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

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**Pre-Treatment**

Progesterone (Depo-Provera)

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**Disruption/Infection**

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

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**Long Term Time Course**

Analysis by Luciferase expression.

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**Short Term Readout**

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

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

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HPV Capsids Don’t Bind Apical Surfaces of Intact Epithelium

Vaginal Mucosa - stratified squamous

Endocervical Mucosa - simple columnar

green = (infectious) dye-coupled HPV capsids

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

In vivo Model of Early Events in HPV Infection

Occurs over several hours

Exposure of cell receptor binding site on L1

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

=Basement Membrane

=X-neut Epitopes

=L1

=L2

=HSPG

=Furin
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
Ectocervix

PsV challenge without cytology
PsV challenge with cytology

Transformation Zone

Endocervix
## Mean No. Infectious Events Per Section

<table>
<thead>
<tr>
<th>Protocol</th>
<th>RFP Pos Cells</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>RFP PsV Only</td>
<td>0.05*</td>
<td>0.01, 0.08</td>
</tr>
<tr>
<td>Pap Smear RFP PsV</td>
<td>84.3</td>
<td>45.1, 157.6</td>
</tr>
<tr>
<td>Surgilube</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap Smear RFP PsV</td>
<td>3.5</td>
<td>1.8, 6.9</td>
</tr>
<tr>
<td>Carrrageenan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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 adenocarcinoma in younger women.
U.S. Time Trends in Cervical SCC and Adenocarcinoma

(E) Malignant SCC, white
(F) Malignant SCC, black
(G) Malignant adenocarcinoma, white
(H) Malignant adenocarcinoma, black

Rate per 100,000 person-years

Year of diagnosis

15-34 years
35-54 years
55-74 years
75+ years

SS Wang et al, Cancer 2004;100:1035044
Netherlands Time Trends in Cervical Adenocarcinoma
Does More Aggressive Collection of Endocervical Cells Promote Cx Adenocarcinoma?

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

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