U.S. FOOD & DRUG ADMINISTRATION

June 5, 2020

Siri & Glimstad LLP
Aaron Siri
200 Park Avenue, 17th Floor
New York, NY 10166

In reply refer to file: 2020-316 (Siri & Glimstad LLP IR#0212)

Dear Mr. Siri,

This is in reply to a Freedom of Information Act request submitted by Siri & Glimstad LLP on behalf of Informed Consent Action Network ("ICAN"). The request is dated January 3, 2020 and is for "A copy of the slide presentation presented by Keith Peden at the Science of Vaccine Safety meeting entitled "DNA, Fetal Tissue, Etc." in the timeframe "between March 2019 and August 2019." The subject request was received in the Center for Biologics Evaluation and Research on January 8, 2020.

Enclosed is Dr. Keith Peden's slide presentation titled "Issues Associated with Residual Cell-Substrate DNA in Vaccines: Facilitating the Introduction of New Vaccines by Slaying the "Dragon" (dated May 31, 2019).

If you have any questions or if I can be of further assistance, please let me know by referencing the above file number. I can be reached by phone at 240-402-8001 or by e-mail at Elizabeth.Sly@fda.hhs.gov.

You also have the right to contact:

FDA FOIA Public Liaison
Office of the Executive Secretariat
5630 Fishers Lane
Room-1050
Rockville, MD 20857
E-mail: FDAFOIA@fda.hhs.gov

Sincerely,

Elizabeth A. Sly -S

Digitally signed by Elizabeth A. Sly -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002025196, cn=Elizabeth A. Sly -S
Date: 2020.06.05 17:57:04 -04'00'

Elizabeth Sly
Access Litigation and Freedom of Information Branch
Center for Biologics Evaluation and Research
Food and Drug Administration

Issues Associated with Residual Cell-Substrate DNA in Vaccines: Facilitating the Introduction of New Vaccines by Slaying the "Dragon"

Keith Peden PhD
Laboratory of DNA Viruses
Division of Viral Products
Office of Vaccines Research and Review
CBER/FDA

May 31, 2019

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Outline of Presentation

- A brief history of vaccine-cell substrates
- Genesis of our studies on DNA
- Concerns with the use of tumorigenic cells: DNA and adventitious agents
- Why DNA has been considered a risk factor
- Ways to address DNA risks; allowing new cells substrates while retaining public confidence in vaccines

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Until 2012: Cell Substrates for Licensed Vaccines

Primary Cells:	chick embryo fibroblasts (MMR) embryonated hens' eggs (Influenza) African green monkey kidney cells (OPV)
Diploid Cells:	WI-38 (MMR); MRC-5 (varicella; zoster)
Cell Line:	Non-tumorigenic VERO (IPV, smallpox, rotavirus)

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New Cell Substrates

- New vaccines are required to combat emerging infections and against agents of bioterroism
- Viruses are obligate parasites and require cells ("cell substrates") for their growth
- The current cell substrates are not sufficient to produce these new vaccines
- Many of the new cell substrates are tumorigenic or derived from human cancers
- There has been a reluctance to use such cells to produce prophylactic vaccines, especially those intended for children
- Our program is directed at addressing potential safety issues associated with these types of cell substrates

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Safety concern with such cells: Can materials or agents present in the cell substrate be present in the vaccine and cause disease in vaccinated humans?

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Significant Meetings on Cell Substrates

- 1954: US Armed Services Epidemiology Board
  - Recommended against the use of HeLa cells for adenovirus vaccine production, preferring primary monkey kidney cells ("normal" cells)
- 1967: NIH Conference on Substrates for Vaccine Production
  - Use of human diploid cell strains and development of monkey equivalents
- 1978: Cell Substrates: Their Use in the Production of Vaccines and Other Biologicals (Lake Placid, New York)
  - Use of diploid cell strains was discussed
- 1984: NIH/FDA meeting: Abnormal Cells, New Products, and Risk
  - First conference where DNA limits were posited: 10 pg per vaccine dose

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Significant Meetings on Cell Substrates (cont.)

