



# Cancer risk associated with receipt of vaccines contaminated with simian virus 40: epidemiologic research

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Simian virus (SV)40 was an accidental contaminant of poliovirus vaccines used widely in the USA and other countries in 1955–1962. Exposure to SV40 via contaminated vaccines has led to concern as SV40 causes cancer in laboratory animals. In addition, some laboratories, although not all, have detected SV40 DNA in human tumors including mesothelioma, certain brain tumors, osteosarcoma and non-Hodgkin's lymphoma. This article reviews the data regarding contamination of poliovirus vaccines with SV40 and summarizes the results from epidemiologic studies of vaccine recipients. Long-term follow-up studies have not revealed recipients of SV40-contaminated poliovirus vaccines to be at an increased risk for cancer. Thus, these studies are somewhat reassuring and indicate that either SV40 does not readily infect humans or, following infection, does not cause cancer. Recognizing that the history of SV40 contamination of vaccines highlights an inherent risk of contamination of vaccines with adventitious agents, the Institute of Medicine recently called for the development of a comprehensive US plan to prevent vaccine contamination and respond to potential contamination events when they arise.

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A major challenge to the safety of vaccines and other biologic products is the possibility that extraneous infectious agents may inadvertently be incorporated. Such agents may then be transmitted accidentally to recipients of these products, perhaps causing untoward effects. This unfortunate possibility is obviously greatest when the adventitious agent has not previously been described.

The accidental contamination of early poliovirus vaccines and several other less widely used vaccines with simian virus (SV)40, a primate polyomavirus, represents an example of widespread exposure to an adventitious virus present in a biologic product. In 1960, SV40 was discovered by Sweet and Hilleman as a frequent infection of monkeys and contaminant of harvested kidney tissue, which was used to grow poliovirus vaccine stocks [1]. During the period from 1955, when poliovirus vaccines were first administered in public health campaigns, until

1963, when the vaccine in use was free of SV40, it is estimated that tens of millions of people in the USA and other countries received SV40-contaminated poliovirus vaccines [2].

Exposures to SV40 raises concern as it can cause various malignancies (e.g., sarcomas, brain tumors and leukemia) when injected into laboratory rodents [3], especially in the newborn period [4]. Virtually simultaneously with Sweet and Hillman's identification of SV40, Eddy and colleagues described the induction of soft-tissue sarcomas in newborn hamsters injected subcutaneously with extracts of monkey kidney cells [5] and demonstrated that the tumor-inducing agent was SV40 [6]. Virally induced transformation is driven by the SV40 T-antigen protein, which inhibits the activity of the cellular tumor-suppressor proteins, p53 and pRb [7]. Motivating further interest regarding SV40 as a cause of cancer in humans, some laboratory investigators have used highly sensitive

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polymerase chain reaction (PCR) methods to detect SV40 DNA in human tumors of various types including mesothelioma, certain rare brain tumors (i.e., ependymoma and choroid plexus tumors), osteosarcoma and non-Hodgkin's lymphoma [8–11]. While the frequency of detection has varied widely, in some studies the proportion of SV40-positive tumors has been high (e.g., 42% for non-Hodgkin's lymphoma [11] and 60% for mesothelioma [9]). However, other studies have not detected SV40 DNA in these tumor types [12–16]. Thus, although the positive reports have raised concern, the lack of reproducibility of SV40 DNA detection across laboratories has left it unclear whether SV40 is present in human tumors [3].

While an unfortunate accident, the occurrence of widespread exposure of individuals to SV40 via contaminated vaccines presents an opportunity to examine the possible cancer-inducing effects of this virus in humans. If SV40 causes cancer in humans, recipients of SV40-contaminated vaccines should have an increased risk of cancer. Detection of a heightened cancer risk would have important biologic and public health implications. This review summarizes epidemiologic evidence regarding whether widespread use of SV40-contaminated vaccines has led to such an increase in cancer.

#### Vaccine-related exposures to SV40

A cornerstone of the ongoing conquest of poliomyelitis was the development of poliovirus vaccines and their use in large-scale vaccination campaigns beginning in the 1950s. These vaccines, produced in macaque kidney tissue cells, also represented the most widespread, generally recognized exposure of humans to SV40. As most poliovirus vaccine used in the USA during the 1950s and early 1960s was the Salk inactivated poliovirus vaccine (IPV), it represents the principal known source of SV40 exposure in the USA.

IPV was introduced in the USA in 1955 following a successful field trial involving over 400,000 schoolchildren. By July 1955, IPV had been administered to 6 million US children of 6–8 years of age. Over time, IPV campaigns were implemented much more widely, involving additional age groups. As of September 1961, 98 million individuals under 60 years of age (62% of the population in that age range) had received at least one dose and many had received multiple doses [17]. Among children (0–19 years of age) who were especially targeted by public health officials, 88% had received at least one dose and nearly 50% had received at least four doses of IPV.

