School of Global Health
Faculty of Medicine
Chulalongkorn University

Chula VRC
Chula Vaccine Research Center
Faculty of Medicine, Chulalongkorn University

HPV Therapeutic Vaccine

For Chronic Infection and Premalignacies

Kiat Ruxrungtham

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

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HPV Therapeutic Vaccine: Outlines

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

HPV Vaccine

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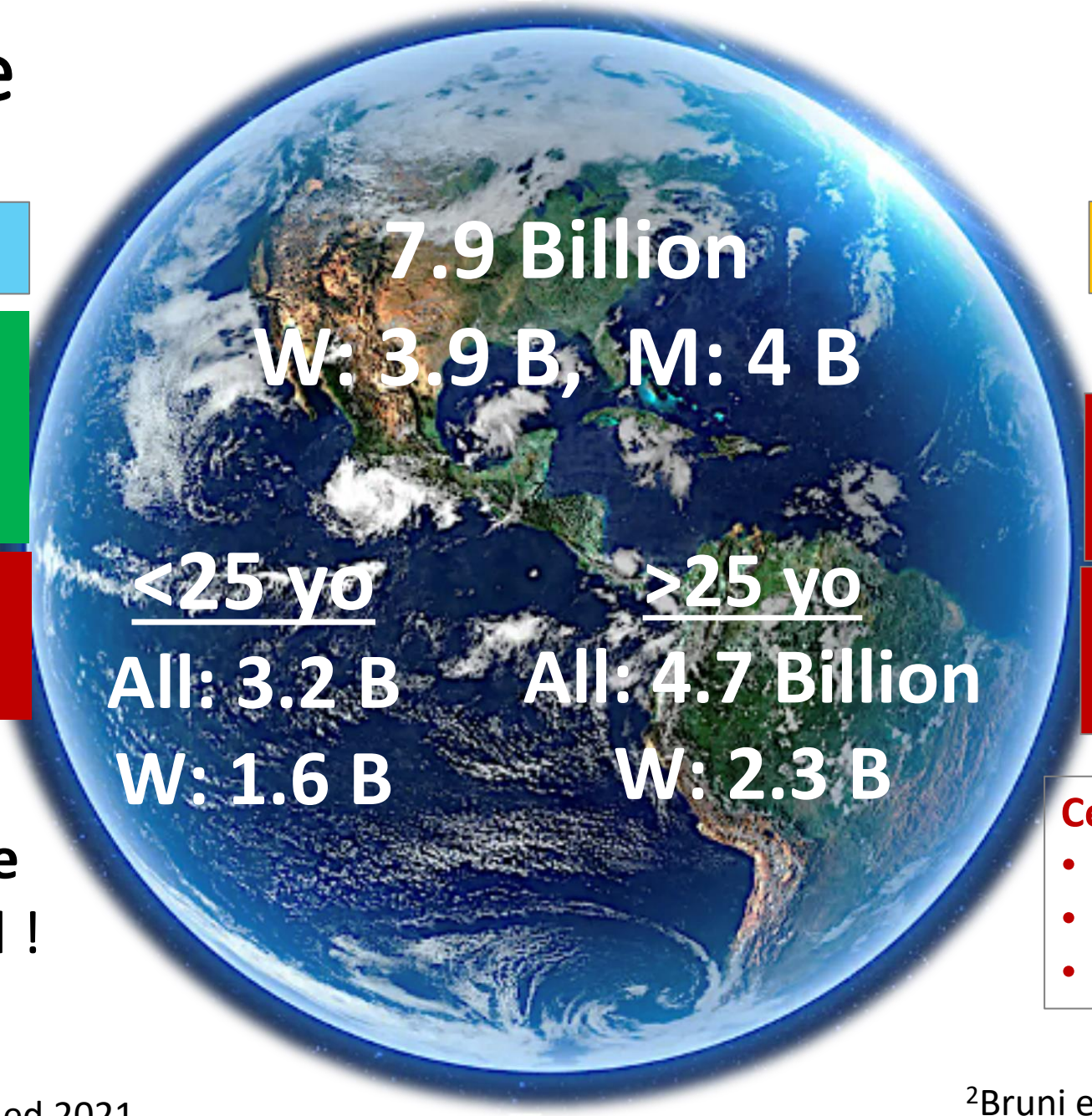
PREVENTIVE

Highly Efffective HPV Vaccine
Avialable since **2014**

Global 2019[1]
15 % coverage !!

L1-VLP
Affordable Vaccine
Is definitely needed !