- 1986: WHO Study Group convened in Geneva
  - Assembled a group of experts in the fields of molecular biology, genetics, cancer biology, oncogenic viruses, and vaccine development
  - Identified which components of cell substrates could represent a risk

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WHO Study Group on Biologicals Geneva 1986 Which Cell Components Could be a Risk?

- Transforming proteins
- Transforming RNA
- Transforming DNA

- Dismissed as could not replicate themselves; thus effect would be transient
- Dismissed as being unstable
- Transforming DNA was considered the only risk factor

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Significant Meetings on Cell Substrates (cont.)

- 1986: WHO Study Group convened in Geneva
  - Assembled a group of experts in the fields of molecular biology, genetics, cancer biology, oncogenic viruses, and vaccine development
  - Identified which components of cell substrates could represent a risk
  - Based on the oncogenic dose of polyoma virus DNA, the amount of DNA in a vaccine from a continuous cell line was put at 100 pg/dose

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Significant Meetings on Cell Substrates (cont.)

- 1995: WHO/IABS Consultation; Fondation Mérieux, Annecy
  - DNA issues revisited
  - Amount of DNA raised to 10 ng/dose for non-tumorigenic cell lines
    - Human cancers require multiple separate events
    - Manufacturers could not meet the 100 pg/dose level for certain enveloped viruses

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Significant Meetings on Cell Substrates (cont.)

- 1999: FDA/NIH/IABS/NVPO/WHO Meeting Evolving Scientific and Regulatory Perspectives on Cell Substrates for Vaccine Development
  - Identified gaps in knowledge and where data needed to be generated
  - DVP initiated studies on DNA oncogenicity and infectivity
- 2004: NIAID/IABS Meeting Vaccine Cell Substrates 2004
  - Progress was presented on DNA oncogenicity and infectivity
  - Discussion of the remaining issues with the use of cell lines

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VRBPAC Meetings on Cell Substrates

- 1998: Discussed issues associated with tumorigenic cell substrates
  - DVP presented its approach to addressing issues – Defined Risk Approach
  - Three concerns were raised: Residual cells, adventitious agents, and DNA
- 2000: VERO cells; VRBPAC was concerned that VERO cells had the propensity to become tumorigenic; DVP was recommended to establish a program to understand how VERO cells become tumorigenic
- 2001: PER.C6 and 293 cells; cells that allow replication-defective adenoviruses to grow; both cell lines are tumorigenic. Issues were:
  - History – TSE concerns
  - Adventitious oncogenic viruses
  - Residual DNA
  - Recommended that DNA and cell lysates be tested in:
    - Newborn nude mice
    - Newborn rats
    - Newborn hamsters

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VRBPAC Meetings on Cell Substrates (cont)

- 2005: Tumorigenic MDCK cells for seasonal inactivated influenza vaccine
  - Recommended to move into clinical trials based on the clearance studies and DVP studies on DNA
- 2008: Non-tumorigenic MDCK cells for LAIV vaccine
  - Recommended to move into clinical trials based on the non-tumorigenicity of the cells
- 2012: Issues with use of cell substrates derived from human tumors
  - CEM, HeLa, A549
  - Adventitious agents and residual DNA were discussed
  - Committee concluded that adventitious viruses were the major concern, and DNA issues could be dealt with by reducing its amount and size

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Major Potential Safety Concerns with Novel Cell Substrates

- Presence of adventitious viruses
- Residual cell-substrate DNA

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How to Address Potential Safety Concerns While Retaining the Public Trust in Vaccines?

- Identify potential risks
- Establish assays to quantify the risk
- Develop methods to mitigate risks

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Does Residual Cell-Substrate DNA in Vaccines Represent a Risk?