Poliovirus for vaccine production was grown in kidneys harvested from both rhesus and cynomolgus macaques. SV40 commonly infects rhesus macaques in the wild and SV40 is readily transmitted to cynomolgus macaques in captivity [2]. Therefore, SV40 was frequently present in kidneys harvested for poliovirus vaccine production. Two tissue culture methods were used to grow pools of vaccine virus [2]. In the Maitland method, poliovirus was grown in a cell culture prepared from the minced kidney of a single monkey. In the monolayer method, virus was produced in a cell monolayer derived from the kidneys of dozens of monkeys, which would be expected to

lead to universal contamination of the culture with SV40. The final step in IPV production involved formalin inactivation of poliovirus. SV40 is relatively resistant to formalin inactivation and thus low levels of live SV40 frequently remained present in lots of IPV [18].

As most US lots of vaccine were produced using the Maitland culture method and because formalin inactivated much of the live SV40 in the finished product, it has remained uncertain how much live SV40 was present in US IPV. Testing of lots released in the first year of public vaccination campaigns indicated that 71% of American states used lots of IPV that contained live SV40 [19]. The titer of live SV40 present in IPV varied but was substantially lower than in the cultures from which it was derived. Gerber and colleagues estimated that some IPV doses contained up to 2000 viral particles/ml [18]. The amount of live SV40 that neonates received by injection in a vaccine immunogenicity trial was 10–50 TCID<sub>50</sub> (where TCID<sub>50</sub> is defined as the amount of virus needed to infect 50% of a tissue culture [20]) less than the amount typically required to induce tumors in experimental animals [4].

Another indication that SV40 was present in IPV is provided by data on SV40 seroconversions following vaccinations. Seroconversion rates provide an upper bound on the proportion of people infected with SV40, because some individuals could also have seroconverted due to the presence of formalin-inactivated virus proteins or rapid clearance of live SV40. Estimates of the proportion of individuals who seroconverted following one or more doses of pre-1963 IPV range widely (4–92%) in studies based in the USA and UK [1,21–23]. This wide variability might reflect uneven contamination of IPV produced using the Maitland method, variable amounts of live SV40 in vaccines or different assay methods. It has been difficult to synthesize these limited data to estimate the overall frequency of SV40 contamination of IPV in the USA. However, Shah and Nathanson estimated that 10–30% of the 98 million people who had received IPV by 1961 were exposed to live SV40 through vaccination [2].

The Sabin oral poliovirus vaccine (OPV) was not licensed in the USA until 1963 and thus was not administered as widely as IPV during the period associated with SV40 contamination of vaccines (1955–1962). As OPV is a live vaccine not treated with formalin, OPV lots that contained SV40 had titers of SV40 that were much higher than those present in IPV. Mortimer and colleagues reported titers of live SV40 of 10<sup>4.5</sup>–10<sup>7</sup> TCID<sub>50</sub> units/ml of OPV [20]. Some studies, but not others, reported that SV40 was shed in stool for several weeks following OPV ingestion, suggesting that SV40 can transiently infect cells at enteric sites [2]. However, SV40 seroconversion was not observed following receipt of OPV, indicating that systemic infection probably did not occur after oral exposure [1,21].

Another type of vaccine that was widely contaminated with SV40 was the adenovirus vaccine developed by the US military. Several versions of the vaccine were developed and evaluated in field trials in the US Army and Navy [24–26]. In vaccine production, adenovirus was propagated in macaque kidney tissue. Importantly, adenoviruses grow poorly in this culture

system unless SV40 was also present as a helper virus [27]. Therefore, as a result of strong positive selection, nearly all adenovirus seeds and vaccine pools grown from them, became contaminated with SV40 [1,28]. This situation is unlike poliovirus vaccine contamination, which was not uniform, as SV40 is not a cofactor for poliovirus replication *in vitro*. As for poliovirus vaccines, formalin treatment of the final vaccine product would not have inactivated all of the live SV40 present. In 1961, Gerber and colleagues tested three samples of formalin-inactivated adenovirus vaccine and all contained live SV40 [18]. In addition, 100% of nine evaluated subjects seroconverted to SV40 in one early Army adenovirus vaccine trial [1]. Therefore, available evidence points to frequent SV40 contamination of this vaccine. However, because the vaccine was used only in the US military over a limited period, it has not been considered a major source for SV40 exposure in the general US population.

The discovery of SV40 led to changes in the manufacture and testing of vaccines which have eliminated SV40 from US vaccine products [2]. A June 1961 memorandum from the director of the Division of Biologics Standards, National Institutes of Health (MD, USA) stated that no lots of IPV could subsequently be released without a negative tissue culture test for SV40. However, previously released vaccine lots were not withdrawn. Accounting for storage time and shelf life, it is estimated that SV40-contaminated IPV is likely to have been in use in the USA until the end of 1962, but from 1963, IPV in use in the USA was free of SV40. The Army's adenovirus vaccine was withdrawn in mid-1961 due to manufacturing difficulties and concerns regarding SV40 contamination. An oral adenovirus vaccine, free of SV40, was later reintroduced into US military use.