THERAPEUTIC

Vaccine- not available

291 Million Women are HPV DNA carriers

21% men are HR-HPV Infected[2]

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

[1]Bruni et al. Preventive Med 2021

[2]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)

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Natural History of HPV Infection

Rational of Therapeutic Vaccine

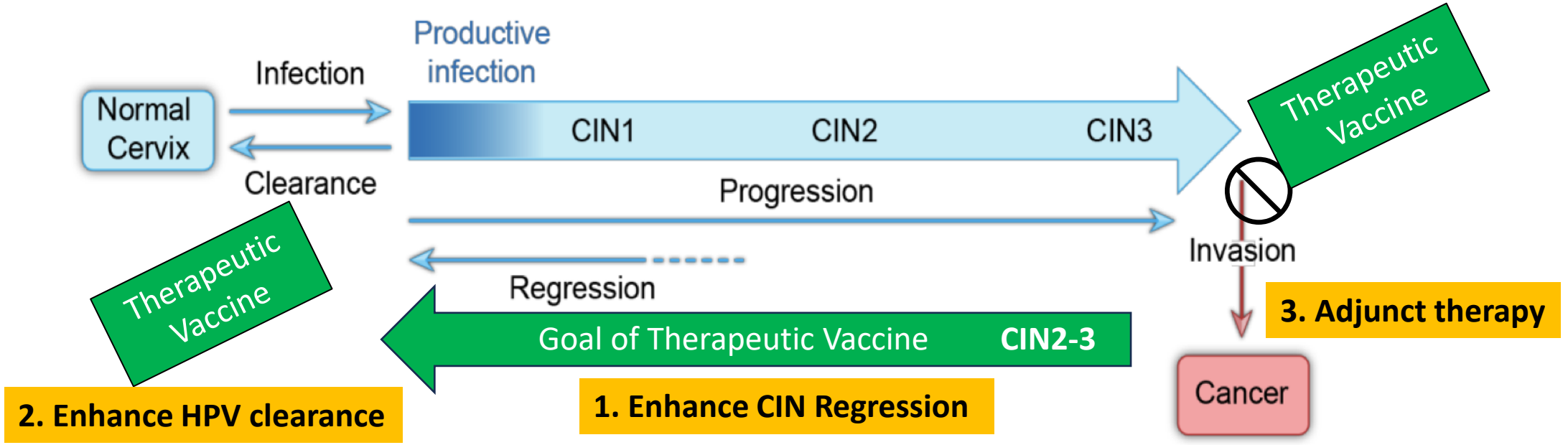

Figure 1. Natural history of oncogenic human papillomavirus infection. A model showing the progression of HPV infection to invasive cancer. Infection with HPVs is usually cleared by the immune system within a couple of years. Persistently infected cells can regress, but over time can progress to invasive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

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

Yan et al. Curr Otolaryngology Report 2023: 11(1)

Kenter et al NEJM 2009

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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**.

Kenter et al NEJM 2009

The NEW ENGLAND JOURNAL of MEDICINE

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Kenter et al NEJM 2009

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:

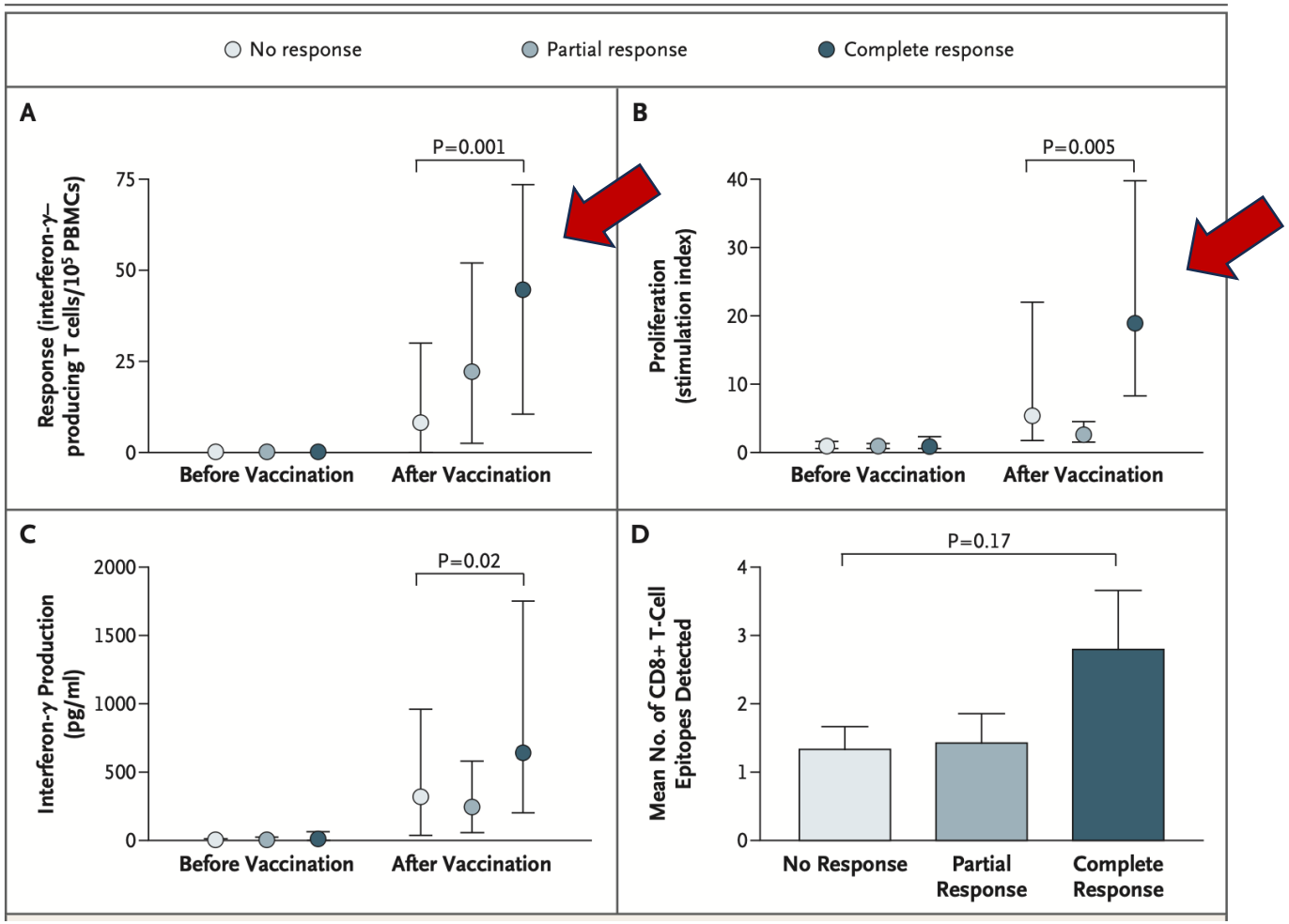
Local swelling 100%

Fever 64%

All <grade 2 AES

***Adjuvant**: *Incomplete Freund's Adjuvant*

Immune Response before and after Vaccination



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- 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.

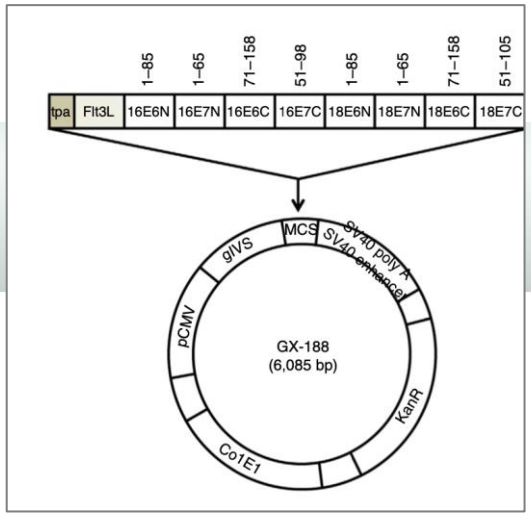
Kenter et al NEJM 2009

nature COMMUNICATIONS

ARTICLE

Received 9 May 2014 | Accepted 19 Sep 2014 | Published 30 Oct 2014

DOI: 10.1038/ncomms6317 OPEN



Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients

Tae Jin Kim[1,*], Hyun-Tak Jin[2,*], Soo-Young Hur[3], Hyun Gul Yang[4], Yong Bok Seo[4], Sung Ran Hong[5], Chang-Woo Lee[6], Suhyeon Kim[6], Jung-Won Woo[2], Ki Seok Park[2], Youn-Young Hwang[2], Jaehan Park[2], In-Ho Lee[1], Kyung-Taek Lim[1], Ki-Heon Lee[1], Mi Seon Jeong[7], Charles D. Surh[4,8], You Suk Suh[2], Jong Sup Park[3] & Young Chul Sung[2,4]

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

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Vaccine Type	Vaccine - antigens	Study Phase	Sample size Vac/Placebo	Route	Eligibility	Regression Vac vs Pla
DNA VGX-3100	HPV16/18 E6/E7	IIb	**125 : 42**	IM-EP	HPV16/18 CIN2/3	**9 months:** 49 % vs 30 %, p=0.034
DNA ZYC101a	HPV16 E6/E7	II	**53 : 58 : 50**	IM Lateral Thigh	HPV CIN2/3	**6 months:** 43% vs 27%, p=0.12 (ns)
Viral Vector TG4001	HPV16 E6/E7 (+ IL12)	II	**136 : 70**	SQ	HPV16 CIN2/3	**6 months** HPV16 mono-infection: 18% vs 4%, p<0.05
Peptide CVLP-L1E7	VLP HPV16 L1-E7	NR	**14 : 12 : 13**	SQ Upper arm	HPV16 CIN2/3	**6 months** 39% vs 24%, p=ns

