Review of the health consequences of SV40 contamination of poliomyelitis vaccines, and in particular a possible association with cancers.

Prepared by:

Professor Yvonne Cossart, AO
Bosch Professor of Infectious Diseases,
University of Sydney

14th December, 2004
Abstract

The published papers concerning the human health risk of vaccines contaminated with SV40 virus falls into three groups: those published in the 1960s when the virus was discovered, a second group dating from the period when the two related human viruses BK and JC were described and the third recent period when molecular techniques were applied to the problem.

Group 1:
SV40 was discovered in 1960 and shown
- To be a common infection in healthy rhesus monkeys
- To belong to the polyoma virus family
- To cause tumours (especially ependymomas, osteosarcomas, mesotheliomas and lymphomas) when injected into baby hamsters
- To be incompletely killed by the heat and formalin treatment used to inactivate polioviruses during “Salk” vaccine manufacture
- To be capable of infecting human recipients of contaminated vaccine
- To be capable of transforming human cells into cancer cells in the laboratory

Immediate steps were taken to free the vaccine seed cultures of SV40 and to ensure that all future batches of vaccine (both the inactivated “Salk” and the then prototype attenuated “Sabin” types) were made in monkey kidney cultures free of SV40. This was accomplished in 1963.

Many millions of children and adults had already been inoculated with polio vaccine before these measures were fully effective. It is not known which of the early batches actually contained infectious doses of SV40, but tests of recipients showed that many produced SV40 antibodies. This could be the result of either SV40 infection or “immunisation” by the killed SV40 in the vaccine.

Concern focussed on the risk to very young children but no increased risk of cancer was found in follow up of over 1000 vaccinees. As the tumour types induced in hamsters are rare these studies were supplemented with much larger studies comparing cancer registry data for children born (and presumably mostly immunised) during the period between introduction of polio vaccine and eradication of SV40 from manufacture (ie 1957-63) and children born within the preceding or subsequent five year periods. These studies were reassuring, although it was recognised that the follow up was not sufficiently long term to detect a risk of the cancers such as mesothelioma which occur in middle age and beyond. There were also some discrepant reports but in retrospect these (including the single Australian study) have significant design limitations.

Group 2:

The issue was revived in the 1970s when two new human polyomaviruses (BK and JC) were discovered. These cause tumours and degenerative neurological disease in humans. They also share antigens and DNA sequences with SV40 which may cause cross reactions leading to false positive results in diagnostic tests. Surveys showed that serological evidence of infection with the two new agents was common in healthy people and that disease emerged almost exclusively in immune deficient individuals. Attempts to isolate SV40 from human tumours, even by explanting the cells in culture, were generally unsuccessful, but one typical SV40 strain was obtained form a melanoma and two others from diseased brain tissue.
Serological surveys showed that earlier findings that up to 5% of the population had low titre SV40 antibody were mostly, if not entirely, due to cross reactions with the much commoner human polyoma viruses.

It was thus concluded that while SV40 involvement in human tumours could not be absolutely denied it must be very rare indeed.

Group 3:

The most recent group of publications has reported the use of molecular techniques to detect SV40 DNA in tumours. The results have been conflicting, some studies showing no positives others a high proportion. Most workers have focussed on detection of the viral oncogene (T antigen) and/or its expression. Persistence of these sequences integrated into the host cell genome would be expected from experimental studies of polyomavirus induced tumours of other species. Unfortunately the SV40 sequences of interest are widely used as tools in molecular laboratories creating a very substantial risk of cross contamination when testing tumour samples. This casts doubt on these studies which has not yet been resolved. Another new avenue of research has revealed that the SV40 oncogene (Tag) acts through complexing with p53 and affects the pRb pathway of cell cycle control. Genetic mutations of these control elements makes the cell exquisitely sensitive to SV40 transformation. These mutations occur naturally in the population, and confer cancer susceptibility on individuals who often develop tumours of similar type to those associated with SV40. This may need to be taken into account in future epidemiological studies.