OPV was required to be SV40-free upon its licensure in 1963. The absence of SV40 contamination is supported by steps in vaccine production and evaluations specified in US regulations [29]. Using sensitive PCR methods, Sierra-Honigman and Krause evaluated 30 OPV lots released in the USA from 1972 to 1996 [30]. Likewise, Sangar and colleagues evaluated OPV utilized in the UK from 1966 through to the 1990s [31]. Both studies found no evidence of SV40 contamination in these lots of poliovirus vaccine.

#### Cancer risk in recipients of SV40-contaminated vaccines

Epidemiologic studies of individuals who received SV40-contaminated vaccines provide valuable data regarding the potential relationship between SV40 and cancer. The premise of these studies is that, if SV40 causes cancer, cancer incidence or mortality will be higher in vaccinated than in unvaccinated groups. Thus, the studies rely on documentation that the vaccine under consideration was contaminated by SV40 and on the ability to determine which individuals actually received the vaccine. These epidemiologic studies have ascertained cancer outcomes in vaccine-exposed and -unexposed individuals, through active follow-up or the use of population-based cancer registries. The major studies are summarized in TABLE 1 and several are reviewed in more detail in the following paragraphs.

#### *Mortimer & colleagues and Carroll-Pankhurst & colleagues*

In 1960–1962, 1073 neonates in Cleveland (OH, USA) participated in a study of the immunogenicity of poliovirus vaccine administered in the first few days of life [20]. In the study, 86% received OPV, while 14% received IPV. Subsequent testing revealed that all vaccine lots used in the trial contained live SV40 at varying titers [20]. With the documented exposure to live SV40 at a very young age, this cohort provides valuable information on the effects of early life exposure to SV40.

Two long-term follow-up studies of these individuals have been conducted [20,32]. In the first, investigators contacted parents about the health status of the vaccinated children in 1976–1979, when the children were 13–19 years of age [20]. They also matched a list of the vaccinated children against medical and registry records. They found that 15 children had died but no death was from cancer. One child had developed a salivary gland tumor reported to have a low degree of malignancy. No other cancers were noted.

In the second study, identifying information on these individuals was matched against the US National Death Certificate Registry to ascertain additional deaths up to 1996 [32]. Only four cancer-related deaths were found (relative risk 1.3 compared with the general population, 95% confidence interval [CI]: 0.3–3.2). Two deaths were due to leukemia, but these were of different types and did not represent a significant excess (relative risk 4.2, 95% CI: 0.5–16) and two deaths were attributed to testicular cancer (relative risk 37, 95% CI: 4.5–130). The increased mortality from testicular cancer was unexpected, since no data from animal experiments support an etiologic link with SV40. The investigators speculated that this increase arose because of late diagnosis or poor treatment of testicular cancer in this largely inner-city population [32].

#### *Engels & colleagues*

Highly informative data regarding the cancer risk associated with receipt of SV40-contaminated poliovirus have come from Denmark [33]. IPV was first administered in Denmark in April 1955. Danish public health officials mounted a concerted campaign especially targeted at children and young adults, and these efforts were maintained through the early 1960s. By April 1962, approximately 90% of children 9 months of age or older had received at least one dose of IPV and most had received three to four doses. Of note, Danish vaccine, unlike US vaccine, was grown using a monolayer tissue culture method, greatly increasing the likelihood of SV40 contamination. Indeed, in late 1961, testing revealed that all nine evaluated lots of IPV previously used in vaccination campaigns contained live SV40. Vaccine production was halted and from 1963, all Danish IPV was free from SV40. Hence, almost all Danish children alive in 1955–1962 were exposed to live SV40 on multiple occasions through injection. Engels and colleagues examined the cancer incidence in Denmark for three birth cohorts with varying exposures to SV40-contaminated IPV: the 1946–1952 birth cohort, who were vaccinated in 1955 when the vaccine first became available (exposed as young children); the

Table 1. Selected epidemiologic studies of recipients of SV40-contaminated vaccines.

