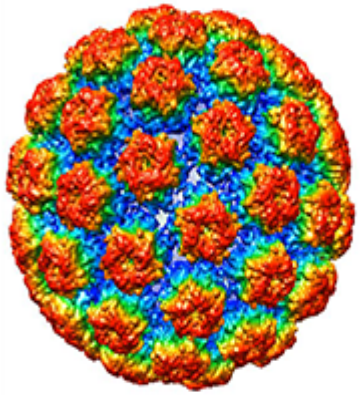
Cervical Cancer

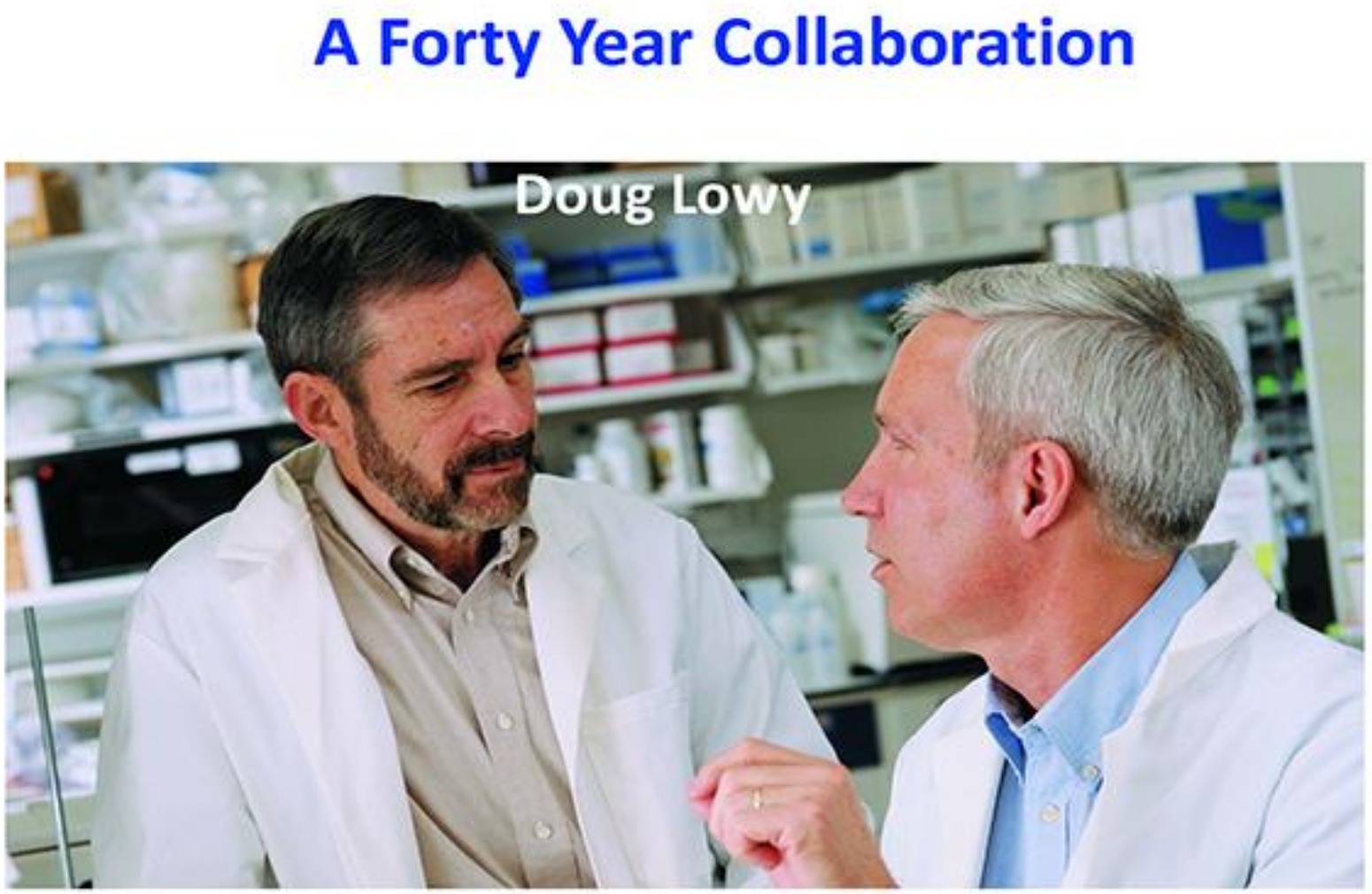
HPV Vaccines to Prevent Cervical Cancer and other HPV-associated Diseases

John Schiller, Center for Cancer Research, NCI



- HPV and Cancer
- Vaccine Efficacy/Effectiveness
- Key Implementation Issues
- Why they work so well

Collaboration



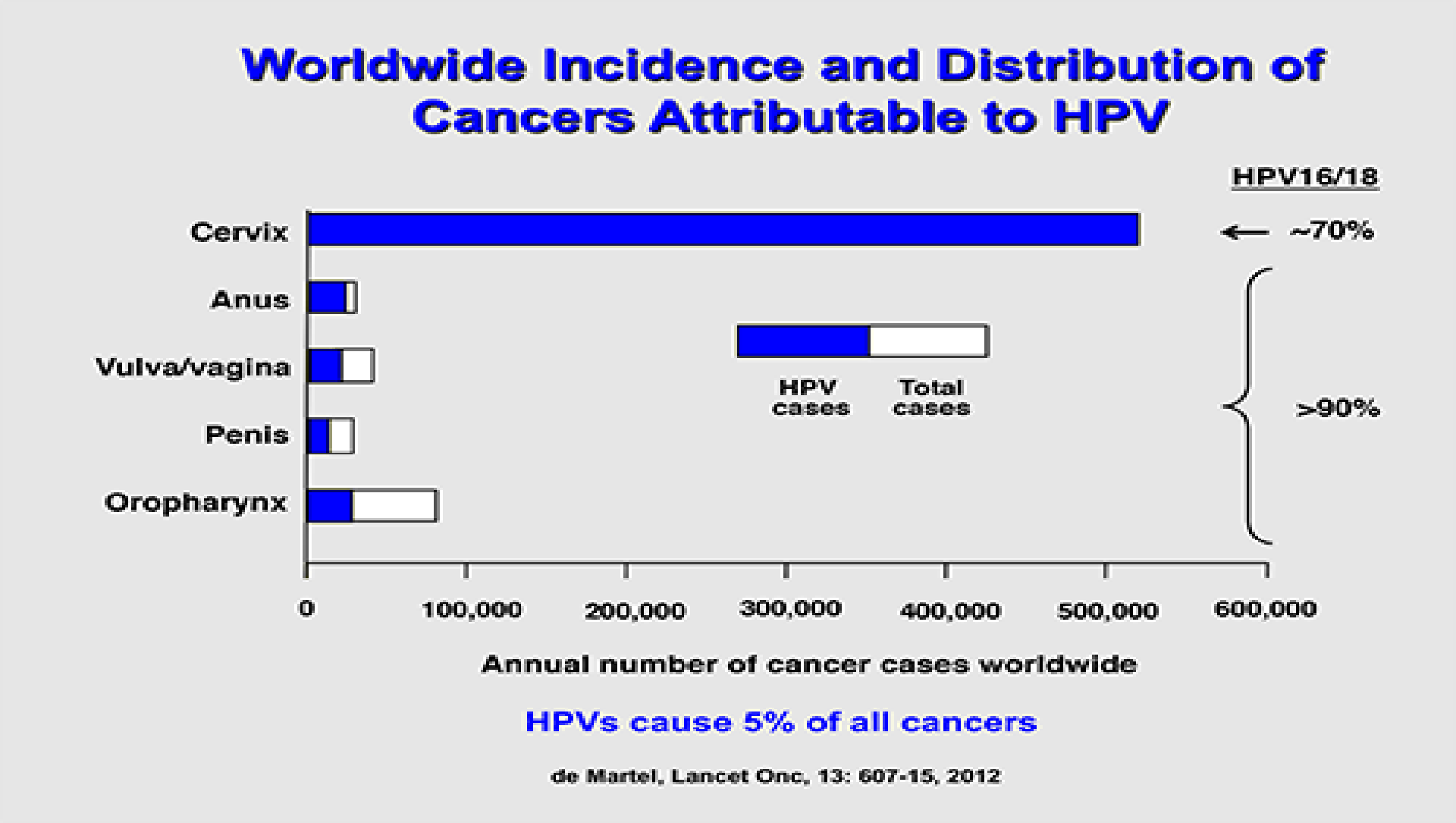
Lessons learned

Lesson Learned

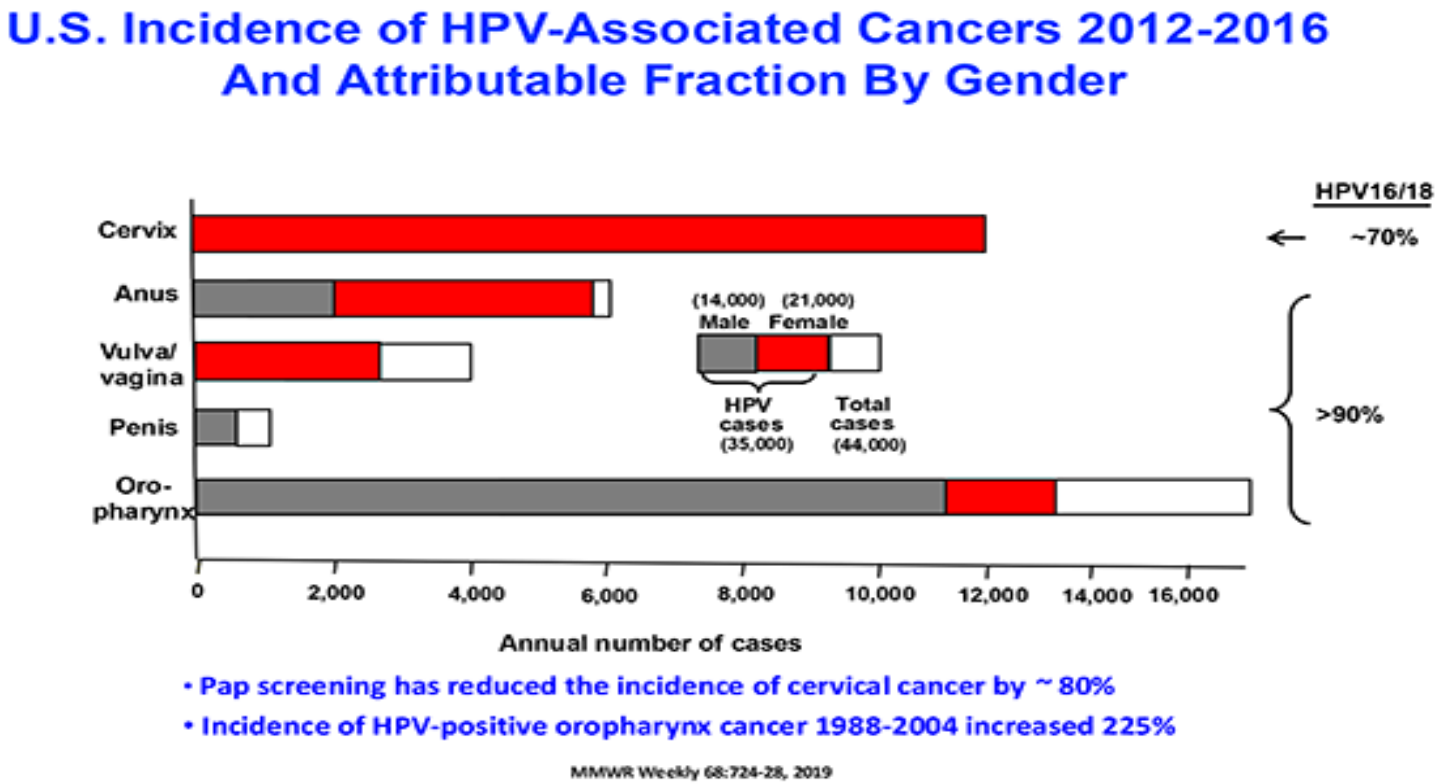
Establish strong and durable collaborations with exceptionally bright and generous scientists.

It’s a lot more fun and your chances of success go way up.

