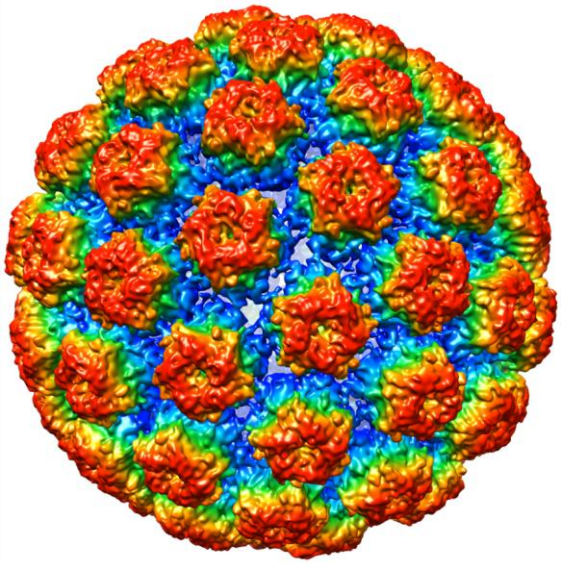
# Effect of Pap Smear Collection on Cervicovaginal HPV16 Infection in a Rhesus Macaque Model



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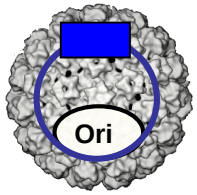
# A Mouse Model of Genital Tract HPV Infection

HPV Pseudovirus




***Pre-Treatment***

**Progesterone (Depo-Provera)**

**Day 0**

**Day 4**

**Day 7+**

***Disruption/Infection***

**Physical/Chemical disruption, followed by PsV 5-6 hours later**

***Long Term Time Course***

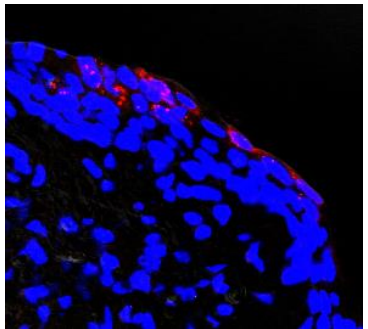
Analysis by **Luciferase** expression.

***Short Term Readout***

Sacrifice mouse; dissect out genital tissues. Analyze for **RFP** expression.

