HPV Therapeutic Vaccine
For Chronic Infection and Premalignancies

Kiat Ruxrunghetham
Chula Vaccine Research Center, and
School of Global Health,
Chulalongkorn University

WHO/MPP mRNA Technology Transfer Programme, Regional meeting in South-East Asia
Shangri-la hotel, Bangkok, Thailand 31 Oct-1 Nov 2023
HPV Therapeutic Vaccine: Outlines

• Overview Epidemiology
• HPV Therapeutic Vaccine Trials
• What have we learned?
• Research Gaps
HPV Vaccine

PREVENTIVE

Highly Effective HPV Vaccine
Available since 2014

Global 2019<sup>1</sup>
15% coverage !!!!

L1-VLP
Affordable Vaccine
Is definitely needed!

7.9 Billion
W: 3.9 B, M: 4 B

<25 yo
All: 3.2 B
W: 1.6 B

>25 yo
All: 4.7 Billion
W: 2.3 B

THERAPEUTIC

Vaccine- not available

291 Million Women are HPV DNA carriers

21% men are HR-HPV Infected<sup>2</sup>

Cervical Cancer
• 3.1% of all cancers
• 604,127 new cases
• 341,831 deaths annually

<sup>1</sup>Bruni et al. Preventive Med 2021

<sup>2</sup>Bruni et al. Lancet Global Health 2023
HPV Type Epidemiology

• HPV16 and HPV18 are the most common causes of HPV-mediated cancers.

• Among the over 200 known types of HPV, 13 in the Alpha-papillomavirus genus are high-risk, or oncogenic.
  • 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66.

• 57% and 18% of cervical cancers are caused by HPV16 and HPV18.

• HPV16 causes 82% of HPV-associated head and neck squamous cell carcinomas.

Yan et al. Current Otorhinolaryngology Reports (2023)
Immune response after HPV infection

- HPV infections are restricted to the epithelial layer of the mucosa and do not induce a vigorous immune response.
- The median time from HPV infection to seroconversion is approximately 8–12 months, although immunological response varies by individual, HPV type and duration of infection.
- After natural infection,
  - 70–80% of women seroconvert; their antibody responses are typically slow to develop and of low titre and avidity.
  - In men there is less response to natural HPV infection; few men seroconvert and any antibodies produced may not be protective.

WHO position paper (2022 update)
Natural History of HPV Infection
Rational of Therapeutic Vaccine

**Figure 1.** Natural history of oncogenic human papillomavirus infection. A model showing the progression of HPV infection to invasive cancer. Infection with HPVs is usually cleared by the immune system within a couple of years. Persistently infected cells can regress, but over time can progress to invasive cancer.

Modified from Della Fera et al. Viruses. 2021
Therapeutic HPV-Vaccine Trial Results
from Past to Present

- **Limited evidence** of therapeutic vaccine efficacy in patients with **advanced, recurrent, or metastatic** HPV-mediated malignancies

- Most of the **double blinded RCTs** demonstrate that therapeutic HPV vaccination **either trends towards efficacy or is effective** in patients with **cervical intraepithelial neoplasia (CIN)**

Yan et al. Curr Otolaryngology Report, Feb 2023
### HPV Therapeutic Vaccine in Precancerous and Cancers

#### Early Landmark Trials Results (Non-RCT, single arm)

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Vaccine - antigens</th>
<th>Study Phase</th>
<th>Sample size</th>
<th>Published</th>
<th>Eligibility</th>
<th>Regression Vac vs Pla</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>DNA GX188E HPV16/18 E6/E7+FLT3L</td>
<td>I</td>
<td>9</td>
<td>2014</td>
<td>CIN3</td>
<td>9 months: 7/9 with complete regression + viral clearance</td>
</tr>
<tr>
<td>Peptides</td>
<td>Peptides ISA101 HPV16 E6/E7 epitopes</td>
<td>II</td>
<td>8</td>
<td>2009</td>
<td>HPV6 VIN3</td>
<td>12 months: 79% clinical response (15/19) 47% complete response (9/19)</td>
</tr>
<tr>
<td>Protein</td>
<td>Protein TA-CIN HPV16 L2/E6/E7</td>
<td>II</td>
<td>15</td>
<td>2010</td>
<td>VIN2/3</td>
<td>5 months 58% complete regression (11/19)</td>
</tr>
<tr>
<td>Viral Vector</td>
<td>Viral Vector TA-HPV HPV16/18 E6/E7</td>
<td>I/II</td>
<td>22</td>
<td>1996</td>
<td>Invasive Cervical CA</td>
<td>15-21 months 2/8 disease-free</td>
</tr>
<tr>
<td>Bacterial Vector</td>
<td>Bacterial Vector ADXS11-001 HPV16 E7</td>
<td>I</td>
<td>19</td>
<td>2009</td>
<td>A/R Cervical CA</td>
<td>100% Flu-like syndrome 40% grade 3 AEs 1/15 with partial response</td>
</tr>
</tbody>
</table>