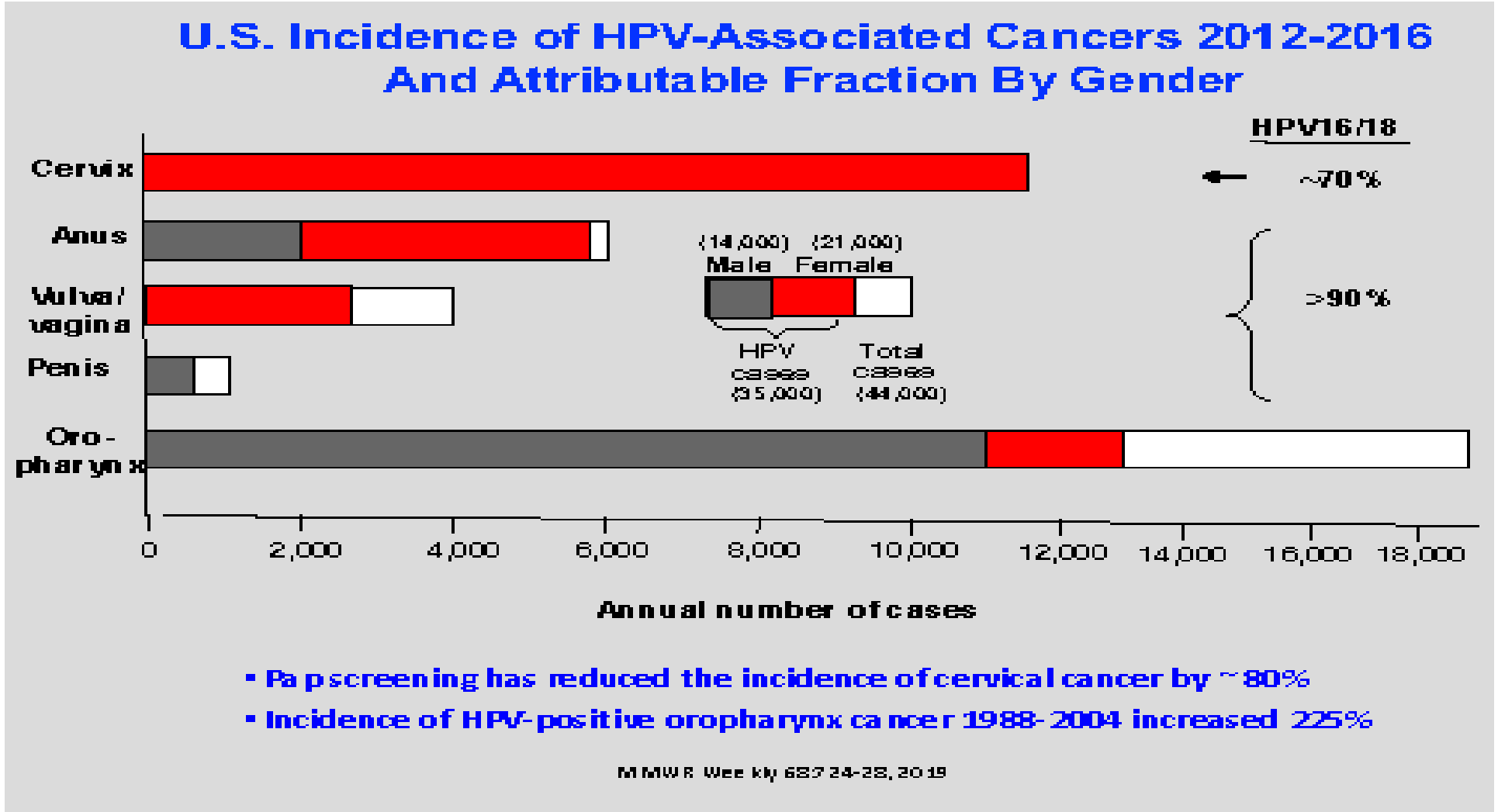
Cancers attributable to HPV



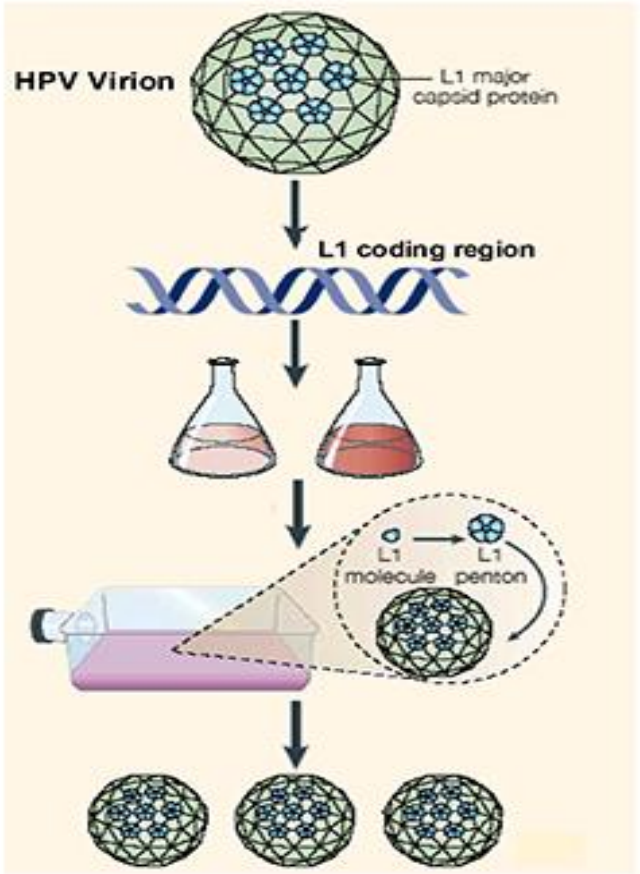
HPV cancers


U.S. Incidence of HPV-Associated Cancers 2012-2016
And Attributable Fraction By Gender
HPV16/18
~70%
>90%
Cervix
Anus
Vulva/
vagina
Penis
Oro-
pharynx
(14,000) (21,000)
Male Female
HPV
cases
(35,000)
Total
cases
(44,000)
0
2,000
4,000
6,000
8,000
10,000
12,000
14,000
16,000
Annual number of cases
• Pap screening has reduced the incidence of cervical cancer by ~ 80%
• Incidence of HPV-positive oropharynx cancer 1988-2004 increased 225%
MMWR Weekly 68:724-28, 2019

HPV incidence



Virus like particles



Reinhard Kirnbauer et al. PNAS 1992

Prophylactic HPV Vaccines Are L1 Virus Like Particles (VLPs)

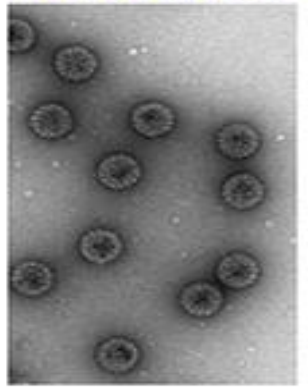
L1 Insertion into a Baculovirus Expression Vector

Production in Insect Cells

Spontaneous assembly of 360 copies of L1 into a VLP

Induce high titers of virion neutralizing antibodies (100,000!)

HPV16 L1 VLPs





Reinhard Kirnbauer

Protected in animal challenge models

Non-infectious, Non-oncogenic

Lesson learned

Lesson Learned

CARPE DIEM!

You don’t need to an expert to make an important contribution to a field.

- **Our experience in Immunology and Vaccinology: ZERO**
- **Our experience in studying the virion proteins: ZERO**
- **Our experience in translational research: ZERO**
- **The NIH intramural program funds scientist, not projects.**

Commercial vaccines

Six Distinct Commercial HPV L1 VLP Vaccines

Name	Producer	VLP Types	Adjuvant	Production	Licensed
Cervarix	GSK	16,18	AS04*	Insect Cells	2007
Gardasil	Merck	6,11,16,18	Alum	S. cerevisiae	2006
Gardasil-9	Merck	6,11,16,18, 31,33,45,52,58	Alum	S. cerevisiae	2014
Cecolin**	Innovax	16,18	Alum	E. coli	2020
CervaVax***	Serum Inst.	6,11,16,18	Alum	Hansenula	2022
Walrinvax**	Walvax	16,18	Alum	Pichia	2022

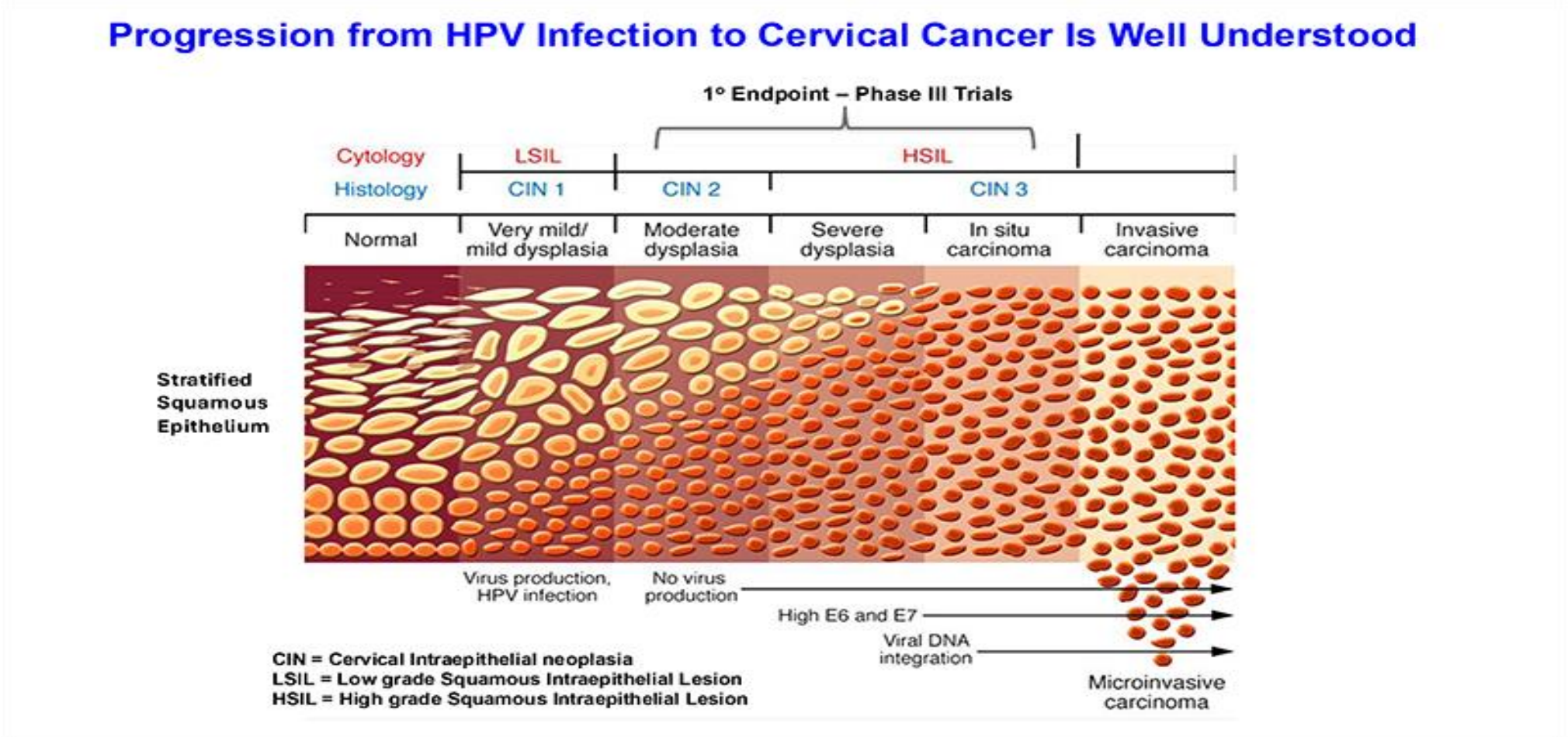
Only one sold In the U.S.

IM Injections at 0, 1 or 2, and 6 months
Then 1, 6 months for ages <15 yrs in U.S. and elsewhere

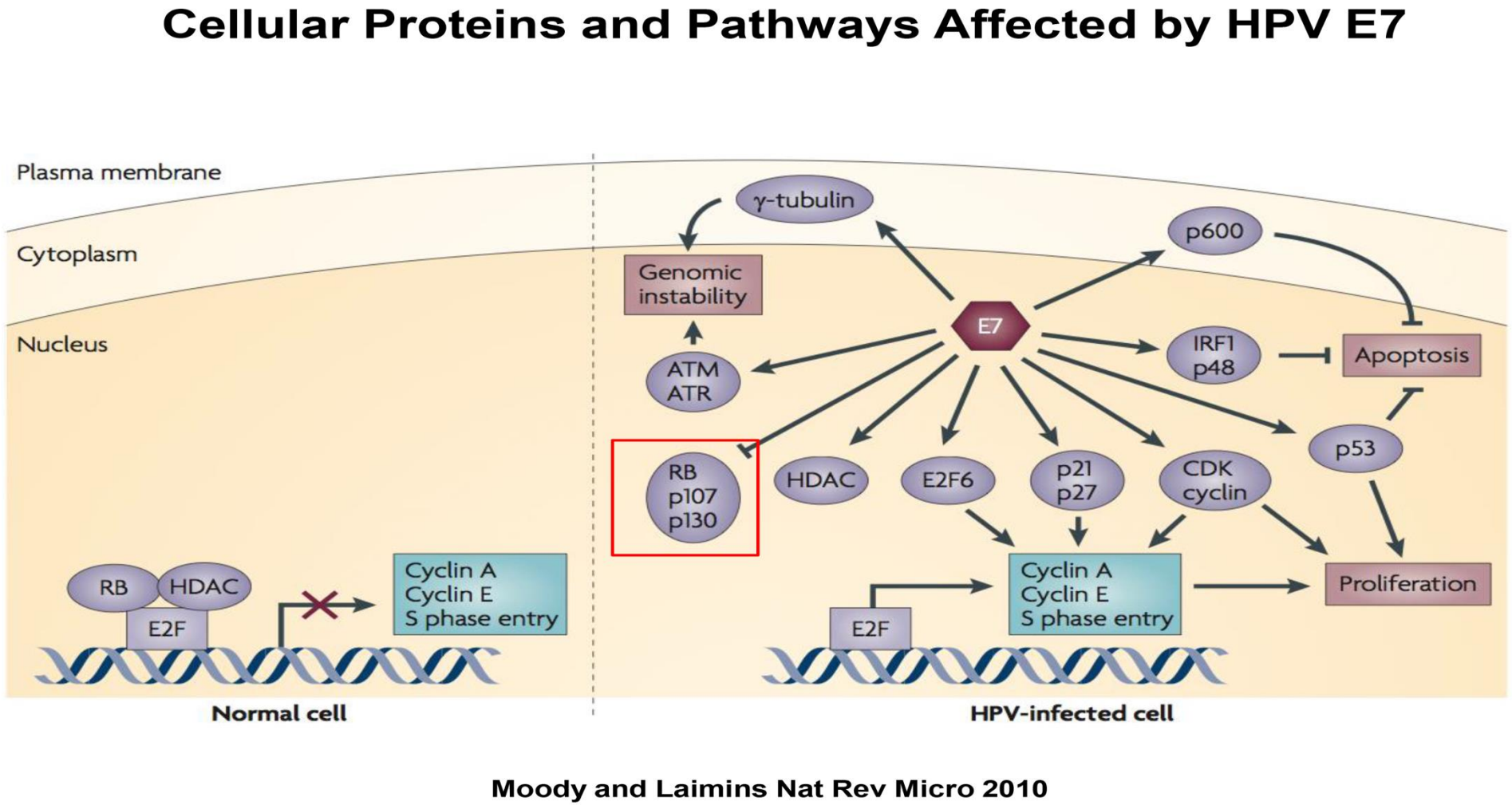
* MPL First TLR Agonist Adjuvant to be FDA Approved
** Only approved in China
*** Only approved in India

