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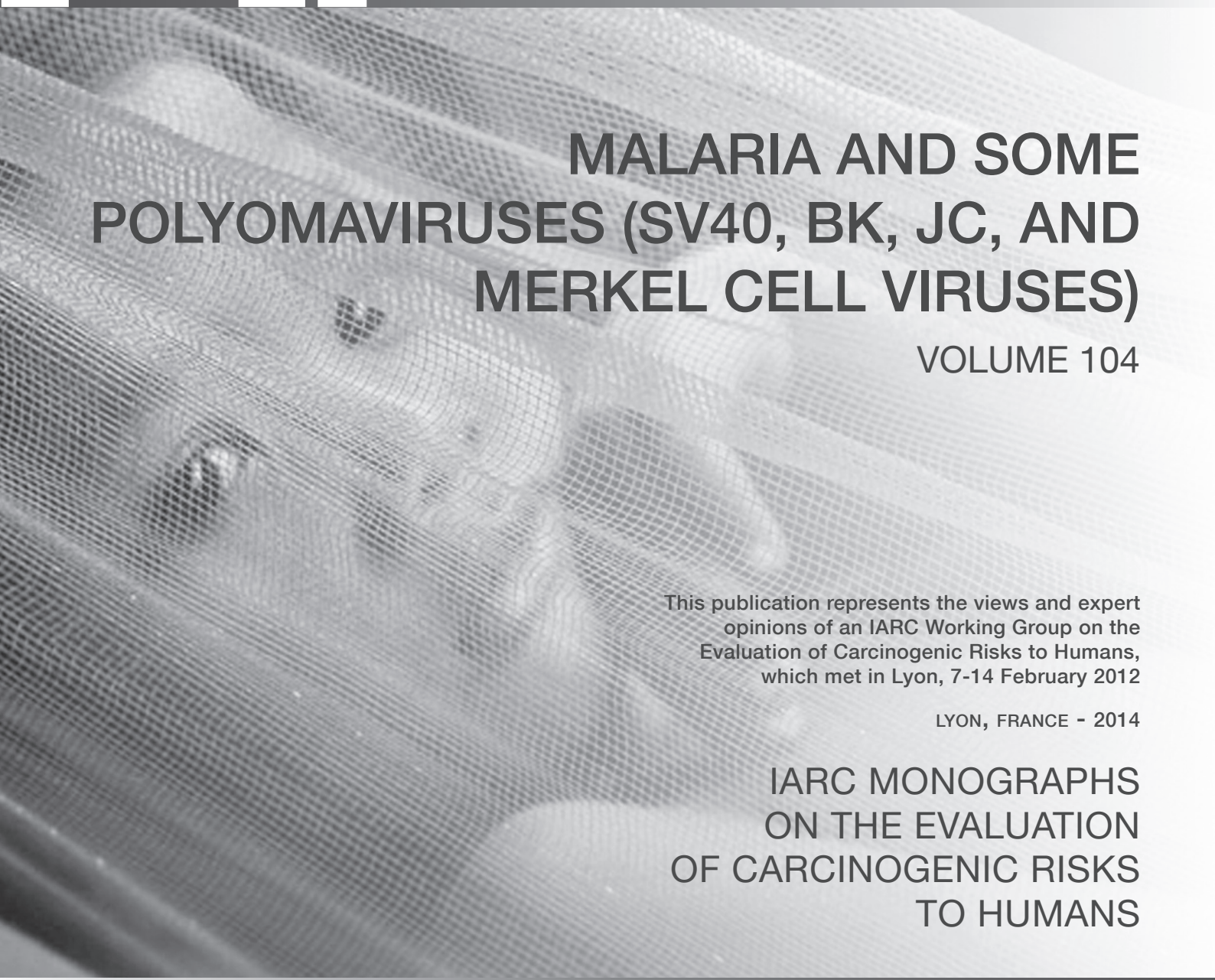

# MALARIA AND SOME POLYOMAVIRUSES (SV40, BK, JC, AND MERKEL CELL VIRUSES)

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# SIMIAN VIRUS 40

## 1. Exposure Data

### 1.1 Host range and tissue tropism

The natural host of simian virus 40 (SV40) is the rhesus macaque, in which the virus is commonly maintained as a chronic infection of the kidney epithelium and other tissues. Although SV40 infection is not associated with known disease sequelae in immunocompetent animals, in immunodeficient animals SV40 can cause central nervous system disease as well as renal and pulmonary disease (Sheffield *et al.*, 1980; Lednicky *et al.*, 1998; Axthelm *et al.*, 2004; Dang *et al.*, 2008). Under laboratory conditions, SV40 can be transmitted to a variety of non-native hosts. The outcome of SV40 infection is classified as either permissive or non-permissive. In the permissive case, the full replicative life-cycle of the virus is observed, with lysis of infected cells. In the non-permissive case, viruses gain entry and may begin to express early viral proteins, but the full infection cycle is blocked at viral DNA replication steps and late gene expression does not occur. Whether the outcome of SV40 infection is permissive or non-permissive is determined largely by the species of host cells used. Productive infections are elicited in African green monkey kidney cell lines (BSC or CV1) and some human cell lines, whereas non-permissive infections are seen in mice and mouse-derived cell lines (reviewed in Atkin *et al.*, 2009). The inability of SV40 to complete its life-cycle and lyse infected cells from some species can have important biological consequences. For example, non-permissive mouse and rat cells can survive SV40 infection and go on to be stably transformed by the virus. The ability to support virus replication is also cell-type-specific, even within susceptible hosts. Detection of SV40 or SV40-like DNA sequences and proteins in human tissues is discussed in Section 1.2.

### 1.2 Methods for the detection of SV40 infection

The presence of SV40 infection has been examined by detection of the SV40 genome by polymerase chain reaction (PCR) techniques, and by detection of anti-capsid antibodies by neutralization tests or by enzyme-linked immunosorbent assay (ELISA) using recombinant virus-like particles (VLPs). Immunohistochemical staining and in situ hybridization methods are important to confirm the presence of SV40; however, these methods have not been used in epidemiological studies on SV40.

#### *1.2.1 PCR-based methods*

The methods for detection of SV40 DNA are not standardized, and differences in methods may be responsible for some of the discrepancies observed between studies (Strickler, 2001, see also Section 4.3.1). Laboratory contamination, lack of specificity of PCR techniques, or

cross-amplification with other polyomaviruses might also explain some of the varying results. To reduce false-positive results, PCR primers should be able to differentiate between the SV40 genome and SV40 sequences present in common laboratory plasmids and in some cell lines (López-Ríos *et al.*, 2004). [These lower-risk primers might still amplify contaminating SV40 DNA from cell lines carrying the integrated SV40 genome (Cohen & Enserink, 2011).] Using such primers dramatically reduced the number of samples detected containing SV40 DNA. These findings were confirmed by Manfredi *et al.* (2005). Since most investigators used primer sets (e.g. SV5/SV6, TA1/TA2) that did not amplify BK polyomavirus (BKV) and JC polyomavirus (JCV) DNA but that recognized SV40 sequences present in many laboratory plasmids, contamination from cloning and expression vectors could have occurred if appropriate precautions were not taken (López-Ríos *et al.*, 2004). The absence of standardized PCR methods and quality control procedures, including appropriate blinding and relevant controls, may explain some of the varying findings reported for SV40 DNA detection. When detected, SV40 is present at low copy number (David *et al.*, 2001).

### *1.2.2 Detection of SV40 antibodies*

Serological studies on SV40 have for many years had to rely on neutralization tests (plaque neutralization or microwell neutralization). The recent detection of viral capsid antibodies has been greatly facilitated by the development of recombinant VLPs. ELISAs have been developed using VLPs or capsomers produced in baculovirus systems, and recombinant yeasts or bacteria, and these have become common serological tests for SV40.

In humans, anti-SV40 antibody reactivity is observed largely in BKV- and/or JCV-positive samples, and in those samples with BKV and JCV antibody titres higher than those observed against SV40. The reactivity of rhesus macaque sera and human sera with VLPs of SV40, BKV, and JCV provides unambiguous evidence of immunological cross-reactivity between these viruses (Carter *et al.*, 2003; Engels *et al.*, 2004a).

Competitive inhibition studies have shown that the reactivity to SV40 in human sera is often eliminated by pre-incubation with BKV and JCV VLPs, suggesting that all of the SV40 reactivity observed in humans is due to cross-reactivity to BKV and JCV (Carter *et al.*, 2003; de Sanjosé *et al.*, 2003; Viscidi *et al.*, 2003; Rollison *et al.*, 2005a; Viscidi & Clayman, 2006; Kjaerheim *et al.*, 2007). In contrast, in SV40-infected monkeys, SV40 reactivity was completely blocked by SV40 VLPs but not by BKV and JCV VLPs (Carter *et al.*, 2003; Engels *et al.*, 2004a). To identify specific SV40 reactivity, it is necessary to perform competitive inhibition assays by pre-incubation of SV40-reactive human sera with an excess of BKV and JCV VLPs.

## 1.3 Epidemiology of infection

### *1.3.1 Transmission of SV40 infection to humans*

#### *(a) Transmission from animals to humans*

The understanding of the transmission and pathogenesis of SV40 in humans is poor and largely incomplete. Natural exposure to SV40 in humans is considered a rare event, and antibodies to SV40 can be demonstrated only in people who have been in contact with monkeys (Shah, 1966; Engels *et al.*, 2004a) or who had received, in the past, SV40-contaminated poliovirus vaccine.

Infection with SV40 is common in rhesus macaques, where it causes a silent but lifelong infection (Sweet & Hilleman, 1960). Initial lytic infection is controlled by the immune system, and later the virus persists in the kidney, where it can be reactivated under conditions of immunosuppression (Horvath *et al.*, 1992). There is evidence that SV40 is shed in the urine of infected

animals, which have higher titres of neutralizing antibodies (Shah *et al.*, 1969; Lednicky *et al.*, 1998; Carter *et al.*, 2003; Minor *et al.*, 2003). About 80–100% of captive adult rhesus macaques and 50% of captive baboons (Jones-Engel *et al.*, 2006; Simon 2008; Westfall *et al.*, 2008) are SV40-seropositive. SV40 has been recovered from urine, faeces, and food residues taken from infected animals. Transmission seems to occur through the environment rather than through direct contact between animals (vertical or perinatal transmission or contact within the gang) (Minor *et al.*, 2003; Bofill-Mas *et al.*, 2004).

The route of human exposure to SV40 is unclear. SV40 shedding in macaque urine or occupational injuries or specific incidents (bites, scratches, or splashes) suggest that humans could be at risk of infection with SV40. However, human cells are less susceptible to SV40 replication than are monkey cells (Shein & Enders, 1962a; Shah *et al.*, 1969; O'Neill & Carroll, 1981; O'Neill *et al.*, 1990). Low levels of neutralizing antibodies have been reported in 27% of workers at monkey-export companies in India, suggesting that humans can be exposed through contact with infected animals (Shah, 1966). SV40 seroreactivity confirmed by competitive inhibition experiments was also detected in 10% of zoo workers with regular exposure to monkeys, compared with 3% of workers with infrequent exposure (Engels *et al.*, 2004a). However, Carter *et al.* (2003) showed that although 6.6% of subjects from a normal population were SV40-positive, the reactivity disappeared after pre-incubation with BKV or JCV VLPs; hence, SV40 infection of humans via monkeys remains controversial.

### *(b) Transmission through vaccines to humans*

SV40 was discovered as a contaminant in poliovirus vaccine. Formalin-inactivated poliovirus vaccine (injected) and live poliovirus vaccine (oral) were prepared in primary kidney cells of rhesus and cynomolgus macaques, some of which were from monkeys naturally infected with SV40. Safety testing of the vaccine preparations led to the identification of a new virus called SV40, in 1959 (Sweet & Hilleman, 1960; Eddy *et al.*, 1961, 1962), 5 years after the formalin-inactivated vaccine was licensed. Moreover, although poliovirus vaccine batches approved in 1961 and later were required to be free of SV40, batches approved earlier were not recalled. Thus, the use of inactivated poliovirus vaccine (IPV) containing SV40 may have continued until 1963 (Shah & Nathanson, 1976).

The extent of contamination of poliovirus vaccine stocks with viable SV40 has not been established. For the IPV (Salk vaccine), the formalin inactivation process used for inactivation of poliovirus was shown to also inactivate the SV40 virions (Sweet & Hilleman, 1960), although residual infectious SV40 survived at low levels in some vaccine preparations (Gerber *et al.*, 1961). Live SV40 could still be cultured after formaldehyde treatment, although titres were lower (Gerber *et al.*, 1961; Fraumeni *et al.*, 1963; Engels *et al.*, 2003a). It is estimated that up to 30% of the killed vaccine lots contained live SV40 (Shah & Nathanson, 1976). Pre-licensure candidate oral poliovirus vaccines (OPVs) were presumably all contaminated with SV40. The candidate OPVs were field-tested from 1958 to 1960 at certain sites in various countries, including Mexico (Sabin OPV); Colombia, Costa Rica, Nicaragua, and Uruguay (Lederle Laboratories OPV); and Croatia, Poland, and the Republic of the Congo (Koprowski OPV). Only small trials were carried out in the USA. The commercially licensed Sabin OPV was supposedly SV40-free after 1963.

The Russian OPV was prepared from the pre-licensure Sabin viral strains and was used widely in 1959 and later in the Russian Federation and several countries in eastern Europe (Butel, 2012). The Russian vaccine was subsequently also provided to other countries and was likely contaminated with SV40 until the late 1970s (Cutrone *et al.*, 2005). In these vaccines, the heat treatment in the presence of $MgCl_2$ used

to attenuate poliovirus did not completely inactivate SV40 (Cutrone *et al.*, 2005). In addition, military recruits in the USA from 1959 to 1961 who received SV40-contaminated adenovirus vaccines and the several thousand individuals in the USA who received the experimental live poliovirus vaccine by the oral route in earlier clinical trials were at risk of exposure to live SV40 (Cutrone *et al.*, 2005).

Briefly after vaccination with contaminated OPV, small amounts of infectious SV40 were identified in stools of some of the immunized neonates and infants (Melnick & Stinebaugh, 1962). However, no SV40 antibody response was observed in recipients, suggesting either that the SV40 virions present in the attenuated poliovirus vaccine were not infectious in humans or that the virions had little or no infectivity in humans (Morris *et al.*, 1961; Shah & Nathanson, 1976). Seroconversion was documented after parenteral vaccination with contaminated vaccines (Sweet & Hilleman, 1960; Gerber, 1967; Engels *et al.*, 2003a). [However, it is unclear whether the development of SV40 antibodies represented an infection or instead an exposure to inactivated viral proteins.]

#### *(c) Transmission from humans to humans*

Many routes of SV40 circulation in humans have been speculated, including faecal–oral, respiratory, and mother-to-child, but there is scant evidence to support any of these routes. Patel *et al.* (2008) reported that SV40 DNA was detected in 9.1% of tonsils in immunocompetent children (Table 1.1). However, the detection of SV40 DNA was not confirmed using low-contamination-risk primers as defined by López-Ríos *et al.* (2004), since SV40 DNA was not detected in tonsil tissues from 57 children and was detected in adenoid tissue in only 1 (1.3%) of 80 children and at a very low copy number (Comar *et al.*, 2010). Thus, there are no supporting data that SV40 is transmitted by the respiratory route.

In a recent study (Abedi Kiasari *et al.*, 2011), SV40 was identified in blood and/or urine of two transplant patients, suggesting that SV40 could cause infection in such patients.

### *1.3.2 Prevalence of SV40 infection in the general population*

#### *(a) Prevalence of SV40 DNA*

Detection of SV40 DNA in immunocompromised or immunocompetent subjects was reported in many studies. The most recent studies, all of which used DNA detection by PCR, are presented in Table 1.1.

About half of the studies did not detect SV40 DNA in the examined groups. In other studies, the SV40 prevalence varied from 1.3% to 25.6%, with SV40 being detected in the urine, stools, blood, lungs, and tonsil tissues. The heterogeneity of the findings is illustrated by the results of SV40 DNA detection in the urine or kidneys (Shah *et al.*, 1997; Li *et al.*, 2002a; Manfredi *et al.*, 2005; Vanchiere *et al.*, 2005a). SV40 was not detected in two studies (with 166 and 20 patients, respectively) (Shah *et al.*, 1997; Manfredi *et al.*, 2005) and was detected in 1 (4.5%) of 22 patients and 4 (5.6%) of 72 patients in the other two studies (Li *et al.*, 2002a; Vanchiere *et al.*, 2005a). SV40 DNA has been found in the kidneys, in the urine, in stools, in peripheral blood cells, in the pituitary, and in lung/pleural samples (Woloschak *et al.*, 1995; Galateau-Salle *et al.*, 1998; Martini *et al.*, 1998, 2002; Yamamoto *et al.*, 2000; Li *et al.*, 2002a, b; Vanchiere *et al.*, 2005a, b, 2009). The mean SV40 viral load in the urine of transplant patients was 0.001 times that of BKV and JCV, and the frequency of SV40 viruria was much lower than that of BK and JC viruria (Thomas *et al.*, 2009).

[One reason for the differences between studies may be that the numbers of subjects investigated were generally low, with characteristics that were variable due to the fact that most

**Table 1.1 Detection of SV40 DNA in control subjects from recent studies (past 15 years)**

| Reference | Study location | Type of specimen | Method | No. of samples | Detection of SV40 DNA *n* (%) |
|---|---|---|---|---|---|
| Shah *et al.* (1997) | USA | Urine | PCR + SB | 166 | 0 |
| Galateau-Salle *et al.* (1998) | France | Lung/pleural | PCR + SB | 25 | 4 (16.0%) |
| Griffiths *et al.* (1998) | United Kingdom | Blood | PCR + SB + Seq | 10 | 0 |
| Martini *et al.* (1998) | Italy | Blood | PCR + SB + Seq | 50 | 3 (6.0%) |
| Procopio *et al.* (1998) | Italy | Pleural | PCR + SB | 20 | 0 |
| Strizzi *et al.* (2000) | Italy | Pleural | PCR + SB | 7 | 0 |
| Yamamoto *et al.* (2000) | Japan | Blood | PCR + SB + Seq | 64 | 3 (4.7%) |
| David *et al.* (2001) | USA | Blood | PCR + SB + Seq | 115 | 18 (15.7%) |
| Strickler (2001) | USA | Lung tissue | PCR + SB + Seq | 25 | 0 |
| Li *et al.* (2002a) | USA | Blood | PCR + SB + Seq | 22 | 5 (22.7%) |
| Li *et al.* (2002a) | USA | Urine | PCR + SB + Seq | 22 | 1 (4.5%) |
| Martini *et al.* (2002) | Italy | Blood/bone tissue | PCR + SB + Seq | 43 | 11 (25.6%) |
| Shivapurkar *et al.* (2002) | USA | Blood/lymphoid tissue | PCR + SB + Seq | 28 | 0 |
| Vilchez *et al.* (2002) | USA | Blood/lymph nodes | PCR + SB + Seq | 107 | 0 |
| Vivaldi *et al.* (2003) | Italy | Blood | PCR + SB + Seq | 20 | 5 (25.0%) |
| Ozdarendeli *et al.* (2004) | Turkey | Thyroid tissue | PCR + Seq | 83 | 0 |
| Heinsohn *et al.* (2005) | Germany | Blood | qPCR | 149 | 2 (1.3%) |
| Manfredi *et al.* (2005) | United Kingdom | Kidney | PCR + SB | 20 | 0 |
| Vanchiere *et al.* (2005a) | USA | Urine | PCR + Seq | 72 | 4 (5.6%) |
| Meneses *et al.* (2005) | Costa Rica | Lymph nodes, tonsils | PCR + SB | 51 | 0 |
| Kjaerheim *et al.* (2007) | Norway | Blood | PCR + Seq | 147 | 0 |
| Ziegler *et al.* (2007) | Switzerland | Blood | qPCR | 39 | 0 |
| Heinsohn *et al.* (2009) | Hungary | Blood | qPCR + Seq | 166 | 30 (18%) |
| Patel *et al.* (2008) | USA | Tonsils | PCR | 220 | 20 (9.1%) |
| Pancaldi *et al.* (2009) | Italy | Blood | qPCR | 148 | 24 (16.2%) |
| Vanchiere *et al.* (2009) | USA | Stool | qPCR + Seq | 110 | 2 (1.8%) |
| Comar *et al.* (2010) | Italy | Tonsil/adenoid tissues | qPCR | 80 | 1 (1.3%) |
| Campello *et al.* (2010) | Italy | Blood and intestine | RT-qPCR | 91 | 0 (0.0%) |
| Bolognesi *et al.* (2005) | Italy | Lymphocytes | PCR | 22 | 0 (0%) |

PCR, polymerase chain reaction; qPCR, quantitative PCR; RT-qPCR, real-time qPCR; SB, Southern blot hybridization; Seq, sequencing; SV40, simian virus 40

studies were designed for the evaluation of the prevalence of SV40 in different cancers.]