Study	Population, dates of vaccination	Exposure/measure	Major outcomes*	Follow-up duration/method	Association of cancer outcome with vaccine exposure	Ref.
Fraumeni 1963	7.6 million US children, 6–8 years of age, vaccinated in 1955	IPV with varying SV40 levels documented; birth cohort	Leukemia; mortality	4 years, registry	No increase	[19]
Mortimer 1981 Carroll-Pankhurst 2001	1073 US children, neonates, vaccinated in 1960–1962	IPV or OPV with documented SV40, received in trial	All cancer types; incidence and mortality	36 years, direct follow-up and registry	No increased incidence; only four cancer deaths (RR 1.3 vs. general population)	[20,32]
Strickler 1998	US SEER areas, children vaccinated in 1956–1962	IPV with presumed SV40; birth cohort	Ependymoma, osteosarcoma, mesothelioma; incidence	38 years, registry	No increase	[53]
Engels 2003	39,468 US people with AIDS, vaccinated as children in 1955–1962	IPV with presumed SV40; birth cohort	AIDS-associated NHL; incidence	41 years, registry	No increase compared with individuals with AIDS who did not receive vaccine	[54]
Engels 2003	Denmark, children vaccinated 1955–1962	IPV with documented SV40; birth cohort	Ependymoma, osteosarcoma, mesothelioma, NHL; incidence	42 years, registry	No increase	[33]
Strickler 2003	US SEER areas, children and adults vaccinated in 1955–1962	IPV with presumed SV40; birth cohort	Mesothelioma; incidence	42 years, registry	No increase	[55]
Engels 2004	12,334 US children whose mothers received vaccine during pregnancy	IPV or OPV with presumed SV40; maternal interview and records	Hematologic malignancies, neural tumors; incidence	8 years, direct follow-up	Increase in hematologic malignancies, largely leukemia (RR 2.5), and neural tumors, largely neuroblastoma (RR 2.5). See text for additional details	[23]
Rollison 2004	205,000 US Army recruits, vaccinated in 1960–1961	Adenovirus vaccine; dates of entry into US Army	Brain tumors, mesothelioma, NHL; incidence	35 years, hospital records	No increase	[35]

\*Types of cancer evaluated and measure of risk (incidence or mortality). IPV: Inactivated poliovirus vaccine; NHL: Non-Hodgkin's lymphoma; OPV: Oral poliovirus vaccine; RR: Relative risk; SEER: Surveillance, Epidemiology and End Results Program; SV: Simian virus.

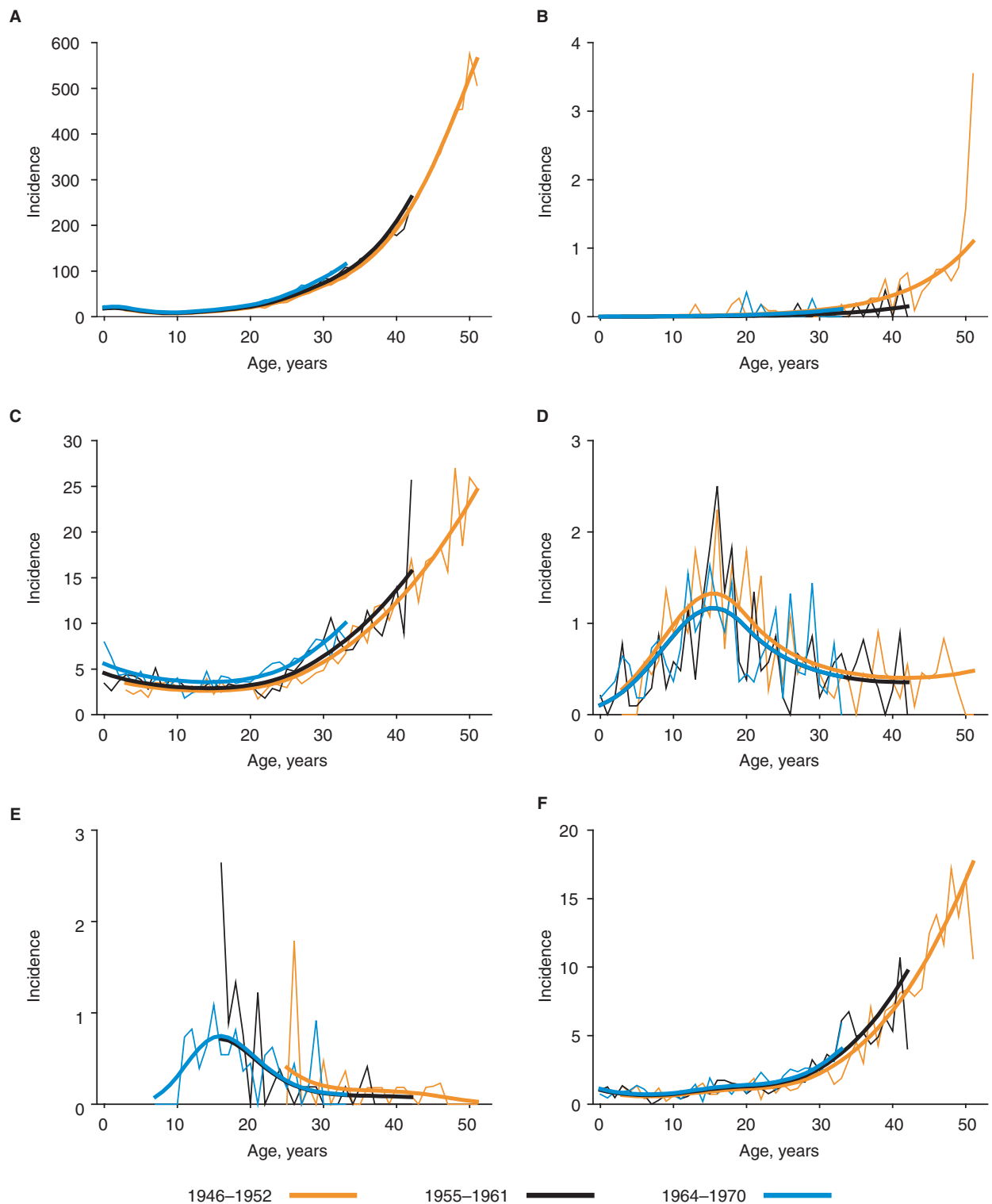
1955–1961 birth cohort, who were vaccinated at approximately 9 months of age or soon thereafter (exposed as infants); and the 1964–1970 birth cohort, who were unexposed as they were born after vaccines were cleared of SV40.