Multispectral imaging

Microscopy

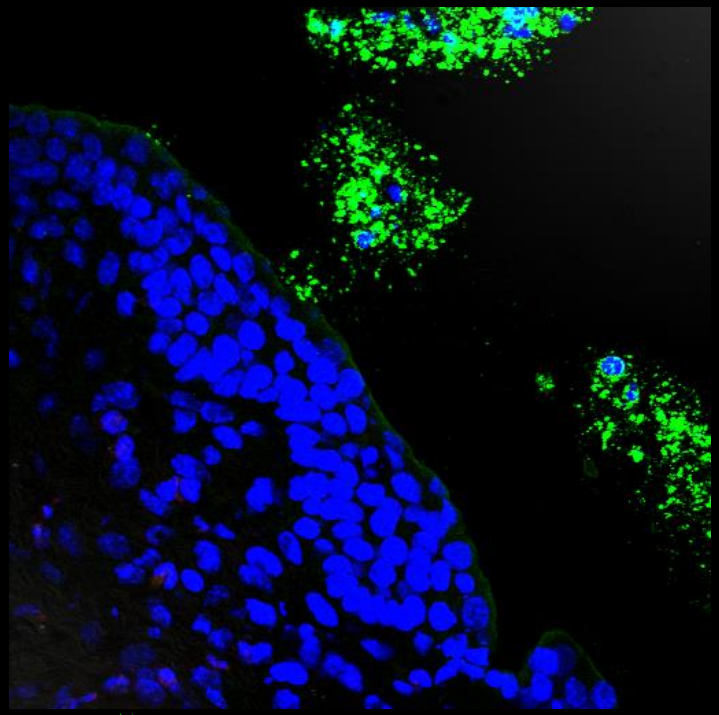


******Pseudovirions display a strict tropism for basal keratinocytes.***

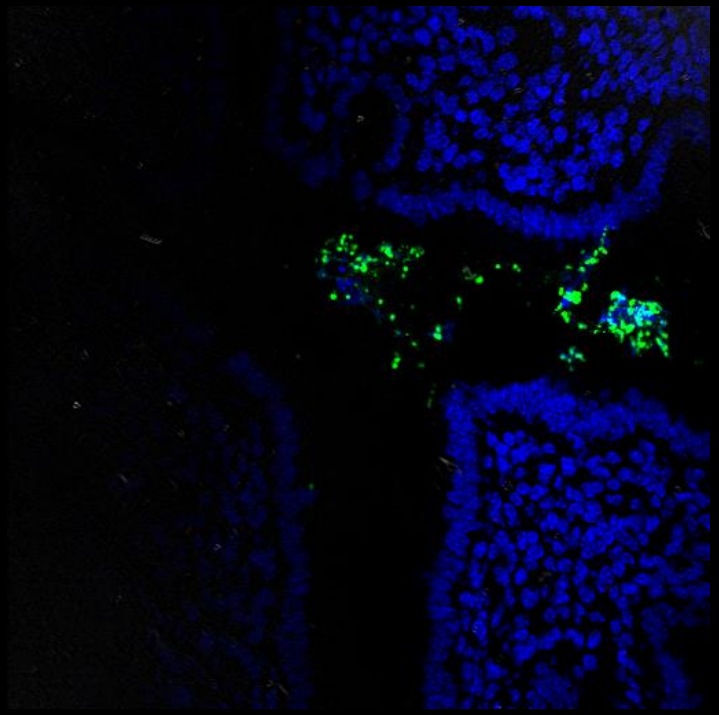
*Roberts, et al. Nat Med. 13(7); 2007.*

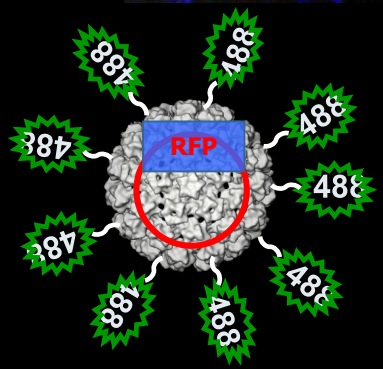
# HPV Capsids Don’t Bind Apical Surfaces of Intact Epithelium