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

Yan et al. Curr Otolaryngology Report 2023

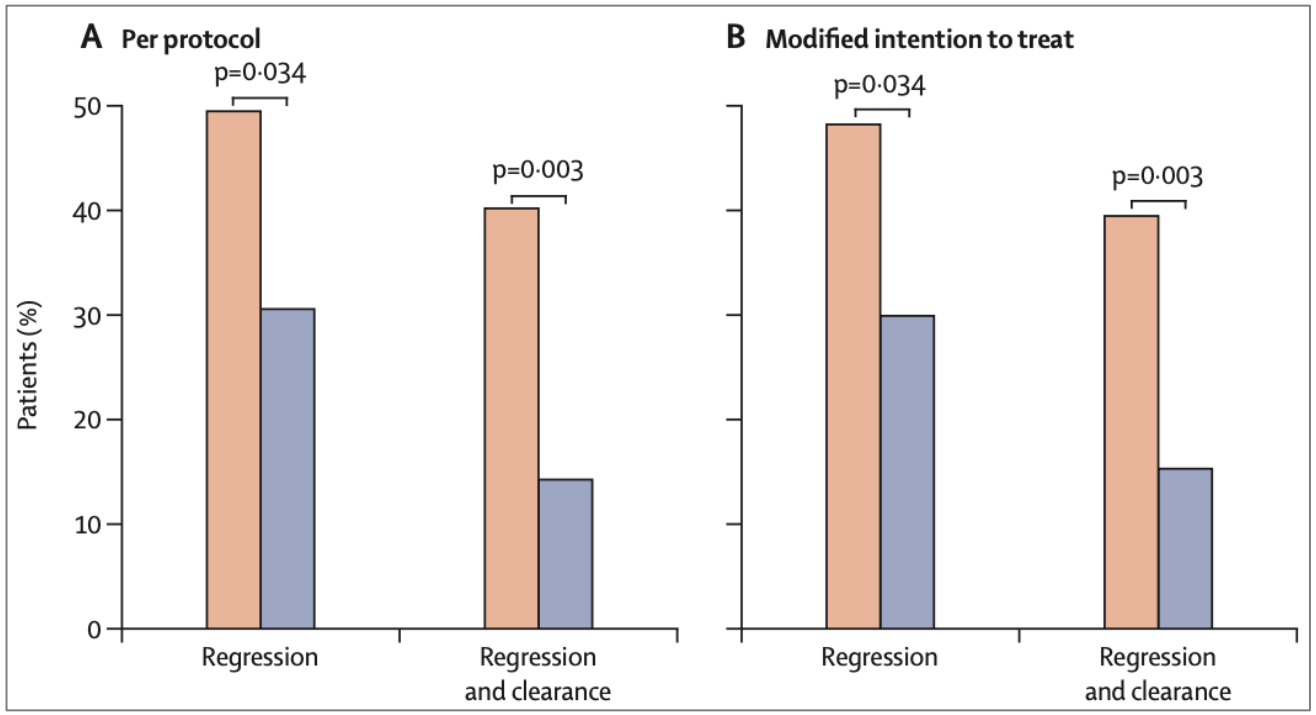
Trimble et al. **Lancet** 2015

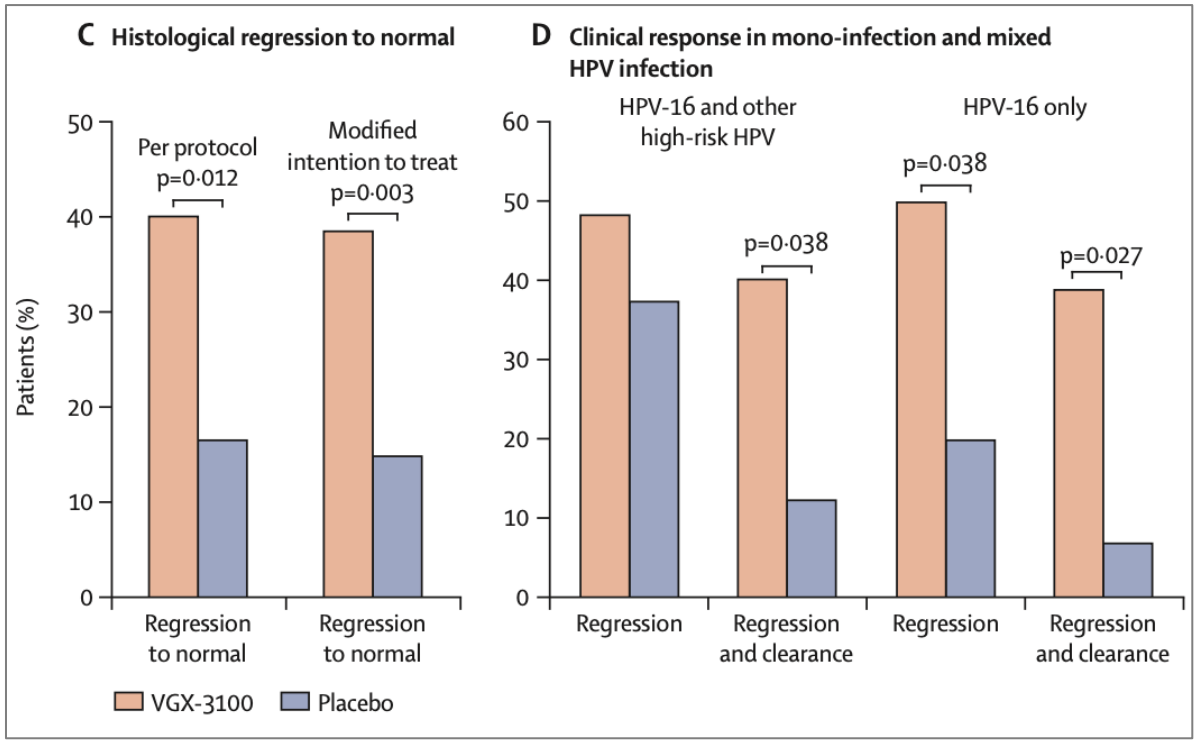
Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial

Cornelia L Trimble, Matthew P Morrow, Kimberly A Kraynyak, Xuefei Shen, Michael Dallas, Jian Yan, Lance Edwards, R Lamar Parker, Lynette Denny, Mary Giffear, Ami Shah Brown, Kathleen Marcozzi-Pierce, Divya Shah, Anna M Slager, Albert J Sylvester, Amir Khan, Kate E Broderick, Robert J Juba, Timothy A Herring, Jean Boyer, Jessica Lee, Niranjan Y Sardesai, David B Weiner, Mark L Bagarazzi

36 Weeks Results

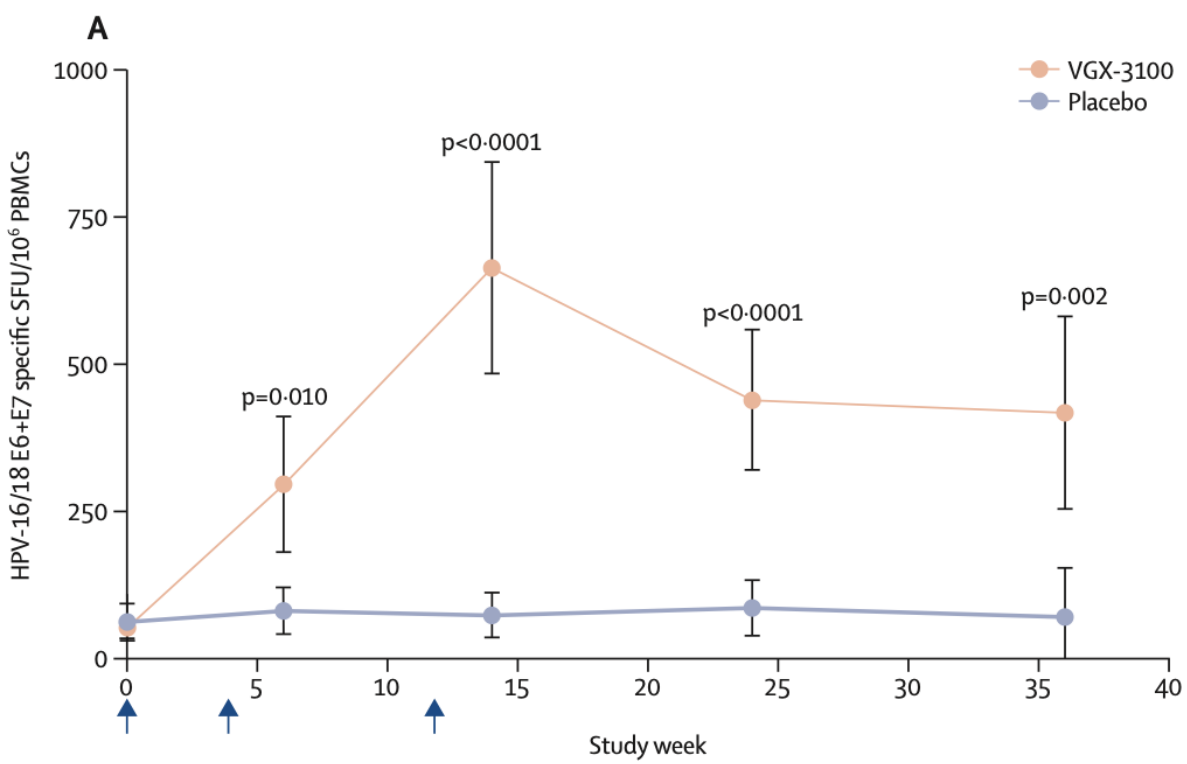
histopathological regression and viral clearance

