

# Biomarkers in Vaccine Research and Development

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# Overview of Talk

- Why a talk about biomarkers in vaccine R&D?
- Biomarkers in vaccine development (by example)
  - Rotavirus: efficacy trial endpoint specificity
  - Human Papilloma Virus: specificity, surrogacy and time
  - HIV: Detecting weak efficacy signals
- Biomarkers in vaccine discovery research
  - Reverse vaccinology
- Discussion

# Introduction

- Question: What is “special” about biomarkers in prophylactic vaccine trials for infectious diseases?

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Answer 1: The Bugs

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- Clear early focal point in causal pathway to disease



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Answer 2: The Vaccines

- The bug as a prototype for vaccine construct
- Response to natural infection a blueprint for mechanism of protective vaccine
- Catalog of highly efficacious vaccines against a variety of bugs



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Answer 2: The Vaccines → Immune response biomarkers\*

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\* Ph I/II trial endpoints, immune correlates of risk, surrogate endpoints in vaccine efficacy trials, basis for



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I will focus on bug genomic biomarkers in situations where

- Access to relevant biological specimens and assay technology provides high sensitivity to detect bug presence and
- Genomic sequencing provides exquisite specificity to bug type



# Bug biomarkers add specificity to clinical endpoints in vaccine efficacy trials

- Each trial endpoint is annotated or “marked” with presence/absence indicator and, if present, the genomic sequence of bug

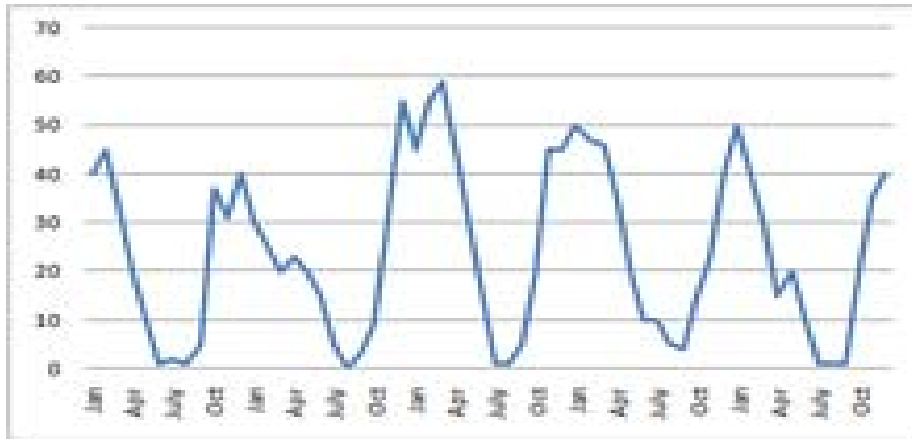
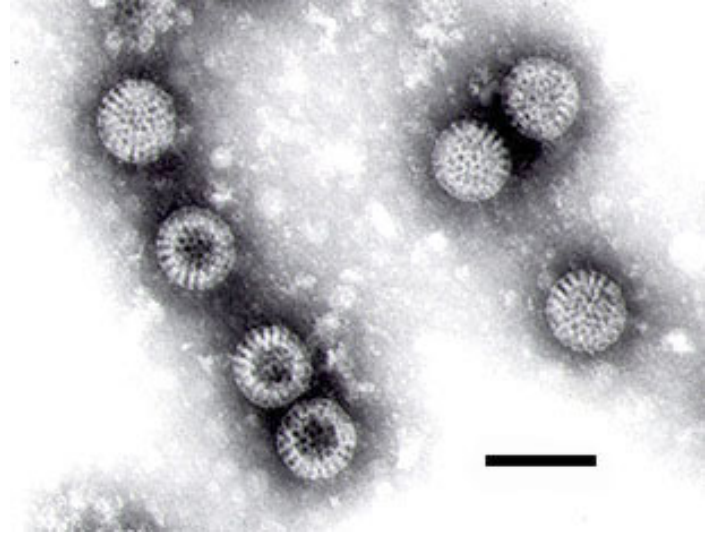


# Rotavirus

Acute GastroEnteritis (AGE) is the second leading cause of mortality in children younger than 5 accounting for 1.9M deaths annually (19% of all child deaths)... 98% of these deaths occur in the developing world.

Multiple causes of AGE including viral, bacterial and parasitic infections and exposure to toxins.