Using data obtained from the Danish Cancer Registry, FIGURE 1 depicts the incidence of all cancers combined and several specific cancer types of interest for the three birth cohorts. For each cancer outcome, the two SV40-exposed cohorts had similar incidence compared with the unexposed cohort. Indeed, the cohort exposed to SV40-contaminated vaccine as infants had slightly lower risk than the unexposed cohort for some outcomes, even though neonatal exposure to SV40 carries the greatest cancer risk in experimental animal models [4]. Data for mesothelioma were limited by small numbers ( $n = 57$  in all three cohorts) but the small number of cases itself indicates a lack of effect of vaccination on risk for this malignancy.

#### Engels & colleagues

A recent study evaluated the relationship between maternal receipt of SV40-contaminated poliovirus vaccines during pregnancy and cancer risk in the subsequently born children [23]. Engels and colleagues used data collected in the Collaborative Perinatal Project (CPP), a US-based cohort study that enrolled pregnant women between 1959 and 1966. The cohort comprised of 54,796 children born to these 44,621 mothers. Overall, 21,649 children (39.5%) had mothers who received poliovirus vaccine (IPV or OPV) during pregnancy; 12,334 children (22.5%) had mothers who received pre-1963 poliovirus vaccine (mostly IPV) and 9315 (17.0%) had mothers who received poliovirus vaccine produced after 1963.

During follow-up to their eighth birthday, 52 children developed cancer, comprising 22 hematologic malignancies, 18 neural tumors and 12 miscellaneous tumors. Compared



**Figure 1.** Age-specific cancer incidence in three Danish cohorts with varying exposure to SV40-contaminated poliovirus vaccine. Incidence data are shown for all cancers combined (A), mesothelioma (B), all brain and nervous system tumors combined (C), osteosarcoma (E), and non-Hodgkin's lymphoma (F). The three birth cohorts are 1946–1952 (exposed to SV40-contaminated poliovirus vaccine as children, orange line), 1955–1961 (exposed to SV40-contaminated poliovirus vaccine as infants, black line) and 1964–1970 (unexposed, blue line). Both observed incidence (thin lines) and fitted estimates derived from a regression model (thick lines) are shown. Incidence is per 100,000 person-years (vertical scales vary). The figure is from Engels and colleagues [33], used with permission from Oxford University Press. SV: Simian virus.

with children whose mothers received no poliovirus vaccine or only post-1963 poliovirus vaccine, children whose mothers had received pre-1963 poliovirus vaccine had an increased risk for hematologic malignancies (relative risk 2.5, 95% CI: 1.1–5.6) and neural tumors (relative risk 2.5, 95% CI: 1.0–6.3). However, 17 of the hematologic malignancies were leukemias, while the most common type of neural tumor was neuroblastoma (seven cases). Laboratory studies have not identified SV40 in childhood leukemia or neuroblastoma specimens [8,34]. Conversely, considering childhood tumors with a proposed relationship to SV40, there was only one CPP child who developed ependymoma and this child's mother had not received poliovirus vaccine during pregnancy; there were no cases of choroid plexus tumor or osteosarcoma.

In order to further explore whether SV40 was responsible for malignancies arising in these children, the investigators obtained paired sera from mothers during pregnancy for 50 of the CPP children with cancer and 200 control children. Two assays were used to measure antibodies to the capsid of SV40. SV40 seroconversion during pregnancy (i.e., the transition from seronegative to seropositive status) was considered a marker for vaccine-related SV40 infection, which was postulated to carry an especially high risk of transmission from mother to child. Notably, SV40 seroconversions in these women were uncommon and not consistently related to either the cancer status of the children or whether the mothers received pre-1963 IPV. Indeed, only six children with cancer had mothers who seroconverted to SV40, according to either assay during pregnancy, and the malignancies that developed in these six children have not otherwise been linked with SV40 (two neuroblastomas, one astrocytoma, two leukemias and one fibrosarcoma).