**Vaginal Mucosa - stratified squamous**



**Endocervical Mucosa - simple columnar**



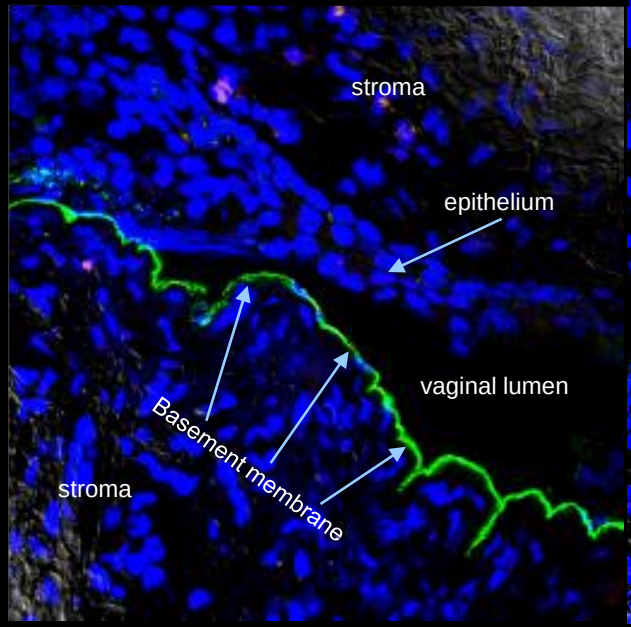



**green = (infectious) dye-coupled HPV capsids**

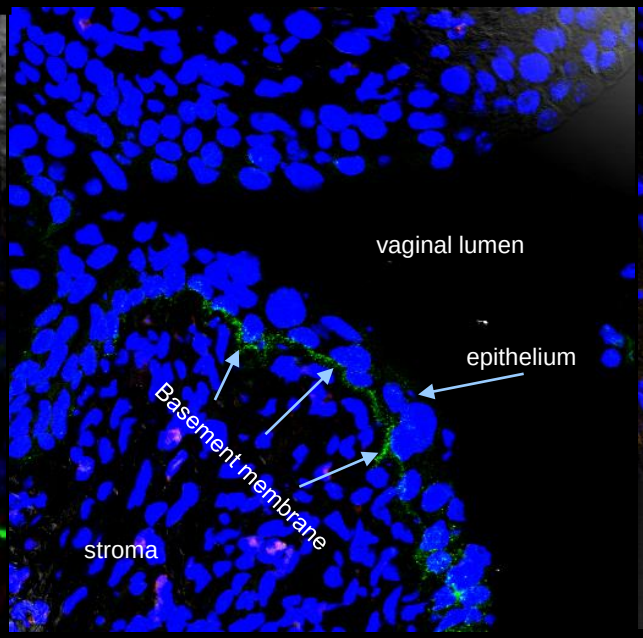
Roberts, et al. Nat Med. 2007 Jul;13(7):857-61