There is no clear evidence of variations in prevalence according to the country, the sex of the subjects, or the presence of immunosuppression. However, in some studies SV40-positive subjects were older than SV40-negative subjects (Patel *et al.*, 2008). Most of the discrepancies observed are thought to be due to variations in the PCR techniques used, either producing false-positive results or not being sensitive to detect SV40 when present. False-positive results have been reported to be due to the use of high-contamination-risk primers as defined by López-Ríos *et al.* (2004). High-contamination-risk primers are primers that amplify a region (nucleotides 4100–4713) of the gene encoding the SV40 large T-antigen (LT) present in many common laboratory plasmids. In contrast, primers not included in this region are considered as low-contamination-risk primers. In particular, the high SV40 prevalence reported in three studies was from one group from Italy that used high-contamination-risk primers (López-Ríos *et al.*, 2004). In two recent studies using low-contamination-risk primers, SV40 was detected in 24 of 148 buffy coats of healthy blood donors (Pancaldi *et al.*, 2009) and in none of 78 samples of peripheral blood mononuclear cells or 57 tonsil tissues from Italian children, but was found in only 1 (1.3%) of 80 adenoid samples (Comar *et al.*, 2010).

With the recent advent of high-throughput sequencing technologies, it has become possible to perform random-primed deep sequencing of microbial nucleic acids from human specimens. This approach can reveal the presence of known, as well as previously undiscovered, viral sequences. Human "metagenomics" studies have not reported the presence of SV40 in a variety of specimen types, including faecal and sewage samples from developing countries, although numerous papillomaviruses and other polyomaviruses, including JCV, human polyomavirus 6 (HPyV6), HPyV7, and HPyV9, have been detected (Jones *et al.*, 2005; Finkbeiner *et al.*, 2008; Blinkova *et al.*, 2009; Victoria *et al.*, 2009; Reyes & Jiang, 2010; Cantalupo *et al.*, 2011; Minot *et al.*, 2011; Sauvage *et al.*, 2011).

### *(b) Prevalence of SV40 antibodies*

#### *(i) Detection by neutralization assays*

In recent years (since 1998), nine studies have investigated anti-SV40 antibodies by plaque neutralization or microwell neutralization tests (Table 1.2). Seroprevalence varied from 2% to 12%, with the highest values observed in the USA (Jafar *et al.*, 1998; Rollison *et al.*, 2003; Viscidi *et al.*, 2003) and with higher values in adults than in infants and children. In addition, significantly lower titres of neutralizing SV40 antibodies are detected in humans compared with monkeys, the natural host. In one study (Kjaerheim *et al.*, 2007), pre-incubation of all positive sera with BKV and JCV VLPs abolished the neutralization activity, indicating that neutralization assays are not specific.

#### *(ii) Detection by ELISA using VLPs or peptides*

In six serological studies using immunoenzymatic assays using VLPs and without pre-incubation or pre-adsorption with BKV and JCV VLPs, SV40 antibodies have been detected in a limited percentage of subjects (7.7–10.5%) (Table 1.3). In one of these studies (Engels *et al.*, 2004c), the SV40 reactivity was further analysed by pre-incubation of sera with SV40, BKV, and JCV VLPs. The findings indicated that specific SV40 reactivity was detected in only 8 (22.9%) of the 35 SV40-reactive human sera investigated, compared with all 8 (100%) of the SV40-reactive macaque sera used as positive controls. In one recent study, SV40 antibodies were investigated using capsid viral protein 1 (VP1) and VP2/VP3 synthetic peptides (Corallini *et al.*, 2012). Although the specificity of the test is unknown, ELISA reactivity was observed in 18% of the blood donors investigated.

## Table 1.2 Detection of SV40 antibodies in healthy control subjects using neutralization tests (1998–2007)

| Reference | Study population | Study location | Method | No. of subjects | Anti-SV40 antibodies | |
|---|---|---|---|---|---|---|
| | | | | | *n* (%) | Titre (range) |
| Jafar *et al.* (1998) | Adults | USA | Plaque neutr. | 180 | 21 (11.7%) | 1:20–1:320 |
| Butel *et al.* (1999) | Children | USA | Plaque neutr. | 337[a] | 20 (5.9%) | 1:40–1:320 |
| Butel *et al.* (2003) | All ages | Hungary | Plaque neutr. | 589 | 17 (3.0%) | 1:20–1:300 |
| Butel *et al.* (2003) | All ages | Czech Republic | Plaque neutr. | 350 | 7 (2.0%) | 1:10–1:50 |
| Knowles *et al.* (2003) | All ages | United Kingdom | Microwell neutr. | 2435 | 79 (3.2%) | 1:8–1:256 |
| Engels *et al.* (2004c) | Mothers | USA | Plaque neutr. | 187 | 8 (4.3%) | ≥ 1:10 |
| Minor *et al.* (2003) | Blood donors (age 9–96 yr) | United Kingdom | Microwell neutr. | 2054 | 93 (4.5%) | ≥ 1:8 |
| Minor *et al.* (2003) | ? | Sierra Leone | Microwell neutr. | 62 | 2 (3.2%) | ≥ 1:8 |
| Minor *et al.* (2003) | Infants (age < 1 yr) | Democratic Republic of the Congo | Microwell neutr. | 168 | 4 (2.4%) | ≥ 1:8 |
| Minor *et al.* (2003) | Children (age 3–4 yr) | Morocco | Microwell neutr. | 419 | 14 (3.3%) | ≥ 1:8 |
| Minor *et al.* (2003) | Children (age 1–6 yr) | Poland | Microwell neutr. | 923 | 26 (2.8%) | ≥ 1:8 |
| Rollison *et al.* (2003) | Adults | USA | Plaque neutr. | 88 | 10 (11.4%) | ≥ 1:10 |
| Viscidi *et al.* (2003) | Blood donors | USA | Plaque neutr. | 130 | 13 (10.0%) | ≥ 1:40 |
| Nurgalieva *et al.* (2005) | Individuals (age 11–69 yr) | Kazakhstan | Plaque neutr. | 307 | (4.9%) | 1:10–1:500 |
| Kjaerheim *et al.* (2007) | Adults | Norway | Microwell neutr. | 147 | 4 (2.7%)[b] | ≥ 1:50 |

[a] All the subjects in this study were patients with a clinical diagnosis of disease.

[b] None of the samples were found positive after pre-incubation with BKV and JCV VLPs.

BKV, BK polyomavirus; JCV, JC polyomavirus; neutr., neutralization; SV40, simian virus 40; VLPs, virus-like particles; yr, year

**Table 1.3 Detection of SV40 antibodies using SV40 VLPs, or capsomers, or peptides in healthy control subjects in recent studies (since 2003)**

| Reference | Study population | Study location | Method | No. of subjects | Anti-SV40 antibodies *n* (%) |
|---|---|---|---|---|---|
| de Sanjosé *et al.* (2003) | Adults | Spain | VLP-based ELISA | 587 | 56 (9.5%) |
| Carter *et al.* (2003) | Adults | USA | VLP-based ELISA | 415 | 32 (7.7%) |
| Viscidi *et al.* (2003) | Healthy adults | USA | VLP-based ELISA | 128 | 13 (10.2%) |
| Engels *et al.* (2004a) | Adults (2 groups) | USA | VLP-based ELISA | 622 | 65 (10.5%) |
| | | | VLP-based ELISA | 615 | 59 (9.6%) |
| Engels *et al.* (2004c) | Pregnant women | USA | VLP-based ELISA | 200 | 6 (3.0%) |
| Rollison *et al.* (2005a) | General population controls | USA | VLP-based ELISA | 434 | 41 (9.5%) |
| Corallini *et al.* (2012) | Blood donors | Italy | Synthetic peptides from capsid, ELISA | 855 | 154 (18.0%) |
| Rollison *et al.* (2005a) | General population controls | USA | VLP-based ELISA adsorbed BKV/JCV | 434 | 7 (1.6%) |
| Lundstig *et al.* (2005) | Pregnant women and hospital-based adults | Sweden | VLP-based ELISA adsorbed BKV/JCV | 241 | 19 (7.9%) |
| Lundstig *et al.* (2005) | Children (age 1–13 yr) | Sweden | VLP-based ELISA adsorbed BKV/JCV | 288 | 22 (7.6%) |
| Kjaerheim *et al.* (2007) | General population and blood donors | Norway | Capsomers/Luminex adsorbed SV40 | 147 | 16 (10.9%) |
| Carter *et al.* (2003) | Adults | USA | VLP-based ELISA blocked BKV/JCV | 415 | 0 (0.0%) |
| Engels *et al.* (2004b) | Adults | North America | VLP-based ELISA blocked SV40/BKV/JCV | 145 | 4 (2.8%) |
| Kean *et al.* (2009) | Adults | USA | Capsomers/Luminex blocked BKV/JCV | 1501 | 32 (2.1%) |
| Kean *et al.* (2009) | Individuals (age 1–20 yr) | USA | Capsomers/Luminex blocked BKV/JCV | 721 | 16 (2.2%) |

BKV, BK polyomavirus; ELISA, enzyme-linked immunosorbent assay; JCV, JC polyomavirus; SV40, simian virus 40; VLP, virus-like particle; yr, year

SV40 antibodies were investigated in three studies by pre-adsorption of the sera with BKV and JCV VLPs, and in one study by pre-adsorption with SV40 (Lundstig *et al.*, 2005; Rollison *et al.*, 2005a; Kjaerheim *et al.*, 2007). The reported seroprevalence of 1.6–10.9% suggests that the tests were not as specific as tests performed by blocking the SV40 reactivity with high concentrations of VLPs.

In four other studies (Carter *et al.*, 2003; Engels *et al.*, 2004a; Kean *et al.*, 2009), the nature of the SV40 immunoreactivity was examined by competitive inhibition studies in which SV40-reactive human sera were pre-incubated with VLPs or capsomers of BKV, JCV, and SV40. Specific SV40 seroprevalence of 0–2.8% was reported in these studies. It should be noted that in the large study by Kean *et al.* (2009), no variation was observed according to age or sex.

### *1.3.3 Diseases associated with SV40 infection*

#### *(a) Rhesus monkeys*

Progressive multifocal leukoencephalopathy (PML) and severe nephritis (Horvath *et al.*, 1992; Chrétien *et al.*, 2000; Dang *et al.*, 2005) as meningoencephalitis (Newman *et al.*, 1998; Simon *et al.*, 1999) were observed in simian immunodeficiency virus (SIV)-infected rhesus macaques that were seropositive for SV40 before SIV inoculation, or in SIV-infected animals that were inoculated with SV40.

#### *(b) Humans*

No disease has been clearly associated with SV40 infection in either immunocompetent or immunocompromised humans. However, SV40 DNA has been identified in PML and other diseases.

Peters *et al.* (1980) identified SV40 by indirect immunofluorescence in one case of PML using anti-virion antibodies. [The cerebrospinal fluid cell sample was not investigated by immunofluorescence with anti-JCV antibodies, and data were not confirmed by virus isolation.] Scherneck *et al.* (1981) identified SV40 in a patient with PML by cell culture with CV1 monkey cells inoculated with homogenates of brain of the patient. SV40 antigen was also detected immunohistochemically in one case of PML (Hayashi *et al.*, 1985), but attempts at viral isolation by cultivation on human brain tumour cells were unsuccessful. Brain tissue from two of the PML cases thought to be associated with SV40 was re-examined for the presence of SV40 VP1 DNA sequences by in situ hybridization and PCR using specific primers (Stoner & Ryschkewitsch, 1998). All these techniques failed to confirm the presence of SV40 but identified the presence of JCV. Eizuru *et al.* (1993) also identified the JCV genome in a previously identified case of SV40-associated PML based on LT immunostaining of cells infected with PML tissue. The original identification of SV40 was suspected to be due to the lack of specificity of the antibody used for immunostaining.

In addition, SV40 was recovered by monkey kidney cell culture inoculated with cerebrospinal fluid from a child with anatomical and neurological anomalies (Brandner *et al.*, 1977). However, the SV40 infection could not be confirmed due to the absence of detection of SV40 neutralizing antibodies up to 60 days after detection of the virus. A lung transplant recipient developed end-stage renal failure potentially related to SV40 infection (Milstone *et al.*, 2004). The diagnosis was documented by detecting SV40 DNA sequences (but not BKV or JCV) in the patient's kidney biopsy and urine sample by PCR, Southern blot, and DNA sequencing. Positive immunohistochemistry for SV40 was found in the kidney, and neutralizing antibodies for SV40 were detected in the serum.

SV40 DNA was also identified by PCR in kidney tissue and urine samples of patients with focal segmental glomerulosclerosis (Li *et al.*, 2002b).

# 2. Cancer in Humans

**Methodological considerations: case–control *versus* case-series study designs**

Numerous studies have reported the prevalence of markers of infection by polyomaviruses in tumour tissues or blood obtained from humans with cancer. Many of these studies included specimens from individuals without cancer as "controls," but such studies were not generally considered by the Working Group as case–control studies, given the convenience sampling strategies used or the lack of comparability of exposure measurement between comparison groups. Specifically, convenience sampling of controls led to the possibility that the control subjects were not representative of the source population. Also, the comparison of tumour tissues in cases with normal tissues (such as blood, urine, or biopsies of normal tissues) in controls may also be biased because it is uncertain whether polyomaviruses are uniformLy present in these normal tissues or can be reliably detected by the assays used. However, because these studies contributed information on cancer sites not investigated by the case–control studies, included comparisons with both normal and pre-malignant control tissues, compared tumour tissue with a convenience sample of controls, compared different tissues in cases or controls, and/or presented findings for susceptible populations (i.e. transplant patients), they are considered here as case series.

## 2.1 Prospective studies

Prospective studies include cohort studies that followed up individuals who received SV40-contaminated vaccines, as well as case–control studies that prospectively evaluated biomarkers of SV40 infection using samples obtained before cancer diagnosis/control selection. These studies are summarized in Table 2.1 (cohort studies) and Table 2.2 (prospective case–control studies) and reviewed below.

The premise of the cohort studies is that if SV40 causes cancer, cancer incidence or mortality will be higher in vaccinated cohorts than in unvaccinated cohorts. These studies are strongest when there is convincing documentation that the vaccine under consideration was contaminated with live SV40 and investigators can determine which individuals received the vaccine.

Some investigators have actively followed cohorts that are known to have received SV40-contaminated vaccines. More commonly, however, researchers have used cancer registry data to evaluate cancer risk for different birth cohorts with varying exposure to SV40-contaminated poliovirus vaccines. Defining cohorts on the basis of birth year is a reliable method to assign vaccination status because SV40 was present in killed poliovirus vaccines for a limited period (i.e. from 1955, when widespread vaccination campaigns were initiated, until early 1963, when the remaining contaminated vaccine lots were last used). Poliovirus vaccination campaigns targeted infants and school-age children during these years, and vaccine coverage rates were typically quite high. Candidate live-attenuated oral poliovirus vaccine (OPV), contaminated with higher titres of infectious SV40, were field-tested from 1958 to 1960 at selected sites in countries in the southern hemisphere, eastern Europe, and Africa; the contaminated Russian OPV was used much more widely and for a longer period of time.