500 Million Doses Administered

HPV infection progression

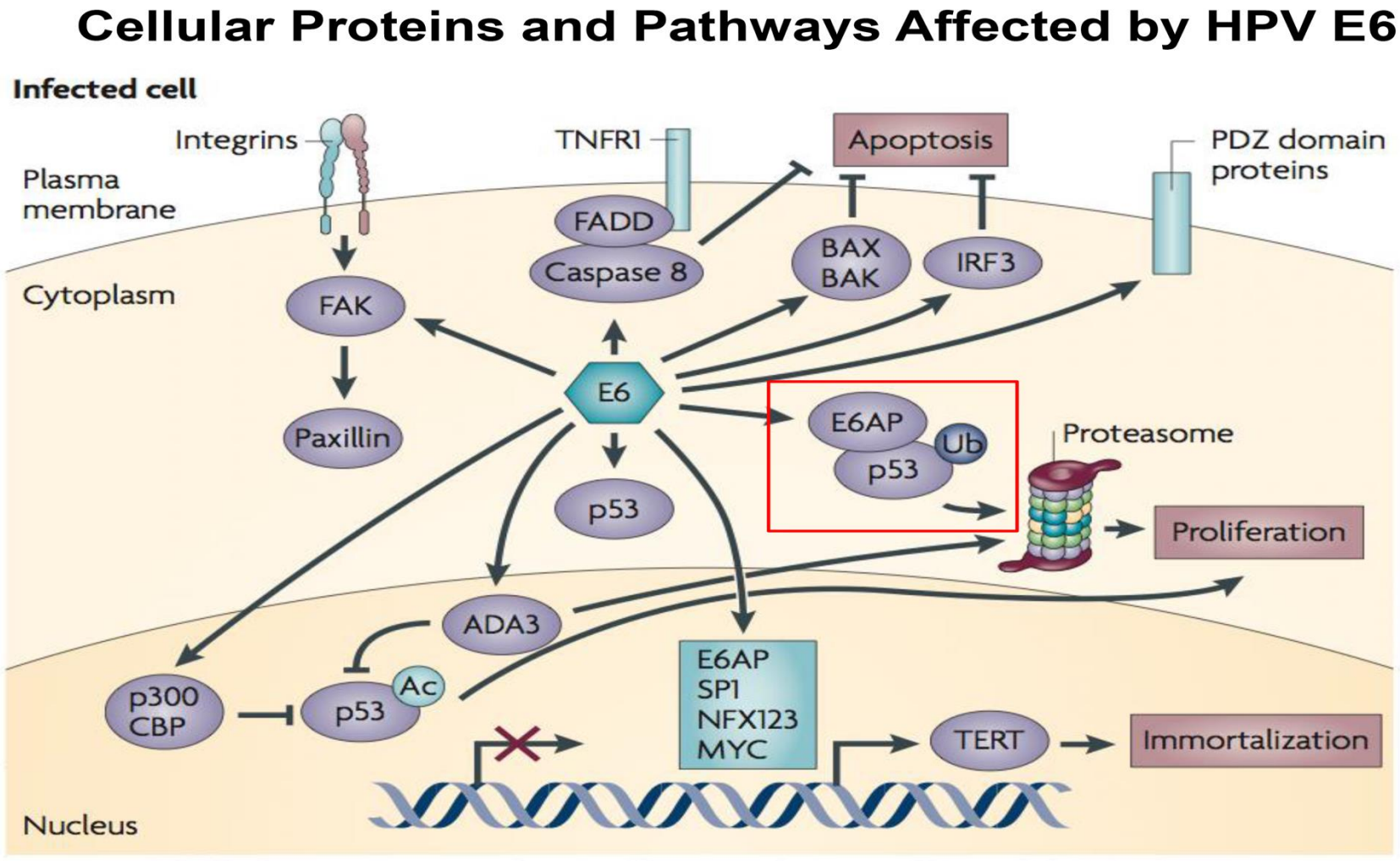


Cellular proteins



Moody and Laimins Nat Rev Micro 2010

HPV pathways



Moody and Laimins Nat Rev Micro 2010

Vaccine efficacy

Summary of Vaccine Efficacy in Randomized Clinical Trials

Phase 3 trials in women ages 15-26
4 year trials with 5,000-19,000 subjects
Primary analyses in those negative for vaccine types at enrollment.

Protection against vaccine targeted types:

- **95-100% against cervical precancers as primary endpoint**
- **95% against genital warts (Gardasil and Gardasil-9)**
- **No indication of waning immunity for at least a decade**

Infection protection

Protection From Initial Infection

- Most Vaccinees never tested positive for HPV infection as measured by sensitive PCR Assays.
- “Breakthrough” infection tended to appear early in the trials suggesting that most were emergence of prevalent infection.
- Results imply that sterilizing immunity normally generated, presumably by inducing antibodies that prevent infection.

What vaccines don’t do

What the HPV Vaccines Don’t Do

- They don’t prevent infection or disease caused by most of the other HPV types that cause cervical cancer.
- They don’t induce regression of established HPV infections or prevent progression of HPV-induced lesions.

Immunization program

How well do the vaccines work in national immunization programs?

Gardasil effects

Reduction in the Gardasil-Targeted Type Infections in the US

Precent reduction in the prevalence of the four Gardasil-targeted types from pre-vaccination (2003-6) to vaccination period (2015-18) in sexually experienced females.

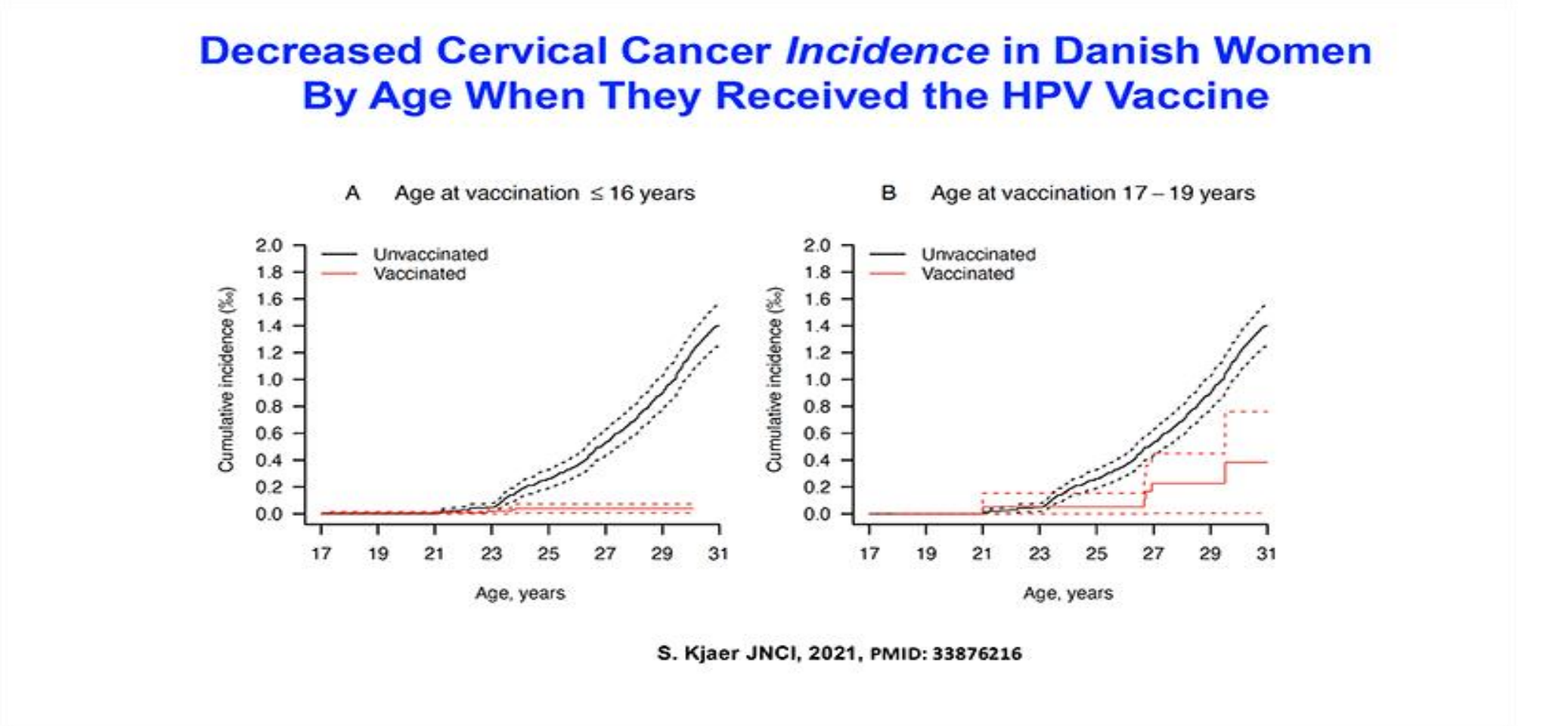
Coverage rate = 60%

Age	Vaccinated	Unvaccinated
14-19	97%	87%*
20-24	86%	65%*

***Evidence of strong herd protection**

MMWR Morb Mortal Wkly Rep 2021 PMID: 33764964

Decreased cervical cancer incidence



Patient deaths in England

Cervical Cancer *Deaths* in England 2001-2024

> Lancet. 2026 Jun 17:S0140-6736(26)00918-9. doi: 10.1016/S0140-6736(26)00918-9.
Online ahead of print.

Cervical cancer mortality trends following HPV vaccination in England, 2001–24: an analysis of population-based mortality data

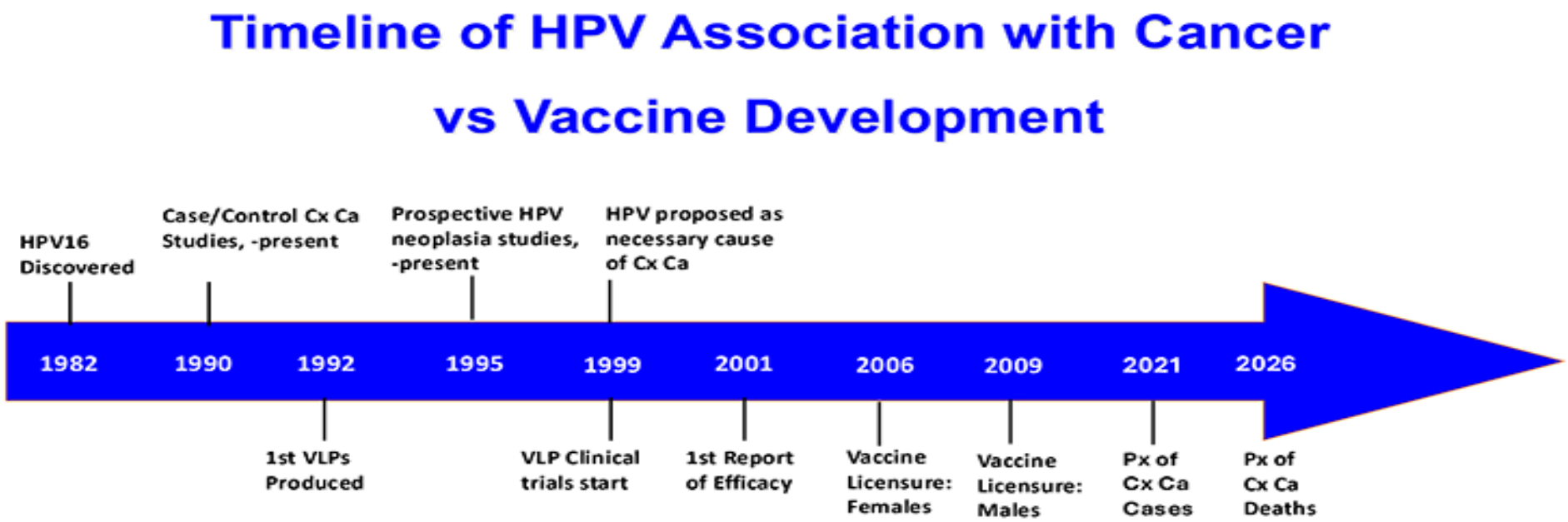
Peter Sasieni [1], Milena Falcaro [2]

Affiliations + expand

PMID: 42309117 DOI: 10.1016/S0140-6736(26)00918-9

- **No deaths in 20-24 year olds between 2020-2024 after 90% vaccination at 12-13 yrs (23 deaths expected).**

Timeline



Lesson: it take a long term commitment to make a public health impact, particularly in disease prevention.

