

# SV40, vaccines, and human cancer:

Epidemiological perspective and  
ongoing research

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# Overview

- Introduction
- Laboratory studies of human tumors
- Cohort studies
- Case control studies
- Prevalence studies
- Conclusion

# SV40: historical background

- SV40 is a macaque polyomavirus
- Contaminant of early polio vaccines manufactured in kidney tissue from infected macaques  
(Sweet 1960)
- SV40 was present during 1955-62
  - ◆ IPV (US and Western Europe): administered to tens of millions of persons, mostly children
  - ◆ Other vaccines: OPV, adenovirus, RSV  
(Shah 1976)

# SV40 and cancer

- SV40 causes cancer in lab animals (newborn rodents)
  - ◆ Mesothelioma (Cicala 1993)
  - ◆ Ependymoma (Kirchstein 1962)
  - ◆ Osteosarcoma (Diamandopoulos 1972)
  - ◆ Hematological malignancies (Diamandopoulos 1972)
- SV40 T-antigen inactivates p53 and pRB
- SV40 immortalizes cells in tissue culture
- SV40 DNA sequences reported in human tumor tissues

# Laboratory studies: human ependymoma

Study	Country	No. positive/ No. tested (%)	PCR cycles	Tissue type	Masking / neg. controls	Quantification of SV40 DNA
Bergsagel, 1992	USA	10 / 11 (91)	45-60	fresh, formalin	no / yes	no
Krainer, 1995	Austria	0 / 10 (0)	45-60	formalin	no / no	no
Martini, 1996	Italy	8 / 11 (73)	35-105	fresh, formalin	no / yes	no
Suzuki, 1997	Japan	4 / 13 (31)	45	formalin	no / yes	no
Huang, 1999	Switzerland	9 / 16 (56)	45	formalin	no / no	no
Ohgaki, 2000	Finland	0 / 10 (0)	45	?	no / no	no
Weggen, 2000	Germany	2 / 27 (7)	40	fresh	no / yes	no
Reuther, 2001	Germany	3 / 62 (5)	36	fresh, formalin	no / no	no

- Substantial variability in results
- Issues in study design

## Laboratory studies: range of human tissues

Tissue type	Reference	Tissue type	Reference
Brain tumors/neural tumors		Hodgkin's lymphoma	Shivapurkar 2002
Ependymoma	Bergsagel 1992	Sarcomas	
Choroid plexus tumor	Bergsagel 1992	Osteosarcoma	Carbone 1996
Astrocytoma/glioma	Huang 1999	Chondrosarcoma	Carbone 1996
Medulloblastoma	Krynska 1999	Ewing's sarcoma	Martini 2002
Pituitary adenoma	Zhen 1999	Giant cell tumor	Carbone 1996
Meningioma	Martini 1996	Normal tissues	
Mesothelioma	Carbone 1994	Blood	Martini 1996
Lung cancer	Galateau-Salle 1998	Urine	Kopp 2001
NHL (various subtypes)	Vilchez 2002	Semen	Martini 1996

- Lack of “specificity of association” (Bradford Hill 1965)

# Laboratory studies: further issues

- SV40 appears to be detected in low copy number in human tumors (Gordon 2002)
  - ◆ PCR is highly sensitive but prone to contamination
  - ◆ Laboratory artifact vs. biological mechanism
  - ◆ In animal tumors, SV40 is integrated into host chromosome, and T-antigen is readily detected (Cicala 1993)
- Not true case control studies
  - ◆ Laboratory studies of control tissues provide no data on prevalence of SV40 infection in control populations
  - ◆ No measure of association (odds ratio)
  - ◆ Impossible to determine cancer risk associated with infection

# Cohort studies

- Retrospective follow-up
  - ◆ SV40-exposed cohorts (vaccine-exposed)
  - ◆ Cancer registries
- Epidemiologically based, with:
  - ◆ Defined exposures
  - ◆ Data on temporal relationship between SV40 exposure and cancer outcome
  - ◆ Measure of association (relative risk)
- Follow-up exposed groups at highest risk
  - ◆ Vaccine-exposed infants

# Previous NCI cohort studies

- U.S., 1973-93 (Strickler 1998)
  - ◆ SEER data on cancer incidence
  - ◆ Studied cancer incidence as a function of birth year and calendar year, as measures of exposure to SV40-contaminated polio vaccine
  - ◆ No association between vaccine exposure and risk of mesothelioma, brain tumors, osteosarcoma
- Cleveland cohort (Mortimer 1981, Carroll-Pankhurst 2001)
  - ◆ 1073 infants exposed in 1960-62 to live SV40 via polio vaccination
    - Exposed during first few days of life
    - Vaccines known to have had live SV40
  - ◆ No excess cancer risk during follow-up through 1996 (age 34-36 y)