A related assumption of the cohort studies of vaccine recipients is that infection with SV40 was less frequent, or had fewer health consequences, in people who did not receive SV40-contaminated vaccines (e.g. individuals born after 1963 for studies of poliovirus vaccination). As reviewed in Section 1, uncertainties remain about routes of SV40 transmission other than via receipt of a contaminated vaccine. Serology-based studies have suggested that SV40 infection

## Table 2.1 Cohort studies of people exposed to SV40-contaminated vaccines

| Reference, location, follow-up period | Total subjects | Exposure assessment | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|
| Fraumeni *et al.* (1963), USA 1950–59 | 9 489 100 | State of residence associated with varying levels of IPV contamination | Leukaemia (mortality) | High-level, low-level, and no contamination | NR | NR<br>Results are presented graphically. Leukaemia mortality was highest in states with vaccine contamination, but differences were apparent before the vaccine was introduced. | Calendar year<br>Results are relevant for short-term risk. Report did not present measures of statistical uncertainty. |
| Geissler (1990), German Democratic Republic 1959–86 | 1 777 104 | Birth cohort with varying exposure to SV40-contaminated OPV | Glioma / glioblastoma<br>Oligodendroglioma<br>Medulloblastoma<br>Spongioblastoma | Born 1959–61 (exposed) *vs* 1962–64 (unexposed) | 52<br>11<br>79<br>93 | NR<br>Results are presented graphically, with suggestion of higher incidence in exposed birth cohort. | Age<br>Report did not present measures of statistical uncertainty. |
| Olin & Giesecke (1998), Sweden 1960–93 | Not stated | Birth cohort with varying exposure to SV40-contaminated IPV | Brain cancers<br>Ependymoma<br>Osteosarcoma<br>Mesothelioma | Born 1946–52 (exposed) *vs* bracketing years (unexposed) | NR | Varying by age and calendar year | Age, calendar year<br>Report did not present measures of statistical uncertainty. |
| Strickler *et al.* (1998), 9 representative areas of USA 1973–93 | NR | Birth cohort with varying exposure to SV40-contaminated IPV | Ependymoma<br>Brain cancers<br>Osteosarcoma<br>Mesothelioma | Born 1956–62 (exposed as infants) *vs* 1964–69 (unexposed) | NR<br>NR<br>NR<br>NR | 1.06 (0.69–1.63)<br>0.90 (0.82–0.99)<br>0.87 (0.71–1.06)<br>3.00 (0.67–13.11) | Age<br>Results for 1947–52 birth cohort (exposed as children) were similar. The number of mesothelioma events was small, limiting the power of this analysis. The same cancer registry data were analysed separately by Fisher *et al.* (1999). |

**Table 2.1 (continued)**

| Reference, location, follow-up period | Total subjects | Exposure assessment | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|
| Strickler *et al.* (1999), 9 representative areas of USA After 1973 | NR | Birth cohort with varying exposure to SV40-contaminated IPV | Medulloblastoma | Born 1956–62 (exposed as infants) *vs* 1964–69 (unexposed) | NR | 0.742 (0.55–1.00) | Age<br>Results for 1947–52 birth cohort (exposed as children) were similar. Report also presents results for Connecticut during 1950–69 for children 0–4 years old. |
| Fisher *et al.* (1999), 9 representative areas of USA 1973–93 | 3 886 342 | Birth cohort with varying exposure to SV40-contaminated IPV | All cancers<br>Ependymoma and choroid plexus tumour<br>Other brain tumours<br>Osteosarcoma<br>Other bone tumours<br>Mesothelioma | Born 1955–59 (exposed) *vs* 1963–67 (unexposed) | 5512<br>18<br>328<br>53<br>89<br>6 | [0.89 (0.86–0.93)]<br>[1.20 (0.60–2.45)]<br>[0.92 (0.79–1.07)]<br>[1.10 (0.74–1.64)]<br>[1.17 (0.85–1.60)]<br>[2.78 (0.64–19.0)] | Age<br>Analysis was limited to ages 18–26 years. Report did not present measures of statistical uncertainty [but these were calculated by the Working Group]. This report used essentially the same cancer registry data as Strickler *et al.* (1999). |
| Carroll-Pankhurst *et al.* (2001), Cleveland, Ohio, USA 1969–96 | 1073 | Culture of live SV40 from IPV and OPV used in trial | All cancers (mortality)<br>Leukaemia (mortality)<br>Testis (mortality) | Receipt of SV40-contaminated vaccine | 4<br>2<br>2 | 1.26 (0.34–3.23)<br>4.19 (0.51–15.73)<br>36.98 (4.47–133.50) | Sex, age, race, calendar year<br>Cancer deaths in cohort compared with general population expected. Study is a follow-up of Fraumeni *et al.* (1970) and Mortimer *et al.* (1981). |

**Table 2.1 (continued)**

| Reference, location, follow-up period | Total subjects | Exposure assessment | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|
| Engels *et al.* (2003a), Denmark | NR | Birth cohort with varying exposure to SV40-contaminated IPV | All cancers | Born 1955–61 (exposed as infants) *vs* 1964–70 (unexposed) | 11 105 | 0.86 (0.81–0.91) | Age<br>Results for 1946–52 birth cohort (exposed as children) were similar. The number of mesothelioma and choroid plexus tumour events was small, limiting the power of this analysis. |
| | | | Mesothelioma | | 6 | 0.48 (0.12–1.83) | |
| | | | Brain cancers | | 630 | 0.81 (0.74–0.90) | |
| | | | Ependymoma | | 45 | 1.25 (0.79–1.98) | |
| | | | Choroid plexus tumour | | 3 | 0.26 (0.06–1.24) | |
| | | | Bone tumour | | 153 | 1.00 (0.78–1.28) | |
| | | | Osteosarcoma | | 26 | 0.95 (0.53–1.71) | |
| | | | Leukaemia | | 711 | 0.96 (0.85–1.08) | |
| | | | NHL | | 480 | 0.93 (0.78–1.11) | |
| | | | Testis | | 1416 | 0.93 (0.84–1.02) | |
| Strickler *et al.* (2003), 9 representative areas of USA 1975–97 | NR | Birth cohort with varying exposure to SV40-contaminated IPV | Mesothelioma | Born 1948–57 *vs* 1936–47 (men)<br>Born 1948–57 *vs* 1936–47 (women) | NR | [0.98 (0.84–1.15)]<br>[1.12 (0.94–1.32)] | Age, calendar year<br>Inclusion of early birth cohorts allowed assessment of mesothelioma risk at older ages. Report includes comparisons of additional birth cohorts not presented in this table. |
| Thu *et al.* (2006), Norway 1953–97 | NR | Birth cohort with varying exposure to SV40-contaminated IPV | NHL | Multiple categories defined by prevalence of vaccine exposure | NR | Differences in birth cohort effects were not correlated with differences in vaccine exposure. | Sex, age, calendar year<br>Results were also null for lymphocytic leukaemia and plasma cell neoplasms. |
| Price *et al.* (2007), Great Britain 1968–2004 | NR | Birth cohort with varying exposure to SV40-contaminated IPV | Mesothelioma (mortality) | Born 1951–55 *vs* 1962–66 (unexposed)<br>Born 1956–60 *vs* 1962–66 (unexposed) | 22<br>9<br>9<br>9 | Men: 2.4 (1.2–5.0)<br>Women: 3.8 (1.0–14)<br>Men: 0.93 (0.39–2.3)<br>Women: 3.5 (0.93–13) | Sex, age<br>The number of mesothelioma deaths was small, limiting the power of this analysis. |

CI, confidence interval; IPV, inactivated poliovirus vaccine; NHL, non-Hodgkin lymphoma; NR, not reported; OPV, oral poliovirus vaccine; SV40, simian virus 40; *vs*, *versus*

## Table 2.2 Nested case–control studies of cancers and SV40

| Reference, study location and period | Total cases Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|---|
| Rollison *et al.* (2003), USA, 1974–2000 | 44<br>88 | Population (same cohort as cases) | Plaque neutralization assay for SV40 antibodies | Brain | Positive | 5 | 1.00 (0.30–3.32) | Age, race, sex, date of blood draw, freeze/thaw history of sample<br>80% of tumours were glioblastomas or astrocytomas. Samples were obtained before cancer diagnosis. |
| Rollison *et al.* (2005a), USA, 1974–2002 | 170<br>340 | Population | ELISA for antibodies against SV40 VLPs | NHL | Positive (any positivity)<br>Positive (low reactivity)<br>Positive (medium reactivity)<br>Positive (high reactivity)<br>Positive after competitive blocking with BKV and JCV VLPs | 33<br>9<br>17<br>7<br>4 | 1.97 (1.03–3.76)<br>1.74 (0.67–4.51)<br>3.30 (1.38–7.91)<br>1.16 (0.43–3.13)<br>1.51 (0.41–5.52) | Sex, race, age, data of blood draw, freeze/thaw status of sample, cohort<br>Some subjects contributed two serum samples. Antibodies were measured before NHL diagnosis. Most SV40 antibodies were non-specific. |

**Table 2.2 (continued)**

| Reference, study location and period | Total cases Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|---|
| Kjaerheim *et al.* (2007), Norway, 1973–2003 | 49<br>147 | Population (same cohort as cases) | ELISA for antibodies to SV40 VP1 | Mesothelioma | Positive | 32 | 1.5 (0.8–2.9) | Age, sex, sample date, county<br>Samples were obtained before cancer diagnosis. |
| | | | | | Positive after competitive blocking with BKV and JCV VP1 | 7 | 1.5 (0.6–3.7) | |
| | | | ELISA for antibodies to SV40 LT | | Positive | 9 | 1.4 (0.6–3.2) | |
| | | | Neutralizing antibodies to SV40 | | Positive | 1 | 0.8 (0.1–6.7) | |
| | | | PCR detection of SV40 DNA in serum | | Positive | 0 | Not defined | |

BKV, BK polyomavirus; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; JCV, JC polyomavirus; LT, large T-antigen; NHL, non-Hodgkin lymphoma; PCR, polymerase chain reaction; SV40, simian virus 40; VLPs, virus-like particles; VP1, capsid viral protein 1

is not common in the general populations of the USA and Europe (see Section 1). To the extent that SV40 does not circulate widely in the general population, receipt of contaminated vaccines represents a uniquely informative exposure. Even if SV40 can be acquired by routes other than vaccine-based exposures, individuals who received SV40-contaminated poliovirus vaccines usually received multiple doses, and exposures to SV40-contaminated poliovirus vaccines typically occurred in infancy or early childhood, when animal models suggest that the greatest susceptibility to cancer occurs (Section 3) (Girardi *et al.*, 1963).

An additional assumption of the cohort studies is that there is no other important cancer risk factor that varies across groups defined by vaccination status. For such confounding to obscure a measurable effect of SV40, the proposed risk factor would have to be common and have a sufficiently strong effect on cancer risk. For example, changes in asbestos exposure over time may affect comparison of mesothelioma incidence rates across birth cohorts. Also, cohort studies that rely on cancer registries for follow-up may be affected by changes over time in the completeness of cancer ascertainment or the classification of cancer subtypes. Finally, most studies have followed cohorts only through the 1990s, and since most poliovirus-vaccine recipients were young children, cohorts of poliovirus vaccine recipients have not reached the age of 50 years. The number of outcomes is thus limited for some cancers, especially mesothelioma, that increase in incidence with age.

For prospective case–control studies, the cases and controls are compared with respect to biomarkers of infection with SV40, typically serum or plasma antibodies. One issue for studies that use assays for SV40 antibodies is that natural infections with BKV and JCV, which are very common in the general population, can lead to production of cross-reacting antibodies. To address this issue, some studies have taken measures to remove cross-reacting BKV and JCV antibodies. The prospective studies have assessed SV40 biomarkers using blood samples before case diagnosis, which eliminates the possibility that the development of cancer affects the biomarkers. However, if the samples were obtained many years before cancer diagnosis, such testing might miss subsequent SV40 infections.

Fraumeni *et al.* (1963) described short-term trends in leukaemia mortality in the USA after exposure to SV40-contaminated inactivated poliovirus vaccine (IPV). In the USA, IPV vaccination campaigns targeting children (mostly aged 6–8 years) began in 1955, and because vaccine supply was limited at that time, only a small number of lots of IPV were distributed to each state. The researchers grouped states in the USA into three categories of SV40 exposure based on subsequently measured contamination of these vaccine lots: none (14 states, with 1.9 million children), low-level (16 states, with 3.7 million children), and high-level (19 states, with 3.9 million children). Among children who were aged 6–8 years in 1955, leukaemia-related mortality during 1950–59 was highest in states with high-level exposure, intermediate in states with low-level exposure, and lowest in states with no exposure to SV40-contaminated vaccine. However, these differences in leukaemia-related mortality were apparent even during 1950–54, before IPV was introduced [pointing to explanations other than vaccine exposure]. After 1955, IPV was made available to individuals outside the age range of 6–8 years. Based on national mortality data, Fraumeni *et al.* did not observe a temporal increase during 1950–59 in leukaemia-related mortality among people in various age groups < 25 years.

Geissler (1990) compared cancer incidence in two birth cohorts in the German Democratic Republic. The 1959–61 birth cohort was considered exposed to SV40, because 86% of these individuals had received OPV as infants, beginning in 1960. No results of testing of OPV lots for

SV40 were presented by Geissler, but if SV40 was present, the titre would have been high, given the absence of any formalin treatment of OPV (Shah & Nathanson, 1976). The 1962–64 birth cohort received OPV beginning in 1963, when SV40 contamination was no longer present. Based on national cancer registry data, the overall incidence of brain tumours was similar in the SV40-exposed birth cohort (28.7 per 10 000) and the unexposed birth cohort (30.1 per 10 000). The incidence also appeared similar in the two cohorts for multiple subtypes of brain tumours. Geissler presented age-specific incidence rates of four types of brain tumour (glioma and glioblastoma, oligodendroglioma, medulloblastoma, and spongioblastoma). The incidence of these cancers appeared higher in the SV40-exposed birth cohort, but the investigator did not present relative risks, confidence intervals, or other data that would allow an assessment of statistical uncertainty. [Cutrone *et al.* (2005) detected SV40 in an OPV used in eastern Europe after the early 1960s, suggesting that people born in 1962–64 in the former German Democratic Republic could have been exposed to SV40 through vaccination.]

Olin & Giesecke (1998) described cancer incidence in Sweden after a 1957 vaccination campaign using IPV from the USA that was potentially contaminated with SV40. This vaccination campaign targeted school-age children born in 1946–53. Beginning in 1958, Sweden used IPV produced in Swedish laboratories, which was claimed to be free of SV40. The investigators assessed cancer registry data for 1960–93, comparing incidence in an exposed birth cohort (born 1946–52) with that in unexposed birth cohorts born slightly earlier or later. The evaluated malignancies included brain cancers (and specifically, ependymoma), osteosarcoma, and mesothelioma. Results were presented for different attained ages, without an overall summary relative risk, and no measures of statistical uncertainty were included. [Therefore, it is not possible to determine whether there is evidence for an effect of SV40 exposure on cancer risk.] Nonetheless, most of the age-specific relative risk estimates were close to 1.00, and the investigators interpreted their findings as indicating no elevation in cancer risk in the SV40-exposed birth cohort. [There were no consistent patterns in the relative risks, and no summary relative risks or confidence intervals were provided. There would likely be very few cases within this cohort of Swedish schoolchildren. It is unknown whether the Swedish vaccine contained SV40.]

Strickler *et al.* (1998) evaluated cancer incidence using cancer registry data from nine areas of the USA that participated in the Surveillance, Epidemiology and End Results (SEER) programme and covered approximately 10% of the national population. The investigators cited the report by Shah & Nathanson (1976), which estimated that live SV40 was present in 10–30% of IPV used in the USA before 1963. By 1961, in the USA 88% of children aged < 20 years, and 55% of infants aged < 1 year, had received one or more doses of IPV. Since 1963, IPV in the USA has been free of SV40 contamination. On this basis, Strickler *et al.* considered three cohorts defined by birth year and thus with varying exposure to SV40-contaminated IPV: the 1947–52 birth cohort (exposed as children in 1955 or soon thereafter), the 1956–62 birth cohort (exposed as infants), and the 1964–69 birth cohort (born after 1963, and thus unexposed). Using SEER data, Strickler *et al.* found that incidence was not elevated for any of the included cancers (ependymoma, brain cancers overall, osteosarcoma, and mesothelioma) in either of the two SV40-exposed birth cohorts compared with the unexposed birth cohort. [The Working Group mentions above that studies that use cancer registries may be affected by changes in the quality of cancer registration over time. Nonetheless, a strength of this study is its use of SEER data, which are believed to be of consistently very high quality over the period evaluated. This study used largely the

same cancer registry data as Fisher *et al.* (1999), although the analysis methods differed.]

In a subsequent brief report, Strickler *et al.* (1999) extended their results, using SEER data to evaluate the association between exposure to SV40-contaminated IPV and risk of medulloblastoma. The 1956–62 birth cohort (exposed as infants to SV40-contaminated IPV) and the 1947–52 birth cohort (exposed as children) did not have elevated risk compared with the 1964–69 birth cohort (unexposed). Using data for the state of Connecticut, the investigators also reported that medulloblastoma incidence among those aged 0–4 years did not increase during 1950–69 in association with use of SV40-contaminated IPV.

Fisher *et al.* (1999) also used SEER data to examine cancer incidence in the USA, for two birth cohorts with varying exposure to SV40-contaminated IPV: the 1955–59 birth cohort (exposed) and the 1963–67 birth cohort (unexposed). To control for differences in ages attained during follow-up, the researchers restricted the analysis to cancer risk among those aged 18–26 years in these cohorts. [The results were similar to those presented in Strickler *et al.* (1998). One limitation of this study is that no confidence limits or *P*-values were presented for the comparisons of cancer incidence in the two birth cohorts.] An increased incidence of mesothelioma was suggested in the exposed cohort (relative risk [RR], 2.78 [95% confidence interval (CI): 0.64–19.0], based on 6 exposed cases); for comparison, Strickler *et al.* (1998) found a relative risk of 3.00 comparing similar exposed and unexposed birth cohorts, with a wide 95% confidence interval (0.67–13.11). [There is significant overlap with the study population of Strickler *et al.* (1999), and the results are consistent.]

Carroll-Pankhurst *et al.* (2001) reported 35–37-year follow-up of a cohort in the USA who were known to have been exposed to SV40-contaminated vaccines as neonates. In 1960–62, a total of 1073 neonates in Cleveland, Ohio, participated in a study of the immunogenicity of poliovirus vaccine. Of them, 86% received OPV and 14% received IPV. Subsequent testing revealed that all vaccine lots used in this trial contained live SV40 at varying titre. An earlier report by Fraumeni *et al.* (1970) noted no deaths from cancer through 1968, and a report by Mortimer *et al.* (1981) described that only one of these individuals had developed a neoplasm (a salivary gland tumour of "low degree of malignancy") through 1979. In their updated report, Carroll-Pankhurst *et al.* (2001) compared cancer mortality in this cohort with expected rates based on the United States national death certificate registry. Only four cancer-related deaths were found, which did not represent an elevation (RR, 1.26; 95% CI, 0.34–3.23). Two deaths were due to leukaemia, but these were of different types and did not comprise a significant excess (RR, 4.19; 95% CI, 0.51–15.73). Two deaths were from testicular cancer (RR, 36.98; 95% CI, 4.47–133.50) [which could be due to late diagnosis or poor treatment of testicular cancer due to socioeconomic factors, because the subjects had been recruited from an urban impoverished community. Testicular cancer has not otherwise been linked to SV40. A limitation of this study is its small size and that there was no replication. Strengths include the prospective documentation of SV40 exposure from contaminated vaccines and that this exposure occurred immediately after birth, when SV40 would be predicted to have the strongest effect on cancer based on animal studies. This study is unique in following people known to have received poliovirus vaccines with specifically documented contamination. The number of deaths from multiple cancers was not increased, nor was the total cancer mortality. A bias is that relative risks are reported only for those cancers that were observed, which would result in a high proportion of elevated risks, and no relative risks are reported for multiple cancers for which there were no observed cases.]

Engels *et al.* (2003a) reported on cancer risk associated with receipt of SV40-contaminated IPV in Denmark. IPV was first administered in Denmark in April 1955. Danish public health officials mounted a concerted vaccination campaign, especially targeted at children, and these efforts were maintained through the early 1960s. As of April 1962, approximately 90% of children aged 9 months or older had received at least one dose of IPV. Danish poliovirus vaccine, unlike the vaccine in the USA, was grown using a monolayer tissue culture method, which pooled kidney tissue from multiple macaques and increased the likelihood of SV40 contamination. Testing of Danish IPV in 1961 revealed that all nine evaluated lots of IPV, previously used in vaccination campaigns, contained live SV40. Beginning in 1963, all Danish IPV was free of SV40. Based on the period when SV40-contaminated IPV was used in Denmark, three birth cohorts of interest were identified: the 1946–52 birth cohort, who were vaccinated in 1955, soon after the vaccine first became available (exposed to SV40-contaminated IPV as young children); the 1955–61 birth cohort, who were vaccinated at age approximately 9 months or soon thereafter (exposed as infants); and the 1964–70 birth cohort (born after vaccines were cleared of SV40, and thus unexposed). Compared with the unexposed cohort, there was similar incidence in the two vaccine-exposed birth cohorts for every examined cancer, including mesothelioma, ependymoma, choroid plexus tumour, bone tumour, leukaemia, and non-Hodgkin lymphoma (NHL). [The strength of this study was the high contamination rate of the vaccines and its contemporaneous documentation.]