# HPV16 Capsids Bind to the Basement Membrane of Disrupted Stratified Squamous Epithelia in the Female Genital Tract

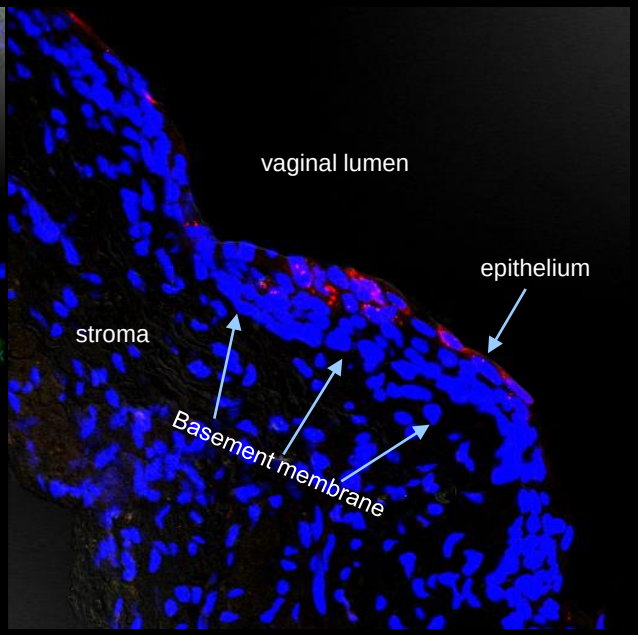
**2hrs post inoculation**

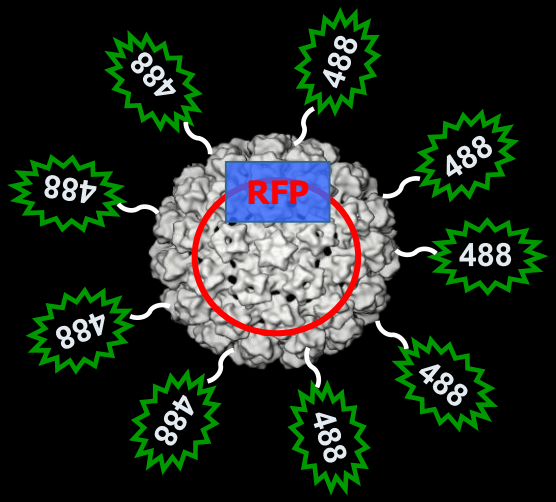



**24hrs post inoculation**




**72hrs post inoculation**



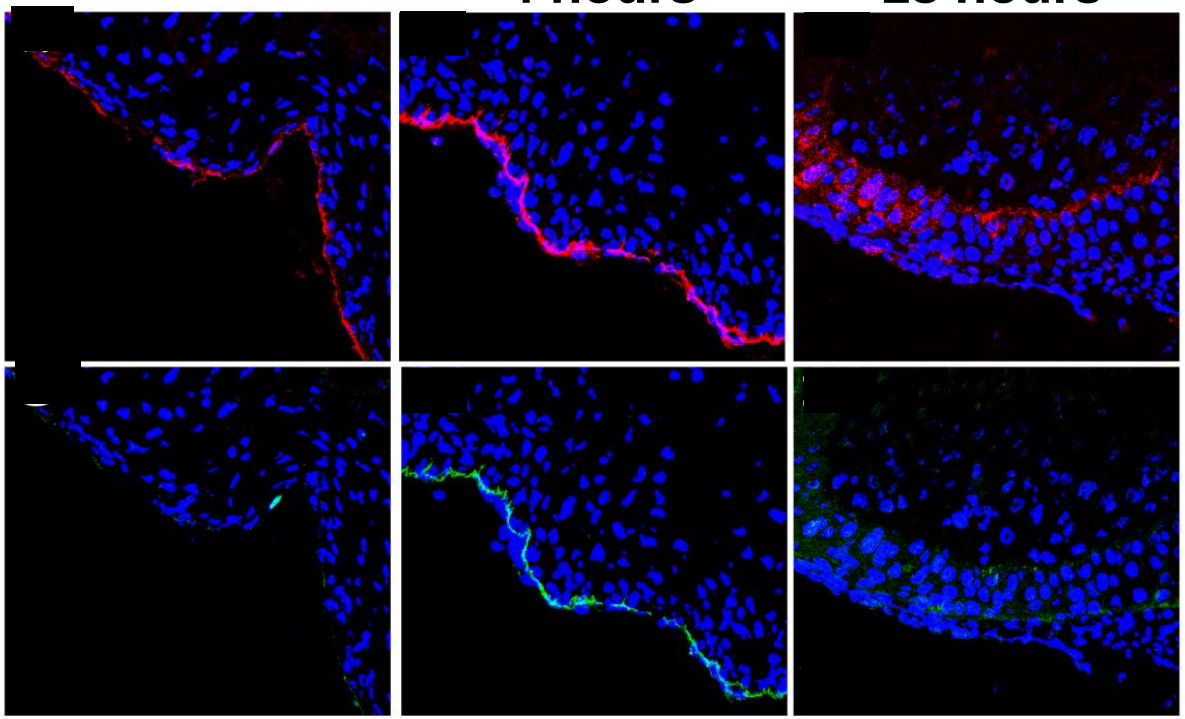