Ongoing research at NCI

# Cancer incidence in Denmark following exposure to SV40-contaminated polio vaccine

E.A. Engels, H.A. Katki, N.M. Nielsen,  
J.F. Winther, H. Hjalgrim, F. Gjerris,  
P.S. Rosenberg, M. Frisch

USA:

National Cancer Institute

Denmark:

Statens Serum Institut

Danish Cancer Society

Neuroscience Center, H:S, Rigshospitalet

# Early IPV in Denmark

- Vaccination campaigns with IPV began in April 1955
- Highly coordinated effort, targeting children
- By mid-1962, at least one dose had been administered to:
  - ◆ 38% of infants <9 months old
  - ◆ 90% of children 9-23 months old
  - ◆ 84-100% of children 2-18 years old

# SV40 contamination of Danish polio vaccine

- Monolayer culture method pooled dozens of monkey kidneys (von Magnus 1955)
- Testing in 1961 revealed that 9/9 IPV lots released in 1957-61 had live SV40
- IPV production halted in January 1962
- Beginning in 1963, IPV was free of SV40

# Follow-up of Danish vaccine recipients

- Danish cancer registry
  - ◆ Population-based
  - ◆ 1943-97
  - ◆ 70 million person-years of follow-up
- Examine cancer incidence as a function of IPV exposure, as measured by birth year and calendar year
- Strengths
  - ◆ Well-documented contamination of Danish IPV (exposure)
  - ◆ Complete cancer registration (outcome)

# Follow-up of U.S. Army recruits exposed to SV40-contaminated adenovirus vaccine

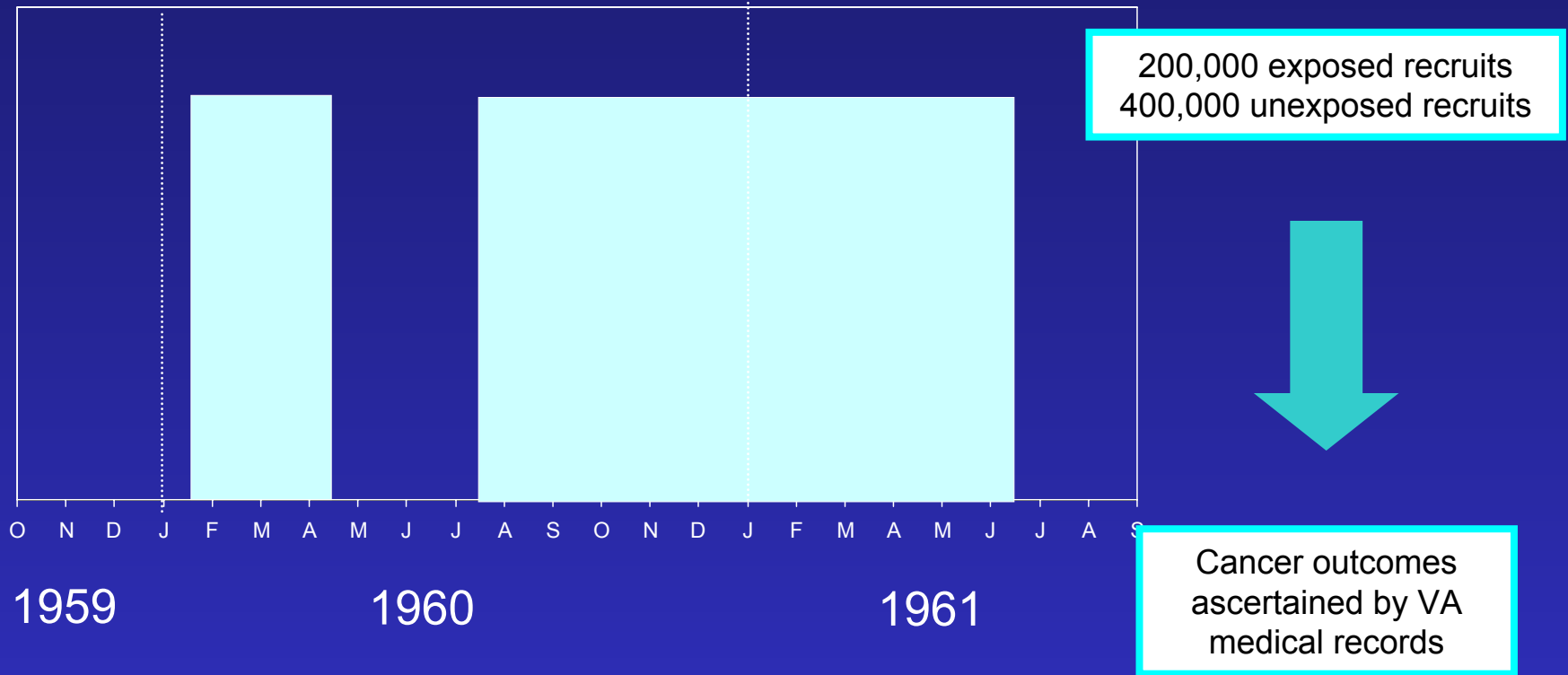
E.A. Engels, G. Gridley, S. Wacholder,  
W. Page, R. Miller