Strickler *et al.* (2003) evaluated mesothelioma incidence in the USA in relation to birth year. Assessment of SV40 exposure was based on a 1961 census describing the age-specific proportions of the USA population that had previously received at least one dose of potentially contaminated IPV. Using cancer registry follow-up for 1975–97, Strickler *et al.* described a temporal increase in mesothelioma incidence among men and women who were aged 75–84 years or > 85 years, although these age groups were unlikely to have received IPV during the years when SV40 contamination was present. In comparison, among younger people, who would have had greater IPV exposure during 1955–61, mesothelioma incidence was substantially lower and either constant or declining over time. For adjacent birth cohorts, differences in mesothelioma incidence were not correlated with differences in the prevalence of exposure to potentially contaminated IPV. For example, a comparison of the 1948–57 birth cohort with the 1936–47 birth cohort, which was less frequently exposed, revealed no difference in mesothelioma incidence [RR, 0.98; 95% CI, 0.84–1.15 for men; RR, 1.12; 95% CI, 0.94–1.32 for women]. Because mesothelioma incidence increases with age, a strength of this study is that it evaluated birth cohorts that had aged into late adulthood during the period of follow-up. [Exposure to asbestos was not assessed in these analyses and thus could obscure the effect of SV40. However, the estimated effect of SV40 was similar in both sexes and therefore the risk of mesothelioma is unlikely to be due to asbestos exposure. The effect of SV40 would be expected to be stronger in women, who were less frequently exposed to asbestos than men were.]

Rollison *et al.* (2003) described results of a prospective case–control study nested in a general population cohort in Washington County, Maryland, USA. The study included 44 cases with brain tumours, most of which were glioblastomas. Serum samples were evaluated for SV40 neutralizing antibodies. The prevalence of antibodies was identical in cases and controls (odds ratio [OR], 1.00; 95% CI, 0.30–3.32). [Serum samples were obtained before cancer diagnosis (in this study, 0.6–22.3 years before diagnosis), so associations with brain tumours could have been missed if some SV40 infections occurred subsequent to the draw date.]

Rollison *et al.* (2005a) described results of a prospective case–control study of NHL nested within the same general population cohort in Maryland, USA, as described in Rollison *et al.* (2003). Serum antibodies to SV40 were measured using a VLP ELISA. Compared with cancer-free controls, NHL cases (ascertained through a cancer registry) were more likely to manifest SV40 seropositivity (OR, 1.97; 95% CI, 1.03–3.76). However, no dose–response relationship between SV40 antibody levels and NHL risk was observed. [Antibody levels were evaluated based on the ELISA absorbance at a single dilution, an approach that does not provide a robust quantitative assessment.] Furthermore, the majority of SV40 antibody reactivity could be blocked by addition of BKV or JCV VLPs. SV40-specific reactivity (i.e. reactivity that could not be blocked by BKV or JCV VLPs) was not significantly associated with NHL (OR, 1.51; 95% CI, 0.41–5.52; based on 4 positive cases). Serum samples were obtained from cases a median of 10.8 years before NHL diagnosis. [As noted below for Kjaerheim *et al.* (2007), it is possible that an association with SV40 infection could have been missed if infection occurred subsequent to the blood draw, closer in time to the development of cancer. Nonetheless, a strength of this study is that the antibody measurements before diagnosis were not affected by disease status.]

Thu *et al.* (2006) reported on cancer incidence in Norway during 1953–97 in relation to pre-1964 exposure to IPV. They noted that in 1956–57, Norway used IPV produced in Denmark, which, as described in Engels *et al.* (2003a), was widely contaminated with SV40. From 1957 until 1963, Norway used IPV from the USA, which also may have been contaminated. Exposure to these vaccines varied according to birth year, with the highest vaccinated proportions (> 85%) for individuals born in 1943–62. The investigators used national cancer registry data to evaluate NHL incidence in relation to birth cohort. They estimated curvature effects for various birth years, which correspond to the ratio of the rates of change in incidence for later *versus* earlier birth cohorts. Thu *et al.* observed a positive curvature effect (i.e. an acceleration) in NHL incidence in the 1928–32 birth cohort among both men (curvature effect, 1.18; 95% CI, 0.97–1.43) and women (curvature effect, 1.34; 95% CI, 1.08–1.66). However, these two birth cohorts had similar prevalence of exposure to SV40-contaminated IPV (i.e. 20–40% prevalence for the 1928–32 birth cohort *versus* 40–50% for the 1933–37 birth cohort). Also, there were no other significant curvature effects observed between adjacent birth cohorts related to differences in exposure to SV40-contaminated IPV. [Therefore, there is no consistent pattern associating curvature effects with differences in the prevalence of exposure to SV40-contaminated IPV]. Likewise, no curvature effects were observed for lymphocytic leukaemia or plasma cell neoplasms in the exposed birth cohorts.

Price *et al.* (2007) describe mesothelioma mortality rates in the United Kingdom for birth cohorts with varying exposure to SV40-contaminated IPV. IPV was used in vaccination campaigns in the United Kingdom during 1956–61. Price *et al.* do not describe testing of British IPV for SV40 contamination. In 1962, the United Kingdom switched to OPV that was free of SV40 contamination. Price *et al.* considered several birth cohorts: 1951–55 and 1956–60, considered as potentially exposed to SV40-contaminated IPV, and 1962–66, considered as unexposed. Using data on mesothelioma deaths in a national registry, they found that the age-standardized mortality from mesothelioma was greater in the 1951–55 birth cohort than the 1962–66 birth cohort, for both men (RR, 2.4; 95% CI, 1.2–5.0) and women (RR, 3.8; 95% CI, 1.0–14). Mesothelioma mortality was also borderline elevated in the 1956–60 birth cohort among women (RR, 3.5; 95% CI, 0.93–13) but not among men (RR, 0.93; 95% CI, 0.39–2.3). [The pattern is not clearly consistent with an increased

risk associated with vaccine exposure. A limitation of this study is that the cohorts were followed only to age 38 years and therefore mesothelioma mortality rates were very low. Also, because exposure to asbestos likely varied by birth year, a confounding effect due to asbestos could not be excluded.]

Kjaerheim *et al.* (2007) reported results of a prospective case–control study in Norway, nested within the Janus Serum Bank, a large cohort drawn from the general population. Mesothelioma cases from this cohort (diagnosed during 1973–2003) were identified using data in the Norwegian cancer registry and were matched to cancer-free controls in a 3:1 ratio on age, sex, date of blood sampling, and county of residence. Several measures of exposure to or infection with SV40 were evaluated using serum samples obtained in 1972, i.e. 0.4–30 years before case diagnosis. Using an ELISA that measured antibodies to the SV40 VP1 protein, the investigators found that a high proportion of subjects exhibited reactive antibodies, but the difference in prevalence was not significant (65% of cases *versus* 56% of controls; OR, 1.5; 95% CI, 0.8–2.9). SV40 VP1 antibodies were weaker than seen for BKV and JCV, and most SV40 antibodies were blocked by competition with VP1 proteins from those viruses. After blocking, the prevalence of SV40 VP1 seroreactivity did not differ between cases and controls (14% *versus* 11%; OR, 1.5; 95% CI, 0.6–3.7). Furthermore, only one case exhibited SV40 neutralizing antibodies. In addition, cases and controls did not differ with respect to the proportion with antibodies against SV40 LT measured by ELISA (18% *versus* 14%; OR, 1.4; 95% CI, 0.6–3.2). Finally, no subject had SV40 DNA detectable in serum by PCR and sequencing. [Strengths of this study include the sampling from a well-defined population and measurement of SV40 infection status before the development of cancer in cases (so that there is no disease effect). Another strength is that the study used multiple complementary measures of SV40 infection, and the associations with cancer were similar according to the different measures. Poliovirus vaccination campaigns using potentially contaminated vaccines were carried out in Norway during 1956–62. Thus, if SV40 was acquired through vaccination, one would have expected cases to manifest serological evidence for infection. However, if SV40 infection occurred closer to mesothelioma diagnosis, after the blood draw in 1972 for some subjects, the study may have missed an association.]

## 2.2 Case–control studies

Two approaches have generally been used by case–control studies that have examined the association between SV40 and cancer. In one approach, some case–control studies have compared the prevalence of prior exposure to SV40-contaminated vaccines between cancer cases and controls. This approach mirrors that taken in cohort studies of vaccine recipients (reviewed in Section 2.1) and, as for the cohort studies, the relevance of these studies depends in large part on the quality of the assessment of vaccine exposure and evidence that the vaccine contained live SV40. In the second approach, cases and controls are compared with respect to biomarkers of infection with SV40, typically serum or plasma antibodies. As noted above for prospective case–control studies, one issue for studies that use assays for SV40 antibodies is that natural infections with BKV and JCV, which are very common in the general population, can lead to production of cross-reacting antibodies. Use of samples obtained after diagnosis of the cancer in the cases may result in artefacts in assessing SV40 infection status (i.e. a disease effect), but it has the advantage of capturing any prior exposure to or infection with SV40.

### 2.2.1 Mesothelioma

Three case–control studies have evaluated the association between SV40 exposure or infection and mesothelioma (Table 2.3). A general limitation of these studies is the small number of mesothelioma cases.

Strickler *et al.* (1996) reported results of a study in the USA that used serum samples from patients with mesothelioma and control subjects who had non-malignant gastrointestinal disease. The prevalence of SV40 neutralizing antibodies did not differ significantly between mesothelioma cases (9%) and controls (3%) [crude OR, 3.2; $P = 0.62$ by Fisher exact test]. [This study did not measure or adjust for asbestos exposure.]

Rollison *et al.* (2004) evaluated United States army veterans who had entered military service in 1959–61. During some intervals of this 3-year enrolment period, all army recruits were inoculated with an inactivated adenovirus vaccine, whereas during other intervals the vaccine was not used. Recruits could be divided into vaccine-exposed and unexposed groups based on date of entry into the army. [It is likely that this adenovirus vaccine contained live SV40, because in the tissue culture system that was used, adenovirus grows poorly without the presence of SV40 as a "helper virus," and because formalin added during manufacture did not completely inactivate SV40. Furthermore, later testing of three lots of adenovirus vaccine yielded live SV40, and 100% of 9 recruits who received the vaccine in an early trial exhibited SV40 seroconversion.] Rollison *et al.* used a national Veterans Administration database to identify cases with mesothelioma and control subjects (patients with colon or lung cancer) during 1969–96, i.e. up to 35 years after entry into military service. Receipt of adenovirus vaccine was not associated with mesothelioma risk (OR, 1.49; 95% CI, 0.38–5.88). [A limitation of this study is that all subjects were likely exposed to SV40-contaminated IPV. However, IPV in the USA was not as uniformLy contaminated with SV40 as adenovirus vaccine was, and as noted in Section 2.1, cohort studies of recipients of SV40-contaminated IPV have not shown clear evidence of elevated risk of cancer, including mesothelioma. Therefore, it is unclear whether IPV exposure would have attenuated the association between receipt of SV40-contaminated adenovirus vaccine and mesothelioma. Lung cancer and, to a lesser extent, colon cancer are associated with asbestos exposure. Thus, use of individuals with these cancers as controls somewhat mitigates the possible confounding effects of asbestos.]

Bolognesi *et al.* (2005) recruited mesothelioma patients in a case–control study in Italy during 1996–2000. The researchers also evaluated control subjects, described as healthy or with benign lung disease, and patients with lung cancer. SV40 DNA was detected by PCR in peripheral blood T cells from 2 (11%) of 19 mesothelioma cases and 0 (0%) of 22 controls, who were healthy or had benign lung disease. The odds ratio is thus infinite. However, this difference in prevalence is not significant [$P = 0.21$ by Fisher exact test]. Also, a similar prevalence of detectable SV40 DNA in peripheral blood T cells (2 of 18, 11%) was found among the lung cancer cases [even though lung cancer has not been thought to be associated with SV40]. [There is possible confounding by age; controls were younger than cases, although differences are not statistically significant. SV40 DNA results were available on only a subset of subjects, and it is not clear how the evaluated subjects were selected. The majority of the cases reported asbestos exposure, but only a minority of the controls reported asbestos exposure.]

### 2.2.2 Haematological malignancies

Case–control studies evaluating the association between SV40 and haematological malignancies are summarized in Table 2.4. Most of these studies have addressed NHL, but limited data are

## Table 2.3 Case–control studies of mesothelioma and SV40

| Reference, study location, and period | Total cases Total controls | Control source (hospital, population) | Detection method | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|
| Strickler *et al.* (1996), USA, 1975–91 | 35<br>35 | Hospital (patients with gastrointestinal disease) | Neutralizing antibodies to SV40 | Positive | 3 | [3.2; $P = 0.62$ by Fisher exact test] | Sex, age<br>Did not measure or adjust for asbestos exposure. |
| Rollison *et al.* (2004), United States army veterans, 1969–96 | 10<br>221 | Hospital (army veterans with colon or lung cancer) | Receipt of SV40-contaminated adenovirus vaccine, based on army enrolment date | Exposed to vaccine | 4 | 1.49 (0.38–5.88) | Age, race<br>Subjects were also exposed to SV40-contaminated poliovirus vaccine, which could have attenuated the association. |
| Bolognesi *et al.* (2005), Italy, 1996–2000 | 19<br>22 | Mixed (healthy individuals, patients with benign lung disease) | PCR for SV40 DNA in peripheral blood T cells | PCR positive | 2 | [Infinity; $P = 0.21$ by Fisher exact test] | None<br>A similar prevalence of PCR positivity was observed in lung cancer cases (2 of 18; 11%). Possible confounding by age. Nearly all mesothelioma cases had asbestos exposure. |

CI, confidence interval; PCR, polymerase chain reaction; SV40, simian virus 40

also available for other lymphoid neoplasms and leukaemias.

In an early study based in England, Stewart & Hewitt (1965) compared vaccination histories for case children who died from leukaemia during 1956–60 and control children from the same period. During the period of evaluation, England used the inactivated poliovirus vaccine (Roden, 1964). A similar proportion of cases and controls had received poliovirus vaccination. [The report is very brief, and few details are provided by the investigators. Based on the dates of death among the cases, all vaccinations would have occurred before 1963, but no information was included on the level of SV40 contamination in poliovirus vaccines.]

Holly & Bracci (2003) reported results of a population-based case–control study in the USA. NHL cases were identified through a cancer registry, and controls were selected from the general population by random-digit dialling or from health administrative records. Study subjects provided questionnaire responses to medical history items, including whether they had received poliovirus vaccine and the date of vaccination. Self-reported history of poliovirus vaccination in 1963 or earlier, when live SV40 could have been present in poliovirus vaccines, was not associated with an elevated risk for any of the NHL subtypes examined, including the two most common subtypes, follicular lymphoma (OR, 1.0; 95% CI, 0.78–1.4) and diffuse large-cell lymphoma (OR, 0.81; 95% CI, 0.63–1.0). [A limitation of this study is that subjects' recall of vaccination events from early childhood could have been inaccurate, which might have biased the results. It is likely that both cases and controls were frequently vaccinated since the USA had a national vaccination programme.]

De Sanjosé *et al.* (2003) conducted a case–control study of lymphoma in Spain. Cases with B-cell neoplasm ($n$ = 485, mostly NHL but also including plasma cell myeloma), Hodgkin lymphoma ($n$ = 57), or T-cell lymphoma ($n$ = 35) were enrolled from four hospitals. Controls ($n$ = 587) were patients treated for other medical conditions at the same hospitals. Antibodies against SV40 were measured in these individuals using an ELISA incorporating SV40 VLPs. Overall, SV40 seroprevalence was lower in cases than controls (OR, 0.61; 95% CI, 0.38–0.95). SV40 seroprevalence was not significantly elevated among cases with individual subtypes of B-cell neoplasm, Hodgkin lymphoma, or T-cell lymphoma. Furthermore, the levels of SV40 antibody present in seropositive subjects were much lower than seen in SV40-infected macaques, and these levels decreased when subjects' sera were incubated with BKV VLPs [suggesting that much of the SV40 reactivity was due to cross-reactivity to BKV].

A USA-based case–control study is described in two related reports, Engels *et al.* (2004b, 2005). In Engels *et al.* (2004b), the investigators evaluated 724 cases with NHL and 622 general population controls. Cases and controls were similar demographically and in terms of education, and analyses were adjusted for sex, race, birth year, and study site. Sera were tested for antibodies using SV40 VLP ELISAs in two independent laboratories. In one laboratory, SV40 seropositivity was less common in cases than controls (OR, 0.68; 95% CI, 0.46–1.00), and in the other laboratory the cases and controls had similar seroprevalence (OR, 1.02; 95% CI, 0.71–1.47). Associations were of comparable magnitude across major NHL subtypes. Among SV40 seropositive subjects, the majority of SV40 antibodies were non-specific, i.e. the SV40 reactivity could be competed away through incubation of sera with BKV or JCV VLPs. The proportion of cases and controls with SV40-specific antibodies was low in the evaluated subjects and did not differ between cases and controls.

In the related case–control study, Engels *et al.* (2005) measured antibodies to the SV40 LT in a sample of the participants from the parent case–control study (Engels *et al.*, 2004b). The

## Table 2.4 Case–control studies of haematological malignancies and SV40

| Reference, study location, and period | Total cases Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|---|
| Stewart & Hewitt (1965), United Kingdom, 1956–60 | 999<br>999 | NR | Immunization history | Leukaemia (mortality) | Poliovirus vaccination | 270 | [1.06 (0.86–1.30)] | NR<br>Brief report provided few details. There was no information on contamination of poliovirus vaccines. |
| Holly & Bracci (2003), USA, 1988–95 | 352<br><br>510<br>2402 controls | Population | Self-report of childhood vaccination | Follicular lymphoma<br>Diffuse large-cell lymphoma | Poliovirus vaccination in 1963 or earlier | 179<br><br>227 | 1.0 (0.78–1.4)<br><br>0.81 (0.63–1.0) | Age, sex<br>Results were similar for less common NHL subtypes. Recall of vaccination may have been inaccurate. |
| de Sanjosé *et al.* (2003), Spain, 1998–2002 | 520<br><br>485<br>57<br><br>35<br>587 controls | Hospital (benign conditions) | ELISA for antibodies against SV40 VLPs | Lymphoid neoplasms overall<br>B-cell neoplasm<br>Hodgkin lymphoma<br>T-cell lymphoma | Positive | 31<br><br>28<br>11<br><br>3 | 0.61 (0.38–0.95)<br><br>0.59 (0.37–0.94)<br>2.04 (0.96–4.33)<br><br>0.76 (0.22–2.57) | Age, sex<br>Most cases were subtypes of NHL. Associations were similar across NHL subtypes. Antibody reactivity was reduced after incubation with BKV VLPs. |

**Table 2.4 (continued)**

| Reference, study location, and period | Total cases Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|---|
| Engels *et al.* (2004c), USA, 1998–2000 | 724<br>622 | Population | ELISA for antibodies against SV40 VLPs in laboratory A | NHL | Positive | 52 | 0.68 (0.46–1.00) | Sex, race, birth year, study site Associations were similar across NHL subtypes. Antibody reactivity was reduced after incubation with BKV or JCV VLPs. |
| | | | ELISA for antibodies against SV40 VLPs in laboratory B | | Positive | 70 | 1.02 (0.71–1.47) | |
| Engels *et al.* (2005), USA, 1998–2000 | 85<br>95 | Population | ELISA for antibodies to SV40 LT | NHL | Positive | 5 | 1.2 (0.3–4.6) | Sex, race, age, study site Antibodies to SV40 LT were low-level. |
| Rollison *et al.* (2004), United States army veterans, 1969–96 | 220<br>221 | Hospital (army veterans with colon or lung cancer) | Receipt of SV40-contaminated adenovirus vaccine, based on army enrolment date | NHL | Exposed to vaccine | 69 | 0.98 (0.65–1.47) | Age, race Subjects were also exposed to SV40-contaminated poliovirus vaccine, which could have attenuated the association. |

BKV, BK polyomavirus; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; JCV, JC polyomavirus; LT, large T-antigen; NHL, non-Hodgkin lymphoma; NR, not reported; SV40, simian virus 40; VLPs, virus-like particles

investigators evaluated all SV40 VLP seropositive subjects, and a sample of SV40 VLP seronegative subjects sampled in strata defined by BKV VLP serostatus and case–control status, who were selected on the basis of SV40 and BKV VLP antibody status. Only 6% of NHL cases had measurable antibodies to LT, and the levels of antibodies were low. Furthermore, the presence of antibodies to SV40 LT was not associated with NHL (OR, 1.2; 95% CI, 0.3–4.6). The ELISA used by the investigators was able to demonstrate strong antibody responses to SV40 LT among hamsters with SV40-related tumours, demonstrating its sensitivity. [A strength of this study is measurement of antibody to LT, expression of which is thought necessary for SV40-mediated transformation.]