Safety profile

The HPV Vaccines Have an Outstanding Safety Profile.

For example:

- Willame C, Pediatr Infect Dis J, 2020, PMID: 31876615
- Donahue JG, Pediatrics, 2019, e20191808
- Shimabukuro, TT, Pediatrics, 2019, e20191791
- Arbyn, M, Expert Rev Vac, 2018, PMID: 30495978

Health impact

Public Health Impact

It has been estimated that current vaccine programs have prevented over 1 million future cases of cervical cancer, with the prospects of preventing 10s of millions of more cases of HPV-induced cancers in the future.

Based on the strong efficacy and herd protection observed, it is expected that the HPV types targeted by the vaccines will be eliminated in populations with reasonably achievable coverage levels (70-80%) in adolescents.

Cancer Prevention

Cancer Prevention is Generally Underappreciated

- **Because it's silent.**
- ***Cure* a hundred cases of a deadly cancer and you have a hundred very grateful patients and the front page of the Washington Post.**
- ***Prevent* a million cases of a deadly cancer and you have a statistic.**

NCI involvement

Key Question in NCI's Involvement

Should we continue public sector development of your technology when first rate companies are already competing to develop it?

Decided yes because:

- **We were concerned that the companies will pull the plug.**
- **We thought that there were important questions that the companies won't address in their trials.**

NCI decision

The NCI's Decision

- **We conducted Phas1/2 trials in collaboration with JHU Center for Immunization Research.**
- **We conducted a Phase 3 efficacy trial in Costa Rica, the site of a long-standing collaboration to study the HPV/cervical cancer link.**

Answer

The Answer to One Question Made it Worthwhile

Does a single dose of the vaccine work as well as the prescribed three doses?

Answer: in post hoc analyses, young women who received a single dose were equally protected against persistent infection by the HPV types targeted by the vaccine as those that received three doses.

This finding went against the dogma that a prime/boost series is required for subunit vaccines.

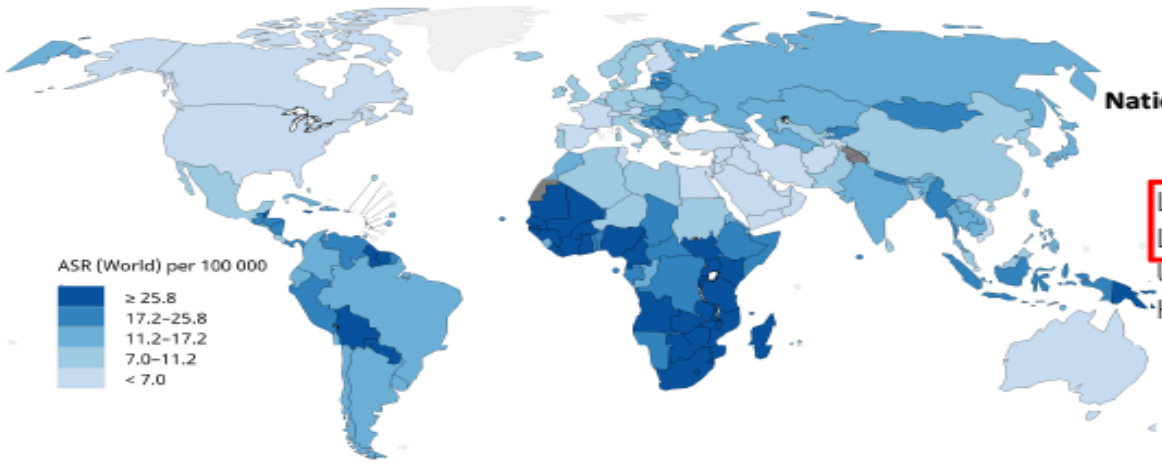
Single dose

Why is this single dose efficacy finding important?

Global cervical cancer incidence

Global Cervical Cancer Incidence

"A Story of Inequality"



National HPV Vaccine Introductions by World Bank Category

*15 years after introduction**

LIC	**7 of 34** have introduced
LMIC	**9 of 47** have introduced
UMIC	**34 of 58** have introduced
HIC	**68 of 86** have introduced

Worldwide coverage peaked at 15% in 2019

Vaccine coverage in 10-20 yr females <3% in LMICs vs 34% in HICs.

**Introduction not always synonymous with nationwide-implementation*

Clinical Trial

Summary of Clinical Trial Finds Supporting Single Dose Efficacy

- NCI Costa Rica: In post hoc analyses, no difference in efficacy against infection after 1, 2, or 3 doses of Cervarix after 11 years[1].
- IARC India trial: In post hoc analyses, no difference in efficacy against infection after 1, 2, or 3 doses of Gardasil after 10 years[2].
- KEN-SHE Kenya trial: In randomized placebo-controlled trial, 97% protection against persistent HPV16/18 infections for 3 years after a single dose Cervarix or Gardasil 9[3] compared to no vaccine. No waning to 5 years.

[1]Kreimer A JNCI 2020. [2]Basu P Lancet Onc 2021. [3]Barnabas R 2022, DOI 10.21203/rs.3.rs-1090565/v1

Landmark decision

An April, 2022 Landmark Decision by the WHO



One-dose Human Papillomavirus (HPV) vaccine offers solid protection against cervical cancer

SAGE recommends updating dose schedules for HPV as follows:

- **one or two-dose schedule** for the primary target of girls aged **9-14**
- **one or two-dose schedule** for young women aged **15-20**
- Two doses with a 6-month interval for women **older than 21**.

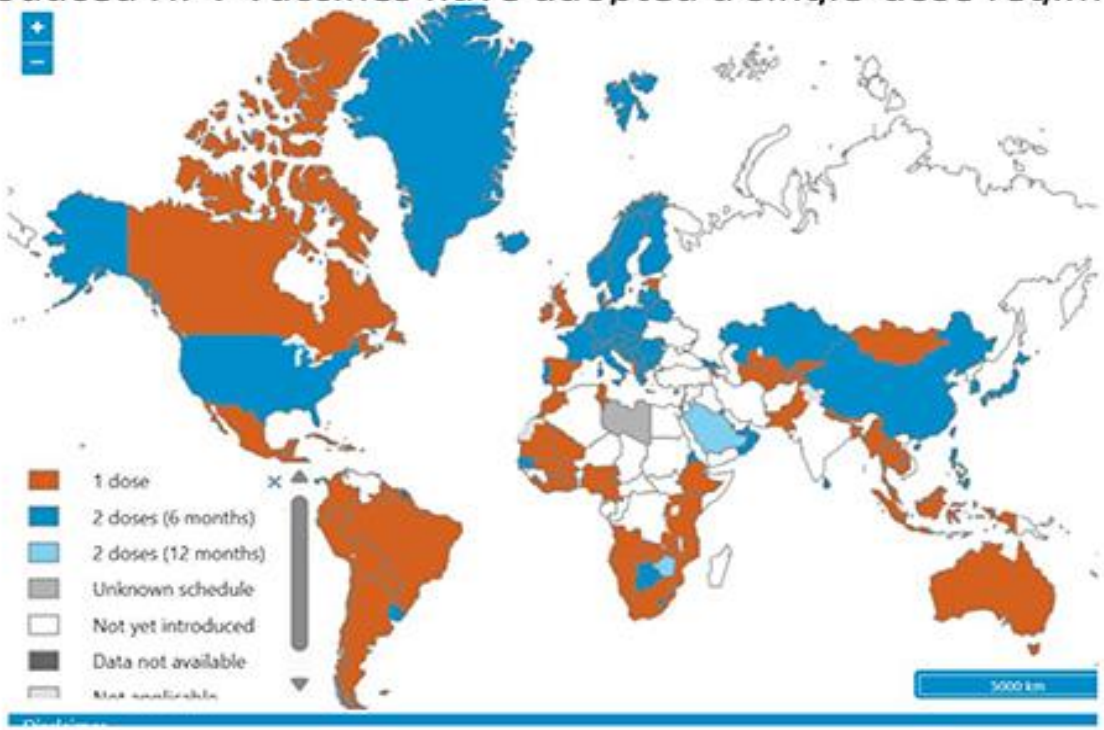
Immunocompromised individuals, including those with HIV, should receive three doses if feasible, and if not at least two doses. There is limited evidence regarding the efficacy of a single dose in this group.

Dose schedules

Dose Schedules for 9-14 Year Old Girls in 2025

> Half of 156 countries that have introduced HPV vaccines have adopted a single-dose regimen

Interval_doses	No. of countries
1 dose	83
2 doses (12 months)	2
2 doses (6 months)	71
Not yet introduced	36
Unknown schedule	2



WHO reported that between 2023–2025, an estimated >30M additional girls have/will receive HPV vaccine due to the adoption of a single-dose schedule

World Health Organization. HPV vaccine dashboard: Schedule (interval between doses). Assessed Nov 17, 2026.
https://app.powerbi.com/view?r=eyJrIjoiNDk2ZTFkZGUtMDQ1Ny00MDZkLTlhZDktYWFlYTdkOGU2NDcwIiwidCI6ImY2MT8jMGI3LWJkMjQtNGIzOS04MTBiLTNkYzI4MGFmYjU5MCIsImMiOjh9

Single dose HPV vaccination

How do we convince the other half of countries to adopt single dose HPV vaccination programs?

Answer: Conduct a formal randomized noninferiority trial comparing one and two dose efficacy.

One or Two doses

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Noninferiority of One HPV Vaccine Dose to Two Doses

A.R. Kreimer,[1] C. Porras,[2] D. Liu,[1] A. Hildesheim,[1] L.J. Carvajal,[2] R. Ocampo,[2] B. Romero,[2] M.H. Gail,[1] B. Cortes,[2] M.S. Sierra,[1] K. Coronado,[2] J. Sampson,[1] C. Coto,[2] C.L. Dagnall,[3] D. Mora,[2] T.J. Kemp,[4] M. Zuniga,[2] L.A. Pinto,[4] G. Barrientos,[2] J. Schussler,[5] Y. Estrada,[2] C. Montero,[2] C. Avila,[2] D. Ruggieri,[5] J.T. Cyr,[5] S. Chanock,[1] D.R. Lowy,[6] J.T. Schiller,[6] and R. Herrero[2,7]

Published Dec 3, 2025

ESCUDDO

ESCUDDO: Costa Rica HPV Vaccine RCT
One vs. Two Doses of Gardasil 9 & Cervarix

Noninferiority

- One dose of Gardasil 9 or Cervarix are noninferior to two doses of the same vaccine in prevention of persistent HPV16/18 infection ($p<0.001$)

- One dose of Gardasil 9 noninferior to two doses in prevention of persistent HPV16/18/31/33/52/58 infection ($p<0.001$)

ESCUDDO

ESCUDDO: Costa Rica HPV Vaccine RCT

Efficacy

In comparison to 5 yr end of study survey

Endpoints	Vaccine	Efficacy
HPV16/18	1D Cervarix	98.2%
HPV16/18	2D Cervarix	97.8%
HPV16/18	1D Gardasil 9	97.0%
HPV16/18	2D Gardasil 9	98.5%

NEJM, Dec 3, 2025

Lessons learned

Lessons Learned

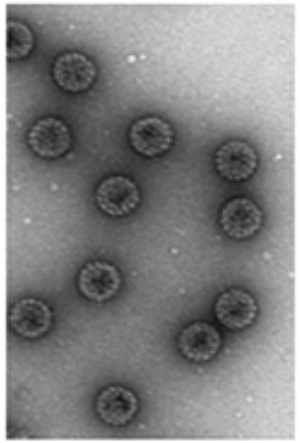
- It is the company's job to do the minimal analyses required for licensure ASAP.
- They are not in the business to lower program costs or their profits.
- Although it can be expensive, it is sometime worthwhile to follow through with public sector trials of your invention.

HPV VLP Vaccine

Why Do HPV VLP Vaccines Work So Well?