National Cancer Institute  
Medical Follow-up Agency, Institute of Medicine

# Inactivated adenovirus vaccine

- Administered to U.S. Army recruits in 1960-61
- Grown in monkey kidney tissue
- Adenovirus grows poorly without presence of SV40 in same culture = positive selection for SV40 (Rabson 1964)
- Live SV40 was found in tested lots of vaccine (Gerber 1961)

# Army adenovirus vaccine study



Sherwood 1961

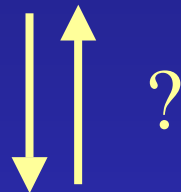
# Cohort studies

- Depend on two assumptions
  - ◆ Ability to identify persons exposed to live SV40
    - High prevalence of vaccination for children, Army recruits
    - Documentation of live SV40 in vaccines
  - ◆ Little or no SV40 exposure outside of vaccinations
    - Effects of repeated exposures additive, through increases in the probability of infection with SV40
    - Vaccine exposures were high inoculum, early-life events
    - What is known about other routes of exposure?

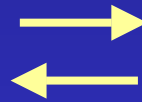
# Comparison of approaches

Laboratory  
studies

Cohort  
studies



SV40 detection in tumors from  
persons born after 1962



*How did these people get  
infected? Does SV40 circulate?*

# SV40 prevalence and transmission

- Polyomavirus excretion
  - ◆ BK and JC virus are present in human urine, but unclear for SV40 (Shah 1997, Kopp 2001)
  - ◆ BK and JC virus are present in human sewage, but SV40 is not (Bofill-Mas, 2000)
- SV40 neutralizing antibodies are present in 3-4% of humans (Shah 1971, Strickler 1996)
  - ◆ At low titer
  - ◆ Cross-reacting antibody

# SV40 and human brain tumors in northern India

E.A. Engels, C. Sarkar, R.W. Daniel, P.E. Gravitt,  
K. Verma, M. Quezado, K.V. Shah

USA:

National Cancer Institute  
Johns Hopkins School of Public Health

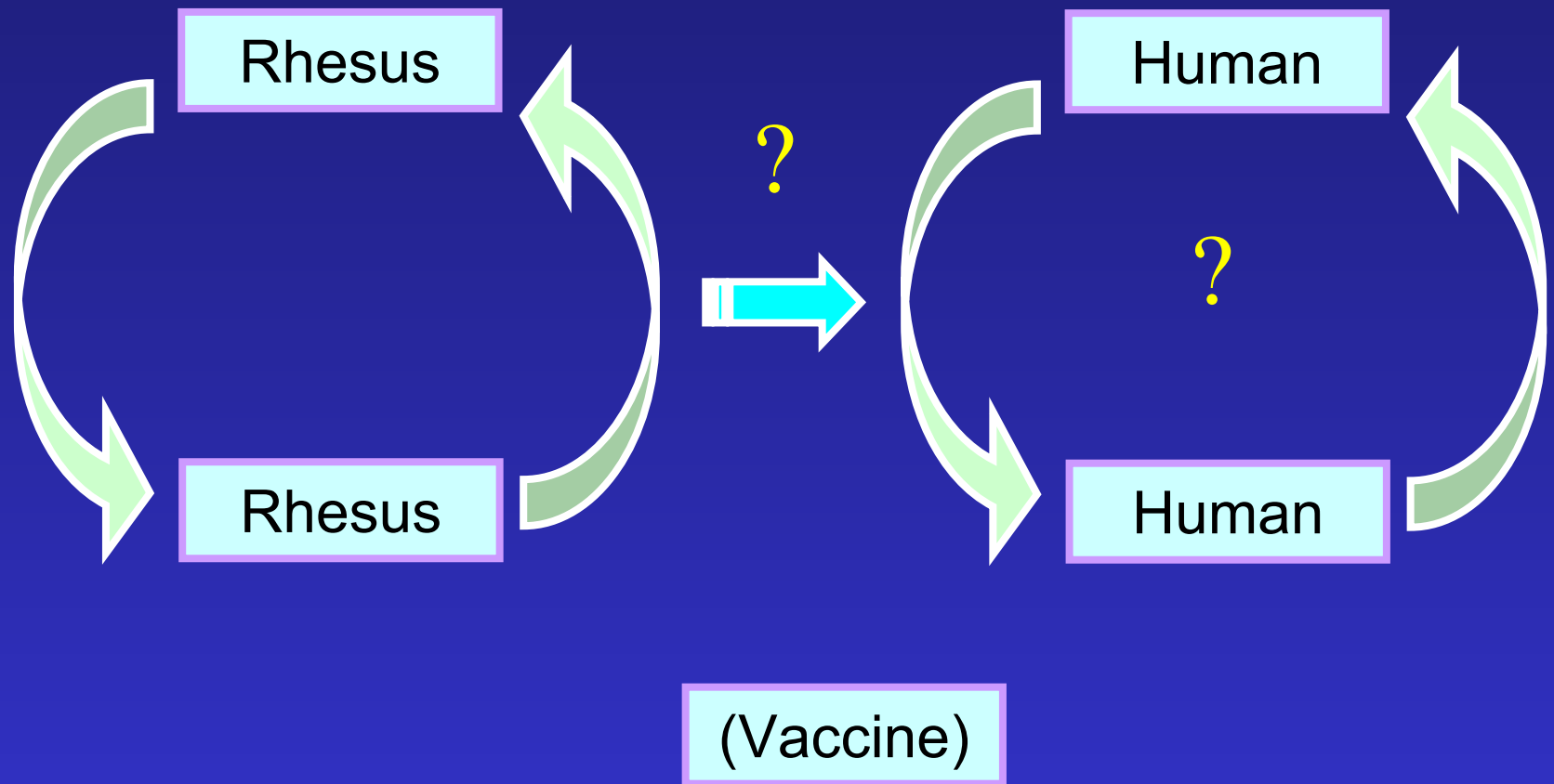
India

All India Institute of Medical Sciences

# Rhesus macaques in northern India

- Large population of rhesus
  - ◆ 800,000 in Uttar Pradesh (Southwick 1965)
  - ◆ Trapping led to decline, but numbers increased after 1978
- Close proximity of rhesus to humans
  - ◆ 75% in villages/cities, 9% along roads/canals, 3% in temples
  - ◆ Destruction of woodlands for farming
- SV40 is highly prevalent in rhesus population (Shah 1965)
- SV40 is detected in rhesus urine (Shah 1969)

# SV40 transmission in northern India



# India study

- Cases (n=47)
  - ◆ Ependymomas and choroid plexus tumors
  - ◆ Treated at AIIMS, 1991-2000
- Controls (n=18)
  - ◆ Patients with contusion or metastases
  - ◆ Treated at AIIMS
  - ◆ Frequency matched to cases by:
    - Age (<18, 18+ yrs)
    - Year of diagnosis (1991-95, 1996-2000)

# India study: strengths

- Tissues extracted and analyzed under masked conditions
- Real-time PCR to quantify SV40 and human DNA sequences detected in tissue specimens
- Novel and epidemiologically grounded setting

# India study

- Negative findings would argue against a role for SV40 in brain tumors
  - ◆ SV40 is present rarely or in low amounts (biological plausibility)
  - ◆ *Either* SV40 is not transmitted to/among humans *or* it does not cause cancer
  - ◆ Results would directly challenge studies from countries where vaccination campaigns were presumably important
- Positive results would provide new avenue to study routes of SV40 infection in humans

# Case control studies

- Compare infection rates in cancer cases and controls
  - ◆ Not possible with tumor tissues
  - ◆ Measure of association (odds ratio)
- Assays for infection
  - ◆ Antibody
  - ◆ PCR to detect viremia
- Limited data for SV40 and human cancer
  - ◆ Mesothelioma, osteosarcoma (Strickler 1996)
  - ◆ Brain tumors (Rollison 2002)