NHL was also evaluated in Rollison *et al.* (2004), the case–control study mentioned in Section 2.2.1 in conjunction with mesothelioma. Cases with NHL and control subjects with colon or lung cancer were identified using a military veterans health database. The investigators then compared cases and controls with respect to date of entry into the United States army, which tracked with the army's use of SV40-contaminated adenovirus vaccine. Receipt of adenovirus vaccine was not associated with NHL risk (OR, 0.98; 95% CI, 0.65–1.47).

### 2.2.3 Brain tumours

Case–control studies evaluating the association between brain tumours and SV40 are presented in Table 2.5. These studies evaluated a range of different brain tumours but did not specifically examine ependymomas or choroid plexus tumours, two rare subtypes suggested by case series to be related to SV40 (Bergsagel *et al.*, 1992).

Brenner *et al.* (2003) conducted a hospital-based case–control study in the USA that included adult patients with glioma, meningioma, or acoustic neuroma. Controls were patients admitted to the same hospitals with various non-malignant conditions. The investigators assessed SV40 exposure status through study questionnaire items regarding history of poliovirus vaccination, including year and route of vaccination. No significant association between poliovirus vaccination and cancer was observed for any of the three types of brain tumours, either overall or according to type of vaccine (inactivated or oral) or calendar year of vaccination. [A limitation of this study is that adult subjects would have had difficulty accurately recalling their vaccination status as young children. Supporting the possibility of inaccurate recall, a substantial proportion of subjects reported receipt of poliovirus vaccine before 1955, although large-scale campaigns did not begin in the USA until that year.]

Rollison *et al.* (2004) examined the association between brain tumours and receipt of SV40-contaminated adenovirus vaccine among United States army veterans. [Additional details are provided in Sections 2.2.1 and 2.2.2 above, as the study also evaluated mesothelioma and NHL.] The investigators found no association between brain tumours and previous receipt of adenovirus vaccine (OR, 0.76; 95% CI, 0.48–1.20). No data were available on histological subtypes of brain tumours.

### 2.2.4 Miscellaneous cancers

Table 2.6 summarizes results of case–control studies that evaluated associations of other malignancies with SV40.

In the same report in which they evaluated leukaemia (see Section 2.2.2), Stewart & Hewitt (1965) also evaluated non-leukaemia cancers in England. Immunization histories were compared for children who had died from cancers other than leukaemia and control children. There was no difference in the proportion of case and control children who had received poliovirus vaccination. This brief report provides no details

## Table 2.5 Case–control studies of brain tumours and SV40

| Reference, study location, and period | Total cases Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|---|
| Brenner *et al.* (2003), USA, 1994–98 | 489<br>197<br>96<br>799 controls | Hospital (non-malignant conditions) | Self-report of childhood vaccination | Glioma<br>Meningioma<br>Acoustic neuroma | Poliovirus vaccination in 1954–62 | 133<br>53<br>33 | 1.08 (0.71–1.66)<br>0.95 (0.53–1.70)<br>1.30 (0.54–3.41) | Age, sex, race/ethnicity, distance of residence from hospital<br>Recall of vaccination may have been inaccurate. |
| Rollison *et al.* (2004), United States army veterans, 1969–96 | 181<br>221 | Hospital (army veterans with colon or lung cancer) | Receipt of SV40-contaminated adenovirus vaccine, based on army enrolment date | Brain tumours | Exposed to vaccine | 50 | 0.76 (0.48–1.20) | Age, race<br>No data were available on brain tumour subtypes. Subjects were also exposed to SV40-contaminated poliovirus vaccine, which could have attenuated the association. |

CI, confidence interval; SV40, simian virus 40

## Table 2.6 Case–control studies of miscellaneous cancers and SV40

| Reference, study location, and period | Total cases Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|---|
| Stewart & Hewitt (1965) United Kingdom, 1956–60 | 1108<br>1108 | NR | Immunization history | Non-leukaemia cancers (mortality) | Poliovirus vaccination | 259 | [0.97 (0.79–1.19)] | NR<br>Brief report provided few details. There was no information on contamination of poliovirus vaccines. Cancer outcomes were not described. |
| Innis (1968), Australia, 1958–67 | 706<br>706 controls<br>110<br>110 controls | Hospital | Poliovirus vaccination documented in medical record | All cancers, children > 1 year old<br>All cancers, children < 1 year old | Receipt | 618<br>29 | [1.69 (1.25–2.29)]<br>[1.61 (0.81–3.25)] | Matched on sex, age, hospital<br>No data on date of poliovirus vaccination and likelihood of SV40 contamination. Cancer outcomes were not described. |
| Strickler *et al.* (1996), USA, 1975–91 | 35<br>35 | Hospital (patients with gastrointestinal disease) | Neutralizing antibodies to SV40 | Osteo-sarcoma | Positive | 1 | [1.00 (0.012–80.77)] | Report also described results for mesothelioma. |
| Carter *et al.* (2003) | 90<br>72 | Population | VLP ELISA for SV40 antibodies | Prostate cancer | Positive | 5 | [0.65 (0.15–2.68)] | NR<br>Report also described results for 122 paediatric osteosarcoma cases. SV40 antibody reactivity could be blocked by BKV and JCV VLPs. |

BKV, BK polyomavirus; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; JCV, JC polyomavirus; NR, not reported; SV40, simian virus 40; VLPs, virus-like particles

on the types of non-leukaemia cancers that were included, the method of selecting controls, or the likelihood that poliovirus vaccines were contaminated with SV40. During the period of evaluation, England used the inactivated poliovirus vaccine (Roden, 1964).

Innis (1968) conducted a hospital-based case–control study during 1959–67 in Australia. Cases included children with a variety of unspecified malignancies. Controls were drawn from children admitted to the same hospital and were matched to cases on sex and age. Review of medical records provided data on vaccine exposure. An association was observed between poliovirus vaccination and cancer risk among children: this association was statistically significant among children aged > 1 year [crude OR, 1.69; 95% CI, 1.25–2.29; $P < 0.0005$] but not among younger children [crude OR, 1.61; 95% CI, 0.81–3.25]. [Although it is likely that some of the children aged > 1 year would have been vaccinated before 1963, a limitation of this study is that the investigator did not describe the dates of poliovirus vaccination, nor did he describe the frequency or level of contamination of poliovirus vaccines in Australia. Therefore, it is unclear whether children received poliovirus vaccine likely contaminated with SV40. Another limitation is that the types of cancer were not characterized. Also, there may be confounding by socioeconomic status if children with lower socioeconomic status were less likely to receive poliovirus vaccination. The control series may also have been biased if hospitalization would have been related to likelihood of vaccination.]

Strickler *et al.* (1996) reported results for osteosarcoma. The researchers evaluated serum samples collected in the USA from osteosarcoma cases and controls with non-malignant gastrointestinal diseases. The prevalence of SV40 neutralizing antibodies was identical in the two groups. [A limitation of this study is the small number of osteosarcoma cases. Also, the osteosarcoma cases were younger than the controls, who had been matched to another case series. See Section 2.2.1.]

Carter *et al.* (2003) measured antibodies against SV40 VLPs in prostate cancer cases and controls in the USA. SV40 seroreactivity was observed in 5.6% of cases and 8.3% of controls. The study also included 122 children with osteosarcoma, among whom 2.5% had SV40 antibodies, but similarly aged controls were not evaluated. In competitive inhibition experiments, the researchers demonstrated that SV40 reactivity could all be blocked by addition of BKV or JCV VLPs, indicating that the SV40 antibodies were non-specific. [Limitations of this study include an inadequate description of how subjects were selected from the parent case–control study of prostate cancer.]

## 2.3 Susceptible populations

### *2.3.1 Children*

Young children may comprise an especially susceptible population for any carcinogenic effects of SV40. As noted in Section 3, experimental results in animals suggest that the carcinogenic effect is strongest when exposure occurs in the neonatal period and drops off sharply with age (Girardi *et al.*, 1963). Such results would suggest that children who received SV40-contaminated poliovirus vaccines, especially as infants, would have an elevated cancer risk. As reviewed in Section 2.2.1 and presented in Table 2.1 and Table 2.2, most cohort studies of young children who received SV40-contaminated IPV have not identified an elevated cancer risk. Many of these children received multiple inoculations (i.e. parenteral exposures) with SV40-contaminated IPV. The study by Carroll-Pankhurst *et al.* (2001) deserves special mention because it described a cohort of 1073 people exposed in the neonatal period (in the first 3 days of life). All of these neonates received poliovirus vaccines later documented to contain live SV40. After 35

years of follow-up, only 4 deaths from cancer were documented, which did not represent an excess, and the cancer deaths that occurred (2 from leukaemia and 2 from testicular cancer) did not suggest an effect of SV40 infection (see also Section 2.2.1 and Table 2.1).

### 2.3.2 *People potentially exposed to SV40* in utero

Cancer risk in children born to women who received potentially contaminated poliovirus vaccines during pregnancy was evaluated in two studies (one case–control study and one cohort study combined with a nested case–control study). It is possible to consider these studies as providing information on cancer risk after *in utero* infection with SV40. [However, it is not established that infection can occur *in utero*.] Case–control studies (both retrospective and nested within a cohort) and cohort studies of this topic are presented in Tables 2.7 and Table 2.8, respectively.

Farwell *et al.* (1979) evaluated the association between childhood brain tumours and maternal exposure to IPV during pregnancy in a case–control study. Children with brain tumours who were born in 1956–62 (the period when children could have been exposed *in utero* through vaccination of the mother) and were aged 0–19 years at diagnosis were identified through the Connecticut Tumor Registry in the USA. Control children were identified through a search of birth certificates. The investigators asked the obstetricians who had delivered these children whether the mothers had received poliovirus vaccination during pregnancy. Based on the birthdates, vaccinations would have all been before 1963 when poliovirus vaccines in the USA were contaminated with SV40. Ten (67%) of 15 children with medulloblastoma, 8 (35%) of 23 children with glioma, and 1 (7%) of 14 children with other brain tumours had mothers who had been vaccinated during pregnancy, compared with 8 (21%) of 38 control children. Overall, there was a borderline association between mothers' vaccine exposure and the occurrence of brain tumours in children [crude OR, 2.15; 95% CI, 0.76–6.54]. The difference in vaccine exposure was significant for medulloblastoma [crude OR, 7.5, 95% CI, 1.68–35.39] but not for glioma [crude OR, 2.0, 95% CI, 0.53–7.45] or other brain tumours.

Engels *et al.* (2004a) included both a cohort analysis in relation to poliovirus vaccine exposure and a nested case–control study evaluating associations with maternal SV40 antibody status. In the cohort study component, the investigators evaluated cancer risk among 54 796 children in the Collaborative Perinatal Project. The Collaborative Perinatal Project is a cohort study that enrolled pregnant women in the USA during 1959–66 and subsequently followed their children. The children were classified according to whether their mothers had received poliovirus vaccine (mostly IPV) during pregnancy and, if so, whether that vaccine had been administered before 1963. During 8 years of follow-up, children whose mothers had received pre-1963 poliovirus vaccine had an increased risk for haematological malignancies (mostly leukaemias; RR, 2.5; 95% CI, 1.1–5.6) and neural tumours (RR, 2.5; 95% CI, 1.0–6.3), compared with children whose mothers had not been vaccinated or had received poliovirus vaccine only in 1963 or later. Risk was not elevated for other miscellaneous types of cancer (RR, 1.2; 95% CI, 0.3–4.6). The most common type of neural tumour in the children was neuroblastoma, and maternal poliovirus vaccine exposure was associated with an elevated risk of this cancer (RR, 8.2; 95% CI, 1.6–43). [A strength of this study is the contemporaneous documentation of poliovirus vaccination during the mother's pregnancy, which eliminates differential recall of vaccination status between mothers of cases and controls.]

Of note, in the same report, Engels *et al.* described a nested case–control study testing sera

## Table 2.7 Case–control studies of cancer in relation to potential exposure *in utero* to SV40

| Reference, study location, and period | Total cases<br>Total controls | Control source (hospital, population) | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates<br>Comments |
|---|---|---|---|---|---|---|---|---|
| Farwell *et al.* (1979), USA, 1956 and later | 15<br>23<br><br>14<br>38 controls | Population | Physician report of maternal vaccination during pregnancy | Medulloblastoma<br>Glioma<br><br>Other brain tumours | Poliovirus vaccination of mother in 1956–62 | 10<br>8<br><br>1 | [7.5 (1.68–35.39)]<br>[2.0 (0.53–7.45)]<br><br>[0.29 (0.006–2.60)] | Sex, birthdate, town of birth |
| Engels *et al.* (2004b), USA, 1959–74 | 50<br>200 | Population (same cohort as cases) | Plaque neutralization assay for SV40 antibodies<br>VLP ELISA for SV40 antibodies | All cancers | Maternal seroconversion during pregnancy | 3<br><br>4 | 1.5 (0.3–6.3)<br><br>4.0 (1.0–15.7) | Maternal receipt of poliovirus vaccine, timing of poliovirus vaccination<br>Cases and controls were drawn from a cohort in which maternal receipt of poliovirus vaccine was associated with cancer. Study extended report by Rosa *et al.* (1988). |

CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; SV40, simian virus 40; VLP, virus-like particle

## Table 2.8 Cohort studies of susceptible populations exposed to SV40

| Reference, location, follow-up period | Total subjects | Detection method | Organ site | Exposure categories | Exposed cases | Relative risk (95% CI) | Covariates Comments |
|---|---|---|---|---|---|---|---|
| *People potentially exposed* in utero | | | | | | | |
| Engels *et al.* (2004a), children in the USA 1959–74 | 54 796 | Vaccination status of mothers followed during pregnancy | Neural tumours<br>Neuroblastoma<br>Haematological malignancies<br>Miscellaneous tumours | Children whose mothers received pre-1963 polio vaccine *vs* children whose mothers received poliovirus vaccine in 1963 or later, or no vaccine. | 8<br>NR<br>10<br>3 | 2.5 (1.0–6.3)<br>8.2 (1.6–43)<br>2.5 (1.1–5.6)<br>1.2 (0.3–4.6) | Sex, race, maternal age at delivery<br>Evaluation of maternal sera did not reveal association of SV40 seroconversion with cancer in children. Study improves on exposure and outcome assessment and extends follow-up of Heinonen *et al.* (1973). |
| *Immunosuppressed populations* | | | | | | | |
| Engels *et al.* (2003b), people with AIDS in the USA 1980–96 | 56 808 | Birth cohort with varying exposure to SV40-contaminated IPV | NHL | Born 1958–61 (exposed) *vs* 1964–67 (unexposed) | 616 | 0.97 (0.79–1.20) | Sex, HIV risk group, ethnicity, age, registry area<br>No association was seen with exposure for any lymphoma subtype. |
| Paracchini *et al.* (2006), Italian transplant recipients NR | 387 | PCR testing of peripheral blood DNA from organ donors | All cancers | Organ donor with PCR-amplifiable SV40 DNA in peripheral blood | 0 | 0 | None<br>The number of exposed transplant recipients was small. |

CI, confidence interval; IPV, inactivated poliovirus vaccine; NHL, non-Hodgkin lymphoma; NR, not reported; PCR, polymerase chain reaction; SV40, simian virus 40; *vs*, *versus*

from these mothers for SV40 antibody seroconversion during pregnancy. Results were presented for all cancer types combined. During pregnancy, 3 of 46 case mothers exhibited seroconversion according to an SV40 plaque neutralization assay (the children developed neuroblastoma, leukaemia, or fibrosarcoma), and 4 of 50 case mothers exhibited seroconversion according to an SV40 VLP ELISA (2 children with neuroblastoma, 1 with astrocytoma, and 1 with leukaemia). Only 1 case mother, whose child developed neuroblastoma, seroconverted according to both assays. The seroconversion rates did not differ significantly between case and control mothers, although the results according to the VLP ELISA were borderline significant (OR, 4.0; 95% CI, 1.0–15.7). [Because SV40 seroconversion was not associated with cancer among the children, and seroconversion was seen in few of the mothers whose children developed cancer, the study does not provide strong support for the conclusion that the positive associations between vaccination and cancer seen in the cohort study were due to SV40 infection transmitted via maternal vaccination. Neuroblastoma is a unique tumour of early childhood and has not otherwise been associated with SV40. This study extends an earlier report by Rosa *et al.* (1988) by including additional cases ascertained during longer follow-up. A limitation of this case–control study was the small number of outcomes, which precluded a separate assessment by cancer type.]

### *2.3.3 Immunosuppressed people*

Immunosuppressed individuals are a third potentially susceptible population. HIV-infected people and solid organ transplant recipients have an increased risk for a range of virus-related malignancies, including NHL (Grulich *et al.*, 2007). Two cohort studies are summarized in Table 2.8.

Engels *et al.* (2003b) examined the association between childhood exposure to potentially contaminated IPV and risk of NHL among HIV-infected people in the USA. The investigators used linked data between HIV and cancer registries in the USA to evaluate NHL risk among 56 808 people with AIDS. People with AIDS were categorized by birth year into two groups based on likely exposure to SV40-contaminated IPV in childhood: an exposed birth cohort (born in 1958–61) and an unexposed birth cohort (born in 1964–67). After adjustment for potentially confounding demographic factors, the SV40-exposed and unexposed birth cohorts had similar NHL risk (RR, 0.97; 95% CI, 0.79–1.20). In addition, the SV40-exposed birth cohort did not have elevated risk for any NHL subtype defined by site or histology. [The Working Group noted that this study is important, showing that in this population with a very high NHL risk, there was no elevated risk for NHL associated with prior vaccination-related exposure.]