- Whether DNA from the cell substrate poses a risk to vaccine recipients has been debated for ~50 years
- Data were needed to resolve the issue
- Biological activities of DNA:
  - Infectious activity: cell DNA contains an infectious viral genome
  - Oncogenic activity: DNA induces a tumor

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Operational Assumptions for DNA Activity

- For a given DNA, the level of the response of a cell to that DNA is proportional to the amount of that DNA
- The activity of a gene/viral genome integrated in chromosomal DNA or as part of plasmid or phage vector is similar
- The amount of uptake of a gene/viral genome by a cell and the expression of this gene/viral genome in the cell is related to the concentration of the gene/virus in the DNA
- The activity of a gene/viral genome inoculated as chromatin is the same or lower than when the same gene/viral genome is inoculated as free DNA

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Approach to Evaluating the Biological Activity of DNA

- Establish a sensitive assay to detect the activity
- Use the assay to quantify the activity to estimate safety/risks (based on conservative estimates)
- Use the assay to quantify the reduction in DNA activity afforded by various treatments (chemical, nuclease digestion, *etc.*)

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Challenges Measuring the Biological Activity of Mammalian DNA: A Matter of Size

- A single-copy mammalian gene is 3,000 to 30,000 bp
- Haploid mammalian genome is 3×10^9 bp
- Therefore, a single-copy gene is 10^5- to 10^6-fold less abundant for equivalent amounts of cellular DNA as compared with a plasmid DNA with the same gene
- That is, the amount of mammalian genomic DNA equivalent to 1 µg of plasmid DNA is $1 \times 10^5 - 1 \times 10^6$ µg (0.1 g to 1 g)
- Translates to 0.1 to 1 trillion cells, or 2 to 20 livers
- What would a negative result mean in the absence of an animal model of known sensitivity?

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DNA Infectivity

The ability of the DNA genome of a DNA virus or the DNA copy of a retrovirus to produce an infection following transfection of permissive cells

DNA infectivity might be a greater risk than DNA oncogenicity

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An *in vitro* Assay to Quantify DNA Infectivity

Chose to quantify HIV DNA infectivity:

- Expertise in HIV biology
- Inactivated HIV vaccines had been submitted to CBER

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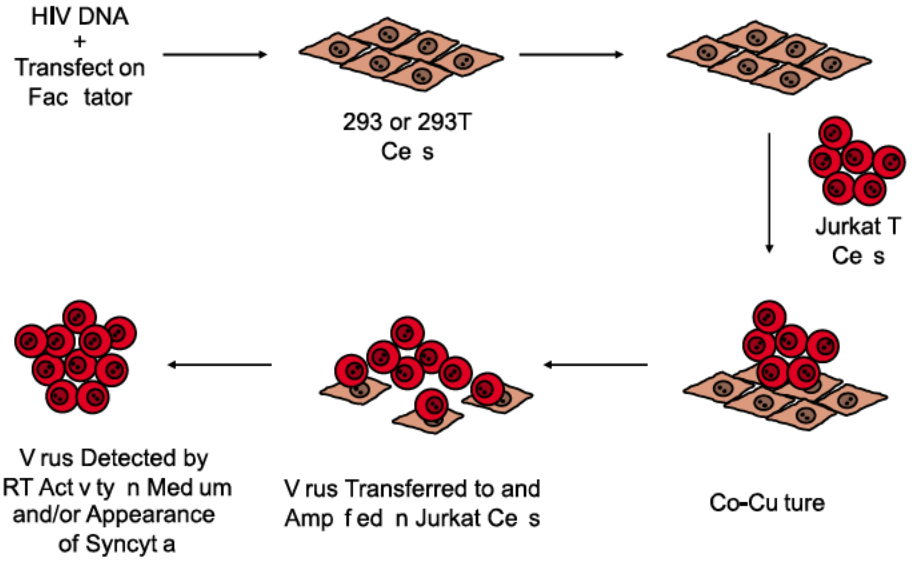
An *in vitro* Assay to Quantify DNA Infectivity