Conclusion:

The literature establishes a plausible mechanism for human carcinogenesis by SV40 virus. Studies of the prevalence of SV40 antibody in the community and the presence of SV40 in human tumours do not absolutely exclude the possibility of rare involvement of the virus in individual cases of cancer, but fail to provide evidence of statistically greater risk for people immunised during the period when SV40 was likely to have been present in polio vaccine. This conclusion has also been reached by several international review panels.
Discovery and Background

In 1960 Sweet and Hilleman\textsuperscript{1} described "vacuolating agent", a previously unrecognised virus derived from monkey kidney cell cultures intended for vaccine production. It was named because of an unusual cytopathic effect on kidney cell cultures from grivet (Cercopithecus) monkeys although most isolates came from apparently normal kidney cultures derived from healthy rhesus monkeys. Soon afterwards the new virus was designated "simian virus 40" (SV40) under a scheme proposed by a group of international collaborators working in research groups, regulatory bodies and vaccine manufacturers to define safety standards for the manufacture of vaccines in cell culture systems (the properties of the first 57 of these are described by Hull RN in "The Simian Viruses" 1968\textsuperscript{2}).

Adventitious agents were a concern because it was not clear whether they might have different inactivation kinetics to the vaccine virus, and, in addition, a number of the newer vaccines consisted of living attenuated virus and could not be subjected to conventional inactivation procedures. Polio vaccine was the first human vaccine to be made in cell culture.

The new virus was soon shown to belong to the papova group of viruses\textsuperscript{3} which included the wart (papilloma) viruses and polyoma, an obscure virus of mice which seldom caused disease in the wild, but once grown to high concentrations in the laboratory could cause many different tumours when injected into mice or hamsters. SV40 was soon shown to produce tumours when injected into hamsters\textsuperscript{4,5}, and to be able to transform human cells in culture\textsuperscript{6}. Surveys of old world monkeys imported into the US and Europe by medical research organisations and vaccine manufacturers showed that almost 70% of rhesus monkeys were SV40 antibody positive, and that high levels (up to $10^9$ infectious doses of virus/ml) could be found in cultures of their kidneys\textsuperscript{7}. This gave rise to considerable concern about the long term risk to millions of children who had received doses of poliovaccine containing SV40 since some viral infectivity might remain even in inactivated (Salk) vaccine treated with formalin for sufficient time to kill polio itself.

Characteristics of SV40 virus

The physical and chemical properties of SV40 are typical of the polyoma family. Table 1. Notable features are the shape and size (icosohedral, 40 nm diameter) and the genetic organisation which encodes 3 different proteins (VP1-3) which are incorporated into the virus particles, as well as two important proteins (the large and small T antigens) which interact with the growth regulatory pathways in the infected cell. These T antigens are potential causes of unregulated growth by infected cells and subsequent tumour production.

SV40 is substantially more resistant to formalin inactivation than poliovirus\textsuperscript{8,9} but the treatment with 1:4000 formalin used to inactivate poliovaccine would reduce the titre by $>99\%$ over 50 hours\textsuperscript{10}.

The polyoma virus family

Many animal species harbour their own specific polyomaviruses, Table 2. In general the polyoma of one species grows poorly or not at all in cells or animals of different species, but there are important exceptions such as the ability of bovine polyomavirus to infect primate cells. Polyomavirus infections have a variety of clinical manifestations. The best known are
nephritis and ureteric stenosis, progressive brain disease and tumours which may be of many different pathological types. However the great majority of infections are asymptomatic. The pathologic potential of human polyomaviruses was not recognised until unexplained disease in immune suppressed patients were investigated and characteristic virus particles were found in the lesions. There proved to be two different human polyomaviruses designated JC (originally found in the brain of patients with progressive multifocal leukoencephalopathy\textsuperscript{11}) and BK (which originated in a ureteric tumour)\textsuperscript{12}. Antibody studies showed that many adults had been infected with these viruses\textsuperscript{13}. This pattern of asymptomatic lifelong infection in most members of the community with disease occurring only in a few individuals, usually with inadequate immune function, is now known to be typical of the entire family of viruses.

When polyomaviruses of different species are compared viruses from closely related hosts (such as humans and other primates) are more alike than those from evolutionarily distance hosts eg mice and birds. These resemblances mean that there is significant cross reactivity between diagnostic reagents developed for SV40, BK and JC virus detection (including T antigen detection\textsuperscript{14,15} and antibody measurement\textsuperscript{16,17}).

Growth of SV40

SV40 infection of a cell may lead to three different outcomes.