Rotavirus-associated AGE (RAGE) accounts for about 40% of childhood hospital admissions and 30% of deaths due to AGE



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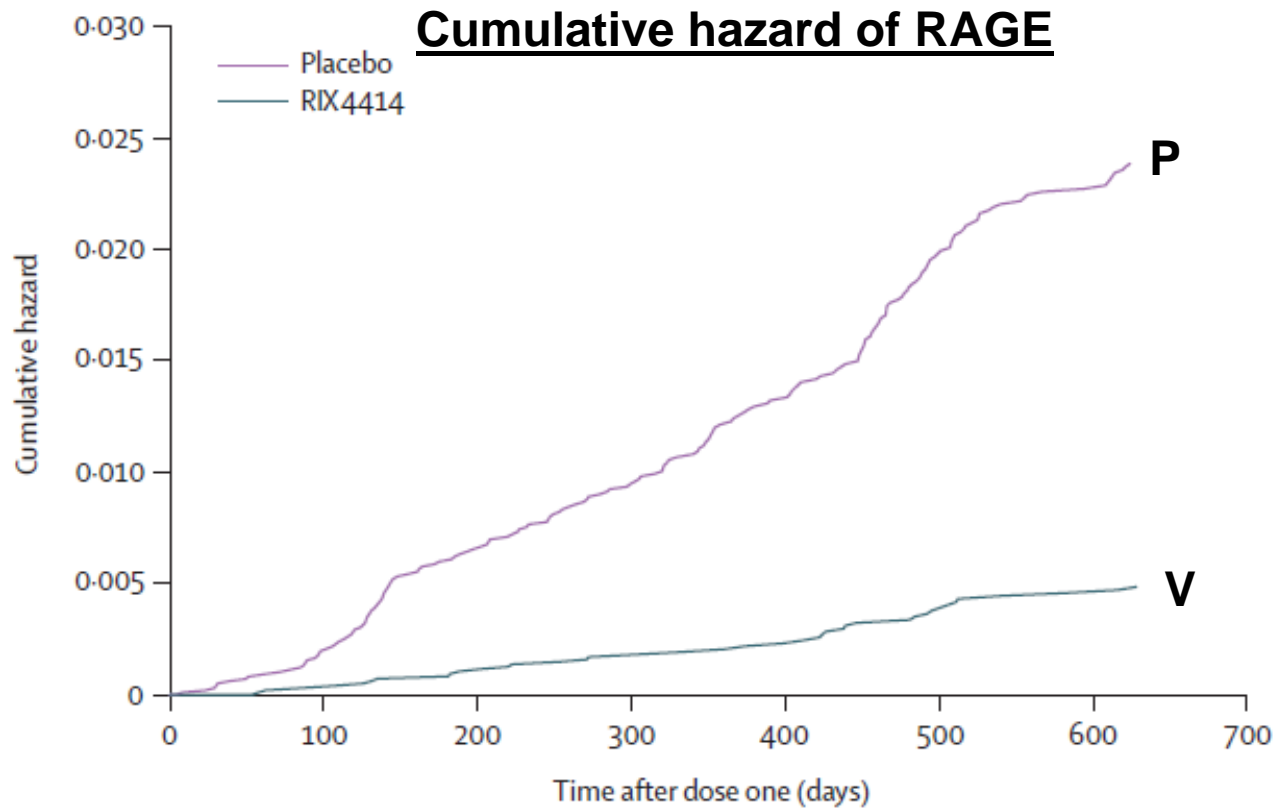
Two licensed rotavirus vaccines exist, Rotateq (Merck) and Rotarix (GSK), that have >90% efficacy in preventing severe RAGE in the developed world but have only 30-40% efficacy in the developing world.... WHY?

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# Rotavirus

- Vaccine efficacy trial primary endpoint composed of:
  - Clinical diagnosis of severe AGE (specifics depend on setting) plus
  - Biomarkers for presence (and type) of rotavirus in stool
    - binding rotavirus-specific antibody (serotype) and
    - RT-PCR assay for rotavirus specific genetic sequence (genotype)
- Analyses of efficacy trial data
  - Primary: % reduction in rates of RAGE
  - Secondary:
    - % reduction in rates of AGE
    - % reduction in rates of type-specific RAGE



**Number at risk**

RX4414	10 159	9 620	9 227	8 903	7 617	7 537	7 450	1366
Placebo	10 010	9 428	9 024	8 719	7 469	7 371	7 272	1370

**Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study**

***Lancet* 2008; 371: 1181-89**

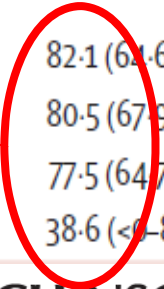
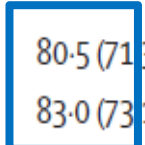
Alexandre C Linhares, F Raúl Velázquez, Irene Pérez-Schnaier, Xavier Saez-Llorens, Hector Abate, Felix Espinoza, Pio Lopez, Mercedes Macías-Parrá, Eduardo Ortega-Barria, Doris Maribel Rivera-Medina, Luis Rivera, Noris Pavía-Ruz, Ernesto Nuñez, Silvia Damaso, Guillermo M Ruiz-Palacios, Béatrice De Vos, Miguel O’Ryan, Paul Gillard, Alain Bouckenoghe, and the Human Rotavirus Vaccine Study Group\*

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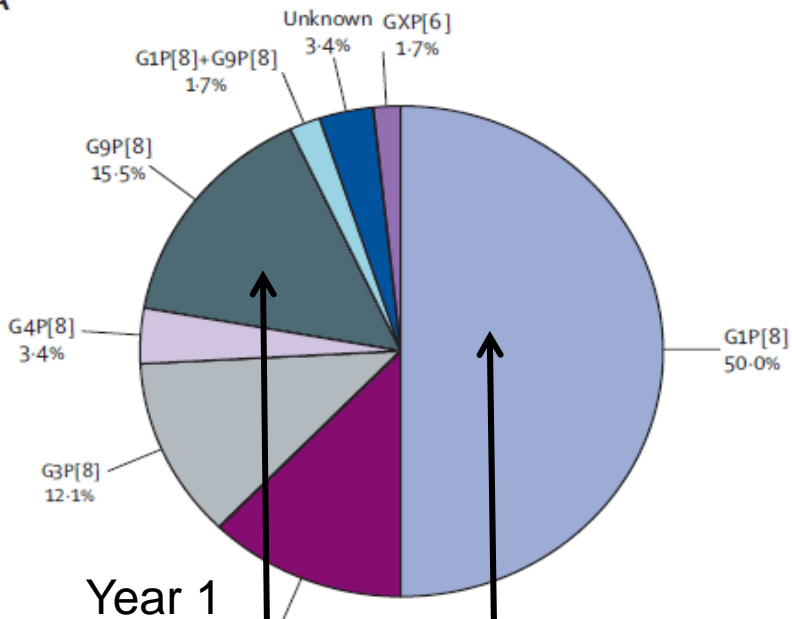
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# Type-specific Vaccine Efficacy

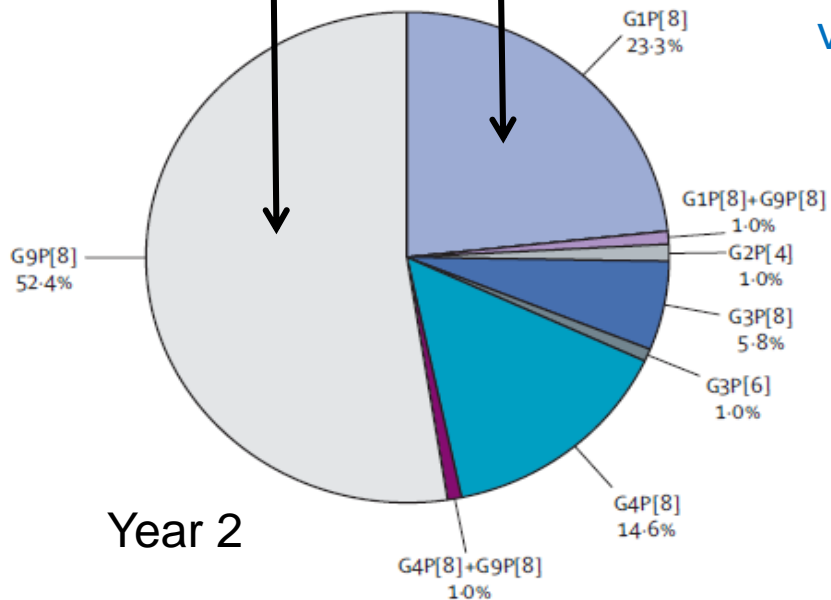
	RIX4414 (N=7205)		Placebo (N=7081)		Relative risk† (95% CI)	Absolute risk	Vaccine efficacy (95% CI)
	Infants with ≥1 episode*	1000 infants-year ratio	Infants with ≥1 episode*	1000 infants-year ratio			
<b>Severe gastroenteritis according to the clinical case definition‡</b>							
<b>All-cause gastroenteritis</b>							
Severe	342	28.5	551	46.7	0.610 (0.531-0.699)	0.078	39.0 (30.1-46.9)
Admission	265	22.1	429	36.4	0.607 (0.519-0.709)	0.061	39.3 (29.1-48.1)
<b>Rotavirus gastroenteritis§</b>							
Severe	32	2.7	161¶	13.6	0.195 (0.129-0.287)	0.023	80.5 (71.3-87.1)
Admission	22	1.8	127	10.8	0.170 (0.103-0.269)	0.018	83.0 (73.1-89.7)
<b>Serotype specific rotavirus gastroenteritis  </b>							
G1P[8]**	10††	0.8	55‡‡	4.7	0.179 (0.081-0.354)	0.008	82.1 (64.6-91.9)
Pooled P[8], non-G1 (G3, G4, G9)	19§§	1.6	96¶¶	8.1	0.195 (0.112-0.321)	0.014	80.5 (67.9-88.8)
Pooled non-G1 (G2, G3, G4, G9)	24	2.0	105	8.9	0.225 (0.138-0.353)	0.015	77.5 (64.7-86.2)
Non-G1, non-P[8] (G2P[4])	5	0.4	8	0.7	0.614 (0.158-2.129)	0.001	38.6 (<0-84.2)