Paracchini *et al.* (2006) described cancer risk in a cohort of 41 transplant recipients in Italy who received organs from 12 donors who had SV40 DNA amplified from peripheral blood. The premise of the study was that SV40 might be transmissible to such transplant recipients and that immunosuppression administered to recipients would facilitate the development of cancer. During an average follow-up of 671 days, none of these recipients developed cancer. In comparison, cancer developed in 11 of 346 recipients who received organs from donors who did not have SV40 DNA detectable in blood. [The 11 cancers in the comparison group were not specified. Nonetheless, it is striking that none of the SV40-exposed recipients developed cancer, especially NHL, which is common in the early period after transplantation. Limitations of this study include the short follow-up and the small number of recipients who received organs from donors considered to be SV40-positive. Also, the sensitivity and specificity of detection of SV40 DNA in donor blood is uncertain, and it

is unknown whether SV40 can be transmitted through organ transplantation.]

### *2.3.4 Asbestos-exposed people*

Asbestos exposure is a recognized cause of mesothelioma, and it would be of interest to assess the contribution of SV40 in asbestos-exposed individuals. However, no epidemiological study has used appropriate measures of SV40 infection to assess whether the effect of SV40 on mesothelioma risk is independent of asbestos exposure, or whether any effect of SV40 varies by asbestos exposure. Mayall *et al.* (1999) and Cristaudo *et al.* (2005) reported on the prevalence of SV40 DNA detected in mesothelioma tumours in relation to asbestos exposure, but the studies were too small to be informative ($n$ = 7 and $n$ = 13 asbestos-exposed cases, respectively).

## 2.4 Case series

Numerous studies have reported on the detection of SV40 in human tumours. These case series are not reviewed systematically in this report, because of methodological issues mentioned below and because other epidemiological study designs were judged to provide a higher level of evidence. Recent reviews that include summaries of case series, and which reached conflicting conclusions regarding the interpretation of those data, include Institute of Medicine of the National Academies (2002), Vilchez & Butel (2004), Shah (2007), and Butel (2010, 2012).

In the published case series, most investigators have used PCR to identify SV40 DNA in archived formalin-fixed or frozen tumour tissues. Positive studies that have been influential in stimulating additional research include Carbone *et al.* (1994), which found SV40 DNA in 60% of mesothelioma tumours; Bergsagel *et al.* (1992), which found SV40 DNA in 91% of ependymomas and 50% of choroid plexus tumours; Vilchez *et al.* (2002) and Shivapurkar *et al.* (2002), which found SV40 DNA in 42–43% of NHL tumours; and Carbone *et al.* (1996), which found SV40 DNA in 32% of osteosarcoma tumours. In subsequent studies, some investigators confirmed detection of SV40 DNA in these tumour types, but some studies were largely or entirely negative, for example, Strickler *et al.* (1996) and Gordon *et al.* (2002) for mesothelioma; Weggen *et al.* (2000), Engels *et al.* (2002), and Rollison *et al.* (2005a) for ependymomas and choroid plexus tumours; and MacKenzie *et al.* (2003) and Schüler *et al.* (2006) for NHL.

Reasons for the discrepant results across these case series are uncertain. One possibility is that geographical variability in the epidemiology of SV40 has resulted in differing contributions of SV40 to cancer in different regions. However, no clear geographical pattern has been identified, and studies conducted using tissues from the same country (e.g. USA) have produced conflicting results. Some studies were poorly designed or conducted (e.g. lacking negative or positive controls, testing of samples under unblinded conditions) or inadequately described in the published reports. The discrepancies across studies have prompted debate about whether appropriately sensitive methods were used in the negative studies, or whether false-positive results could explain the results of positive studies (see also Section 4.3.1). Few studies have used quantitative PCR to assess the amount of SV40 DNA present in tumour samples, but several studies have found that SV40 DNA, when detectable, is present at less than one copy per tumour cell (Gordon *et al.*, 2002; Rollison *et al.*, 2005b; Ziegler *et al.*, 2007). Such low-level detection is inconsistent with a model whereby SV40 DNA is integrated into host DNA and present in every tumour cell.

Likewise, it has been difficult to interpret reports of the detection of SV40 DNA in a wide range of other tumour types, including, in addition to the types already mentioned, thyroid cancer (Vivaldi *et al.*, 2003), colon cancer

(Shivapurkar *et al.*, 2002; Campello *et al.*, 2010), breast cancer (Shivapurkar *et al.*, 2002), lung cancer (Shivapurkar *et al.*, 2002), and prostate cancer (Shivapurkar *et al.*, 2002). SV40 DNA has also reportedly been detected in additional histologically defined tumour subtypes, including astrocytomas and gliomas (Martini *et al.*, 1996; Suzuki *et al.*, 1997), Hodgkin lymphoma (Shivapurkar *et al.*, 2002), and chondrosarcomas and giant cell bone tumours (Carbone *et al.*, 1996; Gamberi *et al.*, 2000).

Three multicentre studies have evaluated the reproducibility of SV40 DNA detection in human tumour tissues. In the first such study, Testa *et al.* (1998) reported results for four laboratories that tested DNA extracted from 12 mesothelioma cases. The PCR testing was performed in a blinded fashion, although the panel did not include tissues other than the mesothelioma cases as negative controls. Using each of two sets of PCR primers, 9 (75%) of the 12 tumours were positive in all four laboratories, and the remaining 3 tumours were variably positive.

The second interlaboratory comparison was reported by Strickler (2001). Nine testing laboratories performed blinded PCR evaluation of specimens prepared by a separate processing laboratory. The specimens included DNA extracts from 25 mesothelioma tumours (included in duplicate) and 25 specimens of normal lung, which were randomLy sorted within the test batch. All samples were PCR positive for DNA from the human β-globin gene, indicating the presence of amplifiable DNA. Across the nine laboratories, the proportion of mesothelioma specimens that were PCR positive for SV40 DNA, using a variety of testing protocols, ranged from 0% to 40%. In the majority of cases, PCR positive reactions were not confirmed in the blinded testing of the replicate mesothelioma sample by the same laboratory or by testing in other laboratories. The highest detection rate (40%) was found by a laboratory that reported contamination of its PCR primers, and this laboratory also found 28% of the normal lung extracts to be PCR positive for SV40. Unexpectedly, eight of the nine testing laboratories found some samples from a negative control batch to be PCR positive for SV40 DNA. This SV40 positivity was traced to contamination of the negative control batch during preparation by the processing laboratory.

In the third study, Rollison *et al.* (2005a) compared detection of SV40 DNA by PCR in two laboratories that independently assessed a panel of tumours under masked conditions. Among a total of 225 human brain tumours evaluated, most were astrocytomas or glioblastomas, but 29 cases were ependymomas and 14 were choroid plexus tumours. The majority of tumours had amplifiable human cellular DNA, but SV40 detection was uncommon. One laboratory detected SV40 DNA in three cases, and the second laboratory detected SV40 DNA in a different case. The second laboratory quantified the amount of SV40 DNA in the single PCR positive tumour as 0.12 copies in approximately 7000 cells, inconsistent with the clonal presence of SV40 in each tumour cell. Blinded positive and negative controls yielded expected results and were concordant in both laboratories.

The possibility that false-positive results arising from PCR contamination could explain some published positive reports is supported by López-Ríos *et al.* (2004). The investigators described results for 71 mesothelioma tumours. Application of two widely used PCR primer sets yielded positive results for SV40 DNA in 56–62% of cases. However, results were discordant in 14 cases, and the authors noted occasional PCR positivity of negative controls. These results led López-Ríos *et al.* to sequence the PCR products, and comparison with GenBank revealed that the amplified sequences were also present in commonly used laboratory plasmids. Repeated experiments, using alternative PCR primers that would be less likely to amplify plasmid sequences, yielded only 4 mesothelioma cases (6%) with weakly positive results. Finally, in

experiments that used PCR primers engineered to span a junction site between SV40 and plasmid DNA sequences, 16 (23%) of 69 mesothelioma cases were positive for the junction site found in plasmids rather than naturally occurring viral sequences. López-Ríos *et al.* also re-analysed data from a previously published study and demonstrated that the SV40 sequences amplified from human tumours in that study were unknowingly plasmid-derived contaminants.

Studies that have used other detection methods for SV40 in human tumour tissues have also produced inconsistent results. Examples of studies that have yielded differing results for detection of SV40 RNA or proteins include Carbone *et al.* (1994), Testa *et al.* (1998), López-Ríos *et al.* (2004), and Brousset *et al.* (2005) for mesothelioma; Bergsagel *et al.* (1992) and Sabatier *et al.* (2005) for brain tumours; and Brousset *et al.* (2004) and Vilchez *et al.* (2005) for NHL. No study has reported on whether patients who have tumours that manifest molecular evidence for SV40 also exhibit serum antibodies for SV40 infection.

[Although case series that have reported on detection of SV40 DNA in human tumours have been influential in motivating research on the role of SV40 in causing human malignancies, there are difficulties in interpreting these results that preclude giving them substantial weight in the assessment of the human evidence. Studies have produced widely conflicting results on the presence of SV40 DNA in human tumours, and many studies are of poor quality. There are few rigorous comparisons of detection of SV40 across laboratories, and the existing comparisons do not support that detection is consistent. Contamination of tumour specimens by low levels of SV40 DNA (present in plasmids or from other sources) cannot be ruled out as an explanation for detection.]

# 3. Cancer in Experimental Animals

## 3.1 Infection with SV40

Animal models for SV40 include hamsters (Syrian golden hamsters [*Mesocricetus auratus*], unless otherwise specified) and transgenic mice. Experimental studies of SV40 tumorigenicity *in vivo* dating from the 1960s (when the virus was first studied) to the present are summarized below.

### *3.1.1 Hamster*

The oncogenic potential of SV40 was first demonstrated by Eddy *et al.* (1961) by the subcutaneous inoculation of rhesus monkey kidney cell extracts into newborn (NB) hamsters. [NB hamsters in this and other studies are ≤ 3 days old.] Among the injected animals ($n$ = 154), 70% developed subcutaneous tumours, over 3.5–9 months, as well as occasional lung and kidney tumours. Tumours were identified as undifferentiated sarcomas. Control hamsters ($n$ = 65), inoculated with extracts of human and feline tumours, failed to develop any tumours. The oncogenic substance was soon identified as SV40 (Eddy *et al.*, 1962). That follow-up study showed that SV40 strain 776 inoculated subcutaneously in NB hamsters ($n$ = 13) induced subcutaneous sarcomas with latency periods of 7–9 months in 85% of animals. Some lung tumours were also induced (33%). Controls included animals injected with SV40 mixed with SV40 neutralizing antibody ($n$ = 17); none of those controls developed tumours.

The oncogenic potential of SV40 has been confirmed and characterized in numerous reports in the decades after the studies of Eddy *et al.* (1961, 1962). It has been established that both host and viral factors influence the development of SV40-mediated neoplasia. Factors include age at the time of infection, route of exposure, dose of inoculum (amount of infectious virus), and

viral genetic variation. Reported studies have varied in the numbers of experimental animals and the duration of observation, but they have consistently and reproducibly shown that SV40 has the capacity to transform a variety of target cell types *in vivo*. Subcutaneous, intravenous, and intraperitoneal routes of injection with different strains of SV40 give rise to specific types of tumours. In these studies, cumulative controls numbering more than 1000 have shown that hamsters did not develop these tumours spontaneously.

### *(a) Subcutaneous inoculation*

See Table 3.1

Subcutaneous inoculation of NB hamsters with SV40 usually results in the formation of subcutaneous tumours identified as fibrosarcomas. SV40 strain VA45-54 induced tumours in 45% of animals ($n$ = 94), as well as peritoneal mesothelioma with lung metastases (1%) over a time period of 4–10 months (Girardi *et al.*, 1962). Controls, uninoculated or inoculated with medium or tissue culture fluids ($n$ = 409), remained tumour-free. In a subsequent study, Girardi *et al.* (1963) showed that NB hamsters were more susceptible than older animals to SV40-induced tumours after subcutaneous inoculation. With a uniform inoculum of $3 \times 10^5$ $TCID_{50}$ (50% tissue culture infective dose) of SV40 strain VA45-54 and with groups ranging in size from $n$ = 54 to $n$ = 11, 96% of animals inoculated as newborns developed tumours, as did 60% of animals inoculated at age 7 days, 23% of those inoculated at age 1 month, and none of those inoculated at age 3 months. In addition, tumour latencies were shorter in younger animals. The effect of virus dose on tumour induction in NB animals was demonstrated, and the following subcutaneous tumour incidences were observed: $3 \times 10^5$ $TCID_{50}$, 96%; $3 \times 10^3$ $TCID_{50}$, 18%; and 30 $TCID_{50}$, 0%. Also, whereas SV40 treated with pre-immune serum remained tumorigenic (96%), SV40 neutralized with SV40 antiserum induced no tumours. The subcutaneous route of SV40 inoculation in NB hamsters resulted in more tumorigenic activity (86%) than the intraperitoneal (4%) or intrapulmonary (11%) route. Control animals ($n$ = 371) remained tumour-free. Girardi (1965) also tested the potential inhibitory effect of SV40-transformed human cells on SV40-induced tumorigenesis. NB hamsters were inoculated subcutaneously with SV40 strain Rh911 at $10^7$–$10^8$ $TCID_{50}$ ($n$ = 46). Then, 35 days later, half were inoculated intraperitoneally with $7 \times 10^6$ control human WI-26 cells and half with SV40-transformed WI-26 cells. Ninety-two percent of the SV40-infected hamsters treated with the control cells developed subcutaneous tumours, whereas only 4% of those treated with the SV40-transformed human cells developed tumours.

Ashkenazi & Melnick (1963) showed that SV40 that had been purified by terminal dilution and inoculated at $10^6$ $TCID_{50}$ subcutaneously into NB hamsters induced undifferentiated subcutaneous sarcomas in 79% of animals, with latency periods of 4–8 months. Black & Rowe (1964) tested SV40 strain 777; subcutaneous inoculation of $10^{6.5}$ $TCID_{50}$ into NB hamsters ($n$ = 56) resulted in subcutaneous sarcomas in 63% of recipients. Uninoculated control hamsters ($n$ = 24) did not develop tumours. Three doses of three plaque variants of SV40 received from the Eddy laboratory (Eddy *et al.*, 1961, 1962) were tested in groups of 17–23 NB hamsters for relative tumorigenicity (Takemoto *et al.*, 1966). The dose of virus able to cause tumours in 50% of animals over an 11-month period after subcutaneous injection was as follows: large-plaque variant, $10^{4.2}$ plaque-forming units (PFU) (tumour incidence, 32/60); small-plaque variant, $10^{5.2}$ PFU (tumour incidence, 20/70); and minute-plaque variant, $10^{5.8}$ PFU (tumour incidence, 5/60). These differences in the values of doses causing tumours in 50% of the animals were statistically significant ($P$ = 0.01).

Defendi & Jensen (1967) used a subcutaneous inoculum of $10^7$ PFU into NB hamsters to test the

## Table 3.1 Carcinogenicity studies in hamsters[a] given SV40 by subcutaneous inoculation

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster (NB, < 1–3 d) (NR)<br>Up to 500 d<br>Eddy *et al.* (1961) | SV40 strain, 0.2 mL primary RMKC extracts, subcutaneous ($n$ = 154)<br>Controls: extracts of human and feline tumours ($n$ = 65) | Subcutaneous tumours (70%), lung (2%), kidney (1%), all undifferentiated sarcomas; 3.5–9 mo<br>Controls (0%) | NR | Oncogenic agent subsequently identified as SV40 (Eddy *et al.*, 1962).<br>First report of oncogenicity by SV40 in animals. |
| Hamster (NB, 1–3 d) (NR)<br>Up to 391 d<br>Eddy *et al.* (1962) | SV40 strain 776, 0.2 mL, subcutaneous<br>$n$ = 13<br>SV40 rescued from tumours (Eddy *et al.*, 1961), 0.2 mL, subcutaneous ($n$ = 54)<br>Controls: extracts of AGMK, SV40 + anti-SV40 serum ($n$ = 17) | Strain 776: subcutaneous tumours (85%), lung (33%), all sarcomas; 7–9 mo<br>Rescued virus: subcutaneous tumours (70%); 5 mo<br>Controls (0%) | NR | |
| Hamster (NB, < 1 d) (M, F)<br>Up to 10.5 mo<br>Girardi *et al.* (1962) | SV40 strain VA45-54, 0.2 mL, subcutaneous ($n$ = 94)<br>Controls: uninfected GMK cell fluid, medium, or uninoculated ($n$ = 409) | Subcutaneous tumours (45%), fibrosarcomas, peritoneal mesothelioma with lung metastases (1%); 4–10 mo<br>Controls (0%) | NR | Tumours were serially transplanted into hamsters (age, 4–6 wk). |
| Hamster (different ages) (NR)<br>Up to 17 mo<br>Girardi *et al.* (1963) | SV40 strain VA45-54, 0.2 mL, subcutaneous<br>Ages, NB to 3 mo; doses, $10^1$ $TCID_{50}$ to $3 \times 10^5$ $TCID_{50}$; groups ranged from $n$ = 54 to $n$ = 11<br>Virus treated with pre-immune serum ($n$ = 23) and anti-SV40 rabbit serum ($n$ = 24)<br>Controls: uninfected GMK cell fluid, medium, or uninoculated ($n$ = 371) | Subcutaneous tumours. Age at inoculation of high dose: NB (52/54, 96%), 7 d (24/40, 60%), 1 mo (6/26, 23%), 3 mo (0/35, 0%); 3.8–16 mo, shorter times to tumours in younger animals.<br>Varying virus dose in NB: $3 \times 10^5$ $TCID_{50}$ (96%), $3 \times 10^3$ $TCID_{50}$ (18%), $3 \times 10^1$ $TCID_{50}$ (0%); 3.8–14 mo<br>Virus + normal (pre-immune) serum (96%), virus + anti-SV40 serum (0%); 10 mo<br>Controls (0%) | NR | Subcutaneous route of inoculation (66/77, 86%) in NB hamsters more tumorigenic than intraperitoneal (2/44, 4%) or intrapulmonary (2/18, 11%). |
| Hamster (NB, < 1 d) (NR)<br>Up to 8 mo<br>Ashkenazi & Melnick (1963) | SV40 purified by terminal dilution, 0.05 mL ($10^6$ $TCID_{50}$), subcutaneous | Subcutaneous tumours (79%), undifferentiated sarcomas; 4–8 mo | NR | Tumours were serially transplanted into weanling hamsters (cheek pouch). Hamster embryo fibroblasts were transformed *in vitro* by SV40 and were tumorigenic in weanling hamsters (cheek pouch). |