Given the distribution of cancer types arising in CPP children and the lack of frequent seroconversions among their mothers, it is difficult to attribute these cancers to SV40 infections acquired by the children following their mothers' vaccinations. It appears likely that the relationship between maternal receipt of the poliovirus vaccine and risk of cancer in children was related to risk factors other than SV40, that are yet to be identified.

#### *Rollison & colleagues*

A single study has examined the cancer risk associated with receipt of the US Army's adenovirus vaccine [35]. As described, there is substantial evidence suggesting that early adenovirus vaccines were widely contaminated with SV40. The US Army began routine use of a parenteral adenovirus vaccine in 1960. Adenovirus vaccine was given to all recruits entering Army service between February and April 1960, but production difficulties then interrupted vaccination until August 1960 when it resumed [26]. Recruits were again routinely vaccinated from August 1960 through to May 1961, after which vaccination ceased because supplies once more became limited. Other evidence indicates that this vaccine was withdrawn specifically due to concerns regarding contamination with SV40 [36].

In order to examine the possible relationship between receipt of SV40-contaminated adenovirus vaccine and cancer risk, Rollison and colleagues used Veterans Administration records to identify cases of mesothelioma, brain tumors, non-Hodgkin's lymphoma, colon cancer and lung cancer among men entering the US Army between 1959 and 1961 [35]. The first three diagnoses were considered as types of cases, while colon and lung cancer patients were treated as controls, since these malignancies are not generally thought to be related to SV40. Linkage between Veterans Administration and military databases allowed the assignment of exposure status (i.e., entry into the Army during a period of use or nonuse of SV40-contaminated adenovirus vaccine) to these individuals. Compared with controls, a similar prevalence of exposure to adenovirus vaccine was found in individuals with mesothelioma (odds ratio 1.49, 95% CI: 0.38–5.88), brain tumors (odds ratio 0.76, 95% CI: 0.48–1.20), or non-Hodgkin's lymphoma (odds ratio 0.98, 95% CI: 0.65–1.47). These results indicate that exposure to adenovirus vaccine did not lead to a measurable increase in cancer risk.

#### *Expert opinion*

As described above, epidemiologic studies that have followed recipients of SV40-contaminated vaccines (mostly IPV) have not revealed these individuals to have increased cancer risk. There are several possible biologic explanations of these consistently negative results, which have different implications regarding the possibility that SV40 was transmitted from vaccines and whether the virus causes cancer in humans. One explanation is that SV40 is a common infection in humans, such that a slight increase in the prevalence of infection conveyed by vaccination did not result in a measurable increase in cancer incidence. A related explanation is that SV40 was initially an uncommon infection of humans but vaccination campaigns introduced SV40 into the population, that is, the spread of SV40 from vaccinated to unvaccinated individuals has blunted the differences in subsequent cancer risk.

This explanation is not supported by existing data. First, SV40 does not appear to be a frequent infection of humans. Antibodies to the SV40 capsid are detected in only approximately 5–10% of the general population in the USA and Europe [37]. Furthermore, SV40 antibody responses are mostly low level and appear to arise from cross-reactive antibodies to the common human polyomaviruses BK and JC [37,38]. BK and JC viruses are shed in human urine, while SV40 is shed in macaque urine [39]. However, SV40 is not found in human urine or sewage [40,41]. The lack of detection of SV40 in human urine, while BK and JC are readily detected, argues against frequent replicative infection and ready transmission between humans.

Second, there is no evidence to suggest that recipients of SV40-contaminated vaccines transmitted the virus to others. In the CPP study [23], SV40 antibody responses were somewhat weak, even in mothers who received IPV during pregnancy and whose children developed cancer. This observation is not consistent with transmission of vaccine-acquired SV40 to children, since primary infection of mothers would be expected to be accompanied by a robust humoral antibody response. Without viral replication in

the vaccine recipients, subsequent spread to secondary hosts would not occur. Likewise, OPV recipients may have excreted low levels of SV40 in stool following vaccination [2]. However, as receipt of SV40-contaminated OPV did not cause systemic infection of the vaccinees themselves, it seems unlikely that low-level shedding would have led to fecal–oral transmission of SV40.

Another explanation is that SV40 infection was not conveyed by exposure to contaminated vaccines, either because live SV40 was not present or exposure by this route did not lead to infection. As reviewed above, existing data regarding vaccine production methods and contemporaneous testing of vaccine lots revealed that live SV40 was often present in vaccine products administered from 1955 to 1962. Although poliovirus vaccines produced using the Maitland method were inconsistently contaminated, live SV40 was present almost universally in other vaccines (e.g., Danish IPV and US Army adenovirus vaccine).