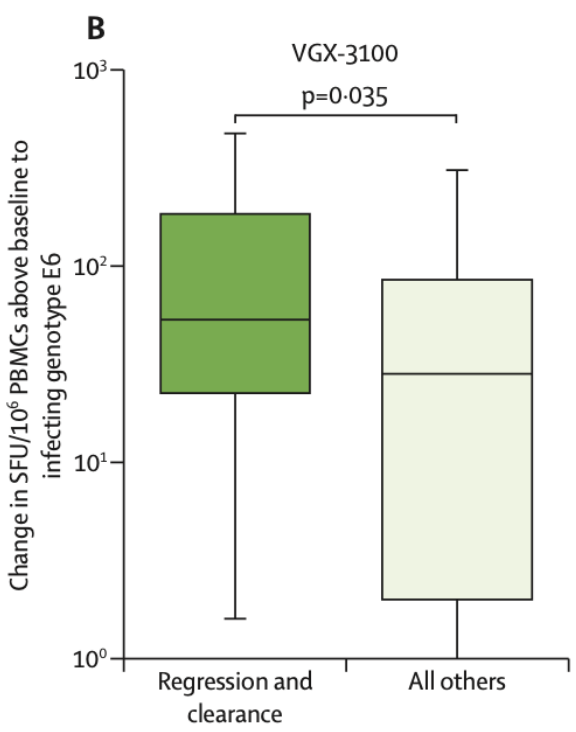


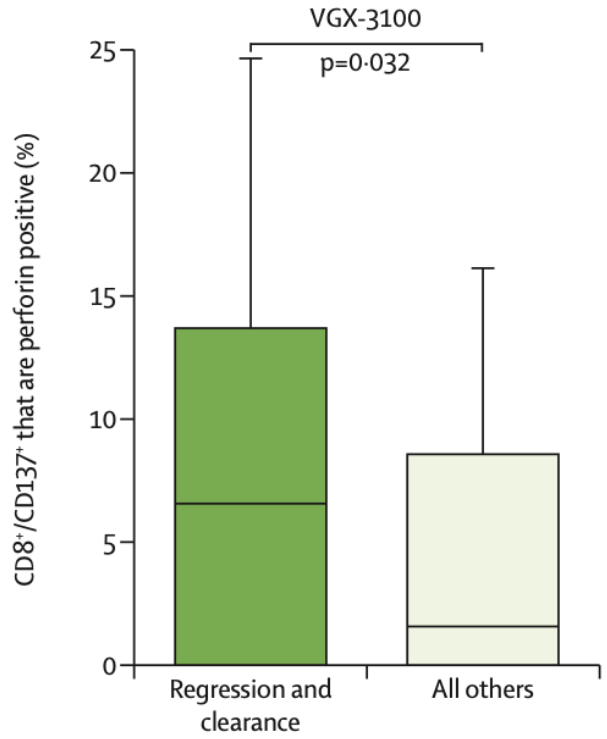


Trimble et al. Lancet 2015

VGX-3001 Induced T-cell Responses that may associate with Regression/Clearance







Trimble et al. Lancet 2015

FIERCE Biotech

Biotech · Research · Medtech · CRO · Special Reports · Fierce 50 ·

Inovio's endpoint switcheroo backfires as phase 3 misses on new measure, hits on old

By **Nick Paul Taylor** · Mar 2, 2023 5:20am

Inovio | clinical trial data | DNA vaccine | Clinical Data

Results from Inovio's phase 3 cervical dysplasia trial "will be used as supportive data in any future regulatory interactions involving VGX-3100." (3D_generator/iStock/Getty Images Plus)

The Phase 3 that changed to biomarker as primary endpoints – failed to show efficacy vs placebo, but significantly improved CIN3 regression and viral clearance rates in the original endpoints !

- 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.

HUMAN GENE THERAPY 25:1035–1049 (December 2014)

DOI: 10.1089/hum.2014.024

Phase 3: MVA bovine E2 Vaccine : Non-RCT

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

Ricardo Rosales,[1] Mario López-Contreras,[2] Carlos Rosales,[3] Jose-Roberto Magallanes-Molina,[4] Roberto Gonzalez-Vergara,[5] Jose Martin Arroyo-Cazarez,[6] Antonio Ricardez-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 Villarreal[11]

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

Rosales et al. Human Gene Therapy 2014

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

Research and decision Gaps

- **Goal**: Eradicate chronic HPV, precanceraous : 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 convining 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