**= PsV capsids**

**= RFP expression, infection**

488 = alexa-fluor488 green fluorescent dye (PsV retains infectivity)

***Roberts et al. Nat Med 13: 857-61, 2007***

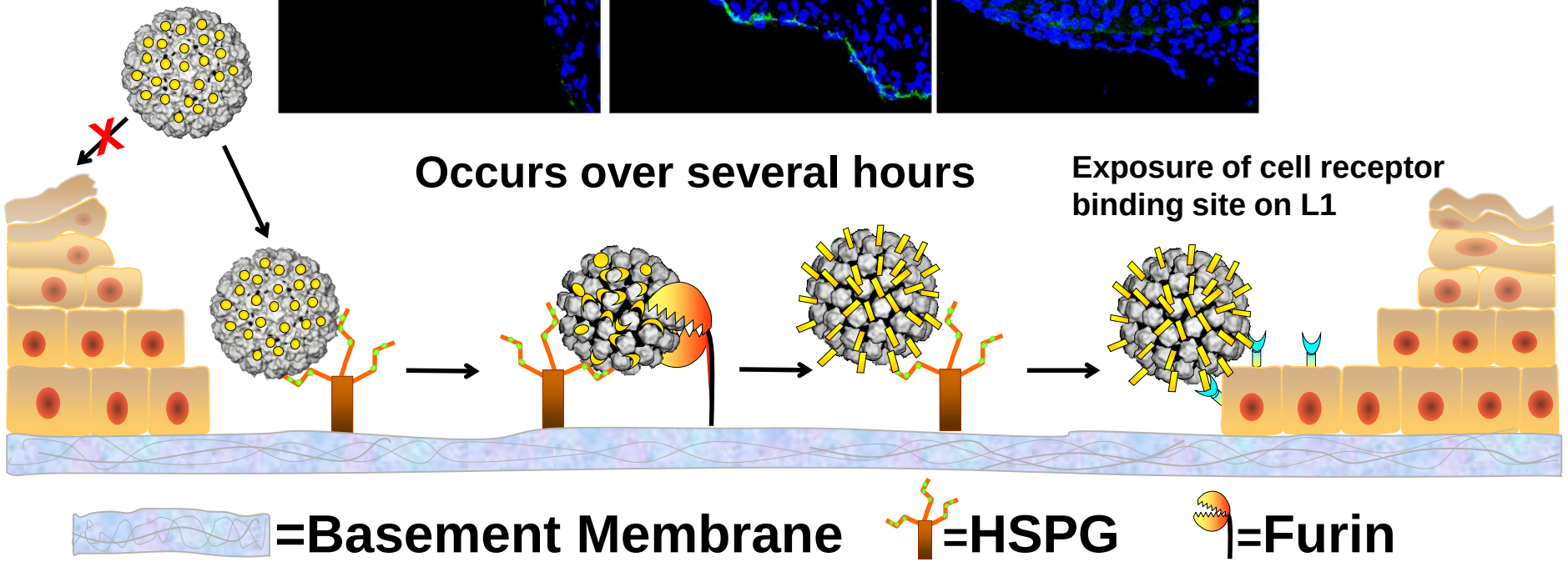
# *In vivo* Model of Early Events in HPV Infection