Yan et al. Curr Otolaryngology Report 2023: 11(1)
Vaccination against HPV-16 Oncoproteins for Vulvar Intraepithelial Neoplasia

Gemma G. Kenter, M.D., Ph.D., Marij J.P. Welters, Ph.D.,
A. Rob P.M. Valentijn, Ph.D., Margriet J.G. Lowik,
Dorien M.A. Berends-van der Meer, Annelies P.G. Vloon, Farah Essahsah,
Lorraine M. Fathers, Rienk Offringa, Ph.D., Jan Wouter Drijfhout, Ph.D.,
Amon R. Wafelman, Ph.D., Jaap Oostendorp, Ph.D., Gert Jan Fleuren, M.D., Ph.D.,
Sjoerd H. van der Burg, Ph.D., and Cornelis J.M. Melief, M.D., Ph.D.

Rational:

Vulvar Intraepithelial Neoplasia - VIN

• Most common – HPV16
• Spontaneous regression occurs in <1.5% of patients
• Recurrence rate after treatment is high.
Study Design:

- **N=20** women with HPV-16–positive, grade 3 vulvar intraepithelial neoplasia (VIN3)
- **Vaccine**: a mix of long peptides from the HPV-16 E6 and E7 in incomplete Freund’s adjuvant.
- **Route**: SQ at 3-wk intervals, each time in a different arm or leg x 3-4 times
- **The end points** were clinical and HPV-16–specific T-cell responses.
HPV16 E6/E7 peptide vaccine trial in **VIN3** Results

**RESULTS**

The most common adverse events were local swelling in 100% of the patients and fever in 64% of the patients; none of these events exceeded grade 2 of the Common Terminology Criteria for Adverse Events of the National Cancer Institute. At 3 months after the last vaccination, 12 of 20 patients (60%; 95% confidence interval [CI], 36 to 81) had clinical responses and reported relief of symptoms. Five women had complete regression of the lesions, and HPV-16 was no longer detectable in four of them. At 12 months of follow-up, 15 of 19 patients had clinical responses (79%; 95% CI, 54 to 94), with a complete response in 9 of 19 patients (47%; 95% CI, 24 to 71). The complete-response rate was maintained at 24 months of follow-up. All patients had vaccine-induced T-cell responses, and post hoc analyses suggested that patients with a complete response at 3 months had a significantly stronger interferon-γ–associated proliferative CD4+ T-cell response and a broad response of CD8+ interferon-γ T cells than did patients without a complete response.

**CONCLUSIONS**

Clinical responses in women with HPV-16–positive, grade 3 vulvar intraepithelial neoplasia can be achieved by vaccination with a synthetic long-peptide vaccine against the HPV-16 oncoproteins E6 and E7. Complete responses appear to be correlated with induction of HPV-16–specific immunity.

Kenter et al NEJM 2009

---

**HPV-VIN3 patients**

**Response Rate at 12 months:**

- **79%** clinical response (15/19)
- **47%** complete response (9/19)

**Post-hoc analysis**

T-cell responses may play important roles in complete response

**Adverse Events:**

- Local swelling **100%**
- Fever **64%**
- All <grade 2 AES

*Adjuvant: Incomplete Freund’s Adjuvant*
Immune Response before and after Vaccination

- All patients had vaccine-induced T-cell responses
- Post hoc analyses suggested that patients with a complete response at 3 months had a significantly stronger interferon-γ–associated proliferative CD4+ T-cell response and a broad response of CD8+ interferon-γ T cells than did patients without a complete response.