A



B



Vaccine efficacy varies by serotype

Distribution of circulating serotypes varies over time (possibly dependent on vaccination)

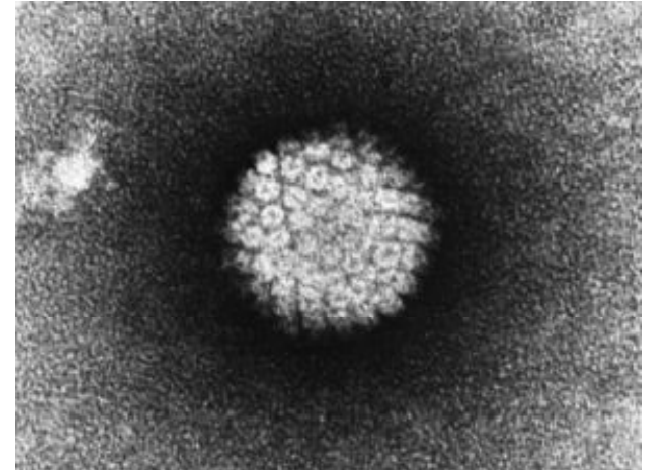
How to define efficacy endpoints and trial designs that deliver compelling evidence for vaccine efficacy that is robust to temporal variations in serotypes?



# HPV

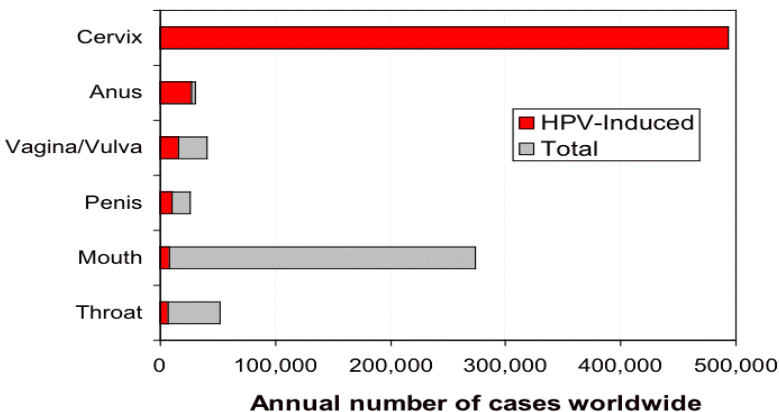
More than 30-40 types of human papilloma virus (HPV) are sexually transmitted and establish productive infections of stratified epithelial cells of skin or mucosa. Most (90%) HPV infections in young females are cleared within in 2 yrs.

When persistent infection with (14+) specific types of HPV occurs there is a high risk for development of precancerous lesions that can then progress to invasive cervical cancer over a 15-20 year time period.



There are 500,000 cases and 270,000 deaths due to cervical cancer per year world wide.

There are two licensed HPV vaccines that prevent persistent infection with HPV Types 16 and 18 to which 70% of cervical cancer cases are attributable.