Rhonda Kines et al.

PNAS 2009;

106:20458-63



# Implications

**Interventions that disrupts or permeablizes the epithelium to the extend that the virus can access the basement membrane will potentiate HPV infection.**

**Pap smear collection disrupts the cervical epithelium by design.**

**Does it potentiate HPV infection in a NHP model?**

# Rhesus Monkey Pap Study Design

Jeff Roberts et al., J. Nat Cancer Inst. 2011; 103(9): 737-43

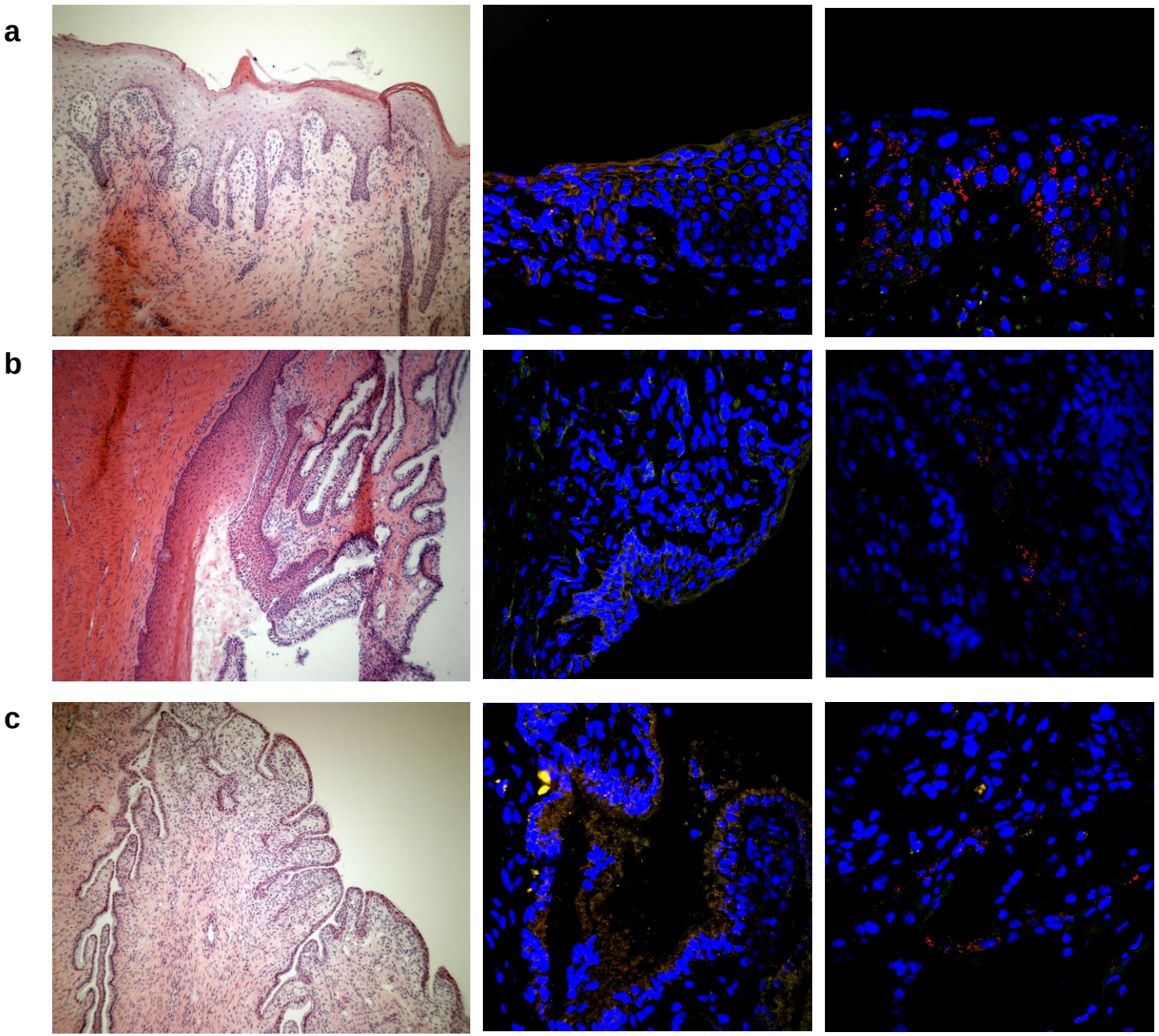
- Speculum Exam w/ or w/o standard cytology collection (cytobrush for endocervix; spatula for extocervix)
- Instillation of HPV16-RFP Pseudovirus ($3.8x10^8$ I.U.)
- Digital Exam with Surgilube or Carrageenan lubricant
- At 3 days, excise reproductive tract, take 6 biopsies, make 5 sections through the transformation zone
- Count number of infected cells by confocal microscopy (660 images per animal, two counters, blinded)

**4 monkeys per group:**

Group 1: instill pseudovirus atraumatically

Group 2: instill pseudovirus, pap test, BME with surgilube

Group 3: instill pseudovirus, pap test, BME with carrageenan


H&E
PsV challenge without cytology
PsV challenge with cytology
a
b
c
Ectocervix
Transformation Zone
Endocervix

# Mean No. Infectious Events Per Section

| Protocol | RFP Pos Cells | 95% CI |
|---|---|---|
| RFP PsV Only | 0.05* | 0.01, 0.08 |
| Pap Smear<br>RFP PsV<br>Surgilube | 84.3 | 45.1, 157.6 |
| Pap Smear<br>RFP PsV<br>Carrrageenan | 3.5 | 1.8, 6.9 |