Kenter et al NEJM 2009
Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients

Tae Jin Kim1,*, Hyun-Tak Jin2,*, Soo-Young Hur3, Hyun Gul Yang4, Yong Bok Seo4, Sung Ran Hong5, Chang-Woo Lee6, Suhyeon Kim6, Jung-Won Woo2, Ki Seok Park2, Youn-Young Hwang2, Jaehan Park2, In-Ho Lee1, Kyung-Taek Lim1, Ki-Heon Lee1, Mi Seon Jeong7, Charles D. Surh4,8, You Suk Suh2, Jong Sup Park3 & Young Chul Sung2,4
Key Findings of GX-188E HPV DNA Vaccine

Phase 1 study, N=9 CIN3 patients

Study Vaccine: HPV16/18 E6/E7 DNA vaccine (GX-188E) x 3 doses

Results

- 9/9 showed E6/E7-specific IFN-γ-producing T-cell responses
- 8/9 patients exhibit an enhanced polyfunctional HPV-specific CD8 T-cell response as shown by an increase in cytolytic activity, proliferative capacity and secretion of effector molecules.
- 7/9 display complete regression of their lesions and viral clearance within 36 weeks of follow up.
- GX-188E administration does not elicit serious vaccine-associated adverse events at all administered doses.

Kim et al Nature Comm 2014
# HPV Therapeutic Vaccine in CIN2/3

**RCT-placebo-controlled Results**

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Vaccine - antigens</th>
<th>Study Phase</th>
<th>Sample size Vac/Placebo</th>
<th>Route</th>
<th>Eligibility</th>
<th>Regression Vac vs Pla</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA VGX-3100</td>
<td>HPV16/18 E6/E7</td>
<td>IIb</td>
<td>125 : 42</td>
<td>IM-EP</td>
<td>HPV16/18</td>
<td>9 months: 49 % vs 30 %, p=0.034</td>
</tr>
<tr>
<td>DNA ZYC101a</td>
<td>HPV16 E6/E7</td>
<td>II</td>
<td>53 : 58 : 50</td>
<td>IM Lateral Thigh</td>
<td>HPV CIN2/3</td>
<td>6 months: 43% vs 27%, p=0.12 (ns)</td>
</tr>
<tr>
<td>Viral Vector</td>
<td>HPV16 E6/E7 (+ IL12)</td>
<td>II</td>
<td>136 : 70</td>
<td>SQ</td>
<td>HPV16</td>
<td>6 months HPV16 mono-infection: 18% vs 4%, p&lt;0.05</td>
</tr>
<tr>
<td>Peptide CVLP-L1E7</td>
<td>VLP HPV16 L1-E7</td>
<td>NR</td>
<td>14 : 12 : 13</td>
<td>SQ Upper arm</td>
<td>HPV16</td>
<td>6 months 39% vs 24%, p=ns</td>
</tr>
</tbody>
</table>

Remark: number of given doses: 3-4, Vac=vaccine, Pla =Placebo

Yan et al. Curr Otolaryngology Report 2023
Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial

36 Weeks Results
histopathological regression and viral clearance

Trimble et al. Lancet 2015
VGX-3001 Induced T-cell Responses that may associate with Regression/Clearance

Trimble et al. Lancet 2015
• VGX-3100 was no better than placebo at improving lesion regression and viral clearance in the biomarker-based subpopulation of women with high-grade squamous intraepithelial lesions. However, VGX-3100 did significantly improve lesion regression and viral clearance in the original all-participants primary endpoint population.
Phase 3: MVA bovine E2 Vaccine: Non-RCT

Regression of Human Papillomavirus Intraepithelial Lesions Is Induced by MVA E2 Therapeutic Vaccine

Ricardo Rosales,² Mario López-Contreras,² Carlos Rosales,³ Jose-Roberto Magallanes-Molina,⁴ Roberto Gonzalez-Vergara,⁵ Jose Martin Arroyo-Cazarez,⁶ Antonio Ricardoz-Arenas,⁷ Armando del Follo-Valencia,⁸ Santiago Padilla-Arriaga,⁹ Miriam Veronica Guerrero,¹⁰ Miguel Angel Pirez,¹ Claudia Arellano-Fiore,¹ and Freddy Villarreal¹¹
Phase 3: non-RCT

N = 1356 patients (1176 female, 180 male) with intraepithelial lesions
- Female CIN 1-3 (25% CIN3) or condyloma
- Male - condyloma lesions.

**Control**: 166

**Vaccine**: MVA Bovine E2

**Route**: Intralesional /wk x 6 wk

**Results**

- **Complete regression rate**
  - 89% female patients
  - 100% male patients

- HPV DNA clearance: 83%

**Immune Responses**

All developed antibodies and specific cytotoxic responses

---

**MVA Bovine E2 Vaccine**

**Rational of E2 Antigen Selection**

- **E2** is negatively regulate Expression of E6/E7 proteins in infected cells
- **E2 protein** can also promote cell arrest and apoptosis in HeLa cells.
- **E2** induced macrophage antibody-dependent cytotoxicity that may enhance tumor/CIN regression

Research and decision Gaps

• **Goal**: Eradicate chronic HPV, precancerous: CIN1-3, very early cancer? And prevent recurrent

• **Antigens**:
  • E6/E7 or plus?: E2, L1, L2,
  • mono-Ag vs multi-Ag: E2, or E7 only

• **Vaccine platform**? Will mRNA work or better?