Virus growth:

In “permissive” cells SV40 grows relatively slowly and the distinctive vacuolation of the host cell and presence of nuclear inclusion bodies do not develop until 1-2 weeks after inoculation. Electron microscopy reveals packed arrays of virus particles in the nucleus. Virus production leads to cell death. Virus growth has two phases, the “early” phase when the virus “large T” and “small t” genes are translated and the late phase when VP1-3 and new viral DNA are produced and assembled into infectious particles. All the virus proteins are synthesised in the cytoplasm then quickly transported to the nucleus where they can be detected by specific staining.

Abortive infection:

SV40 DNA may persist in cells which are unable to support production of new viral particles. The early antigens are produced, but both viral DNA and antigens may be in low concentration. Infected cells may however become highly permissive and produce large amounts of virus if cultured \textit{in vitro}. Such viral DNA can sometimes be rescued by transfection of the cell with the T antigen gene from a different but closely related member of the polyomavirus family. This has been shown experimentally for SV40, BK and JC viruses.

Transformation:

SV40 DNA may become integrated into the genome of the host cell. If the sequences encoding the viral T antigen are intact they can be translated and the unregulated antigen expression leads to increased cell turnover. When multiple copies are integrated malignant transformation of the cell is especially likely to ensue.
Mechanism of oncogenesis by polyomaviruses – role of the T antigen

The large T antigen performs two main functions during virus replication. The first involves binding with a specific “origin of replication” in the virus DNA to establish virus DNA synthesis and the second is to promote cell division by interaction with P53 a tumour suppressor protein important in the control of cell division. New virus DNA is then synthesised by the cellular DNA polymerases in concert with the copying of the cellular DNA prior to cell division. In rare instances the combination of T antigen and P53 becomes “fixed” and normal control of the cell cycle is lost. This is usually a result of over-expression of T antigen from multiple copies of the virus gene sequences integrated in the host cell genome. Other molecular pathways for transformation by T antigen have been proposed where only part of the gene is transcribed and Tag is not detectable\(^1\). However these refer to very artificial experimental systems and remain unproven under natural conditions\(^2\).

Other virus antigens also play a significant role in virus replication and transformation at least under some circumstances, but they do not appear to act without large T activity.

Immune response to polyomavirus infection

Infected individuals produce antibodies against the virus structural proteins and the T antigens. Tumour-bearing animals have very high levels of antibody to the large T antigen.

Specific antibodies neutralise infection and have been used to free vaccine seed stocks of the virus. The production of neutralising antibody by infected animals down regulates virus production but does not eliminate infection\(^3\).

Cell mediated antibody responses have been poorly characterised despite the significance implied by the emergence of polyoma viruses and clinical symptoms in immune suppressed subjects.

Methods of detection of SV40 in polio vaccine and tumours

The original discovery of SV40 was made by growing the virus in cell culture, and this remains the gold standard because it correlates with infectivity. It is laborious, time consuming and requires availability of cells which are known to be highly permissive. In situations where the virus is down regulated explanting the cells in laboratory culture will often activate virus growth which can then be much more readily detected.

Other ways of detecting productive infection are by staining cells with labelled antibodies against the virus proteins (VP1 is especially useful as it bears the receptor through which the virus and cell interact) and by finding virus particles by electron microscopy. These methods require fairly high level of virus presence, but are particularly valuable for showing what proportion, and which cell types are infected.

T antigen and viral DNA are also present but do not define virus production or infectivity. These last two markers (viral DNA and T antigen) are present in abortively infected and transformed cells, where viral particles and structural proteins are absent.
Integration of viral DNA is the hallmark of transformed cells where it characteristically causes over-expression of T antigen. Because of its stability, viral DNA can be detected even after effective formalin inactivation of all infectivity as in routine paraffin blocks prepared for histopathology. The Southern blot methods which demonstrate integration require high levels of viral DNA in the specimen. Newer methods which amplify DNA sequences form parts of the virus (eg the polymerase chain reaction, PCR) are much more sensitive but can seldom be adapted to show whether the virus sequences are integrated.