ORIGINAL ARTICLE

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

Zahra Shiravani[1,5] · Zinab Nazari[2] · Freshteh Yazdani[1] · Fatemeh Sadat Najib[3] · Mojgan Akbarzadeh Jahromi[4] · Mozhdeh Momtahan[1] · Sara Pourseyed[1] · Shaghayegh Moradialamdarloo[5] · Mojgan Hajisafari Tafti[6]

Received: 18 February 2022 / Accepted: 19 September 2022 / Published online: 1 October 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**

Assessment	Year 2 Outcome		
	Quadrivalent HPV Vaccination (N=150)	**Control Group (N=150)**	P value
Pap smear	91% normal	53% normal	<0.001
Colposcopy	92% normal	53% normal	<0.001
Pathology	93% normal	50% normal	<0.001

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

Shiravani et al. Indian J Surg Oncol 2023

Karimi-Zarchi *et al. BMC Public Health* (2020) 20:274
https://doi.org/10.1186/s12889-020-8371-z

BMC Public Health

RESEARCH ARTICLE Open Access

Can the prophylactic quadrivalent HPV vaccine be used as a therapeutic agent in women with CIN? A randomized trial

Mojgan Karimi-Zarchi[1,2], Leila Allahqoli[1], Ameneh Nehmati[3], Abolfazl Mehdizadeh Kashi[1], Shokouh Taghipour-Zahir[2] and Ibrahim Alkatout[4*]

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

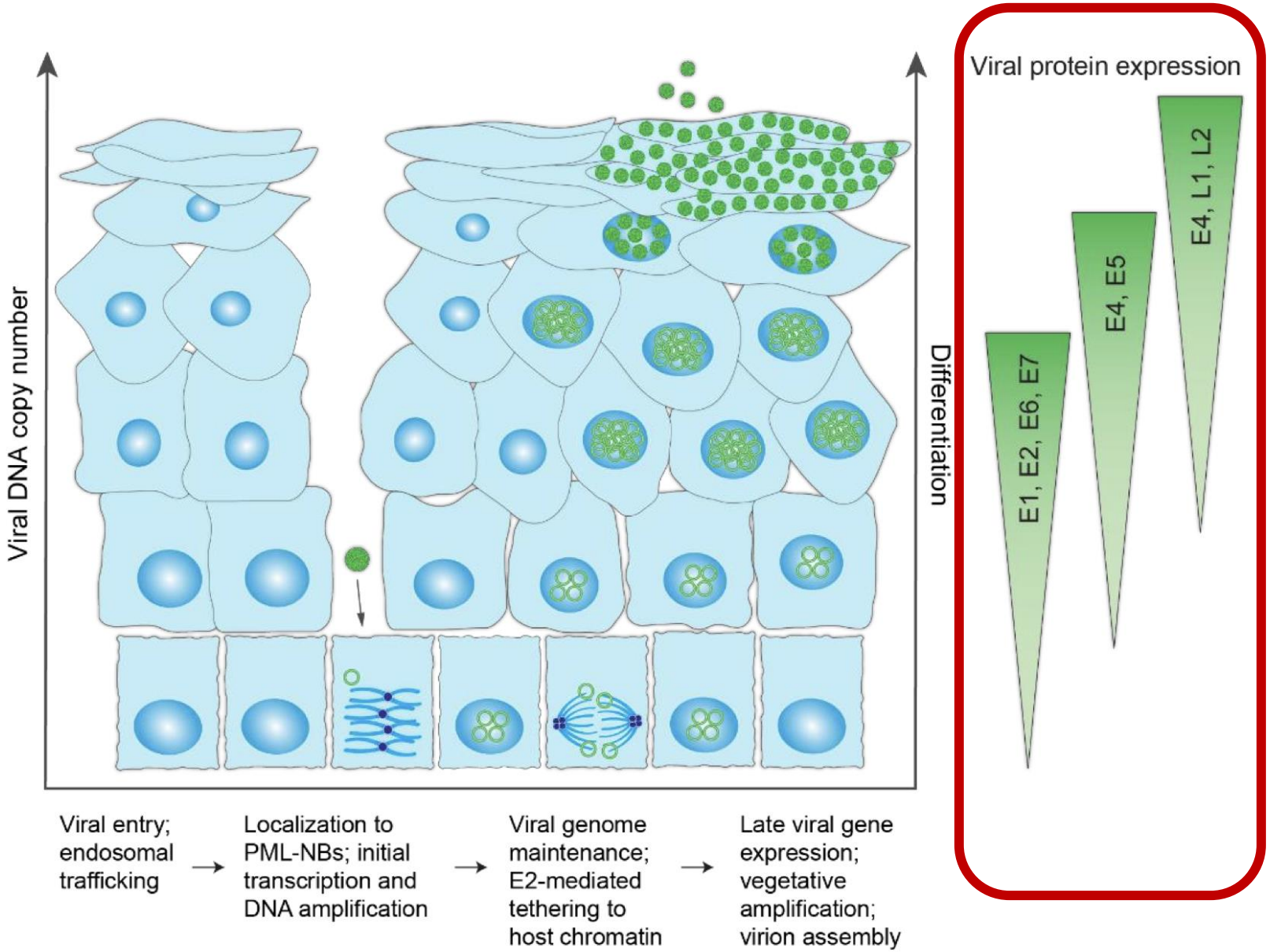
Variable		Post-injection condition of the lesion after 2 years of follow-up			Efficacy (%)	*P value
		Normal	CIN 1	CIN 2–3		
		N (%)				
CIN 1	Control (*N* = 35)	16 (45.7)	19 (54.3)	–	54.9	0.02
	Two more doses of vaccination (*N* = 45)	34 (75.6)	11 (24.4)	–		
CIN 2	Controls (*N* = 35)	14 (40)	–	21 (60)	63.3	0.01
	Two more doses of vaccination (*N* = 50)	39 (78)	–	11 (22)		
CIN 3	**Controls (*N* = 34)	14 (41.2)	–	20 (58.2)	52.5	0.03
	***Two more doses of vaccination (*N* = 43)	31 (72.1)	–	12 (27.9)		