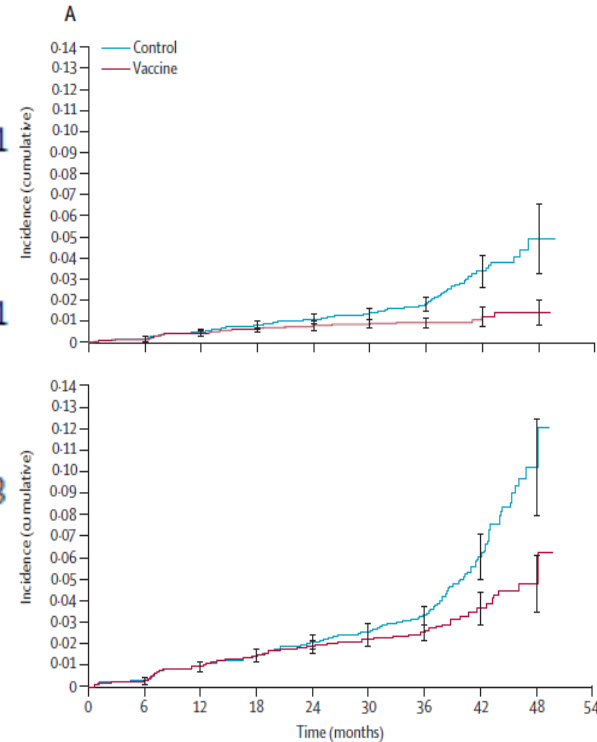
# HPV

- Vaccine efficacy trial primary endpoint composed of:
  - Histologic diagnosis of Cervical Intraepithelial Neoplasia Grade 2+ (CIN2+) *plus*
  - Biomarkers for presence of vaccine-type HPV DNA in cervical lesion
    - RT-PCR assay for HPV16 or HPV18 specific genetic sequence
- Analyses of efficacy trial data
  - Primary: % reduction in rates of HPV16/18-associated CIN2+
  - Secondary:
    - % reduction in rates of any CIN2+
    - % reduction in rates of non-vaccine type-specific CIN2+



N	HPV-16 or HPV-18 DNA In lesion			
	n	Event rate (96.1% CI)†	Vaccine efficacy (96.1% CI)	p value

ATP-E					
CIN2+					
HPV-16/18‡					
Vaccine	7344	4	0.02 (0.01 to 0.06)	92.9% (79.9 to 98.3)	<0.0001
Control	7312	56	0.32 (0.24 to 0.42)	..	..
HPV-16					
Vaccine	6303	2	0.01 (0.00 to 0.05)	95.7% (82.9 to 99.6)	<0.0001
Control	6165	46	0.31 (0.22 to 0.42)	..	..
HPV-18					
Vaccine	6794	2	0.01 (0.00 to 0.05)	86.7% (39.7 to 98.7)	0.0013
Control	6746	15	0.09 (0.05 to 0.16)	..	..



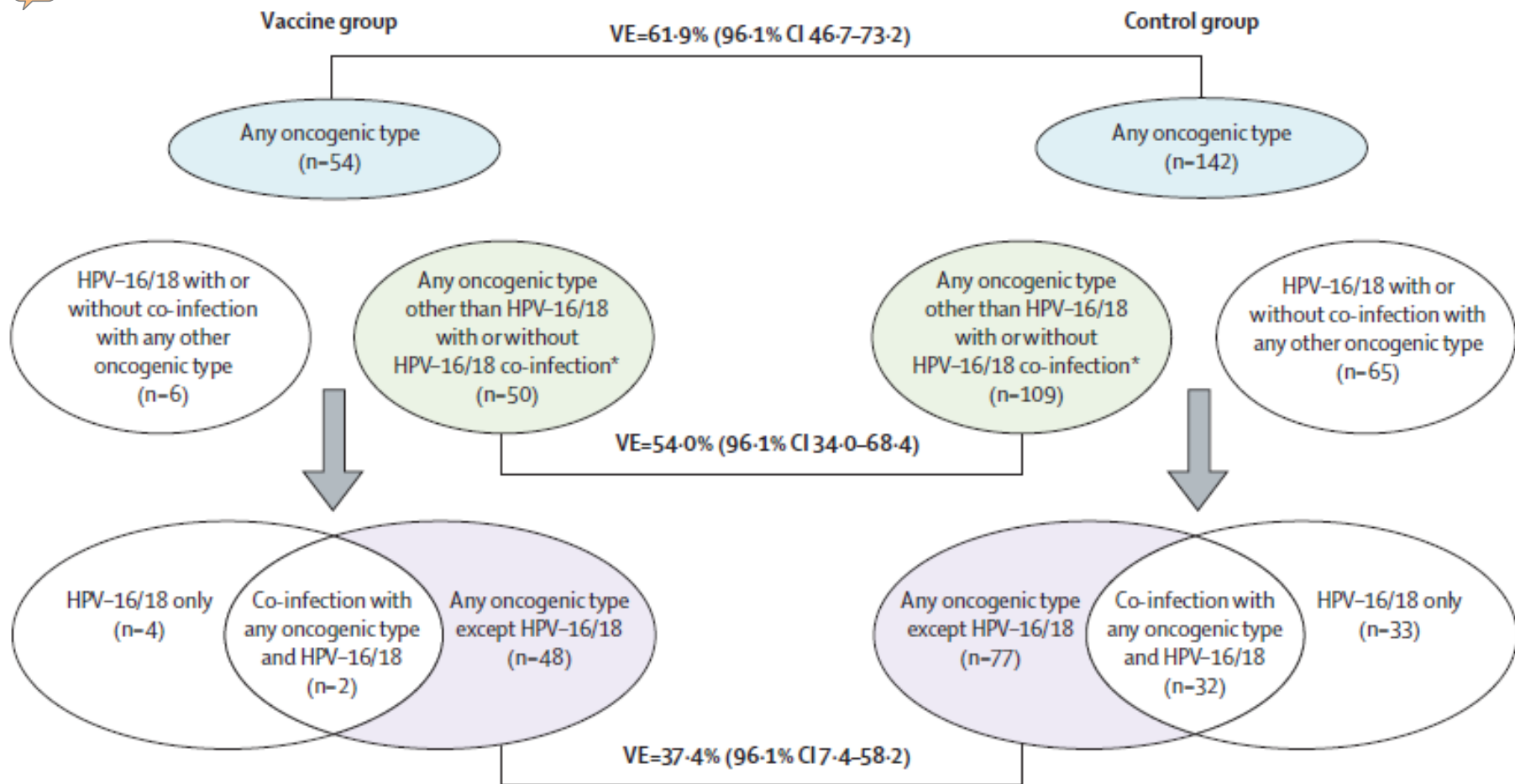
**Efficacy of human papillomavirus (HPV)-16/18 AS04-  
adjuvanted vaccine against cervical infection and precancer  
caused by oncogenic HPV types (PATRICIA): final analysis of a  
double-blind, randomised study in young women**