* The transformation zone was not exceptionally susceptible to infection

Jeff Roberts et al., J. Nat Cancer Inst., 2011

# Implications of Monkey Pap Results

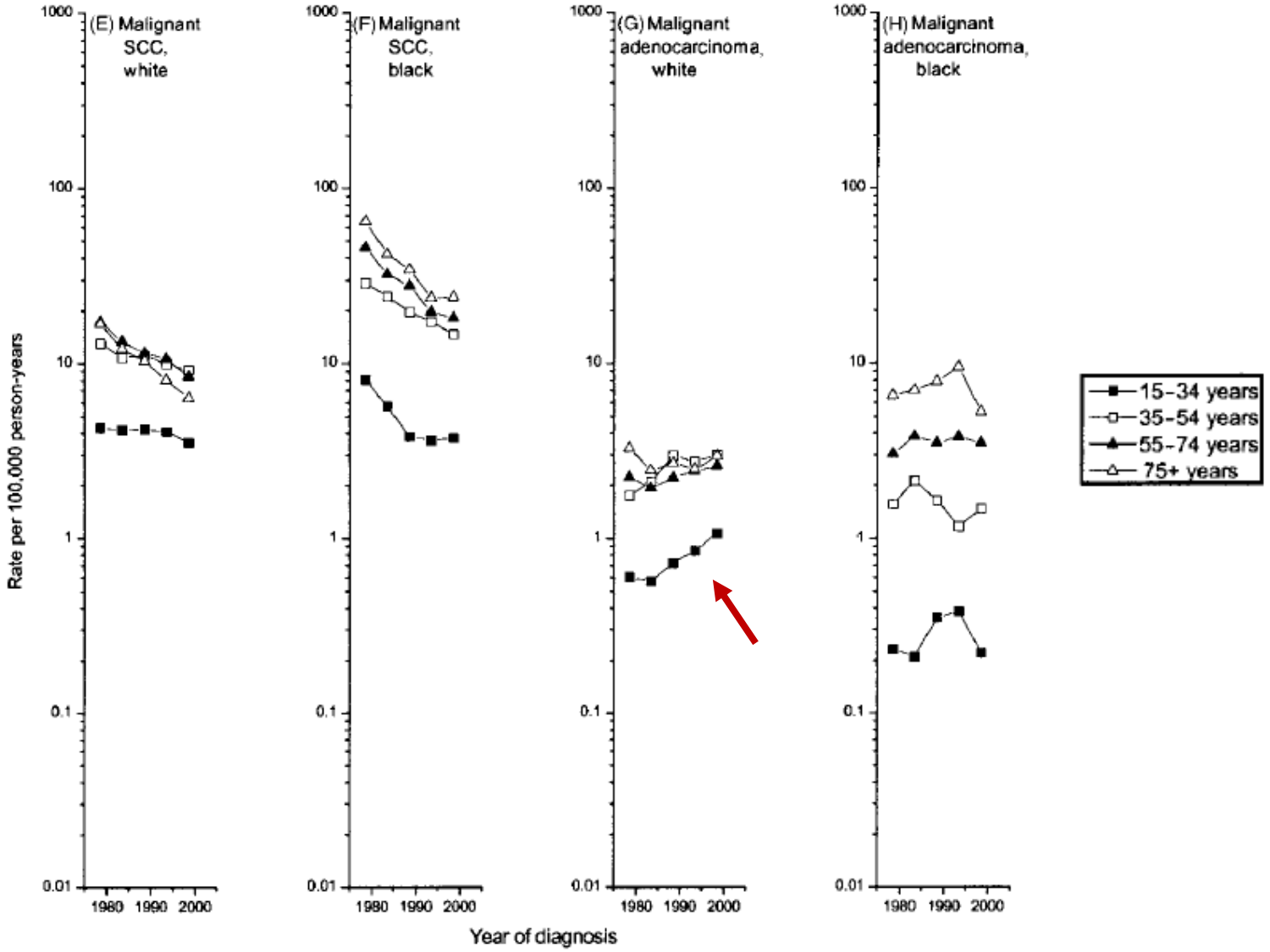
- **Not a call for changing Pap smear recommendations.**