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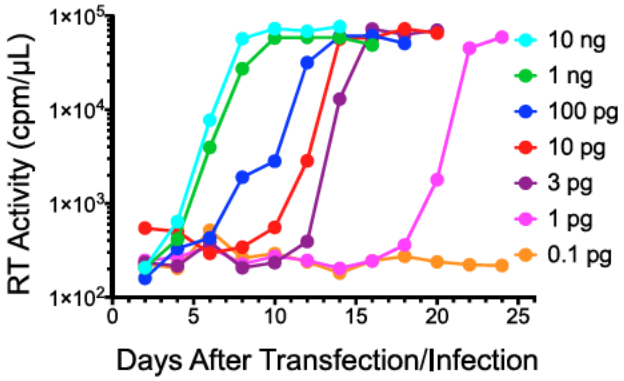
Dose Response of HIV DNA Infectivity



Assay can detect 1 pg of HIV DNA and 2 µg cellular DNA from HIV-infected cells

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Assay Can be Used to Monitor Reduction of Infectivity Following Treatments

- Nuclease digestion (with live or inactivated vaccines)
- Chemical treatment (with inactivated vaccines)
- Irradiation (with inactivated vaccines)

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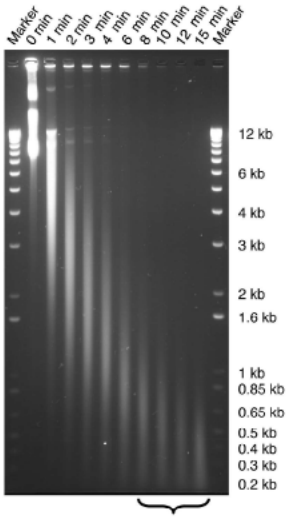
Digestion of HIV DNA to Below 650 bp Eliminated Infectivity



Digestions that eliminated infectivity with 0.3 µg

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Conclusions from DNA Infectivity Experiments

- Digestion of DNA with DNase or treatment with BPL or BEI can reduce the activity of the DNA by $\geq 10^5$ fold
- Combined with an amount of DNA of 10 ng per vaccine dose, safety margins of $\geq 10^7$ can be achieved

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Evaluation of DNA Oncogenicity

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Oncogenic Activity of DNA

- Consequence of DNA integration
  - Activation of a cellular oncogene
  - Inactivation of a tumor-suppressor gene
- Introduction of dominant activated oncogene

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Integration of DNA into the Host Chromosome

- DNA integration is very inefficient
- Thus, consequences of an oncogenic event by DNA integration is predicted to be rare
- Predicted to be a low-frequency event
  - Reinhardt Kürth: 1 in 10^{19}
  - Howard Temin: 1 in 10^{16} to 1 in 10^{19}

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Oncogenic Activity of DNA

- Consequence of DNA integration
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Oncogenic Activity of DNA

- Consequence of DNA integration
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- Introduction of dominant activated oncogene

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Development of Sensitive and Quantitative Animal Models to Assess DNA Oncogenicity

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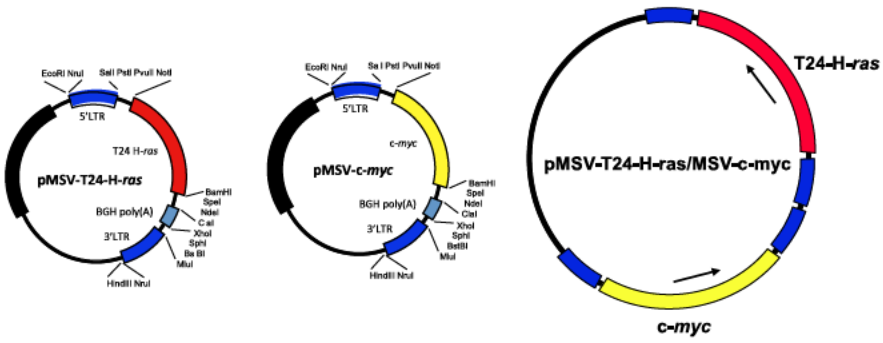
Development of Sensitive and Quantitative Animal Models to Assess DNA Oncogenicity