While it is clear that vaccines contained live SV40, did exposures to these vaccines lead to actual infections, that is, persistent presence of SV40 within the host? The answer to this question is unknown. Although vaccine-related exposures to SV40 by respiratory or oral routes reportedly led to short-lived shedding of virus [2,42], the shed SV40 could have represented direct passage of inoculated virus without systemic infection. Formalin-inactivated vaccines contained relatively low amounts of live SV40. While SV40 seroconversion rates associated with vaccination generally paralleled the frequency of contamination of the vaccines, seroconversion might have reflected an immune response to an abortive SV40 infection. These considerations raise an overarching question, as yet unanswered, of whether humans can be infected with SV40 at all. However, one might expect that, if humans can be infected with SV40 by any route, exposure by injection on multiple occasions at a very young age, as experienced by many IPV recipients in the 1955–1962 period, would have led to infection.

A final possible explanation is that SV40 does not cause cancer in humans. If receipt of SV40-contaminated vaccines could lead to infection and if SV40 causes cancer in humans, then one would expect vaccine recipients to have an increased risk of cancer. Many IPV recipients were very young children, for whom SV40 exposures might be postulated to convey substantial cancer risk and many received multiple doses of contaminated vaccine [4]. Therefore, one tenable interpretation of the negative data from epidemiologic studies of vaccine recipients is that SV40 does not cause cancer in humans.

In reviewing research published before early 2002, the Institute of Medicine Immunization (IOM) Safety Review Committee recently concluded that the ‘evidence is inadequate to accept or reject a causal relationship between SV40-containing polio vaccines and cancer’ [3]. In particular, the Committee was concerned that epidemiologic studies of vaccine recipients were ecologic, that is, exposure to SV40 or SV40-contaminated vaccines was not actually measured but was instead inferred from group characteristics, such as birth year, that serve as surrogates for vaccine exposure. As these surrogates are imperfect, ecologic studies are often considered inadequate for guiding inference.

However, it can be argued that the epidemiologic studies described above are not actually ecologic studies. As noted, certain vaccines during 1955 to 1962 were widely contaminated with live SV40. Exposure to these vaccines was either documented explicitly (e.g., in the study of Cleveland neonates or the CPP study) or could be reliably determined because almost all individuals in well-defined demographic groups received these vaccines (e.g., Denmark, where almost all children were vaccinated with IPV, or the US Army, where all Army recruits in certain periods received adenovirus vaccine). No other route of infection has been demonstrated for SV40. If exposure and lack of exposure to SV40-contaminated vaccines can be accurately identified on the basis of birth year or other characteristics, then follow-up studies of vaccine-exposed cohorts cease to be ecologic studies. Three of the epidemiologic studies discussed above were published after the Committee’s report and thus were unavailable for their review.

Epidemiologic studies of recipients of SV40-contaminated vaccines thus do have etiologic implications; they indicate that either SV40 does not readily infect humans or, following infection, does not cause cancer. These conclusions are in accord with those reached in case-control studies of cancer that have used serologic markers of SV40 exposure or infection [38,43,44]. In those studies, the prevalence of antibodies to SV40 capsid was similarly low in cases and controls (approximately 5–10%), demonstrating an absence of association between SV40 and cancer. Furthermore, among seropositive individuals, the level of SV40 antibody was low and could mostly be attributed to cross-reactive antibodies to BK and JC viruses [37]. In another study, antibodies to the SV40 T-antigen were measured in non-Hodgkin’s lymphoma cases and controls [45]. Only 6% of cases and 5% of controls had detectable antibody. Antibodies were again weak compared with animals with SV40-induced tumors, who made much stronger responses to T-antigen.

It has been difficult to reconcile these convincingly negative epidemiologic studies with laboratory studies describing the detection of SV40 DNA in human tumors [8–11]. As mentioned, other studies have not detected SV40 DNA in these same tumor types [12–16]. Unfortunately, methodologic difficulties with some studies have clouded the interpretation of the laboratory data [3]. Many studies have not incorporated negative controls into their experiments or masked the case-control status of specimens. When detected, SV40 DNA appears to be present at a very low copy number (i.e., substantially less than one copy per tumor cell) [14,15,46]. This observation is inconsistent with animal models for SV40-induced malignancies, where SV40 T-antigen expression is detected within each tumor cell [47–49], indicating the presence of viral DNA in each tumor cell. Of note, SV40 DNA has been incorporated into over 200 plasmids used in laboratories worldwide and contamination of PCR experiments by plasmids remains a possible explanation for positive findings [50]. Only two multicenter studies have attempted to evaluate SV40 DNA in a masked experiment. In one study, the participating laboratories failed to reproducibly find SV40 DNA

in any mesothelioma specimen [16]. In the second study, SV40 DNA was detected in most mesothelioma specimens, but the positive specimens varied among laboratories and masked negative controls were not included [51].