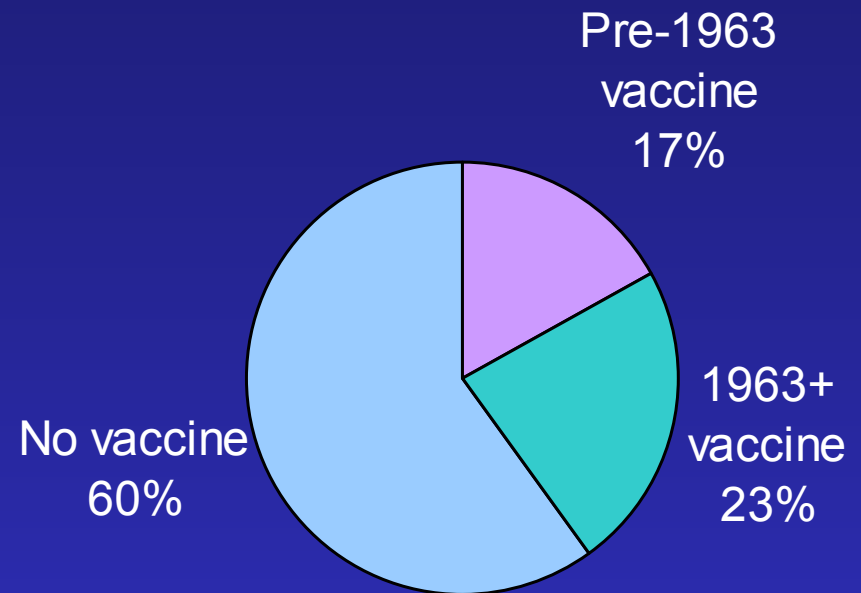
# Case control study of cancer in children potentially exposed *in utero* to SV40-contaminated polio vaccine

E.A. Engels, N. Chatterjee, R. Daniel, K. Shah,  
R. Viscidi, M. Klebanoff

National Cancer Institute  
National Institute of Child Health and Human Development  
Johns Hopkins School of Public Health

# Collaborative Perinatal Project

- Pregnant women and their subsequently born children, enrolled 1959-66 (n=54,796 children)
- During pregnancy, women were interviewed about exposures and provided serial blood specimens



*In utero polio vaccine exposure*

# Collaborative Perinatal Project

- 52 cancers in children up to 8 years of age
  - ◆ 18 neural tumors
  - ◆ 22 hematologic malignancies
  - ◆ 12 miscellaneous tumors
- Nested case control study of childhood cancer
  - ◆ 50 cancer cases with serum, 200 controls
  - ◆ Examine whether SV40 seroconversion in mothers during pregnancy is associated with:
    - Case-control status of children
    - Receipt of pre-1963 polio vaccine
- Strengths
  - ◆ Firm epidemiological grounding, with documented exposures to SV40-contaminated vaccines
  - ◆ Cutting-edge antibody assays

# Prevalence studies

- Prevalence studies can:
  - ◆ Provide clues on whether/how SV40 is transmitted
  - ◆ Help resolve conflicting laboratory and cohort studies
- Challenges
  - ◆ Studies depend on highly sensitive and specific assays
    - Absence of gold standard
    - Need to validate assays with
      - epidemiologic correlates of infection (exposures, outcomes)
      - multiple measures of infection
  - ◆ Antibody assays may be particularly suitable
    - High throughput
    - Avoid problems with PCR contamination
    - Need to avoid cross-reactivity with BK and JC

# Evidence for causality

	<u>SV40</u>	vs.	<u>HHV8</u>
<b>Laboratory evidence</b>			
Detection of viral DNA in human tumor	Bergsagel 1992		Chang 1994
Specificity of association	Many malignancies		Few malignancies
Localization of virus	?		In tumor cells
Amount of viral DNA in tissue specimens	Small		Large
Animal model	Yes		No
Understanding viral carcinogenesis in humans	Limited		Limited
<b>Epidemiologic evidence</b>			
Cohort studies	Negative		Positive
Case control studies	Negative ?		Positive (antibody, PCR)
Infection precedes/predicts disease	?		Yes
Prevalence studies	?		Prevalence reflects transmission routes

# Suggestions for future work

- Cohort studies
  - ◆ Identify and follow-up cohorts with unique exposures to SV40
- Case control studies
  - ◆ New antibody assays
- Laboratory studies
  - ◆ Transparent and rigorous design
  - ◆ Well-defined positive and negative controls
  - ◆ Masking
  - ◆ PCR quantification, *in situ* studies, RNA expression
- Prevalence studies
  - ◆ Highly sensitive, specific, and reliable tests
  - ◆ Epidemiologically relevant correlates of infection