**Table 3.1 (continued)**

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster (NB, 2 d) (NR)<br>Up to 259 d<br>Black & Rowe (1964) | SV40 strain 777, 0.1 mL ($10^{6.5}$ $TCID_{50}$), subcutaneous ($n$ = 56)<br>Controls: uninoculated ($n$ = 24) | Subcutaneous tumours (63%), sarcomas<br>Controls (0%) | NR | |
| Hamster (NB, < 1 d) (NR)<br>Up to 325 d<br>Girardi (1965) | SV40 strain Rh911, 0.2 mL ($10^{7}$–$10^{8}$ $TCID_{50}$), subcutaneous ($n$ = 46)<br>Then, 35 d later, inoculated intraperitoneally with 7 × $10^{6}$ control human WI-26 cells ($n$ = 24) or SV40-transformed WI-26 cells ($n$ = 22) | Subcutaneous tumours in animals treated with control human cells (92%); SV40-transformed human cells prevented tumours (4%) | NR | |
| Hamster (NB) (NR)<br>11 mo<br>Takemoto *et al.* (1966) | SV40 virus from Dr Bernice Eddy.<br>Recovered plaque variants: SV40-L (large), SV40-S (small), SV40-M (minute).<br>Serial dilutions, subcutaneous.<br>Doses tested: 3.7, 37, and 370 × $10^{4}$ PFU.<br>($n$ = 17–23); SV40-L ($n$ = 60), SV40-S ($n$ = 70), SV40-M ($n$ = 60) | Dose of virus to cause tumours in 50% of animals:<br>SV40-L: $10^{4.2}$ PFU<br>SV40-S: $10^{5.2}$ PFU<br>SV40-M: $10^{5.8}$ PFU | $P$ = 0.01 | |
| Hamster (NB, 1–2 d) (NR)<br>240 d<br>Defendi & Jensen (1967) | SV40, $10^{7}$ PFU/0.1 mL, subcutaneous<br>Some aliquots of virus were irradiated with cobalt-60 source or ultraviolet radiation, which reduced infectious titres by 2–3 logs, before inoculation ($n$ = 13–39) | Although irradiation reduced infectivity, tumorigenicity was not inactivated but actually increased: ultraviolet radiation, 8/13 (62%) to 15/17 (88%); cobalt-60 source, 18/27 (67%) to 21/25 (84%) | NR | Histological features of tumours induced by untreated and irradiated virus were similar. |
| Hamster (NB, 1 d; adult, 2.5–3 mo) (NR)<br>18 mo<br>Deichman *et al.* (1978) | SV40, $10^{6.5}$–$10^{8.25}$ $TCID_{50}$/mL, WT SV40 and ts mutants, subcutaneous, 0.2 mL/NB and 1 mL/adult<br>$n$ = 73 NB and $n$ = 64 adult | Tumour incidence – WT SV40: NB (13/16, 81%), adult (0/10, 0%). tsA, tsB, tsBC mutants: NB (42%), adult (9%).<br>Latency in NB: 103–314 d (WT SV40), 120–495 d (ts SV40) | NR | Relatively fewer tumours induced in NB animals by SV40 ts mutants A209 and A239 = 8/30 (26%). |
| Hamster, strain LSH (NB, < 1 d) (NR)<br>1 yr<br>Lewis & Martin (1979) | WT SV40, strain 776, and derivative sT gene dl mutants, dl883, dl885, dl886, and dl890. Subcutaneous, $10^{6.0}$–$10^{7.9}$ PFU (depending on virus); groups ranged from $n$ = 9 to $n$ = 20. | WT SV40, 90% tumours; dl mutants, 21% to 92%. Tumours were fibrosarcomas. WT SV40 tumours appeared more quickly. | Time to tumour appearance, WT *vs* dl886, $P$ < 0.01. | sT not essential for SV40 oncogenicity.<br>Tumours induced by both WT and mutant viruses contained LT. |

## Table 3.1 (continued)

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster, strain LHC (NB, < 1 d) (NR)<br>1.5 yr<br>Topp *et al.* (1981) | WT SV40, strain 776 ($n$ = 1 group), and sT gene dl mutants ($n$ = 9 groups).<br>Subcutaneous, ~$10^7$ PFU in 0.2 mL.<br>WT SV40 DNA, subcutaneous, 1 µg in 0.1 mL.<br>Groups ranged from $n$ = 4 to $n$ = 14. | WT SV40 DNA, 8/9 (89%) tumours; average latency, 6.5 mo. WT SV40, 14/14 (100%) tumours; average latency, 7.5 mo. dl mutant viruses, from 4/4 (100%) to 0/4 (0%) tumours; average latencies, 10–14 mo. Tumours were fibrosarcomas. WT SV40-induced tumours appeared more quickly. | NR | |
| Hamster (NB, < 2 d) (NR)<br>80 wk<br>Matthews *et al.* (1987) | WT SV40 (strain WT 830), subcutaneous.<br>sT dl mutants, dl884, dl883, dl890, subcutaneous, 2 × $10^8$ PFU<br>WT SV40, ($n$ = 130); dl mutants, $n$ = 106, ($n$ = 71, and $n$ = 65, respectively). | WT: subcutaneous fibrosarcomas (69%), abdominal lymphomas (0.8%). Mean latency: fibrosarcomas, 34 wk; lymphomas, 68 wk.<br>dl mutants: fibrosarcomas (49%), lymphomas (15%) [dl883, 31%; dl884, 12%; dl890, 17%]. Mean latency: fibrosarcomas, 59 wk; lymphomas, 34 wk | NR | Lymphomas induced by dl mutants appeared more quickly than those induced by WT virus. Lung metastases occurred in ~5% of animals with fibrosarcomas and in almost all with lymphomas. Suggested that, in the absence of sT, the mutant virus preferentially transforms proliferating lymphoid cells. |

[a] Hamsters are outbred unless otherwise indicated.
[b] Statistical significance is listed if reported in the study.
d, day; dl, deletion; F, female; GMK/AGMK, African green monkey kidney; LT, large T-antigen; M, male; mo, month; NB, newborn; NR, not reported; PFU, plaque-forming units; RMKC, rhesus monkey kidney cells; sT, small t-antigen; SV40, simian virus 40; $TCID_{50}$, 50% tissue culture infective dose; ts, temperature-sensitive; *vs*, *versus*; wk, week; WT, wild-type; yr, year

effect of irradiation (cobalt-60 source or ultraviolet radiation) on the tumorigenic potential of SV40. Group sizes ranged from $n = 13$ to $n = 39$; animals were observed for 240 days. Irradiation reduced viral infectivity by 2–3 logs before inoculation but did not inhibit tumorigenicity. Tumour frequency not only was not reduced but even appeared to be increased. Before ultraviolet irradiation, the virus induced tumours in 62% of animals, and after irradiation in 88%. Similarly, before cobalt-60 irradiation, the virus produced tumours in 67% of hamsters, and after irradiation in 84%.

Numerous studies have examined the effect of SV40 genetic variation and mutations on tumorigenic potential *in vivo*. Deichman *et al.* (1978) compared wild-type (WT) SV40 and temperature-sensitive (ts) mutants (tsA, tsB, and tsBC) in NB hamsters by subcutaneous inoculation of 0.2 mL of $10^{6.5}$–$10^{8.25}$ $TCID_{50}$/mL(total $n = 73$). Animals were observed for 18 months. WT virus induced tumours in 13/16 (81%) of hamsters, whereas the ts mutants induced tumours in an average of 42% of animals. The latency period of the tumours induced by the ts mutants was extended compared with that of tumours induced by WT SV40 (120–495 days versus 103–314 days). Two mutants in late genes, tsB201 and tsBC210, induced tumours at a high frequency (15/26, 58%), whereas two early mutants, tsA209 and tsA239, induced relatively fewer tumours (8/30, 26%). Adult hamsters (age, 2.5–3 months) were inoculated subcutaneously with $10^{6.5}$–$10^{8.25}$ $TCID_{50}$ of virus ($n = 64$). None of the adult animals exposed to WT SV40 developed tumours, whereas 9% (5/54) of those injected with mutant viruses did (tsA30, 2/12; tsA239, 2/8; tsBC210, 1/14).

WT SV40 strain 776 and derivative small t-antigen (sT) gene deletion mutants dl883, dl885, dl886, and dl890 were tested in strain LSH NB hamsters (Lewis & Martin, 1979). Subcutaneous inoculations of $10^{6.0}$–$10^{7.9}$ PFU of virus were given, with groups ranging in size from $n = 9$ to $n = 20$. WT SV40 yielded tumours in 90% of animals, whereas tumour frequencies for the deletion mutants ranged from 21% to 92%. Tumours were fibrosarcomas. WT SV40-induced tumours appeared more quickly, with a significant difference in the time to tumour appearance between WT SV40 and dl886 ($P < 0.01$). Tumours induced by both WT and mutant viruses contained LT. [This study showed that the viral sT was not essential for SV40 tumorigenicity after subcutaneous inoculation.]

After subcutaneous inoculation of NB LHC hamsters with $10^7$ PFU of SV40 WT strain 776 virus, 100% of recipients ($n = 14$) developed tumours (fibrosarcomas) with an average latency of 7.5 months. sT gene deletion mutants ($n = 9$, different mutants) of the same strain were also injected subcutaneously, and tumour frequencies ranged from 4/4 (100%) to 0/4 (0%); tumour latencies varied from 10 months to 14 months (Topp *et al.*, 1981). [This study suggested that SV40 sT might function as a tumour promoter.]

A later study showed that deletion of the SV40 sT gene altered the tissue specificity of tumour induction (Matthews *et al.*, 1987). WT SV40 strain 830 and mutants dl884, dl883, and dl890 were inoculated subcutaneously into NB hamsters ($2 \times 10^8$ PFU per injection), and the animals were observed for 80 weeks. Group sizes for different viruses were: WT SV40 ($n = 130$), dl884 ($n = 106$), dl883 ($n = 71$), and dl890 ($n = 65$). WT virus induced subcutaneous fibrosarcomas (in 69% of animals) and a single abdominal lymphoma (0.8%). In contrast, the sT deletion mutants led to abdominal lymphomas in ~15% of recipients (dl883, 31%; dl884, 12%; dl890, 17%), as well as subcutaneous fibrosarcomas (49%). Lung metastases were very common in animals with lymphomas. Tumours were LT-positive. The mean latency of fibrosarcomas induced by WT virus and by deletion mutants was 34 weeks and 59 weeks, respectively. In contrast, lymphomas induced by the mutants appeared on average by 34 weeks and the single case induced by WT virus at 68 weeks. [This study suggests that in the

absence of sT, thought to have cell growth-promoting activities, the mutant virus preferentially transforms proliferating lymphoid cells.]

### *(b) Intracerebral inoculation*

See Table 3.2

Intracerebral inoculation of NB hamsters with SV40 can produce ependymomas, as first reported by Kirschstein & Gerber (1962). In a small study, 4 of 9 NB hamsters inoculated intracerebrally with SV40 strain 777 developed brain tumours (44%), identified as ependymomas, over a period of 3–4 months. Control animals inoculated with medium 199 ($n$ = 7) did not develop tumours. In a larger study also using NB animals, SV40 strain 777, and the intracerebral route of inoculation, Gerber & Kirschstein (1962) showed a virus dose dependence for induction of ependymomas. Among a total of 49 inoculated animals, 100% developed brain tumours after inoculation with $10^8$ $TCID_{50}$ of virus, 44% with $10^7$ $TCID_{50}$, 38% with $10^{6.5}$ $TCID_{50}$, and 0% with $10^6$ or $10^5$ $TCID_{50}$. The tumours appeared after about 3 months. Controls ($n$ = 10) inoculated with medium 199 were tumour-free at 7 months. SV40 strain PML-1, inoculated into the temporal bone of the skull of NB hamsters ($n$ = 27) at $2 \times 10^5$ PFU, resulted in a tumour incidence of 85% (Davis *et al.*, 1979). Four choroid plexus papillomas and 21 sarcomas in the region of the temporal bone developed with a latency period of about 4 months. Two tumours were cultured and were found to contain SV40 LT.

Wilkins & Odom (1965) confirmed SV40 induction of ependymomas in NB hamsters while surveying other species for susceptibility to SV40 brain tumours. Using the strain of SV40 from Dr A. Rabson, $10^7$ $TCID_{50}$ inoculated intracerebrally into NB hamsters ($n$ = 30) induced ependymomas in 23%, fibrosarcomas of the scalp in 27%, and cerebral cysts in 10% of hamsters. The brain tumours appeared 3.3–8 months after injection and fibrosarcomas after 5.5–12 months.

### *(c) Intravenous or intracardiac inoculation*

See Table 3.3

Intravenous or intracardiac inoculation of SV40 in weanling/young adult hamsters induces a spectrum of tumours, especially lymphomas, mesotheliomas, and osteosarcomas. Diamandopoulos (1972, 1973) inoculated SV40 strain VA45–54 ($10^{8.5}$ $TCID_{50}$) intravenously using the femoral vein into weanling (age, 21–22 days) male hamsters ($n$ = 100), as well as SV40 mixed with normal rabbit serum ($n$ = 50). Controls included animals inoculated with SV40 mixed with anti-SV40 serum ($n$ = 50) or medium ($n$ = 50), or uninoculated ($n$ = 50). Hamsters exposed to SV40 developed tumours (84%), as did those injected with SV40 mixed with normal rabbit serum (94%). All other groups did not develop any tumours during the 6-month observation period. The peak tumour latency was 5 months. Tumour types observed included reticulum cell sarcomas (72%), lymphosarcomas (4%), lymphocytic leukaemias (1%), and osteosarcomas (55%). It was noted that up to one third of tumour-positive animals had more than one type of malignancy. All tumours tested expressed SV40 LT. [This study showed that a different spectrum of tumour types arises after intravascular exposure to SV40 and provided the first evidence that SV40 can induce lymphomas and leukaemias.]

Diamandopoulos & McLane (1975) showed the effect of both host age and virus dose on tumour induction after intravenous inoculation of virus ($10^{0.5}$–$10^{8.5}$ $TCID_{50}$, $n$ = 8–10 animals per group). The tumour latencies and the morphological types of tumours induced were the same as those described in Diamandopoulos (1972, 1973). Controls received medium or cell lysate, or were uninoculated ($n$ = 70). SV40 strain VA45–54 ($10^{8.5}$ $TCID_{50}$) induced tumours in most male hamsters exposed at age 3 weeks (90%). Tumour frequency decreased with age at time of exposure, as follows: 1 month, 78%; 2 months, 50%; 3 months, 20%;

## Table 3.2 Carcinogenicity studies of SV40 in hamsters[a] by intracerebral inoculation

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ:<br>tumours (%); time to tumours | Comments |
|---|---|---|---|
| Hamster (NB, < 1 d) (NR)<br>Up to 156 d<br>Kirschstein & Gerber (1962) | SV40 strain 777, 0.02 mL, intracerebral<br>*n* = 9<br>Controls: 0.02 mL medium 199<br>*n* = 7 | Brain tumours (44%), ependymomas;<br>3–4 mo<br>Controls (0%) | First report of induction of true glioma by a virus. |
| Hamster (NB, < 1 d) (NR)<br>Up to 220 d<br>Gerber & Kirschstein (1962) | SV40 strain 777, 0.02 mL, intracerebral, $10^5$–$10^8$ $TCID_{50}$<br>*n* = 49<br>Controls: 0.02 mL medium 199 from uninfected GMK<br>*n* = 10 | Brain tumours, ependymomas, dose-dependent: $10^8$ $TCID_{50}$ (100%), $10^7$ $TCID_{50}$ (44%), $10^{6.5}$ $TCID_{50}$ (38%); 3–3.5 mo<br>$10^6$ and $10^5$ $TCID_{50}$ (0%).<br>Controls (0%) after 7 mo | Virus was concentrated by centrifugation. |
| Hamster (1 d) (NR)<br>Up to 1 yr<br>Wilkins & Odom (1965) | SV40 from Dr A. Rabson, titre $10^7$ $TCID_{50}$/0.1 mL, intracerebral inoculation of ~0.01 mL<br>*n* = 30<br>Controls NR | Brain tumours: ependymomas (23%); fibrosarcomas of the scalp (27%); cerebral cysts or hydrocephalus (10%)<br>Latency: 3.3–8 mo (brain tumours), 5.5–12 mo (fibrosarcomas) | Poor survival (71%) reduced the numbers of experimental animals for observation. |
| Hamster (NB) (NR)<br>5 mo<br>Davis *et al.* (1979) | SV40 strain PML-1, $2 \times 10^5$ PFU/0.02 mL, intracerebral (temporal)<br>*n* = 27 | Tumour incidence (85%); ~4 mo<br>21 sarcomas in the region of the temporal bone, 4 choroid plexus papillomas | Two tumours explanted, contained SV40 LT. Two animals had both tumour types. |

[a] Hamsters are outbred unless otherwise indicated.

d, day; dl, deletion; GMK/AGMK, African green monkey kidney; LT, large T-antigen; mo, month; NB, newborn; NR, not reported; PFU, plaque-forming units; SV40, simian virus 40; $TCID_{50}$, 50% tissue culture infective dose; yr, year

## Table 3.3 Carcinogenicity studies of SV40 in hamsters[a] by intravenous or intracardiac inoculation

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster (weanling, 21–22 d) (M)<br>6 mo<br>Diamandopoulos (1972, 1973) | SV40 strain VA45-54, $10^{8.5}$ $TCID_{50}$/mL, intravenous (femoral vein)<br>*n* = 100<br>SV40 + NRS<br>*n* = 50<br>Controls, SV40 + anti-SV40 serum, or culture medium, or uninoculated<br>*n* = 50 each | Tumour incidence: SV40 (84%), SV40 + NRS (94%), all other groups (0%)<br>Peak tumour latency: 5 mo<br>Tumour types: reticulum cell sarcomas (72%), lymphosarcomas (4%), lymphocytic leukaemias (1%), osteosarcomas (55%) | | Up to one third of tumour-positive animals had more than one type of neoplasm.<br>First report that SV40 could induce lymphomas and leukaemias.<br>All tumours tested contained SV40 LT. |
| Hamster (3 wk to 12 mo) (M)<br>12 mo<br>Diamandopoulos & McLane (1975) | SV40 strain VA45-54, $10^{8.5}$–$10^{0.5}$ $TCID_{50}$/mL, intravenous (femoral vein)<br>*n* = 8–10 per group<br>Controls: cell lysate, culture medium, uninoculated<br>*n* = 70 | Tumour incidence decreased with age at time of exposure, dropping sharply for animals older than 2 mo: 3 wk, 90%; 1 mo, 78%; 2 mo, 50%; 3 mo, 20%; 6 mo, 12%; 9 and 12 mo, 0%; average latency increased from 7 mo to 11 mo.<br>Only animals exposed to $10^{8.5}$ $TCID_{50}$ of SV40 at 3 wk developed tumours; $10^{7.5}$ to $10^{0.5}$ $TCID_{50}$ induced no tumours. | | Morphological types of tumour were the same as those described in Diamandopoulos (1972, 1973). |
| Hamster,1 outbred, 3 inbred strains (weanling, 3 wk) (M)<br>12 mo<br>Diamandopoulos (1978) | SV40 strain VA45-54, $10^{7.5}$ $TCID_{50}$/mL, intravenous (femoral vein)<br>*n* = 12 per group<br>Controls: uninoculated<br>*n* = 12 | Tumour incidence: outbred (9%), inbred strains (58–90%), controls (0%); latency = 8–11 mo | | Inbred strains: LSH/SsLak, LHC/Lak, and MHA/SsLak.<br>Morphological types of tumour were the same as those described in (Diamandopoulos, 1972, 1973).<br>Permanent cell lines were established from two lymphocytic leukaemias. |
| Hamster (weanling, 21 d) (NR)<br>9 mo<br>Cicala *et al.* (1992) | WT SV40 strain 830, *n* = 21; and strain 776, *n* = 5, $10^{8.5}$ PFU, intracardiac<br>SV40 sT gene dl mutants dl2006, *n* = 22; dl883, *n* = 12<br>Controls: culture medium, *n* = 20 | WT: mesotheliomas (40–62%), abdominal lymphomas (33–40%), and osteosarcomas (10–40%); 17–22 wk<br>sT dl mutants: abdominal lymphomas (100%); 26 wk<br>Controls (0%) | | Both B-cell lymphomas and true histiocytic lymphomas were induced.<br>Suggested that sT is required for SV40 transformation of non-dividing cells. |

**Table 3.3 (continued)**

| Species, strain (age) (sex) Duration of observation Reference | Virus strain, dose, route Controls Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster (weanling, 21 d) (M, F) 9 mo Cicala *et al.* (1993) | WT SV40 strain 830 and strain 776, $10^{8.5}$ PFU in 0.2 mL sT dl mutants dl883 and dl2006 Intracardiac (WT 776, *n* = 5; WT 830, *n* = 21; dl883, *n* = 12; dl2006, *n* = 22); intrapleural (WT 830, *n* = 6; WT 776, *n* = 5); intraperitoneal (WT 776, *n* = 6) Controls: culture medium *n* = 20 (intracardiac) *n* = 4 (intraperitoneal, intrapleural) | Animals injected with WT SV40 (*n* = 43): all developed tumours (100%). Of these, 70% (100%, 58% and 67% after intrapleural, intracardiac, and intraperitoneal injection, respectively) had mesotheliomas; others had lymphomas (23%) and osteosarcomas (9%). Latency: 3–6 mo. Animals injected with sT gene dl mutants (intracardiac) (*n* = 34): all developed abdominal lymphomas (100%); 1 (3%) had mesothelioma. Latency: ~6 mo Controls (0%) | | First report of virus-induced mesotheliomas in mammals. SV40 LT expressed in mesothelioma tumour cell lines. |
| Hamster (weanling, 21 d) (M, F) 8 mo McNees *et al.* (2009) | WT SV40 strains [VA45-54(2E), VA45-54(1E), SVCPC(1E)] with different regulatory region; SV40 recombinants [776-CPC(2E), 776-VA(2E)]. Intravenous, $1 \times 10^7$ PFU in 0.2 mL *n* = 71 (total) [*n* = 70 (total)] *n* = 28 (controls: cell lysate) | Tumour incidences by different strains ranged from 82% [VA45-54(2E)] to 15% [776-CPC(2E)] (overall, 54%); median tumour latencies, 24–34 wk tumour types: mesothelioma, lymphoma, sarcoma, osteosarcoma. Controls (0%) | Tumour incidences differed for SV40 recombinants: 776-VA(2E) (10/16, 62%) *vs* 776-CPC(2E) (2/13, 15%), *P* = 0.01 | Lymphoma cell lines expressed SV40 LT. Most animals were necropsied. |

[a] Hamsters are outbred unless otherwise indicated.

