary series
  - 4th dose boosts T cell responses up to > 2 years
  - HLA DR+ / CD38+ CD8+ T cells release Granzyme B / perforin for cell killing - more sensitive than ELISpot?
Phase II Study Design for VGX-3100 (CIN2/3)

- Randomized, blinded study: VGX-3100/IM EP vs. placebo
- Patients: CIN2/3 (high grade cervical dysplasia)
- + HPV16 and/or 18 (cause 70% of cervical cancers)
- 1st endpoint: CIN 2/3 or CIN 3 lesion clearance to CIN 1 or less 6 months post 3rd dose (based on biopsy)
- 80% Power to detect efficacy >25% difference in regression rate between Vaccine and Placebo: ~150 patients, 3:1 Vaccine/placebo
- Timeline: total duration: ~2 ½ years

- Launched: 2Q 2011
- Enrollment: 1 - 1½ years
- ~18 month protocol: 3 month treatment + 6 month endpoint + 9 month LTFU
Product Opportunities for HPV Serotypes Beyond 16/18

HPV 33, 45 - Cancer

HPV 6, 11 - Genital Warts
Potential for Broader and/or Targeted Coverage

HPV types 16 or 18 account for > 70% ICC and > 55% HSIL worldwide

Ref: Smith et al Int. J. Cancer (2007)
Potent T-Cell ELISpot Responses to HPV 6, 11 Vaccines

**HPV 6 Vaccine**

![Graph showing IFN-γ spots in splenocytes for HPV 6 vaccine](graph1.png)

**HPV 11 Vaccine**

![Graph showing IFN-γ spots in splenocytes for HPV 11 vaccine](graph2.png)

**Mice model**

**CD8+ Ag Specific T-cells (By ICS)**

Ref: Shin et al. *Hum. Vaccines Immunotherapy* 2012

Ref: *Shin et al. Hum. Vaccines Immunotherapy* 2012
Broad Applicability of INOVIO’s Electroporation Platform

**Electroporation Technology**

**INOVIO’s Nucleotide Delivery**

**Target Tissue**
- Muscle
- Skin
- Mucosa
- Buccal Mucosa
- Tumor

**Nucleotide**
- DNA
- Plasmid
- PCR product
- Linear Expression Cassette
- Cosmids

**RNA**
- mRNA
- siRNA
- srRNA

**Tissue**
- Mucosa
- Skin
- Muscle
- Tumor
Next Generation DNA Delivery Systems for T- and B-cell Targets and Prophylactic Vaccination

EP Devices Target Specific Tissue Regions

- Intramuscular EP
- Intradermal EP

Skin

Skeletal Muscle

Epidermis

Hair Follicle

Dermis

Subdermis
Surface EP Device - Transfection localization

Broderick et al, Gene Therapy 2011
SEP Device GFP Expression Kinetics

1 hour                  2 hours             4 hours           8 hours                  24 hours                48 hours

Upper Panel - Gross guinea pig skin
Middle Panel - Magnified guinea pig skin
Lower Panel - Guinea pig skin sections
Directed and Targeted EP

Guinea Pig Skin

GFP

Co-delivery

RFP

- EP

+ EP

x20

x40

Flu Smallpox
HIV Malaria
Lassa FMD
CHIKV Dengue
HCV HBV
CMV CDiff
Histology and Immunology

HAI titers following immunization with the surface ID device in NHP’s

A/H1N1/Mexico/2009

HI Titer 1:40

Percent survival following immunization with the surface ID device in mice

Broderick et al, Gene Therapy 2011

No associated tissue damage in guinea pig skin biopsies removed 3 days post treatment.

Majority of GFP transfection was observed in upper layers of epidermis.
Not just plasmid DNA – Successful delivery of siRNA

Broderick et al, Molecular Therapy 2012
Some Issues of Conventional Vaccine Technologies

Conventional Vaccines

- Attenuation is an art form - ?
- Safety - Goldilocks paradox
- Atypical responses following vaccination (RSV, Measles)
- STEP: Serology issues not understood
- Manufacturing complexity
- Cell substrates (Egg limitations, Cell line contamination, etc)
- Raw materials
- Product Analytics - Complex
- Stability and Formulation
- Filling models - What is in the vial
- Building production plants at risk

Some Novel At Risk Populations

- Eczema
- Autoimmune Diseases (treated - TNF inhibitors etc.)
- Pregnancy
- Increased elderly population
- Elderly travelers (Unique)
- HIV +, Immune suppressed patients
- Transplant patients

Cancer Immune Therapies

- Older patients many vector immune (Ad, pox, many others)
- Poorer ability to control live replication
- Poorer responses to non live antigens

Development of new vaccine technologies (DNA) are an important driver of Competitive Advantage!
Acknowledgements

**Inovio**
Kate Broderick
Amir Khan
Feng Lin
Iacob Mathiesen
Jessica Lee
Mary Giffear
Mark Bagarazzi
Jian Yan
Chris Knott
J. Joseph Kim
Steve Kemmerer
Xuefei Shen
Gleb Kichaev
Jenna Robles
Janess Mendoza
Maria Yang
Jay McCoy
Phil Armendi
Matthew Morrow

**University of Pennsylvania**
Bernadette Ferraro
Natalie Hutnick, Devon Shedlock
Karupppiah Muthumani, Jean Boyer
David Weiner

**VGXI, Inc. (Mfg.)**
Ying Cai
Dorothy Peterson
Young Park

**Alnylam (SiRNA)**
Amy Chan
Tracy Zimmermann

**Public Health Agency, Canada**
Gary Kobinger

Clinical studies volunteers and sites
Funding:
NIH/NIAID/DAIDS; MVI-PATH; DTRA; CDMRP; DOD
IM v. ID EP: Clinical Tolerability Data (FLU-001/002)

FLU study: Interim data from volunteers who received 2x IMEP Prime followed by 2x IDEP Boost

FLU-IM* (n=56)  
FLU-ID (n=36)

IM v ID
T0 p<.0001  
T5 p<.0001  
T10 p = 0.0084

Inovio DNA-EP:
- Generally well tolerated
- No Grade 3 or 4 AEs/SAEs related to vaccine
- No biodistribution/integration concerns noted to-date