Over the years all these methods have been used to assess the infectivity and oncogenic potential of polio vaccine. Infectious SV40 cannot always be obtained from experimentally induced tumours or from natural tumours in infected hosts but early studies showed that both viral DNA and T antigen were easily found. More recent studies based on PCR amplification of SV40 sequences have detected "viral" DNA in tumours which lack T antigen or its messenger RNA. It is these studies which have reignited concern about the risk of cancer after administration of SV40 contaminated vaccine. No plausible biological mechanism has been put forward to explain how these sequences might transform cells without producing the effector (T antigen) and recently it has been suggested that these PCR results may be false positives due to contamination of the specimens with DNA sequences from molecular experiments which often use SV40 sequences as tools. This is supported by the demonstration that many of the reported positives from tumours have been found to have a deletion mutation which is found in the experimental plasmids but not in infectious virus.21

Detection of SV40 in polio vaccine

Two types of polio vaccine have been used in Australia. Inactivated ("Salk") vaccine was used from 1958 onwards but was almost completely supplanted by live attenuated ("Sabin") vaccine after 1960. These vaccines were manufactured from different virus seeds and in different production facilities. Safety testing involved quite different criteria. The critical factor defining the safety of inactivated vaccine was the demonstration that the vaccine contained no viable poliovirus. For the attenuated vaccine the issue was the lack of virulence when injected intra-cerebrally into monkeys.

However for both vaccines all of the early virus stocks and vaccines were produced in primary rhesus or cynomolgous monkey kidney cell cultures. The large batches of cells needed were made by pooling cells derived from groups of 10-30 animals, and it has been estimated that at least 70% of these pools yielded SV40 in the early 1960s. Direct testing of vaccine lots for viable virus has been very limited and many batches probably contained a mixture of killed and viable virus.22

It should noted that the US requirement for polio vaccine seeds (and production lots) be free of SV40 came into effect in 1961 but existing stocks of the "old" product were used until expiry in 1963. In other countries the date of exclusion of SV 40 from vaccines in use varied, but there was little delay in most western countries.

The natural course of SV40 infection in monkeys

SV 40 infection is common amongst wild caught rhesus monkeys. The proportion of antibody positive animals rises with increasing age. Infectious virus can be recovered from their
issues after they have been grown in culture, but spontaneous disease is very uncommon. Animals which are immunosuppressed or have SIV infection however develop tumours and a ML like syndrome. SV40 virus has been demonstrated in these tumours using primers to detect several different regions of the virus. The mode of transmission is uncertain. Excretion in the urine and inhalation of environmental aerosols is a likely scenario.

Polyoma infection of mice is classically transmitted vertically but there is little evidence about this for SV40 under natural conditions.

SV40 infection of humans

After the discovery of SV40 recipients of polio vaccine were tested for the appearance of neutralising antibody to the virus. This was readily detected but it could be interpreted as the result of antibody response to inactivated SV40 rather than infection. No systematic attempts were made to recover virus so it is not clear whether the persistence of this antibody is due to persistent low level infection or simply an inadvertent “vaccination” against SV40. In both the US and Europe about one third of recipients of attenuated oral vaccine containing 100-1000 plaque forming units of SV40 were shown to excrete virus in the faeces for several weeks but there was little production of antibody. This evidence of infectability of humans by SV40 was directly confirmed by inoculation of volunteers, using the nasal route. Lastly, surveys for SV40 antibody in people from regions where contact with rhesus monkeys is common showed higher seroprevalence of anti-SV40 antibody than surveys in Europe or the New World.

More recent seroprevalence surveys using recombinant virus like particles derived from BK JC and SV40 show a low prevalence of reactors (about 6%) with the SV40 reagents but almost all of these disappear when the sera are absorbed with BK and JC human polyomavirus particles.

There are also reports that about 6% of hospitalised children in 1999 – ie born long after use of the SV40 contamination of vaccine - had neutralising antibody to SV40. This raises the issue of persistence of SV40 in the human population unrelated to current (or even past) use of SV40 contaminated vaccines. There are small scale studies of sera for children prior to the introduction of polio vaccine which show less than 5% reactors. Vertical transmission would be the most probable mechanism, but the published studies of babies born to mothers who were inoculated with SV40 containing vaccines are uninformative because vaccination histories of the infants are not provided.

Molecular studies of SV40 in cancer patients often include normal control subjects and there are reports of detection of SV40 DNA from peripheral blood lymphocytes and other tissues of some antibody positive controls.

There are very few reports of isolation of infectious SV40 from non-vaccinated humans, the most convincing from two patients with progressive multifocal leukoencephalopathy and one with melanoma. None of these three patients had been immunised with inactivated poliovaccine.
Detection of SV40 in human tumours

There is only one clear report of the isolation of SV40 from a human tumour (see above), but there are numerous reports of the demonstration of SV40 antigens or DNA sequences in tumours (including one where full length SV40 DNA was rescued by transfection of susceptible cells). Interest has naturally focussed on the tumour types known to be caused by SV40 in hamsters, the most susceptible species. These are primary brain cancers (especially ependymomas), mesotheliomas, osteosarcomas and non-Hodgkins lymphomas. The studies fall into two groups; observational and case control.