*J Paavonen, P Naud, J Salmerón, CM Wheeler, S-N Chow, D Apter, H Kitchener, X Castellsague, J C Teixeira, SR Skinner, J Hedrick, U Jaisamram, G Limson, S Garland, A Szarewski, B Romanowski, FY Aoki, TF Schwarz, W A J Poppe, FX Bosch, D Jenkins, K Hardt, T Zahaf, D Descamps, F Struyf, M Lehtinen, G Dubin, for the HPV PATRICIA Study Group*



**Lancet 2009; 374: 301-14**





Level of protection against CIN2+ depends on pattern of HPV subtype co-infection at baseline and on pattern of co-infection associated with CIN2+ lesion.

# Persistence of HPV infection

- In addition to complexities of patterns of co-infection, absolute risk of CIN2+ depends strongly on duration of persistent infection with oncogenic type HPV
- Is persistent detection of oncogenic type HPV DNA a better prognostic biomarker for cervical cancer than histopathologic diagnosis of CIN2? .... is it a “better” surrogate endpoint in vaccine trials?

## **Longitudinal Study of Human Papillomavirus Persistence and Cervical Intraepithelial Neoplasia Grade 2/3: Critical Role of Duration of Infection**

Ana Cecilia Rodríguez, Mark Schiffman, Rolando Herrero, Allan Hildesheim, Concepción Bratti, Mark E. Sherman, Diane Solomon, Diego Guillén, Mario Alfaro, Jorge Morales, Martha Hutchinson, Hormuzd Katki, Li Cheung, Sholom Wacholder, Robert D. Burk

Manuscript received March 10, 2009; revised December 30, 2009; accepted January 5, 2010.