• **Dosage, number of doses, interval**

• **Delivery**: IM – later of thigh, IL: intralesional, or combined

• **Animal models**
HPV Antigen selection for a Therapeutic Vaccine
To Eradicate Chronic Infection and Premalignant Lesions

• Currently approach targeting E6/E7
  • In most animal studies-showed convincing efficacy in HPV-tumor regression
  • In well designed RCT clinical trials: only a DNA-vaccine showed promising results as a primary endpoint

• Other antigen data
  • E2 alone in MVA bovine E vaccine, non-RCT phase 3, given intralesional showed high rate of regression of CIN1-3, Condyloma
  • L1 may play role in a therapeutic vaccine?
Roles of L1 in CIN cases?

Indian Journal of Surgical Oncology (June 2023) 14(2):504–509
https://doi.org/10.1007/s13193-022-01657-w

Evaluation of the Therapeutic Effect of Quadrivalent Human Papillomavirus (HPV) Vaccination on Cervical Intraepithelial Neoplasia Lesions

Zahra Shiravani\textsuperscript{1,5} · Zinab Nazari\textsuperscript{2} · Freshteh Yazdani\textsuperscript{1} · Fatemeh Sadat Najib\textsuperscript{3} · Mojgan Akbarzadeh Jahromi\textsuperscript{4} · Mozhdeh Mohtahan\textsuperscript{1} · Sara Pour Eyed\textsuperscript{1} · Shaghayegh Moradialamdarloo\textsuperscript{5} · Mojgan Hajisafa Ti\textsuperscript{1} \textsuperscript{6}

Received: 18 February 2022 / Accepted: 19 September 2022 / Published online: 1 October 2022
© The Author(s), under exclusive licence to Indian Association of Surgical Oncology 2022
Results at Yr 1 and Yr 2 after Quadrivalent HPV Vaccination in Patients with CIN1-3

Note: Limitation of the Study: This is an observational study, not a RCT study

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Year 2 Outcome Quadrivalent HPV Vaccination (N=150)</th>
<th>Control Group (N=150)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap smear</td>
<td>91% normal</td>
<td>53% normal</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colposcopy</td>
<td>92% normal</td>
<td>53% normal</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pathology</td>
<td>93% normal</td>
<td>50% normal</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion: The observation suggests that L1-VLP vaccine may play some role in reversing CIN lesions?

Shiravani et al. Indian J Surg Oncol 2023
Can the prophylactic quadrivalent HPV vaccine be used as a therapeutic agent in women with CIN? A randomized trial

Mojgan Karimi-Zarchi¹,², Leila Allahqoli¹, Ameneh Nehmati³, Abolfazl Mehdizadeh Kashi¹, Shokouh Taghipour-Zahir² and Ibrahim Alkatout¹*
## Efficacy of Quadrivalent HPV Vaccine in Treating CIN1-3

RCT, open-labeled, N=312 (138: 104): 2 years FU Results

### Table 2: Efficacy of the HPV vaccine in women with CIN after 2 years of follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Post-injection condition of the lesion after 2 years of follow-up</th>
<th>Efficacy (%)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (N (%))</td>
<td>CIN 1 (N (%))</td>
<td>CIN 2–3 (N (%))</td>
</tr>
<tr>
<td>CIN 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (N = 35)</td>
<td>16 (45.7)</td>
<td>19 (54.3)</td>
<td>–</td>
</tr>
<tr>
<td>Two more doses of vaccination (N = 45)</td>
<td>34 (75.6)</td>
<td>11 (24.4)</td>
<td>–</td>
</tr>
<tr>
<td>CIN 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (N = 35)</td>
<td>14 (40)</td>
<td>–</td>
<td>21 (60)</td>
</tr>
<tr>
<td>Two more doses of vaccination (N = 50)</td>
<td>39 (78)</td>
<td>–</td>
<td>11 (22)</td>
</tr>
<tr>
<td>CIN 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>**Controls (N = 34)</td>
<td>14 (41.2)</td>
<td>–</td>
<td>20 (58.2)</td>
</tr>
<tr>
<td>***Two more doses of vaccination (N = 43)</td>
<td>31 (72.1)</td>
<td>–</td>
<td>12 (27.9)</td>
</tr>
</tbody>
</table>

Abbreviations: N Number; CIN Cervical intraepithelial neoplasia

* Data were analyzed with Fisher’s exact test

**One woman in the control group actually developed invasive cervical cancer

***All women with CIN 3 received 3 doses of the vaccination
• **E1, E2** proteins initiate viral DNA replication and maintain and partition viral genomes, in concert with the cellular replication machinery.