Expression plasmids for activated human H-ras and murine c-myc
Promoter MSV LTR; not downregulated *in vivo*



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Animal Models to Evaluate DNA Oncogenicity

Newborn mice (immune deficient, tumor prone)
Newborn hamsters
Newborn rats

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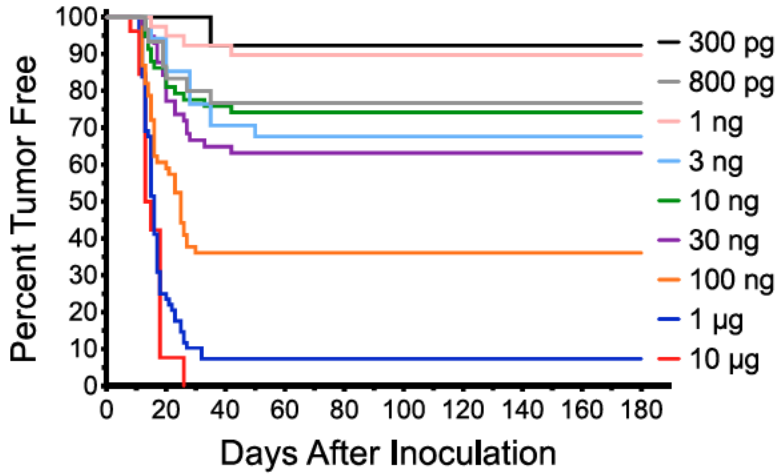
Time of Tumor Appearance in Newborn Rats Inoculated with Linear *ras/myc* Plasmid



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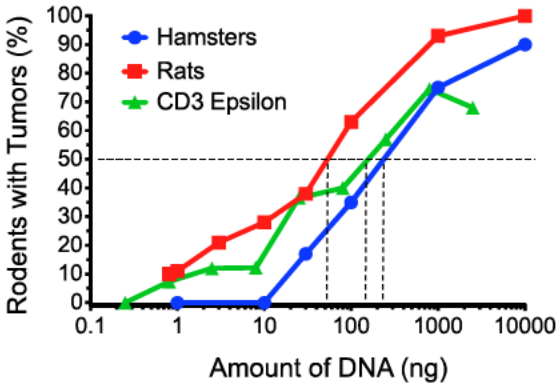
Dose-Response of *ras/myc* in Newborn Rodents: Comparison of CD3 Epsilon Mice, Rats, and Hamsters



Oncogenic $Dose_{50}$
CD3 ε mouse: 130 ng
Hamster: 220 ng
Rat: 50 ng

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Can Single Oncogenes Induce Tumors?

Two dominant activated cellular oncogenes unlikely to be linked closely enough to be taken up by the same cell

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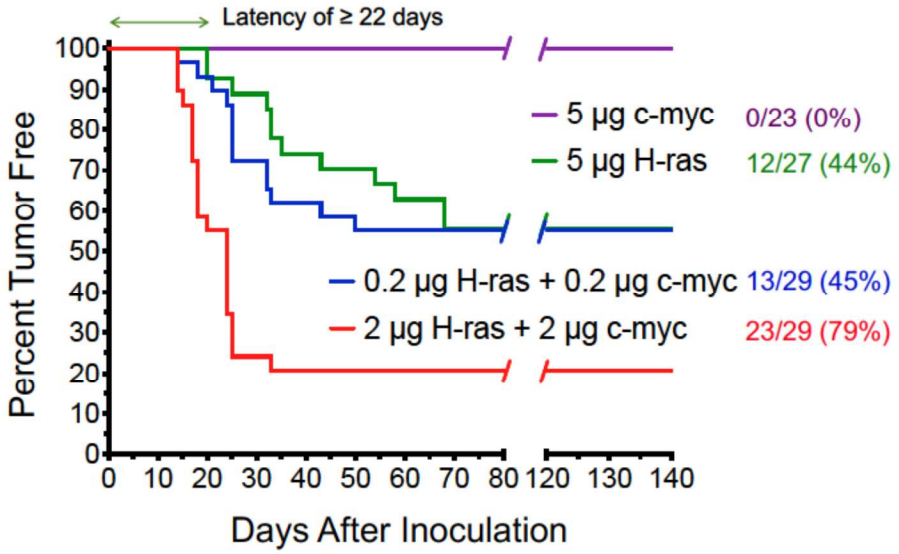
Tumors are Induced in Newborn Rats by H-*ras* but not c-*myc*



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Can Cell DNA from Human Tumors Induce Tumors?