Ependymoma and choroid plexus tumours:

These rare brain tumours occur mainly in infants and very young children. Very few if any patients with these tumours in current US series could have received poliovaccines during the period of known SV40 contamination in 1957-63. All but one of 14 published studies of SV40 in human brain tumours report some positive findings in tumours and far fewer positives in controls. A variety of techniques have been used, ranging from culture of tumour cells to PCR detection of virus sequences (Table 3). This technical disparity in both sensitivity and target makes it hard to compare studies but the positive findings with all methodologies strengthens the overall case for the presence of papovavirus, or at least papovavirus genes in some brain tumours – especially ependymomas and choroid plexus tumours. It is however not absolutely clear that the viral antigens and sequences are derived from SV40 rather than the related human papovaviruses BK and JC. Recently a study which analysed the nucleotide sequences of three different regions of the putative SV40 genome showed that they were all SV40 related. However sequence results generated by other groups have strongly suggested contamination of the samples with amplified product derived from one or other of the widely used experimental vector plasmids, which have incorporated SV40 T antigen gene sequences, leading to false positive results.

Interpretation of findings from different centres is also complicated by the differences in geographical distribution of rhesus monkeys and putative human exposure to SV40. There are also very great discrepancies in the rate of detection of papovavirus sequences when comparable methods are used, and controversy about interpretation of both positive and negative findings.

Osteosarcoma:

Carbone et al studied samples from patients with Li-Fraumeni syndrome, a genetic disorder where only one functional allele of p53 is present. These patients develop many tumours including osteosarcoma and SV 40 T Ag might be especially potent as a carcinogen in this situation since it binds directly to p53. 11/36 of the osteosarcomas were positive for SV40 T antigen sequences. Widening of the study to p53 normal patients in different countries gave mixed results which could be attributable to environmental or technical factors. Osteosarcoma is mainly a disease of children and adolescents so any cohort effect of injections of SV-40 in 1957-63 should already be apparent.

Mesotheliomas:

Detection of SV40 sequences in mesotheliomas has been reported from many centres, but others, including an International Working group report negative findings.
The presence of "SV40 sequences" has been attributed to technical shortcomings of the methods which permit non-specific amplification or hybridisation under the experimental conditions used. On the other hand the sensitivity of many of the methods might be insufficient to detect short segments of viral DNA integrated into the host tumour cell DNA. In any event the presence of the large T protein in these samples is exceptional and even this may be attributable to cross reactivity with BK or JC virus. SV40 detection was reported in asbestos positive and negative specimens.\textsuperscript{54}

Mesothelioma mainly occurs in middle aged or old patients and it is likely that most of the recipients of SV-40 containing polio vaccine are only now entering the age span of greatest risk.

Non-Hodgkin Lymphoma:

Infective causes have long been postulated for lymphomas with Epstein Barr virus and Human Herpes Virus type 8 as prime suspects. Recently SV 40 Tag nucleotide sequences have also been detected in a large minority of non-Hodgkin lymphomas in both HIV positive and negative individuals.\textsuperscript{55} As with the other tumours technical issues about these findings are not fully resolved.\textsuperscript{56} Seroprevalence tests of lymphoma patients and controls in Spain detected SV 40 antibody in 6\% of controls and 9\% of cases. The difference was not significant.

Summary:

Despite the technical disputes about the detection methods there is agreement that a small proportion of each of these four tumour types contain papovavirus DNA sequences which are probably derived from SV40 rather than BK or JC virus.

Far fewer of the tumours show evidence of SV40 large T antigen, the putative effector, so that the role of these sequences in carcinogenesis is unproved. It has even been suggested that they are derived from the chromosomal DNA of the transformed cells rather than any virus.

Seroprevalence studies show that only a low proportion of patients have detectable antibody and even this may mainly reflect cross reactivity with the other human papovaviruses.

\textbf{Is the presence of SV40 in human tumours evidence of a causal association?}

In the laboratory SV40 is able to transform human cells in culture\textsuperscript{58, 59, 60} and these transformed cells were capable of forming tumours when injected into volunteer patients with terminal cancer. Apart from the ethical issues raised by the latter experiments it is likely that these patients were immune suppressed by their disease and/or treatment so the implications for \textit{de novo} establishment of cancer by infection with SV40 are unclear.