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



Della Fera et al. Viruses. 2021

- **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

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

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

Yicheng Mo, Front. Cell. Infect. Microbiol., 04 July 2022

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 monhts. Recurrent rate: Y2,3 ?

Target Populations of HPV Therapeuctic 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

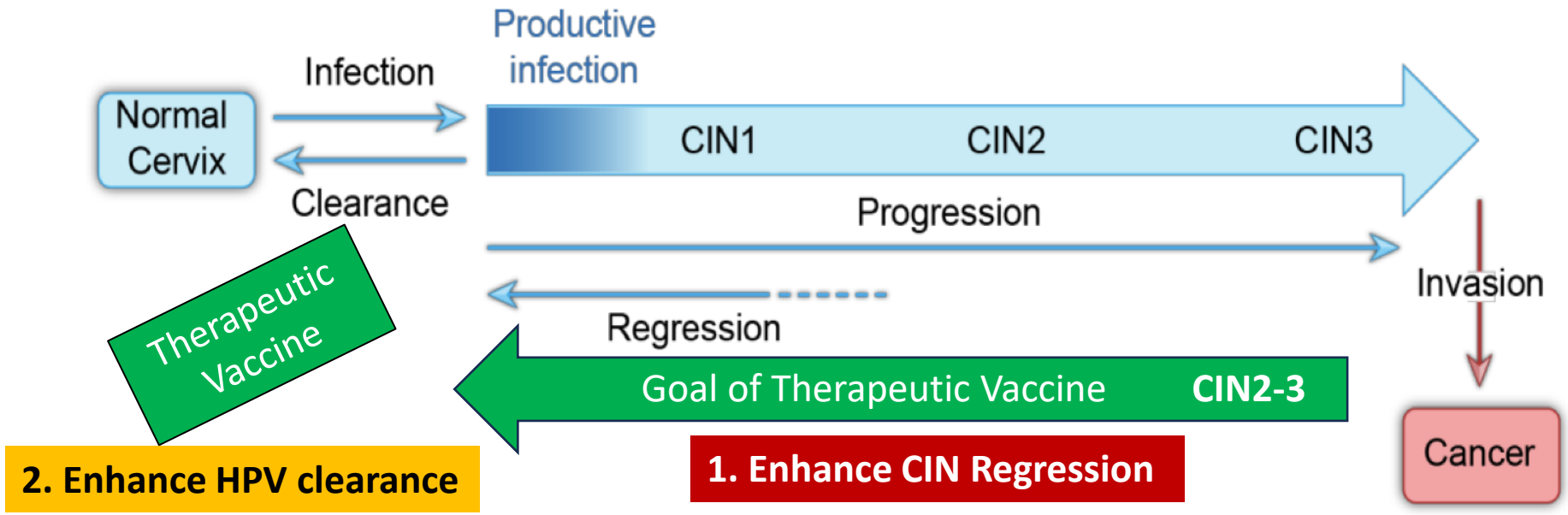


Figure 1. Natural history of oncogenic human papillomavirus infection. A model showing the progression of HPV infection to invasive cancer. Infection with HPVs is usually cleared by the immune system within a couple of years. Persistently infected cells can regress, but over time can progress to invasive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

CIN grade	Regression			Persistent			Progression		
FU Months	6	12	24	6	12	24	6	12	24
CIN 1	**49%**[a]		**66%**[b]	35%[a]		32%[b]	**7%**[a]		**2%**[b]
CIN 2[c]	**50%**	**44%**	**50%**	34%	29%	32%	**13%**	**14%**	**18%**
CIN 3[d]			**32% - 47%**						**12%-40%**

[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