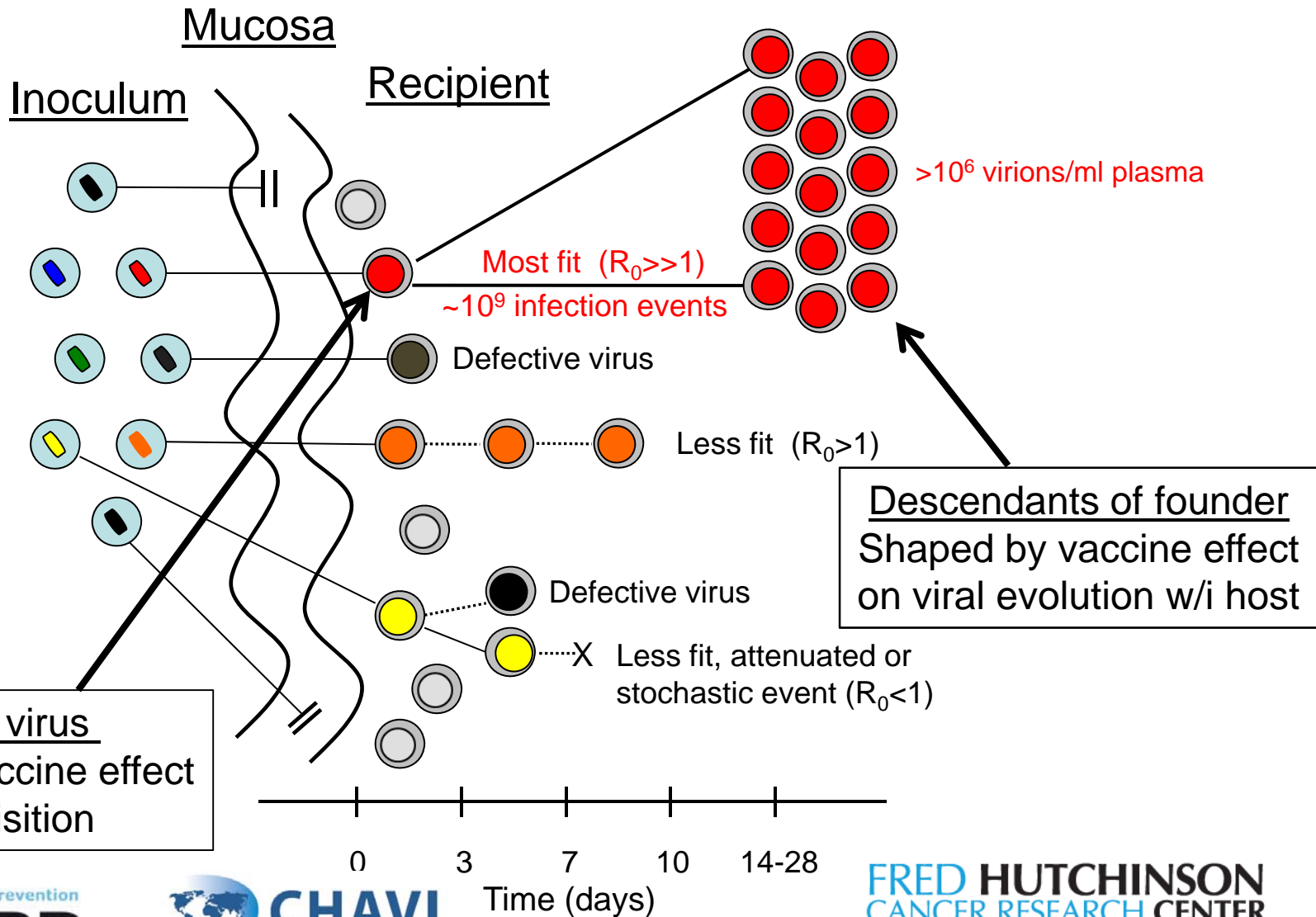
# Vaccine effect on HPV persistence

- Vaccine is highly efficacious at preventing persistent (12 mo) infection by 16/18 types
- Vaccine has variable efficacy at preventing persistent (12 mo) infection by non-16/18 oncogenic types

12-month persistent infection					
HPV-16/18	Vaccine	7035	20	91.4 (86.1, 95.0)	<0.0001
	Control	6984	227		
HPV-16	Vaccine	6052	17	90.4 (83.8, 94.7)	<0.0001
	Control	5903	171		
HPV-18	Vaccine	6508	3	95.5 (85.7, 99.2)	<0.0001
	Control	6440	66		
HPV-31	Vaccine	8141	48	60.6 (43.6, 72.9)	<0.0001
	Control	8169	122		
HPV-33	Vaccine	8255	39	37.0 (2.5, 59.8)	0.0276
	Control	8258	62		
HPV-45	Vaccine	8279	17	51.4 (8.3, 75.3)	0.0125
	Control	8269	35		
HPV-52	Vaccine	8057	187	5.0 (-17.9, 23.5)	0.6058
	Control	8047	197		
HPV-58	Vaccine	8226	61	-28.0 (-94.8, 15.5)	0.2128
	Control	8251	48		
HPV-31/33/45/52/58	Vaccine	8340	337	21.0 (7.9, 32.2)	0.0011
	Control	8336	425		
Any oncogenic type except HPV-16/18 <sup>1</sup>	Vaccine	8340	762	11.1 (1.3, 19.9)	0.0172
	Control	8336	853		
Any oncogenic type	Vaccine	8340	815	27.0 (19.6, 33.7)	<0.0001
	Control	8336	1094		

- Imagine that HPV serotypes were unknown and only HPV DNA sequence was available to annotate endpoints
  - We might have observed that the difference between V and P in rates of endpoints marked with a subset of viral sequences divergent from the vaccine antigen sequence (eg the non-HPV16/18 viruses) was less than that for the subset of viral sequences similar to the vaccine antigen sequence.... a “sieving effect”
  - We might have also observed the same differential effect on persistent type-specific infection
- Could we use those observations to
  - Detect more subtle effects of vaccine on virus?
  - Design better vaccine w/r/t specificity and type of protective immune response?