• **E5, E6, E7** proteins are required to evade host immune responses and support viral DNA replication.

• **E6, E7**: Oncogenesis
## Human Papillomavirus Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E1</strong></td>
<td>Regulation viral DNA replication</td>
</tr>
<tr>
<td><strong>E2</strong></td>
<td>Regulatory factors of viral transcription</td>
</tr>
<tr>
<td><strong>E4</strong></td>
<td>Promote virus maturation and release</td>
</tr>
<tr>
<td><strong>E5</strong></td>
<td>Regulate growth factor signaling pathway</td>
</tr>
<tr>
<td><strong>E6</strong></td>
<td>Promote the degradation of p53 and increase resistance to apoptosis</td>
</tr>
<tr>
<td><strong>E7</strong></td>
<td>Promote retinoblastoma protein (pRb) degradation, affects the cell cycle and stimulates cell proliferation</td>
</tr>
<tr>
<td><strong>L1</strong></td>
<td>Major capsid protein is important for virus assembly and stability</td>
</tr>
<tr>
<td><strong>L2</strong></td>
<td>Secondary capsid protein is important for virus infection</td>
</tr>
</tbody>
</table>

Note: those in green have been investigated and showed potential benefit.
Clinical Trial Design

- **Go/no go criteria** for Phase 1 and for later phase
- **Target Population**: persistent infection, CIN1 ?, CIN2/3, adjunct Rx for early cervical CA
- **Immunogenicity assays**: T-cells – predictive biomarker?
- **Primary endpoints**: Regression, HPV DNA clearance. Timepoint: 6, 12 months. Recurrent rate: Y2,3 ?
Target Populations of HPV Therapeutic Vaccines

- **Persistenc HPV infection**
  - Women worldwide up to 300 million are HPV carriers
  - 1 of 3 to 5 men >200 million are HPV carriers

- **Precancerous lesions**
  - CIN1-3, VIN-13, AIN1-3,
  - 0.1-0.2% of Women Worldwide with CIN1-3

- **HPV-associated Cancer**
  - CA cervix
  - Head and neck cancer
  - CA anus, vulva, vagina, penis
Natural History of HPV Infection
Rational of Therapeutic Vaccine

**Figure 1.** Natural history of oncogenic human papillomavirus infection. A model showing the progression of HPV infection to invasive cancer. Infection with HPVs is usually cleared by the immune system within a couple of years. Persistently infected cells can regress, but over time can progress to invasive cancer.

Modified from Della Fera et al. Viruses. 2021
Cervical Intraepithelial Neoplasia (CIN)

**CIN 1**
- low-grade lesion that has a low potential for progression to malignancy
- high potential for regression

**CIN 2,3**
- high-grade lesion that has a higher potential for progression and
- lower potential for regression.
### Natural History of CIN1-3
#### Clinical Trial Design and Sample Size Estimation

<table>
<thead>
<tr>
<th>CIN grade</th>
<th>Regression</th>
<th>Persistent</th>
<th>Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU Months</td>
<td>6</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>CIN 1</td>
<td>49%(^a)</td>
<td>66%(^b)</td>
<td>35%(^a)</td>
</tr>
<tr>
<td>CIN 2(^c)</td>
<td>50%</td>
<td>44%</td>
<td>50%</td>
</tr>
<tr>
<td>CIN 3(^d)</td>
<td></td>
<td>32% - 47%</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Bansal. Anticancer Res. 2008, \(^b\) Apiwattanasevee. JSHMR 2018. (CIN1 >2 y FU, N=154 Thailand)
\(^c\) Tainio et al. BMJ 2018 (Metaanalysis), \(^d\) Wirght. UptoDate Sep 2023
What have we learned?

• Can therapeutic vaccine treat HPV-mediated pre-malignancies?

• Can therapeutic vaccine treat chronic HPV infection?

Yes, very likely BUT .....  

• A better vaccine design and clinical trial design are needed