\textbf{Direct follow up of individuals known to have received SV40 contaminated polio vaccine}

The number of immunised patients directly followed up is very small. Mortimer \textit{et al}\textsuperscript{61} reported a 17-19 year follow up of 1073 children given contaminated poliovaccine in 1960-
1962. No excess cancer mortality was found and 45 of the females had themselves born children, none of whom had birth defects.

**Incidence of human cancers before and after introduction of polio vaccine**

During a short period of five years SV 40-containing polio vaccine was administered by subcutaneous injection to most members of the community in Western countries. This exposure can be related to cancer registry data in an attempt to show if the virus caused tumours in recipients. There are at least nine studies of this type in the literature. Because the tumours are relatively rare (less than one case/100,000pa) the large population of the US provides the most informative data.

Ependymoma and choroid plexus tumours would be expected to show an effect of SV40 most clearly because they occur in early childhood, but the numbers in the direct follow up studies were insufficient to detect any effect of vaccination. The US birth cohort which received the SV-40-contaminated vaccine (born 1960-1964) had almost the same rate as that observed a decade earlier and actually less than the 1955-9 and 1965-9 cohorts. However in Denmark a more than twofold increase in the incidence of ependymoma but not other cancers was observed in follow up of infant vaccinees.

Osteosarcomas occur mainly in teenage years and they too showed no cohort effect in either the US or other studies.

Most (80%) cases of mesothelioma are associated with asbestos exposure but it has been postulated that SV40 may be important not only in asbestos negative cases, but also as a cofactor in asbestos positive cases. Mesothelioma is mainly a disease of older people so the earlier study was not conclusive. The authors revisited the issue in 2003 when analysis of birth cohort trends in incidence of mesothelioma in relation to the age-specific prevalence of vaccination during the period 1957-61 showed that although both sexes were equally vaccinated mesothelioma was only one fifth as common in women, and that the relative risk was lower in the age groups with the highest exposure to vaccine.

Non-Hodgkin lymphomas are a heterogenous category amongst lymphoproliferative disorders and their high prevalence in immune suppressed individuals means that their prevalence is rising, making interpretation of epidemiological cohort data very difficult.

**Summary:**

The cohort data fails to establish a definite link between receipt of SV-40 containing vaccines and cancer even for the types of malignancy most associated with experimental tumours in hamsters. On the other hand the rarity of the tumours and the likelihood of confounding environmental effects prevent clear rejection of the hypothesis. This conclusion has been reached by multiple individual studies conducted at intervals since 1963 when SV-40 was effectively eliminated from vaccine production. Both the prestigious Vaccine Safety Review Committee of the Institute of Medicine and a more recent meta-analysis also came to this conclusion.
The Australian evidence

There is no doubt that inactivated polio vaccine released in Australia by CSL, the only local manufacturer, or from imported (US) sources contained SV-40 although details from the manufacturers are unavailable. The exact number of individuals immunised is also unavailable, but attenuated vaccine supplanted inactivated vaccine from 1967 onwards. During the intervening period the Australian population has grown substantially, mainly due to immigration from countries where injectable polio vaccine was not used at all and most of the adult immigrants would in any case never have received any polio vaccine. On the other hand many of these immigrants are from SE Asia where there is substantial human contact with rhesus monkeys, the natural reservoir of SV40. Lack of information about the ethnic origin, time of migration and vaccination history impedes interpretation of current cancer registry data about tumour types associated with experimental oncogenesis by SV40.

There are two published studies of importance:

The first, published in 1968, compared immunisation status as recorded from the medical records of 816 children with malignancies treated between January 1958 and May 1967 with controls matched with age, sex and time of hospital admission. Overall the vaccinated children had a 40% greater risk of developing cancer. In retrospect this study could have been biased by exclusion of 87 cancer patients with uncertain vaccination histories as well as a further 110 who were under one year of age on admission. The statistically significant difference between the cancer children aged over 1 year and their controls would completely disappear of the 87 exclusions lacking a clear vaccination history were really unvaccinated against polio. This casts some doubt on the authors assertion of a clear cancer risk in the vaccinated cohort. In retrospect another issue is the high probability that the cancer cases included a high proportion of subjects with p53 or pRb mutations. These were unknown at the time of the study but are both associated with childhood cancer and with susceptibility to the oncogenic effects if SV40 T antigen in experimental systems.