# HIV/SIV Transmission Model



# Sieve Analysis 1.0

- Comparing genetic “distance” of infecting strain from vaccine antigen between infected vaccine and placebo recipients

## Statistical Methods for Assessing Differential Vaccine Protection Against Human Immunodeficiency Virus Types

Peter B. Gilbert,<sup>1,\*</sup> Steven G. Self,<sup>2</sup> and Mark A. Ashby<sup>3</sup>

BIOMETRICS 54, 799–814  
September 1998

Sieve analysis: methods for assessing from vaccine trial data how vaccine efficacy varies with genotypic and phenotypic pathogen variation

Peter Gilbert<sup>a,\*</sup>, Steve Self<sup>b</sup>, Malla Rao<sup>c</sup>, Abdollah Naficy<sup>c</sup>, John Clemens<sup>c</sup>

Journal of Clinical Epidemiology 54 (2001) 68–85

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## Maximum likelihood estimation in semiparametric selection bias models with application to AIDS vaccine trials

BY PETER B. GILBERT

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vardi@stat.rutgers.edu

*Biometrika* (1999), **86**, 1, pp. 27–43

## LARGE SAMPLE THEORY OF MAXIMUM LIKELIHOOD ESTIMATES IN SEMIPARAMETRIC BIASED SAMPLING MODELS<sup>1</sup>

BY PETER B. GILBERT

*The Annals of Statistics*  
2000, Vol. 28, No. 1, 151–194

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# Sieve Analysis 2.0\*

- Integrating data and biological models about immune response to vaccine
  - “Distance” is conceptually immune cross-reactivity of vaccine-induced response to specificity in infecting strain
  - Use a suite of predictive models for immune response as basis for cross-reactivity scores
  - Focus on viral proteome “loci” at which vaccine responses are generated
  - Match proteome “loci” scanned to specific immune effectors:
    - Linear peptides embedded in proteome for CD8+ T-cell epitopes
    - Residues in physical proximity to one another on protein surface for Ab epitopes
- Distinguish sieving effects by epoch
  - Sieving primarily by Ab effects on acquisition
  - Sieving by both Ab and T-cell responses on post-infection viral evolution
- Use missing data methods for dealing with founder virus genomes that can be reconstructed only in subset of subjects

# Bug genomes and vaccine discovery research

## Genome-based vaccine development

A short cut for the future

The reverse vaccinology equation (2006)

Danilo Gomes Moriel, Maria Scarselli, Laura Serino, Marirosa Mora, Rino Rappuoli\* and Vega Masiagnani



## Reverse Vaccinology: Developing Vaccines in the Era of Genomics

Alessandro Sette<sup>1</sup> and Rino Rappuoli<sup>2,\*</sup>

<sup>1</sup>La Jolla Institute for Allergy and Immunology, San Diego, CA 92130, USA

<sup>2</sup>Novartis Vaccines, 53100 Siena, Italy

Immunity 33, October 29, 2010

An alternative to “Pasteur’s rules to isolate, inactivate and inject the microorganism that causes the disease”-. Rappuoli, 2004.

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- From analyses of a population sample of bug genomic sequences
  - All potential antigens can be identified for development even if not highly immunogenic and even if pathogen is fastidious
  - Highly conserved antigens can be identified
  - Antigens can be screened against the human genome to remove self-antigen homologs
  - Models of protein families can identify surface antigens and those associated with pathogenic strains
  - More recently, predictive models of T-cell epitopes (conditioned on HLA) allow for some *in silico* screening of antigens for human T-cell reactivity
- Increasing opportunities for computational biology and biostatistical modeling in vaccine discovery research

# Discussion

- Biomarker and surrogate endpoint problems are hard... and vaccine research is no exception
- However, the specifics of vaccine research and current measurement technologies provide some unique opportunities to make headway on these problems
- A combination of statistical and (computational) biology modeling is required to make progress but that necessitates a considerable investment in learning immunobiology
- Too few statisticians are working in this area relative to the opportunity and importance of the problems