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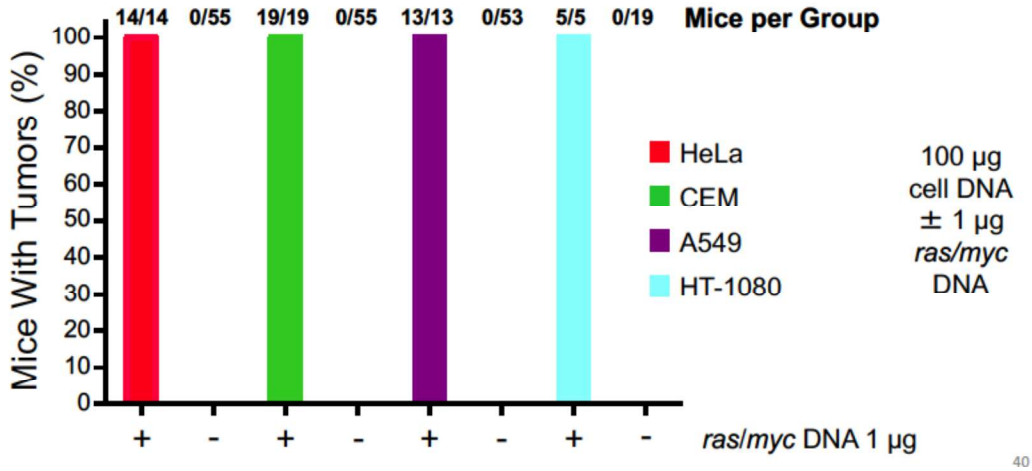
DNA From Four Tumorigenic Cell Lines Failed to Induce Tumors in Newborn CD3 Epsilon Mice



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Why is HeLa DNA Not Oncogenic?

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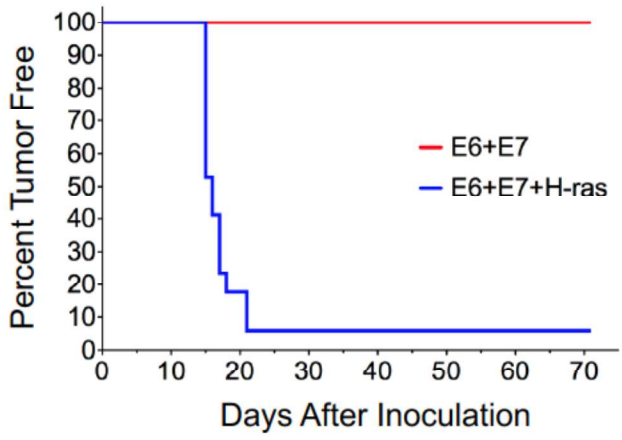
Activated H-*ras* Complements HPV16 E6 Plus E7 for Tumor Induction in Newborn Hamsters



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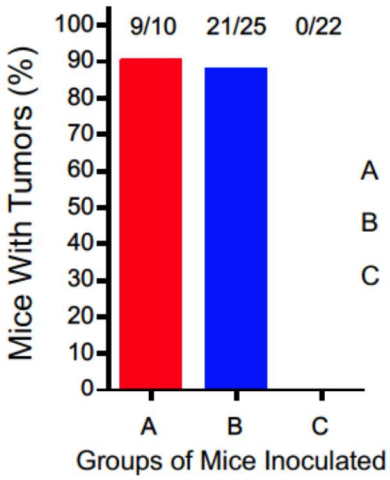
H-*ras* But Not c-*myc* Complements High-Risk HPV E6 and E7