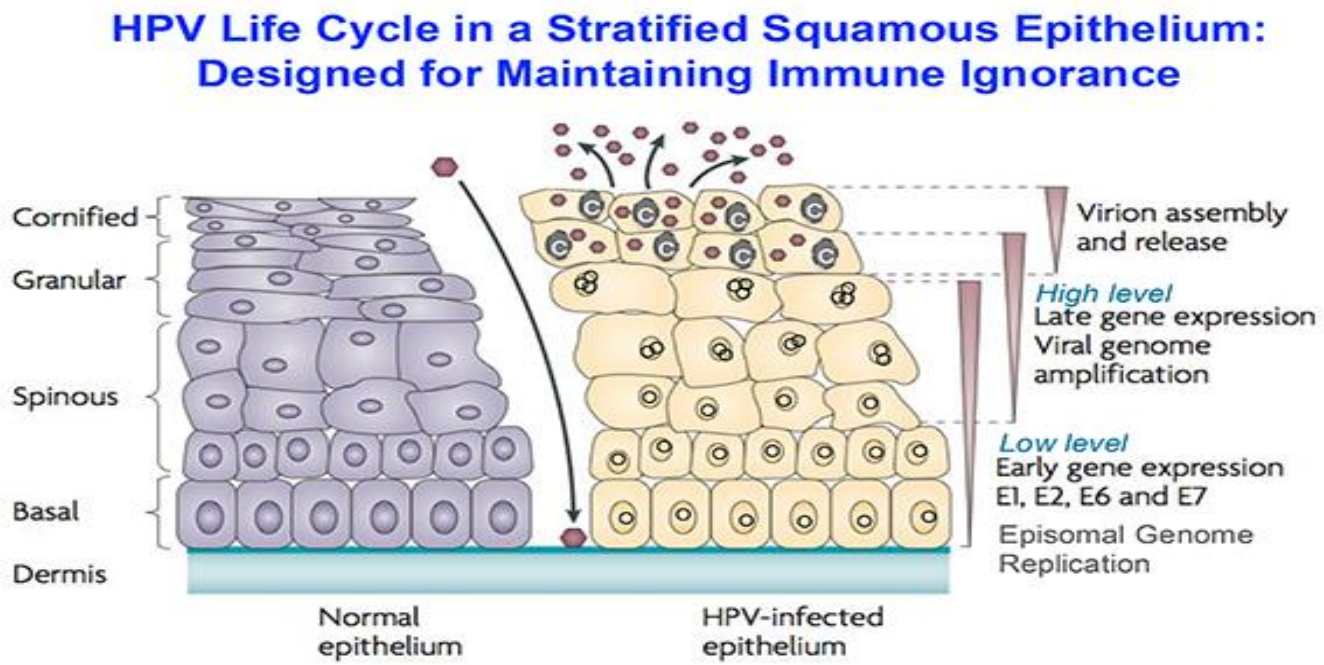
Evolutional Argument:

They exploit the virus' evolutionary Achilles' Heel.



HPV16 L1 VLPs

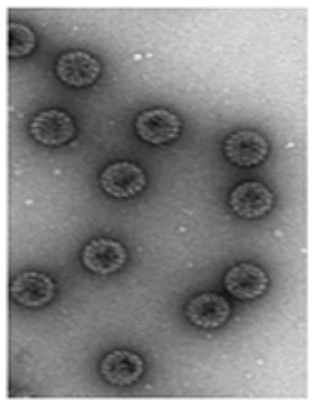
HPV life cycle



HPV VLP vaccines

Why Do HPV VLP Vaccines Work So Well?

- The vaccines are exceptionally good at inducing neutralizing antibodies.
- Infection mechanism make HPVs exceptionally susceptible to neutralizing antibodies.
- HPVs have DNA genomes so don't evolve rapidly to evade nAb responses.



HPV16 L1 VLPs

Provides biological plausibility for HPV VLPs being:

- The first subunit vaccine to induce long term protection after a single dose.
- The only successful STI vaccine.

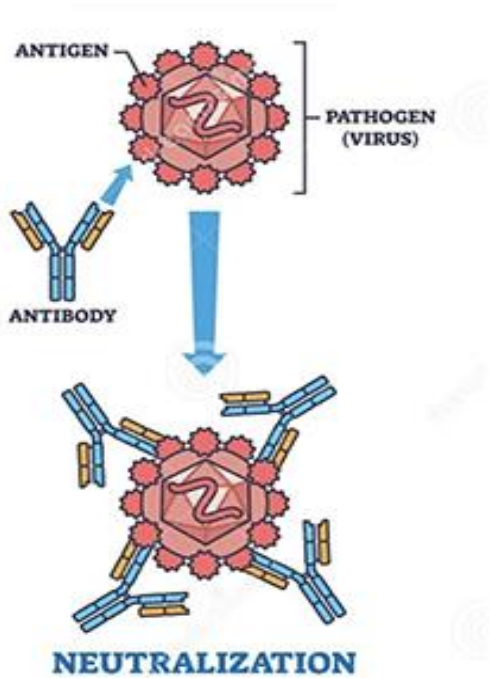
Immunology

Immunologic Considerations

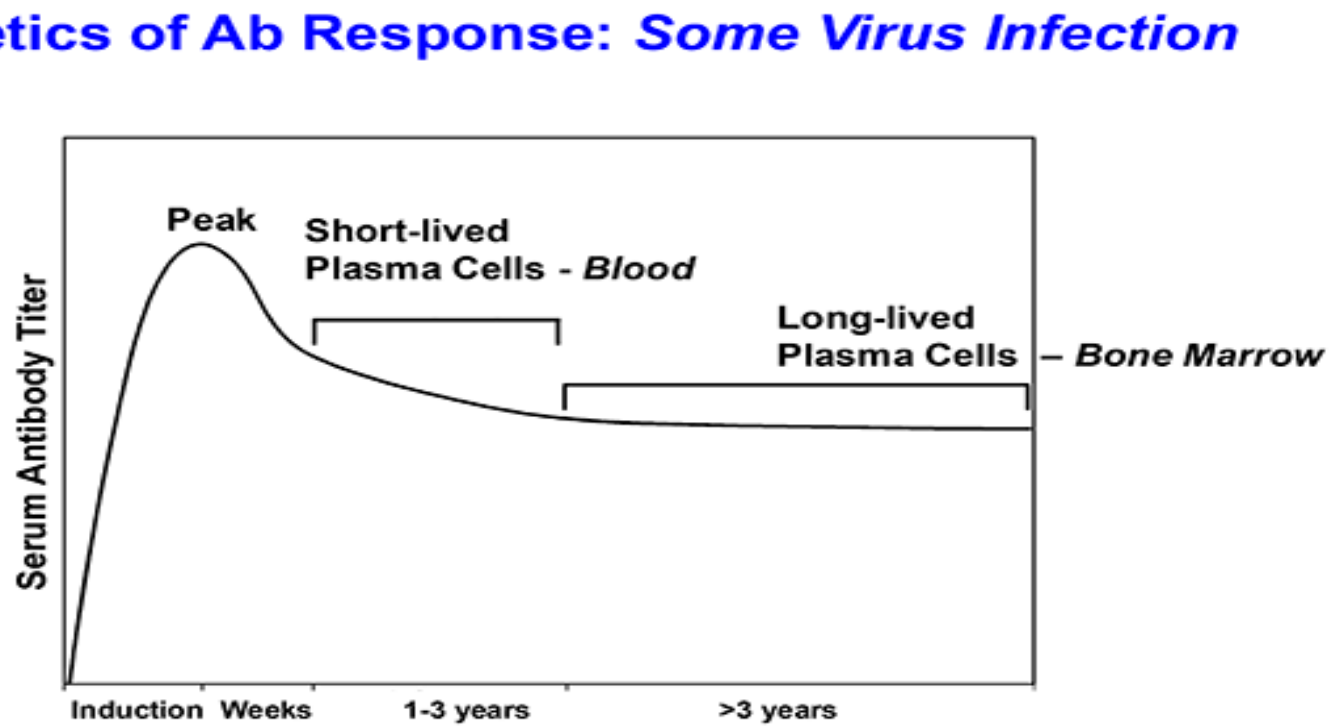
Vaccine effectiveness

Antibodies and Vaccine Effectiveness

- Antibodies that bind the virion and prevent infection are the principal mechanism of protection for viral prophylactic vaccines, including HPV.
- Over 98% of subjects generate an antibody response to the HPV VLPs, even after a single dose of the vaccine. No other protein-based vaccine has this consistent of a response.
- But how long do the antibody responses last?

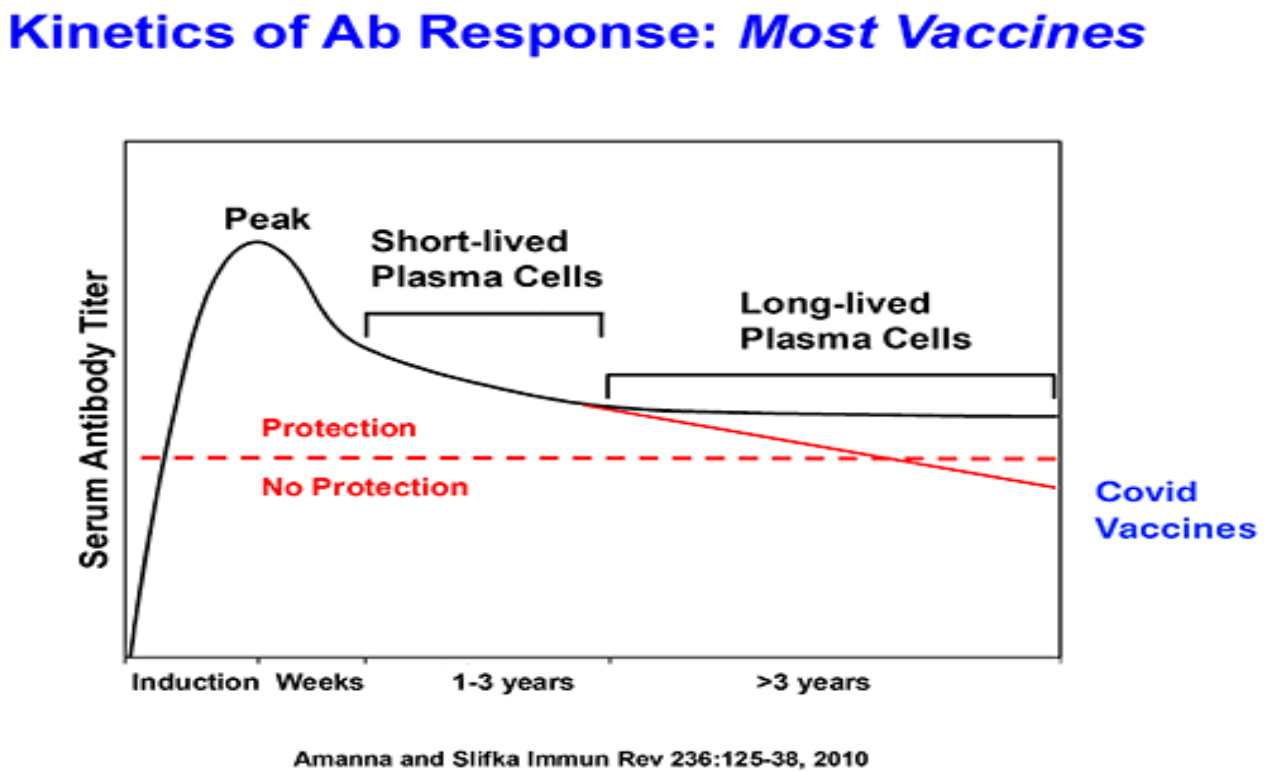


Ab response



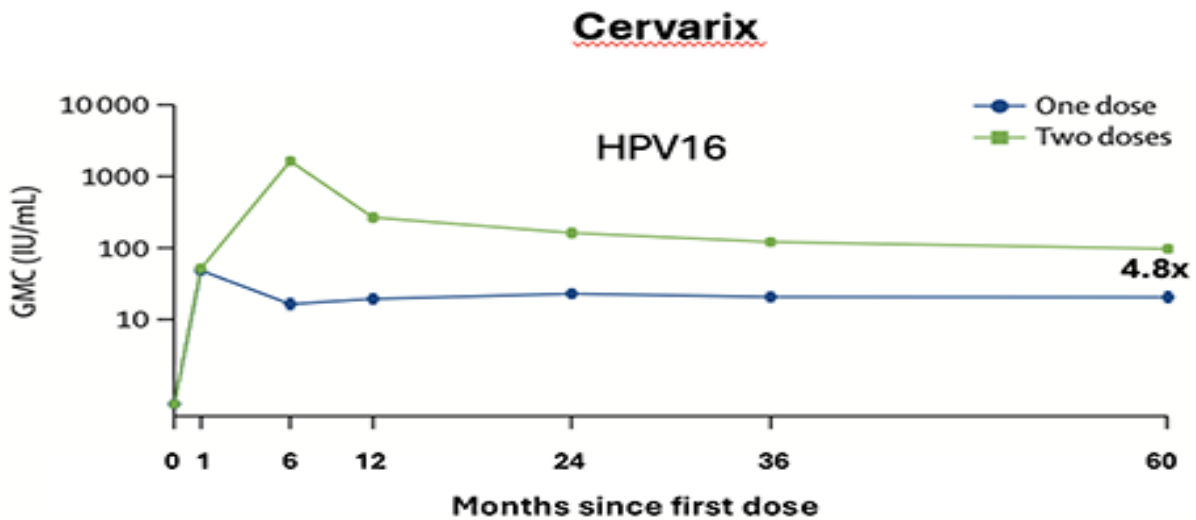
Amanna and Slifka Immun Rev 236:125-38, 2010

Ab kinetics


Kinetics of Ab Response: *Most Vaccines*
Peak
Short-lived Plasma Cells
Long-lived Plasma Cells
Serum Antibody Titer
Protection
No Protection
Covid Vaccines
Induction
Weeks
1-3 years
>3 years
Amanna and Slifka Immun Rev 236:125-38, 2010

Ab responses

The Kinetics of Antibody Responses to HPV VLP Vaccination: Virus Infection or Typical Vaccine?



Stabilized antibody levels have now been measured out to 16 years.