  **- increase in susceptibility expected to be transient**

  **- organized screening programs clearly decrease rates of cervical squamous cell carcinoma**

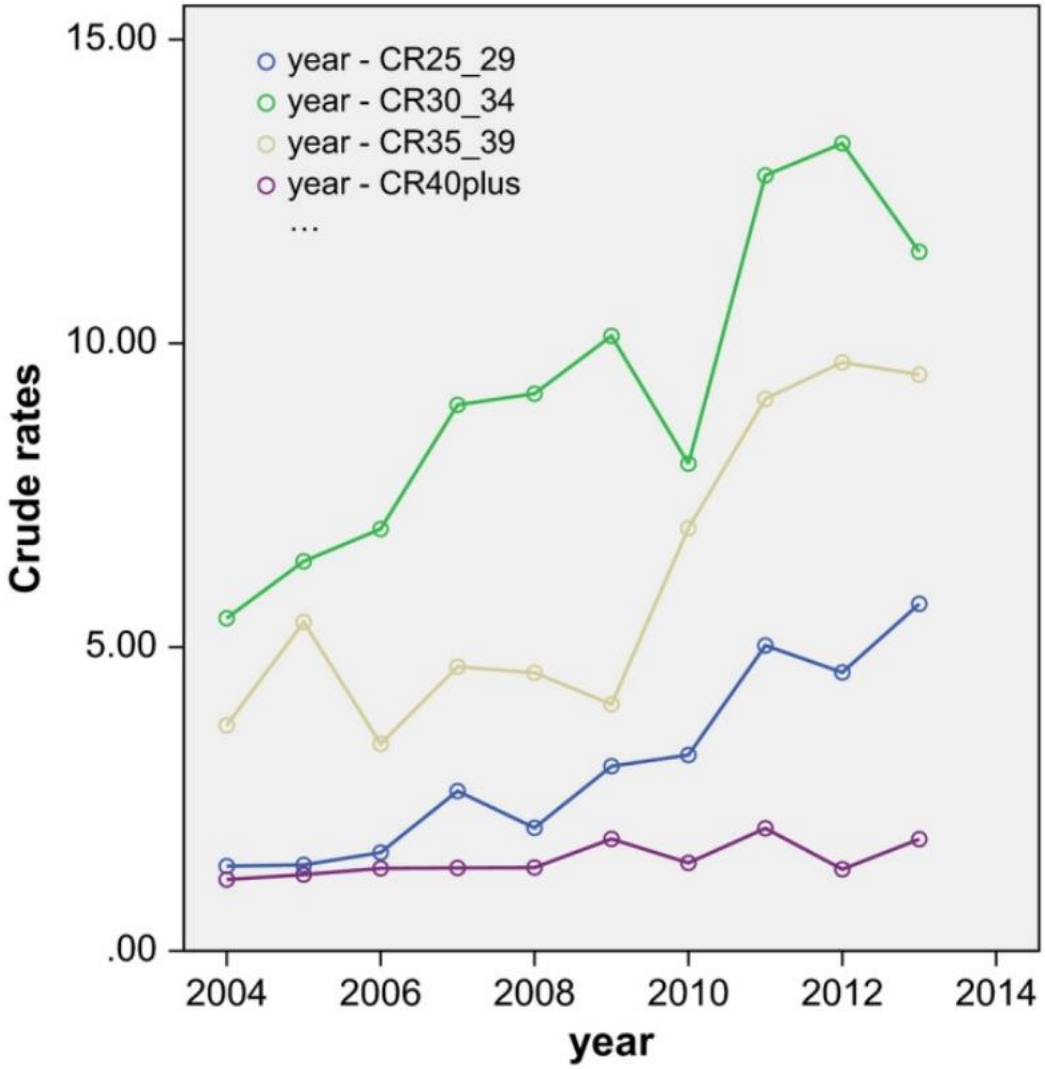
  **- but unexplained increase in rates in adenocarinoma in younger women.**

# U.S. Time Trends in Cervical SCC and Adenocarcinoma




SS Wang et al, Cancer 2004;100:1035044

# Netherlands Time Trends in Cervical Adenocarcinoma




Van der Horst Cancer Medicine 2017; 6(2):416-23

# Does More Aggressive Collection of Endocervical Cells Promote Cx Adenocarcinoma?

Endocervical Brush

**Is there an association between increased frequency and aggressiveness of Pap screen collection and subsequent rates of Cx Adenocarcinomas?**

# Questions Raised by the Study

- **Conduct a trial comparing rates of HPV infection after Pap smear +/- carrageenan?**
- **Use carrageenan gel for pelvic exam as standard practice?**
- **Do the results support changes to atraumatic sample collection for HPV DNA testing, esp in natural history studies?**
- **Would vaccination of mid-adult women prevent autoinoculation of the endocervix after Pap, thereby reducing the rates of cervical adenocarcinoma?**

# Key Collaborators

**NCI – CCR:** **Jeff Roberts**
**Doug Lowy**
**Rhonda Kines**
**Patricia Day**
**Katy Johnson**
**Cindy Thompson**
**Susana Pang**
**Chris Buck**

**DCEG:** **Hormuzd Katki**