Recently, Lopez-Rios and colleagues described results of experiments supporting PCR contamination as a plausible explanation for at least some of the many reported detections of SV40 DNA [15]. Using two sets of PCR primers from the T-antigen region of SV40, they amplified SV40 DNA sequences from 56–62% of mesothelioma specimens. However, they observed that their results were inconsistent across experiments and that negative control specimens were occasionally positive as well. Realizing that their PCR primers would amplify SV40 sequences that had been incorporated into multiple cloning vectors, Lopez-Rios and colleagues then selected alternative PCR primers from an intron region of T-antigen absent from plasmids [50]. Using this new set of primers, only 6% of mesotheliomas were positive for SV40 DNA and the amount of SV40 appeared to be very low. Finally, they utilized an additional set of primers that spanned two discontinuous regions of SV40 DNA that had artificially been spliced together in constructing plasmids. This set of PCR primers amplified SV40 sequences in 36% of mesothelioma specimens. Remarkably, however, in each case of amplification, the DNA fragment length corresponded to that predicted by the joining together of the two distant regions in plasmids, rather than the much longer sequence, including the intervening region, that was found in SV40 itself. The authors concluded that the inconsistent, low-level detection of SV40 DNA in mesothelioma specimens in their laboratory, and perhaps other laboratories, was likely to be the result of contamination of PCR reagents or tissue specimens by cloning vectors [15]. Likewise, recent studies that have used immunohistochemistry have failed to identify the presence of T-antigen in tumor cells [15,52]. Therefore, it remains uncertain whether SV40 DNA is actually present in human tumors. As this question is important in resolving whether SV40 might cause cancer in humans, the IOM urged the development of improved, standardized methods for SV40 detection in tumors and the application of these methods in rigorously designed experiments [3].

In conclusion, the epidemiologic studies of vaccine recipients offer useful biologic information and a somewhat reassuring public health message, namely, that contamination of vaccines with SV40 did not lead to a measurable increase in cancer risk. However, consideration of these epidemiologic data also provides an opportunity to pause and reflect on what might have been. Indeed, if the inadvertent widespread contamination of the vaccine supply with this virus (which causes cancer in laboratory animals) did not cause an increase in cancer risk, then we have truly been very fortunate.

Recognizing that the history of SV40 contamination of vaccines highlights an inherent risk of contamination of vaccines and other biologic products with adventitious agents, the IOM called for the development of a Vaccine Contamination Prevention and Response Plan in the USA [3]. While the US Food and Drug Administration regulates licensed vaccines to ensure general safety and sterility, in calling for this plan, the IOM noted that a comprehensive US response and communication system for dealing with vaccine contamination does not exist at present. The IOM suggested that the plan have several components: routine assessment of vaccines for possible contamination; notification of public health officials, healthcare providers and the public if contamination occurs; and identification and surveillance of recipients of contaminated vaccines for adverse health outcomes. The development of a comprehensive plan will require substantial discussions among government officials and policy makers, researchers, vaccine manufacturers and the general public.

#### Five-year view

As the question of whether SV40 causes cancer in humans remains controversial, it is difficult to predict the state of the field in 5 years time. Additional epidemiologic studies of recipients of contaminated vaccines are possible and would have longer follow-up for cancer outcomes, but these seem unlikely to resolve the controversy. Newly developed assays for antibodies against SV40 capsid and T-antigen should be very useful for additional case-control studies of cancer. Further negative data from case-control studies may prove very convincing. It is hoped that we will also have improved standardized methods for the detection of SV40 in human tissues, which are needed to determine whether SV40 is truly present in tumors.

#### Key issues

- Simian virus (SV)40 was a frequent contaminant of poliovirus vaccine and other less widely used vaccines during the period of 1955 to 1962. Receipt of these vaccines is the major known route of human exposure to SV40.
- SV40 transforms cells *in vitro* and causes cancer in animals. Some laboratories have detected SV40 DNA in human tumors but other laboratories have not confirmed the findings.
- Follow-up studies of individuals who received SV40-contaminated poliovirus vaccine have not found an increased risk of cancer.
- These epidemiologic studies of vaccine recipients indicate that SV40 exposures do not readily lead to infection or that SV40 infection does not cause cancer in humans.
- The negative findings of epidemiologic studies of vaccine recipients are supported by case-control studies, which demonstrate a lack of SV40 antibodies in individuals with cancer.
- The Institute of Medicine noted a need for the development of improved assays for detection of SV40 in human tissues.
- The Institute of Medicine called for the development of a comprehensive US plan to prevent vaccine contamination and respond to potential contamination events when they arise.

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