Watson-Jones, et al. *Glob Health* 13, e319-e328 (2025)

Ab Conclusion

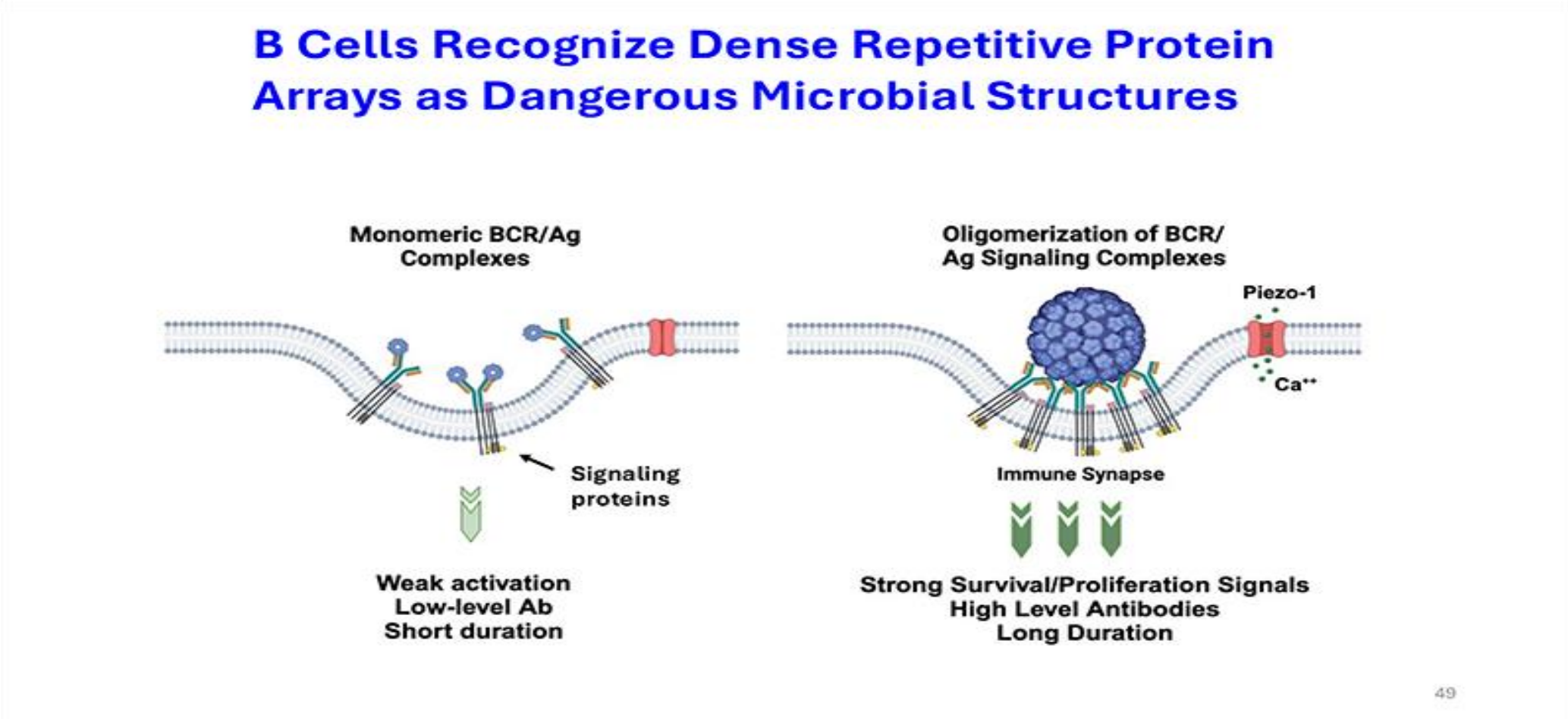
Immunogenicity Conclusion

A single priming dose of an HPV vaccine consistently induces a high quality and durable antibody response, presumable by the induction of long-lived plasma cells.

Durable Ab response

Why do HPV VLPs induce such potent and durable antibody responses?

B cells


B Cells Recognize Dense Repetitive Protein Arrays as Dangerous Microbial Structures
Monomeric BCR/Ag Complexes
Signaling proteins
Weak activation
Low-level Ab
Short duration
Oligomerization of BCR/ Ag Signaling Complexes
Piezo-1
Ca++
Immune Synapse
Strong Survival/Proliferation Signals
High Level Antibodies
Long Duration
49

Virology

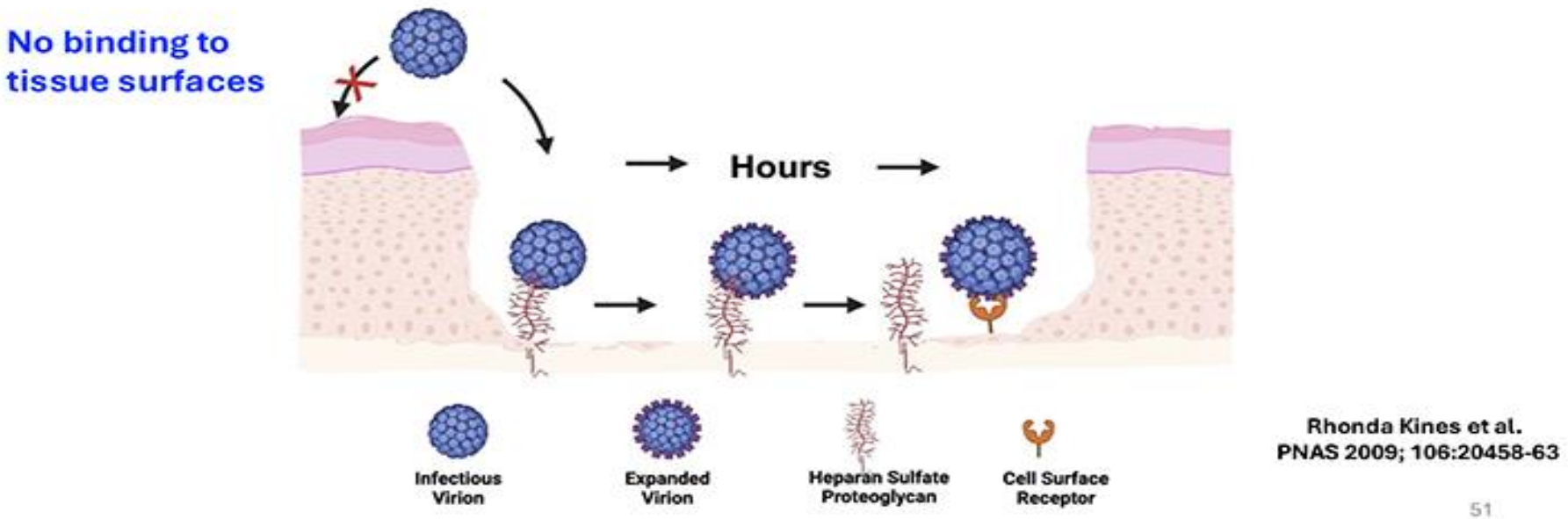
Virologic Considerations

Efficacy

Virologic Aspects Contributing to Efficacy

In vivo Murine Model of Cervicovaginal HPV Infection

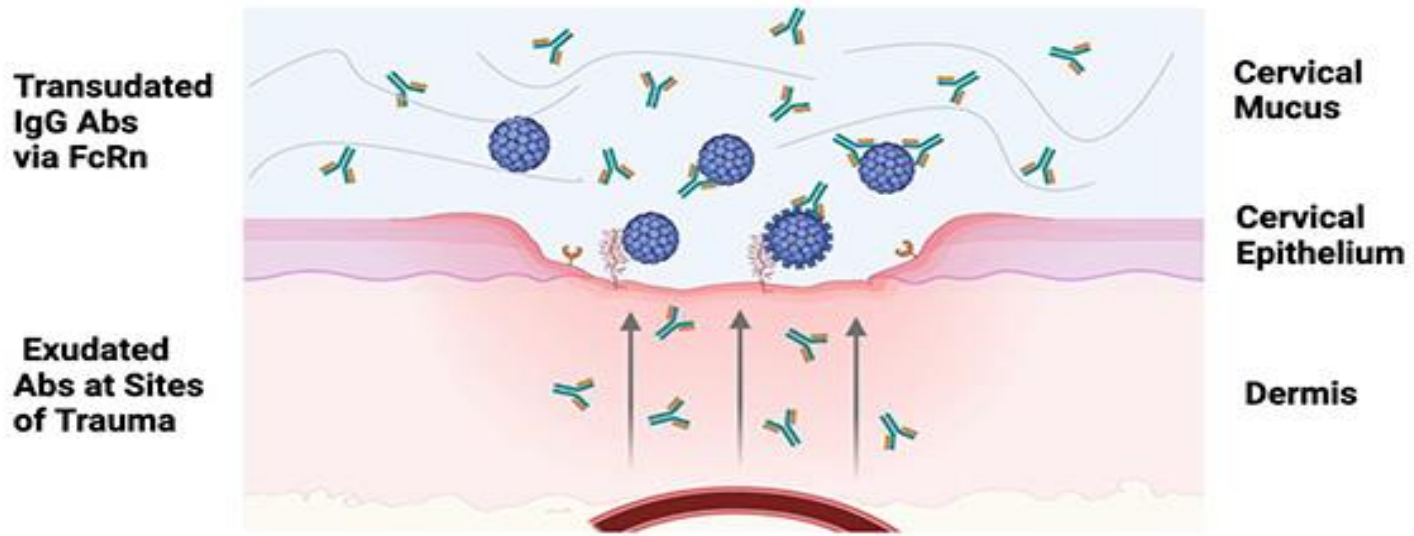
The remarkably slow process of infection makes HPVs exceptionally susceptible to inhibition by antibodies



Rhonda Kines et al.
PNAS 2009; 106:20458-63

Protective Ab response

How Can IM Injection of a VLP Vaccine Induce a Protective Ab Response at the Cervix?



VLP-specific IgG in women's cervical mucus after IM vaccination: but 10-100X less than in serum - ***Nardelli et al. JNCI, 2003***

Interstitial Ab levels are similar to those in blood.
Francian et. NPJ Vaccines 2025

Ab titers

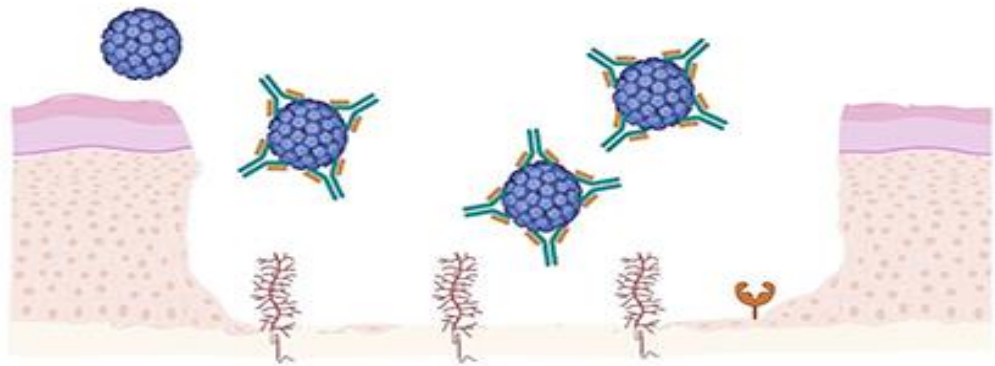
Antibody Titers and Protection

- The minimum level of Abs needed for protection has not been identified in clinical studies; too few breakthrough infections.
- Are the plateau titers after vaccination near the minimum needed for protection?
- Will the 4-fold difference between Ab titers after three vs one dose influence long-term protection?

Infection inhibition

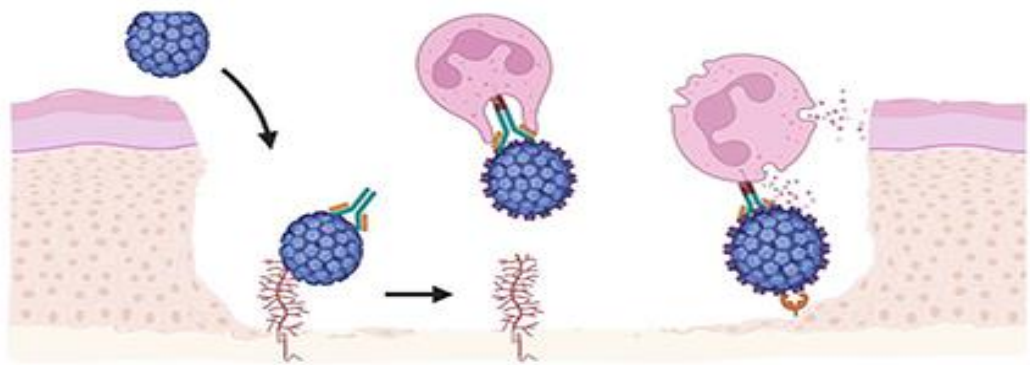
Mechanisms of In Vivo Infection Inhibition by VLP Abs

High Level Abs:
Block HSPG
BM binding



No conformation change.
No keratinocyte binding.

Low level Abs:
Virion still bind HSPGs.
Virion seen on Neutrophils but not keratinocytes.