The second reports detection of SV40 DNA from mesothelioma cell lines and biopsies. All the lines and patients yielded positive results and no confirmatory sequencing was done so it is not possible to rule out contamination or other false positives as discussed above.

The Australian evidence is consistent with the studies reported in the International literature.
Table 1

**Properties of polyomaviruses**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>approx 40nm diameter</td>
</tr>
<tr>
<td>Structure</td>
<td>icosahedral capsid composed of 72 capsomeres made up of 3 virus proteins (VP1-3). These VP encoded proteins are species specific but on denaturation of the particles a 'group antigen' is revealed which is common to all polyomaviruses</td>
</tr>
<tr>
<td>Genome</td>
<td>ds circular DNA approx 5000 base pairs</td>
</tr>
<tr>
<td>Density</td>
<td>1.34</td>
</tr>
</tbody>
</table>
### Table 2
Polyomaviruses and their natural hosts

<table>
<thead>
<tr>
<th>Host species</th>
<th>Virus</th>
<th>Natural Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>K</td>
<td>pneumonitis</td>
</tr>
<tr>
<td>Mouse</td>
<td>Polyoma</td>
<td>tumours</td>
</tr>
<tr>
<td>Rabbit</td>
<td>rabbit kidney vacuolating virus</td>
<td>?nil</td>
</tr>
<tr>
<td>Bovine*</td>
<td>bovine polyoma</td>
<td>?nil</td>
</tr>
<tr>
<td>Rhesus monkeys</td>
<td>SV40</td>
<td>?nil</td>
</tr>
<tr>
<td>Cynomolgous mokleys</td>
<td>CPV</td>
<td>nephritis, enterit</td>
</tr>
<tr>
<td>Baboon</td>
<td>SA12</td>
<td></td>
</tr>
<tr>
<td>African green monkey</td>
<td>lymphotropic papovavirus</td>
<td></td>
</tr>
<tr>
<td>Budgerigar</td>
<td>avian polyomavirus</td>
<td>fledgling disease</td>
</tr>
<tr>
<td>Geese</td>
<td>goose polyoma</td>
<td>haemorrhagic nephritis</td>
</tr>
<tr>
<td>Human</td>
<td>BK virus</td>
<td>nephritis etc</td>
</tr>
<tr>
<td>Human</td>
<td>JC virus</td>
<td>neurological disease</td>
</tr>
</tbody>
</table>

*originally called "stump tailed macaque virus – but it had contaminated the primate cultures through use of bovine serum in the growth medium."
### Table 3

**Detection of SV40 in Brain Tumours**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Date samples</th>
<th>Country</th>
<th>NAT* method</th>
<th>NAT result</th>
<th>Tag** method</th>
<th>Tag result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergsagel59</td>
<td>?1990s</td>
<td>USA</td>
<td>PCR conserved T region</td>
<td>10/32 cases</td>
<td>Polycl rabbit</td>
<td>7/11 cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/12 neuroblastoma</td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td>Lednicky35</td>
<td>? subset prev study</td>
<td>USA</td>
<td>PCR T and VP1</td>
<td>14/17 choroid plexus and 10/11 ependymomas pos with both primer sets</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Zhen70</td>
<td>frozen tumour samples</td>
<td>China</td>
<td></td>
<td>8 ependymomas + 2 choroid plexus and 55 other brain tumours 8 normals</td>
<td>Monocl mouse anti-T Pab101 western blot plus silverstain</td>
<td>10/10 33/55 0/8</td>
</tr>
<tr>
<td>Suzuki71</td>
<td>Frozen tumour samples</td>
<td>Japan</td>
<td>Tag primers Sequence product</td>
<td>4/13 ependymomas 3/18 other tumour 1/22 1/22 controls</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* nucleic acid detection (NAT)  
** SV40 T antigen detection (Tag)
References

1 Sweet BH and Hilleman MR (1960) The Vacuolating Virus, SV40. PSEBM 105:420-27
2 Hull RN The Simian Viruses (1968) Springer-Verlag Vienna
21 Lopez-Rios F Illei PB Rausch V Landanyi M (2004) Evidence against a role for SV40 infection in Human mesotheliomas and high risk of false positive PCR results owing to presence of SV40 sequences in common laboratory plasmids. Lancet 364:1157-66
67 Innis MD (1968) Oncogenesis and polyomavirus vaccine Nature 219:973-5