A *H-ras* plus *c-myc* plasmids

B High-risk HPV E6 + E7 + H-*ras*

C High-risk HPV E6 + E7 + c-*myc*

Doses *ras or myc* 1 µg
E6 E7 2 µg each
Calf DNA 40 µg

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Evaluating the Oncogenicity of DNA from HeLa Cells with Complementing Oncogenes

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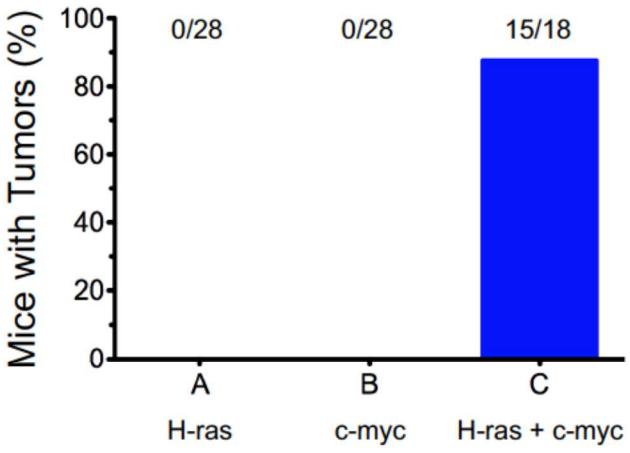
Neither H-*ras* nor c-*myc* Complements HeLa DNA for Tumor Induction in Newborn CD3 Epsilon Mice



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What is the Range of Oncogenes that can be Detected in the Animal Models?

<u>Positive</u> in the *in vivo* assay
Activated H-ras
vSrc
SV40 LT + ST (long latency)

<u>Negative</u> in the *in vivo* assay
c-myc
HPV E6 + E7

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Limitations with *in vivo* Oncogenicity Assays

- Not all dominant oncogenes score positive; *e.g.*, the *ras* family oncogenes are positive, while *myc* and viral oncogenes are not
- Even in the most sensitive system, two oncogenes are required for efficient tumor induction
- However, two activated, dominant oncogenes are not likely to be linked close enough in cell-substrate DNA to be on the same molecule and thus enter the same cell
- A negative result might provide a false sense of security with using a novel cell substrate
- Reducing the amount and size of DNA is likely the best solution to addressing concerns with DNA

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After 2012: Cell Substrates for Licensed Vaccines

Primary Cells:	chick embryo fibroblasts (MMR) embryonated hens' eggs (influenza) African green monkey kidney cells (OPV)
Diploid Cells:	WI-38 (MMR); MRC-5 (varicella; zoster)
Cell Lines:	Non-tumorigenic VERO (IPV, smallpox, rotavirus) Tumorigenic MDCK cells (inactivated influenza-virus vaccine) Tumorigenic CHO cells (Shingrix vaccine; gE in AS01$_B$)

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Some Outstanding Issues with Cell-Substrate DNA

- What is the range of oncogenes that can and cannot be detected by the *in vivo* assays?
- Is any model sensitive enough to detect activated oncogenes in cellular DNA? If so, at what level?
- Experiments so far have been negative, likely because:
  - Amount of oncogenes in cell DNA is too low
  - Two activated oncogenes are not linked
- Routes of inoculation
- Activity of chromatin needs to be determined
- Could a single oncogene induce vaccine-recipient cells to be predisposed to cancer development?

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Cell Substrates and WHO Recommended DNA Limits

▪ Primary Cells:	No limits
▪ Diploid Cells:	No limits
▪ Continuous Cell Lines:	
– Parenteral vaccines	≤ 10 ng per dose
– Oral vaccines (not encapsulated)	100 µg per dose
– Intra-nasal vaccines	no data

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John Dennis

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MCMi

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Thank You

"C'mon, c'mon—it's either one or the other."

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