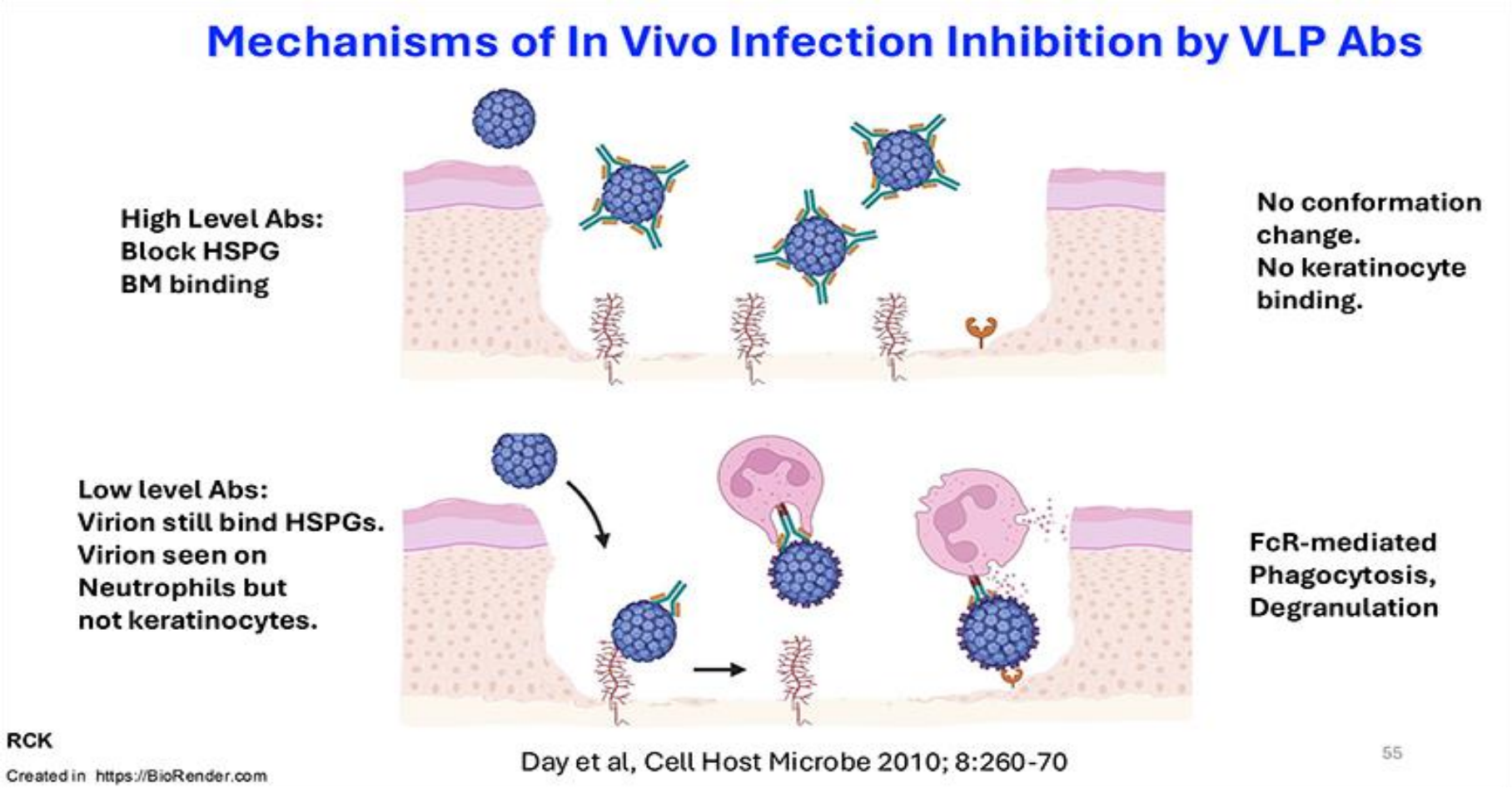
FcR-mediated Phagocytosis, Degranulation

RCK

Created in https://BioRender.com

Day et al, Cell Host Microbe 2010; 8:260-70

Ab infection



Considerations

Virologic Considerations

Learnings

Lessons Learned

- **You don’t have to fully understand how your technology works before evaluating it in trials.**
- **Insight into the mechanisms of action can provide critical information for acceptance of your intervention.**

VLPs bind to tumor cells

One Last Surprise:
HPV VLPs Bind Directly to Most Tumor Cell Surfaces!



- No binding to apical surfaces of normal tissues
- Initial binding only to basement membrane HSPGs
- Bind to most cancer cell lines
- Have BM-like HSPG modifications

Kines RC, Proc Natl Acad Sci U S A. 2009

VLPs to treat cancer

HPV VLPs as Guided Missiles to Treat Cancer:
A Long-Standing Collaboration with Aura BioSciences



- **We do preclinical studies under a CRADA**
- **Aura responsible for GMP product development and clinical trials**

Big Pharma

Lesson Learned

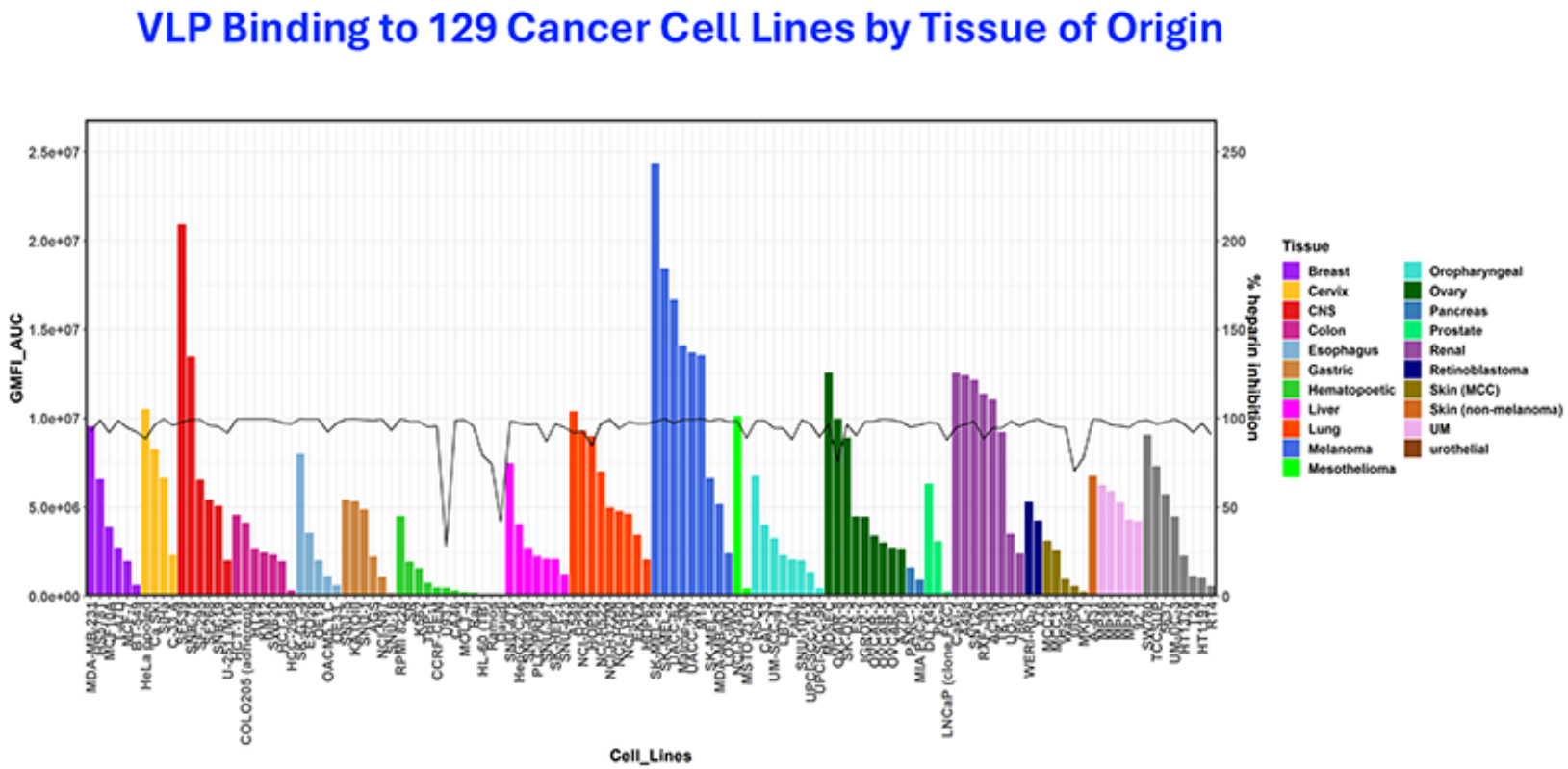
Working with Biotech startup is completely different from ”working” with big Pharma.

FINANCIAL DISCLOSURE

Financial Disclosure

I'm an inventor of U.S. Government-owned patents and Aura joint patents that have been licensed to Aura.

VLP BINDING

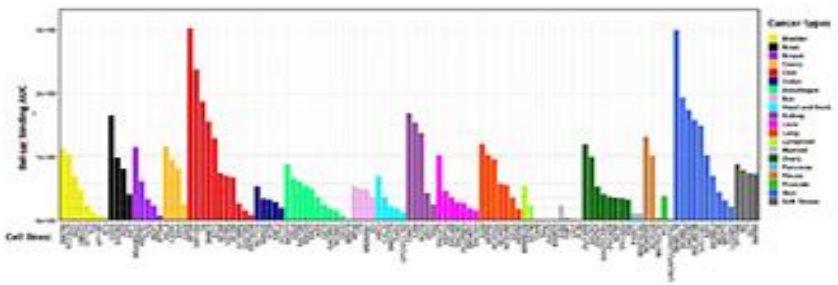


What is driving the consistent change of cell surface HSPGs during cancer development?

EMT transition

VLP Binding is Correlated with EMT* Transition

*Epithelial to Mesenchymal Transition

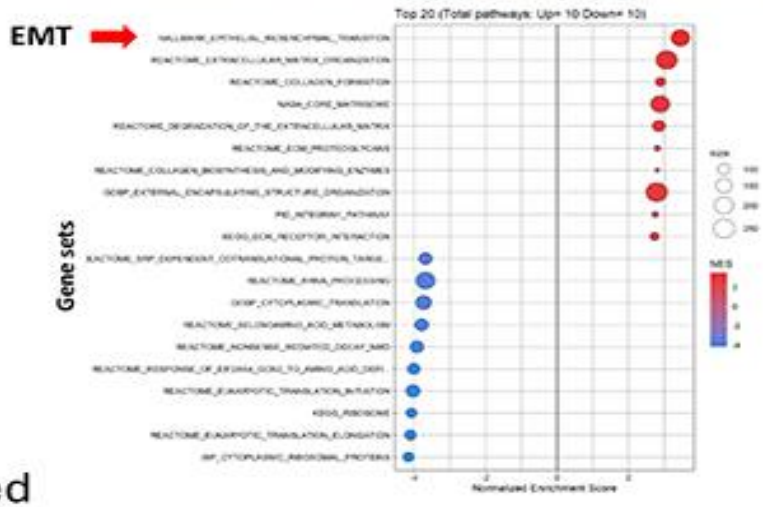


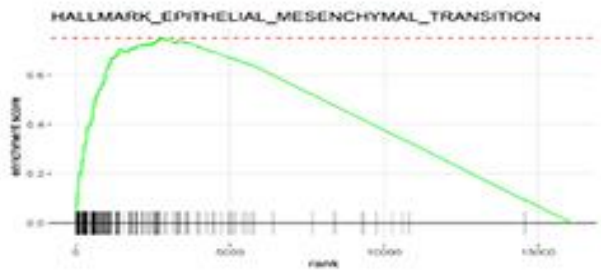
CCLE Cancer Cell Line Encyclopedia

Genetic, gene expression, proteomic, methylation data from over 1800 commonly used cancer cell lines

Unpublished

Gene set enrichment analysis (GSEA) of ranked-gene correlates





HSPG modifications are a fundamental driver for cancer cells to dedifferentiate.

VLPs bind cancer stem cells.

Cytotoxic drug conjugate

A Cytotoxic Drug Conjugate: VLP + IRDye® 700DX = AU-011

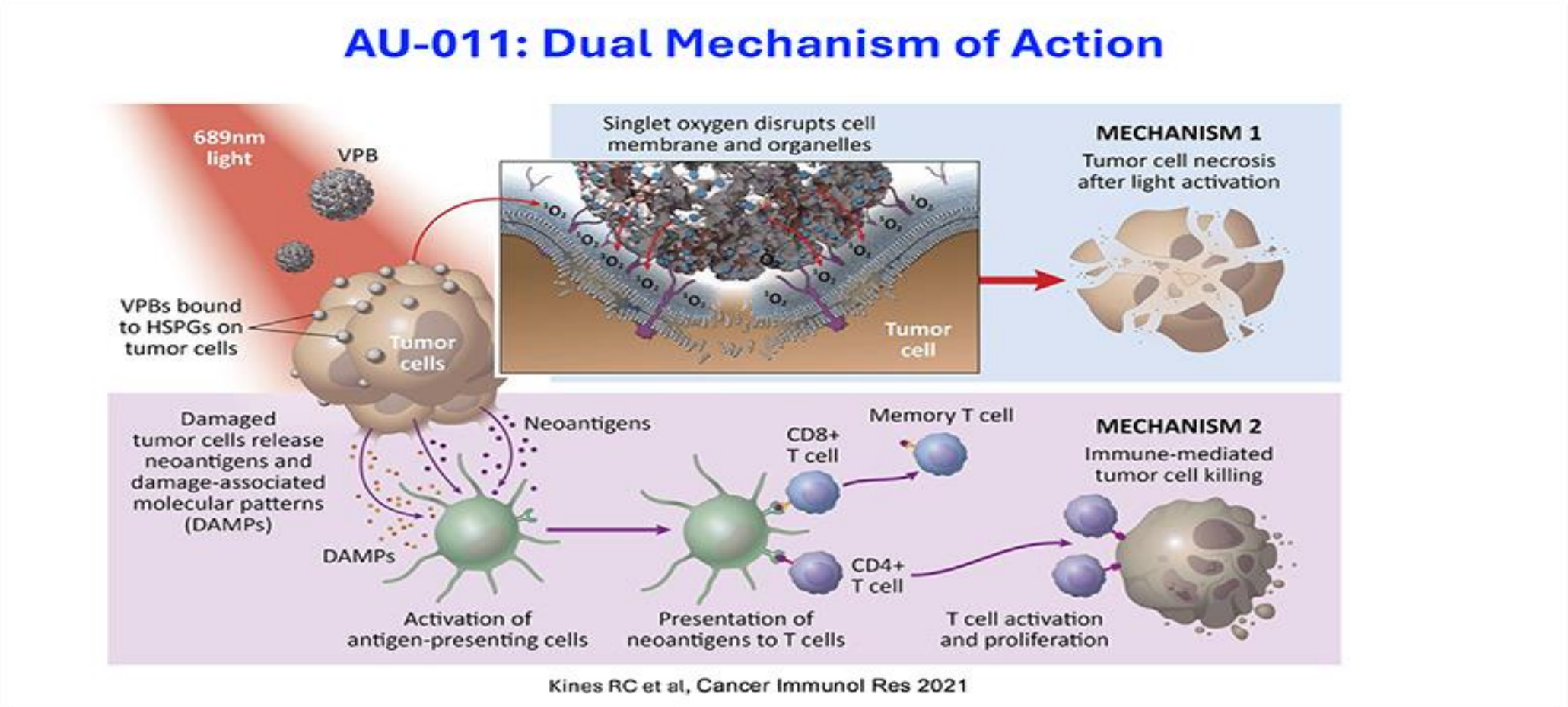
- HPV 16 L1/L2 VLPs
- Infrared activated IR700DX dye
- ~ 200 Dye molecules are conjugated to each VLP



Dual Specificity: Cytotoxic only if bound to cell surface and illuminated with infrared light

Kines RC, 2018, Mol Cancer Ther.

AU-011

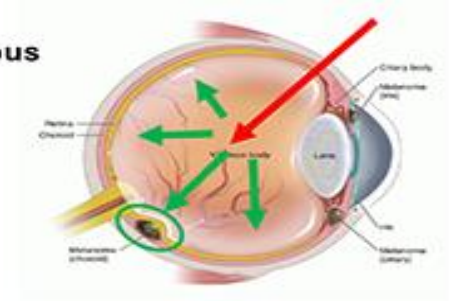

AU-011: Dual Mechanism of Action
689nm light
VPB
VPBs bound to HSPGs on tumor cells
Tumor cells
Singlet oxygen disrupts cell membrane and organelles
Tumor cell
MECHANISM 1
Tumor cell necrosis after light activation
Damaged tumor cells release neoantigens and damage-associated molecular patterns (DAMPs)
DAMPs
Neoantigens
Activation of antigen-presenting cells
CD8+ T cell
Memory T cell
CD4+ T cell
Presentation of neoantigens to T cells
MECHANISM 2
Immune-mediated tumor cell killing
T cell activation and proliferation
Kines RC et al, Cancer Immunol Res 2021

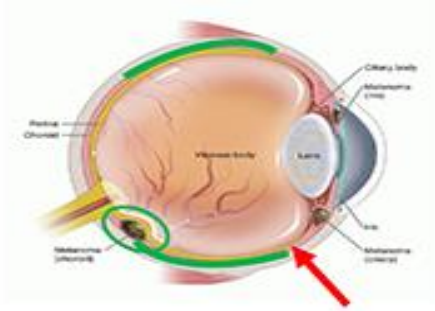
Clinical trials

AU-011 Clinical Trials of Small Choroidal Melanomas

- **Phase 2 trial of intra-vitreous injection complete. Some vitritus.**
- **Phase 2 trial of supra-choroidal injection complete. No vitritus.**
- **Phase 3 trial of supra-choroidal injection is fully enrolled, 100 patients.**
- **Primary Endpoints: vision preservation**
  delay in referral to standard of care cf no treatment

Intra-vitreous





Supra-choroidal

Phase 3 regimen

Phase 2 Data Using Phase 3 Regimen

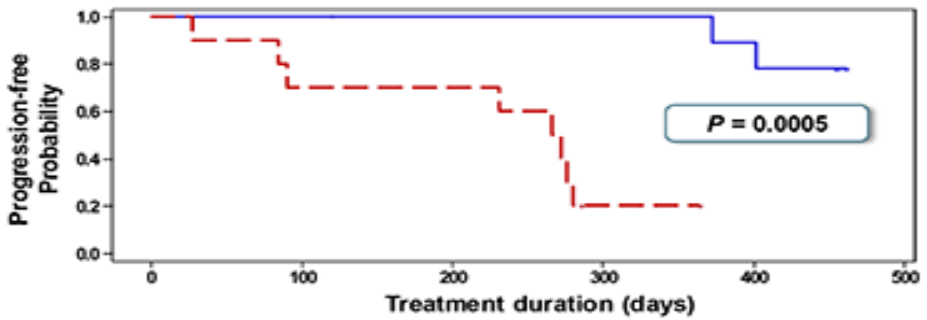
Kaplan-Meier analysis simulation of time-to-event "Therapeutic"* vs Subtherapeutic" treatment regimen

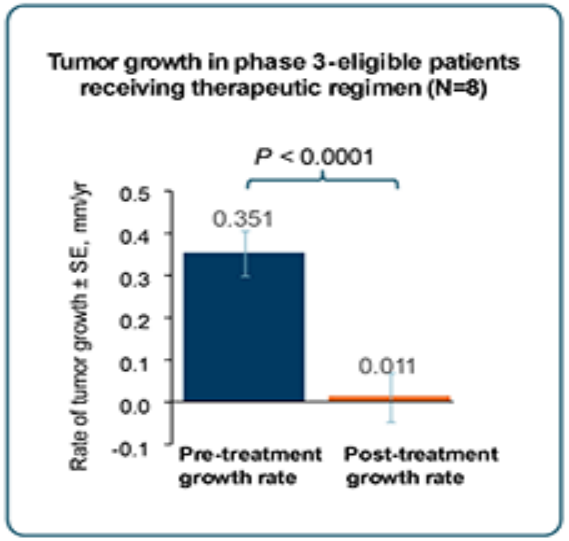
Time to tumor progression

Change from **baseline in thickness** ≥0.5 mm; or in LBD ≥1.5 mm confirmed by at least one repeat assessment

— Therapeutic n=10

--- Subtherapeutic n=10





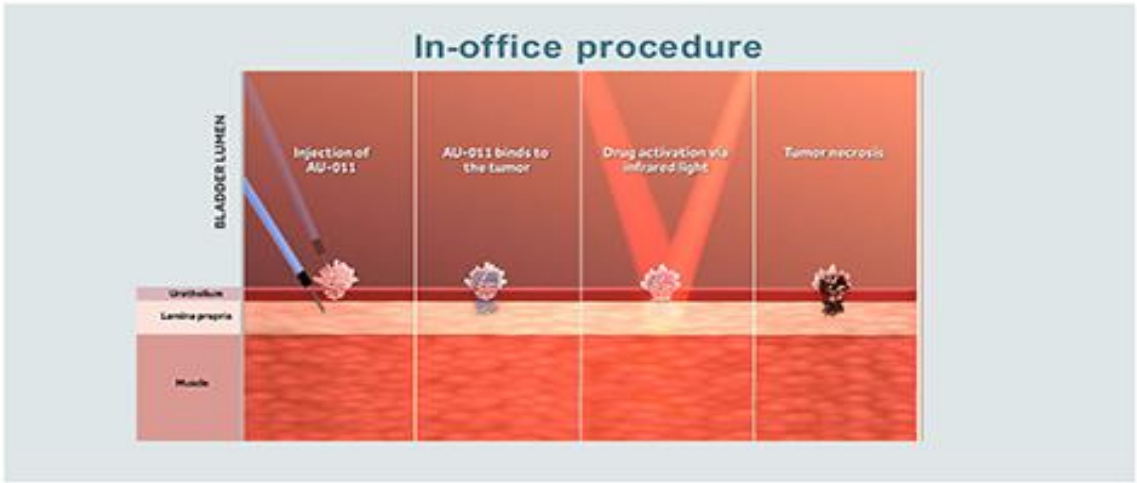
*3 cycles of 3 weekly injections

Also vision preservation

Invasive bladder cancer

Second Indication: Non-Muscle Invasive Bladder Cancer

Phase 1/2 Trial



Local administration and light activation with standard cystoscope

Linehan et al, 2026 DOI: 10.1016/j.euros.2026.05.017

Lesion outcomes

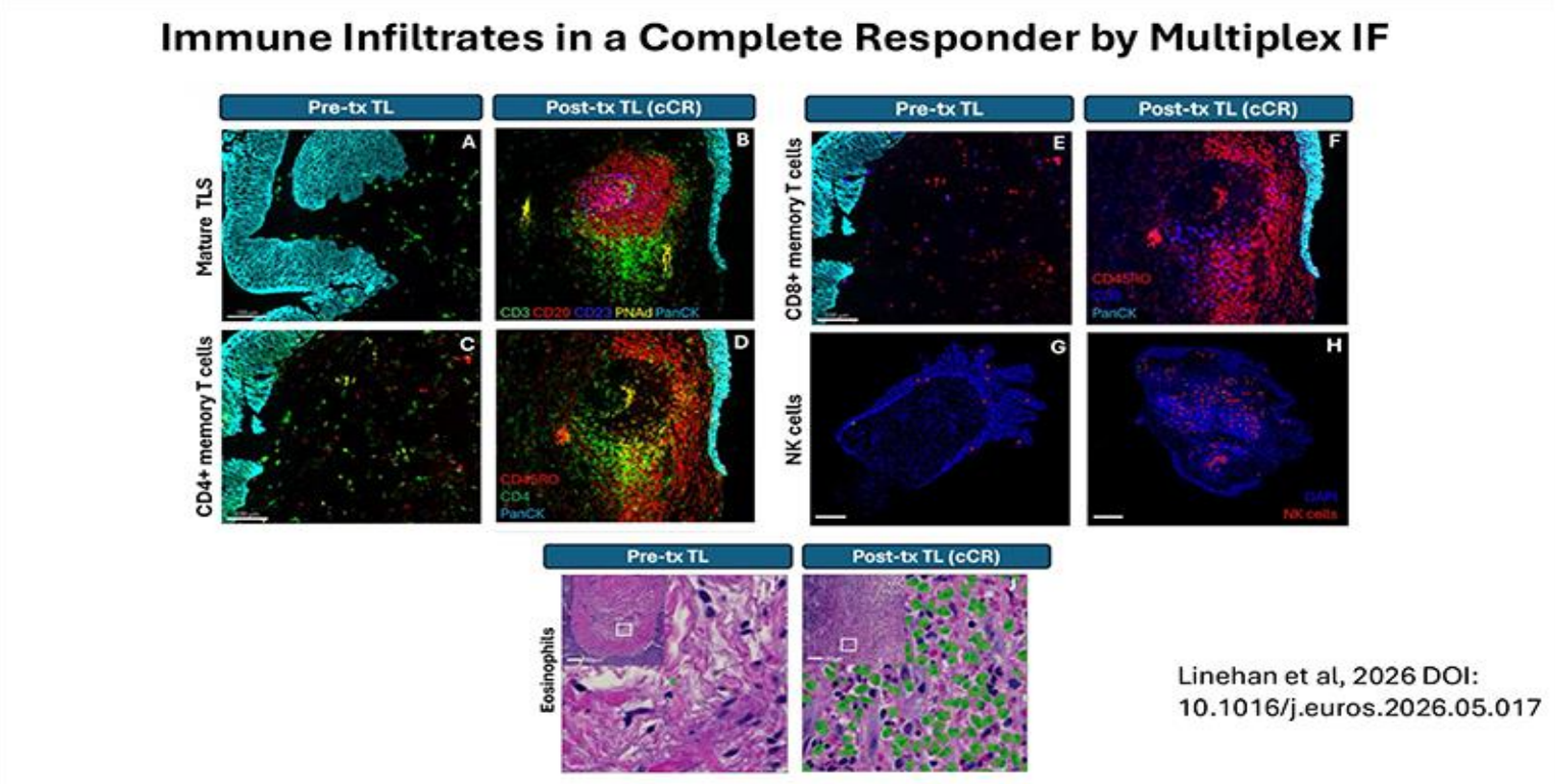
Summary of Lesion Outcomes by IBCG Risk Group

Single injection; light activate at 24 hrs; resection 7-12 days later.

IBCG Risk Group	Dose (µg)	Response-Tx Tumor	Response- UnTx Tumor
Intermediate Risk	100	Complete Response	CR (2/2 tumors)
	100	Complete Response	CR (1/2 tumors)
	100	Complete Response	CR (1/1 tumors)
	100	Compleat Response	None (0/1 tumors)
	200	Tumor Shrinkage	None (0/1 tumors)
High Risk	100	Tumor Shrinkage	Single Tumor Only
	100	Tumor Shrinkage	None (0/1 tumors)
	100	None	Single Tumor Only
	100	Tumor Shrinkage	Single Tumor Only
	200	Complete Response	CR (1/3 tumors)

Why does it work so well, so fast?

Immune filtrates



LESSONS LEARNED

Lessons Learned

Investigating the basic biology of your technology can lead to unexpected prospects for expanding its utility.

Collaborators

Thanks to Some of My Many Collaborators

Present and Past Members of the Lab:

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Universitaire Vaudois, Lausanne: Denise Nardelli

Aura BioSciences: Eli de los Pinos, Jill Hopkins, Sabine Brookman-May

Ips

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