[b] Statistical significance is listed if reported in the study.

1E, simple regulatory region, SV40; 2E, complex regulatory region, SV40; d, day; dl, deletion; F, female; LT, large T-antigen; M, male; mo, month; NB, newborn; NR, not reported; NRS, normal rabbit serum; PFU, plaque-forming units; sT, small t-antigen; SV40, simian virus 40; $TCID_{50}$, 50% tissue culture infective dose; *vs*, *versus*; wk, week; WT, wild-type

6 months, 12%; and 9 or 12 months, 0%. Average tumour latency increased from 7 months to 11 months as older animals were inoculated. Only those exposed to $10^{8.5}$ $TCID_{50}$ of SV40 developed tumours; lower doses of virus resulted in no tumours during the 12-month observation period. Diamandopoulos (1978) also found that certain inbred strains of hamsters were more susceptible than outbred hamsters to tumour induction by SV40. Tumours were the same as those described in Diamandopoulos (1972, 1973). An inoculum of $10^{7.5}$ $TCID_{50}$ intravenously (in the femoral vein) was used in weanling (age, 3 weeks) male hamsters ($n$ = 12 per group). The tumour incidence was 9% in the outbred animals and 58–90% in three inbred strains, based on a 12-month observation period. Uninoculated controls did not develop tumours.

Cicala *et al.* (1992) used the intracardiac route to inoculate weanling (age, 21 days) hamsters with WT SV40 strain 830 ($n$ = 21), WT SV40 strain 776 ($n$ = 5), or sT deletion mutants dl2006 ($n$ = 22) and dl883 ($n$ = 12). The WT viruses (an inoculum of $10^{8.5}$ PFU) induced mesotheliomas (40–62%), abdominal lymphomas (33–40%), and osteosarcomas (10–40%) with tumour latencies of 17–22 weeks. In contrast, the sT deletion mutants induced abdominal lymphomas (B-cell lymphomas or true histiocytic lymphomas) in 100% of recipients, with a latency of 26 weeks. [This observation suggests a requirement for sT for SV40 transformation of non-dividing cells.] In a follow-up report, Cicala *et al.* (1993) expanded the intracardiac group and added groups that were intrapleurally or intraperitoneally inoculated with the WT SV40 830 or WT SV40 776 strains. Of those inoculated by the intrapleural route, 11 (100%) of 11 developed mesotheliomas, compared with 15 (58%) of 26 by the intracardiac route and 4 (67%) of 6 by the intraperitoneal route. All control animals injected with medium by the intracardiac ($n$ = 20), intrapleural ($n$ = 4), or intraperitoneal ($n$ = 4) route were tumour-free after the 9-month observation period. SV40 LT was expressed in mesothelioma tumour cell lines. [This was the first description of virus-induced mesotheliomas in mammals.]

McNees *et al.* (2009) used the intravenous route to expose weanling hamsters to WT SV40 strains VA45–54(2E) ($n$ = 17), VA45–54(1E) ($n$ = 15), and SVCPC(1E) ($n$ = 9), and two recombinant strains, 776-CPC(2E) ($n$ = 13) and 776-VA(2E) ($n$ = 16). Tumour frequencies ranged from 82% for VA45–54(2E) to 15% for 776-CPC(2E), with an overall incidence of 54%. Median tumour latencies were 24–34 weeks. There was a significant difference ($P$ = 0.01) between tumour frequencies after exposure to two different SV40 recombinant strains: 776-VA(2E) (62%) and 776-CPC(2E) (15%). Control animals inoculated with cell lysate ($n$ = 28) did not develop tumours during the 8-month observation period. Tumour types identified histologically included mesotheliomas, lymphomas, sarcomas, and osteosarcomas. Lymphoma-derived cell lines were shown to express SV40 LT. [This study suggests that different SV40 strains can differ in tumorigenic potential *in vivo* after intravenous inoculation.]

### *(d) Intraperitoneal or intrapleural inoculation*

See Table 3.4

Introduction of SV40 into pregnant hamsters resulted in the appearance of SV40-positive tumours in their offspring (Rachlin *et al.*, 1988). SV40 strain 776 was inoculated intraperitoneally at doses ranging from $10^7$ PFU to $10^3$ PFU into pregnant hamsters on day 4–12 of gestation. Tumours developed in the offspring of mothers inoculated with $10^7$ PFU of virus on day 8 (13/24, 54%) and day 12 (13/30, 43%) of gestation. Litters from mothers exposed to other doses of virus remained tumour-free, as did all the inoculated mother hamsters. The subcutaneous tumours were classified as sarcomas, and all expressed SV40 LT. Of the mothers inoculated on day 4 of gestation, 63% (12/19) of their progeny died within the first 4 weeks after birth. [This study showed that vertical transmission of SV40 can

## Table 3.4 Carcinogenicity studies of SV40 in hamsters[a] by intrapleural or intraperitoneal inoculation

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster (offspring)<br>Up to 1 yr<br>Rachlin *et al.* (1988) | SV40 strain 776, $10^3$–$10^7$ PFU, intraperitoneal (dams), d 4–12 of gestation; *n* = 16–30/group<br>Controls: medium only<br>*n* = 23–31/group | Subcutaneous sarcomas in offspring of dams inoculated with $10^7$ PFU on<br>d 8 (13/24, 54%) and<br>d 12 (13/30, 43%).<br>Lower doses, no tumours. | | First report that vertical transmission of SV40 can lead to tumour formation in progeny. No tumours in dams. Effect of age of fetus: 63% (12/19) of progeny of mothers inoculated on day 4 of gestation died in first 4 wk after birth. All tumours contained SV40 LT. |
| Hamster (weanling, 21 d) (M, F)<br>9 mo<br>Cicala *et al.* (1993) | See Table 3.3 | | | |
| Hamster (weanling, 21 d) (M, F)<br>8 mo and 12 mo<br>Vilchez *et al.* (2004) | SV40 strains VA45-54(2E), SVCPC(1E),<br>$10^7$ PFU in 0.5 mL, intraperitoneal, *n* = 33 and *n* = 37<br>Controls: cell lysate, *n* = 78 | VA45-54(2E), 21%; SVCPC(1E), 49%. Similar types of abdominal tumour in both strains (sarcomas, mesotheliomas, osteosarcomas); mean, 6–8 mo<br>Controls: 0% tumours | | Metastases found in lung and lymph nodes. SV40 LT expressed in tumour cells. |
| Hamster (weanling, 21 d) (M, F)<br>12 mo<br>Sroller *et al.* (2008) | Eight SV40 WT strains and eight SV40 recombinants consisting of different regulatory regions and LT with variable T-ag–C domains, $1 \times 10^7$ PFU in 0.5 mL, intraperitoneal, *n* = 307 (total)<br>Controls, *n* = 187 | Tumour frequencies by different WT strains: 83% (Baylor 1E), 54% (SVCPC), 31% (776 1E), 26% (777), 23% (VA45-54), 10% (776 2E), 6% (Baylor 2E), 0% (SVPML).<br>Tumour frequencies by different recombinants: 58% (CPC-PML 1E), 37% (CPC-Baylor 1E), 35% (CPC-776 1E), 33% (776-Baylor 2E), 24% (CPC-VA 1E), 13% (776-VA 2E), 7% (776-CPC 2E), 6% (776-PML 2E) | SV40-exposed (84/307, 27%) *vs* controls (0/187, 0%), *P* = 0.0001.<br>Tumour induction higher by viruses with simple 1E regulatory regions (38/80, 48%) *vs* viruses with complex 2E regulatory regions (7/76, 9%), *P* = 0.0001 | Tumour type unspecified.<br>LT variable domain (T-ag–C) showed little influence on SV40 tumorigenicity.<br>Non-tumorigenic/weakly tumorigenic strains and highly tumorigenic strains transformed mouse cells *in vitro* with similar frequencies; differences in tumorigenicity *in vivo* must reflect host responses to viral infection.<br>First report of influence of SV40 regulatory region on tumorigenic potential *in vivo*. |

[a] Hamsters are outbred unless otherwise indicated.

[b] Statistical significance is listed if reported in the study.

1E, simple viral regulatory region, single enhancer; 2E, complex, rearranged viral regulatory region; d, day; F, female; LT, large T-antigen; mo, month; M, male; NB, newborn; NR: not reported; PFU, plaque-forming units; T-ag–C, SV40 large LT C-terminal variable domain; $TCID_{50}$, 50% tissue culture infective dose; ts, temperature-sensitive; *vs*, *versus*; wk, week; WT, wild-type; yr, year

occur, followed by expression of viral oncogenicity in progeny animals.]

Hamsters were intrapleurally or intraperitoneally inoculated with the WT SV40 830 or WT SV40 776 strains by Cicala *et al.* (1993). This study is described in Section 3.1.1(c) and Table 3.3.

Vilchez *et al.* (2004) compared the tumorigenicity of two SV40 strains by intraperitoneal inoculation of weanling hamsters with a uniform inoculum of $1 \times 10^7$ PFU. Strain VA45–54(2E) contains a complex regulatory region, whereas strain SVCPC(1E) contains a simple regulatory region with a single enhancer. The numbers of animals per virus group were $n = 33$ [VA45–54(2E)] and $n = 37$ [SVCPC(1E)]. Controls ($n = 78$) were inoculated with cell lysate. Tumour frequencies were 21% for VA45–54(2E), 49% for SVCPC(1E), and 0% in controls after observation periods of 8–12 months. The two viruses induced similar types of abdominal tumours (sarcomas, mesotheliomas, and osteosarcomas) with similar mean latencies of 6–8 months. Metastases were observed in lung and lymph nodes.

It has been demonstrated that the viral regulatory region is a genetic determinant of SV40 oncogenic potential, whereas the LT *C*-terminal variable domain (T-ag–C) exerts negligible influence (Sroller *et al.*, 2008). These two genomic regions are sites of variation among SV40 isolates. Weanling hamsters were inoculated by the intraperitoneal route using $1 \times 10^7$ PFU per injection. Eight different strains of WT SV40 and eight recombinants consisting of different viral regulatory regions linked to LT with variable T-ag–C domains were included. The total numbers of experimental animals were $n = 307$ (SV40-inoculated) and $n = 187$ (controls). Tumour frequencies by different SV40 strains after a 12-month observation period ranged from 83% (Baylor 1E) to 0% (SVPML) [tumour type unspecified]. Tumour incidence was significantly increased ($P = 0.0001$) in SV40-exposed hamsters (84/307, 27%) *versus* controls (0/187, 0%). Tumour incidence was significantly increased ($P = 0.0001$) by viruses with simple 1E regulatory regions (38/80, 48%) *versus* viruses with complex 2E regulatory regions (7/76, 9%). However, there was no difference in the median time to tumours by 1E and 2E viruses (32.5 weeks *versus* 27 weeks). The LT variable domain showed little influence on SV40 tumorigenicity. The highly tumorigenic and non-tumorigenic/weakly tumorigenic strains of SV40 transformed mouse cells *in vitro* with similar frequencies, suggesting that the differences in tumorigenicity observed *in vivo* reflected host responses to viral infection. [This was the first report of the influence of the SV40 regulatory region on oncogenic potential *in vivo*.]

### 3.1.2 Mouse

See Table 3.5

In the original study by Eddy *et al.* (1962) that demonstrated the tumorigenicity of SV40 in NB hamsters, NB NIH white mice were also inoculated subcutaneously with SV40 ($n = 10$) or rhesus monkey kidney cell oncogenic extracts ($n = 17$). No tumours were observed. [The Working Group noted the absence of a control group.]

The effect of co-infection with murine malaria parasites, *Plasmodium berghei yoelii,* on SV40 tumorigenicity in NB mice was examined. NB CFW mice were inoculated intravenously via the anterior facial vein with $10^4$ $TCID_{50}$ of SV40 strain VA45–54 (Hargis & Malkiel, 1979). After 3 months, some virus-infected animals ($n = 11$) were given $5 \times 10^6$ malaria parasite-infected mouse red blood cells, whereas others were not co-infected ($n = 10$). Control animals were injected with parasites only ($n = 12$). Mice exposed to SV40 developed sarcomas of the liver and/or spleen, including 70% of those infected with SV40 only and 100% of those co-infected with virus and parasites. Tumours contained SV40 LT. Tumour latency was shorter in the co-infected animals compared with virus alone (9 months *versus* 11 months). Control animals

did not develop tumours. Adult CFW mice ($n$ = 31) were treated in parallel with the virus, with or without parasites, but no tumours were observed.

### 3.1.3 Other species

See Table 3.6

Subcutaneous inoculation of SV40 into NB *Mastomys natalensis*, an African rodent, induced brain tumours (Rabson *et al.*, 1962). SV40 strain 777 at $10^7$ $TCID_{50}$ produced brain tumours (papillary ependymomas and maybe some choroid plexus papillomas) after 3.7–7.5 months in 8 of 10 animals (80%). Controls ($n$ = 29) inoculated with medium or uninfected African green monkey kidney cell fluid did not develop tumours. [This was the first report of SV40 oncogenicity in species other than hamsters and of subcutaneous inoculation of SV40 producing brain tumours in animals in the absence of other tumours.]

Wilkins & Odom (1965) gave intracerebral inoculations of SV40 strain from Dr A. Rabson at $10^7$ $TCID_{50}$ to Fischer rats ($n$ = 27; age, 26 days and 7 weeks), rabbits ($n$ = 8; age, 3 days), kittens ($n$ = 5; age, 1 day), and puppies ($n$ = 22; age, 1 day). Some rats and rabbits were exposed to total body irradiation (175 röntgen [45.15 mC/kg]) before virus inoculation. A group of puppies was uninoculated ($n$ = 9). None of the animals developed brain tumours. [The Working Group noted that poor survival reduced the numbers of experimental animals for observation, so this study cannot be considered informative.]

Adult female dogs were injected with $10^9$ $TCID_{50}$ of SV40 into the bladder submucosa, either alone ($n$ = 1) or mixed with Freund's complete adjuvant ($n$ = 4) (Shah & Pond, 1972). No neoplastic changes were observed in the bladder after observation periods of 31–57 weeks.

## 3.2 Infection with SV40 DNA

### *Hamster*

See Table 3.7

Induction of tumour formation after infection with SV40 DNA was demonstrated by Sol & Noordaa (1977). They introduced DNA (1–2 μg) from SV40 strain VA45–54 with an infectivity titre of $1.5 \times 10^6$ PFU/μg DNA into NB hamsters ($n$ = 33) by subcutaneous inoculation. Tumours (sarcomas) [type not further specified] were produced in 33% of the animals. Linearization of the viral DNA by the restriction enzyme *Eco*RI before inoculation ($n$ = 8) resulted in tumours in 50% of hamsters. Control DNase-treated SV40 DNA was non-tumorigenic ($n$ = 32 animals).

When NB LHC hamsters ($n$ = 9) were injected subcutaneously with WT SV40 DNA (1 μg), 8 of the 9 animals developed tumours, with an average latency of 6.5 months (Topp *et al.*, 1981).

DNAs from WT SV40 strain 777 and from deletion mutant dl2005 lacking the sT gene were tested in parallel (Bouchard *et al.*, 1984). Subcutaneous injection of 2 μg of WT DNA that was linearized by the enzyme *Sal*I [with or without carrier DNA (10 μg)] into NB hamsters ($n$ = 7) produced fibrosarcomas at the site of inoculation in 57% of animals, with latency periods of 6–7 months. The mutant viral DNA ($n$ = 22) produced tumours in 23% of hamsters, with latencies of 11–12 months.

## 3.3 Transgenic mouse models

### 3.3.1 SV40 DNA with natural viral promoter

See Table 3.8 and Table 3.9

The development of transgenic mouse technology added a new dimension to studies of viral oncogenes as it became possible to analyse tumour virus proteins in host animals. SV40 LT under the control of its natural regulatory region was the first viral oncoprotein expressed in transgenic mice (Brinster *et al.*, 1984). Since that

## Table 3.5 Carcinogenicity studies of SV40 in mice

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: Tumours (%); time to tumours | Comments |
|---|---|---|---|
| Mouse, NIH white (NB) (NR)<br>Up to 344 d<br>Eddy *et al.* (1962) | SV40 strain 776, 0.1 mL, subcutaneous<br>*n* = 10<br>RMKC oncogenic extracts (Eddy *et al.*, 1961), 0.1 mL, subcutaneous<br>*n* = 17 | 0% | No controls. |
| Mouse, strain CFW (NB and adult) (NR)<br>Up to 12 mo<br>Hargis & Malkiel (1979) | SV40 strain VA45-54, $10^4$ $TCID_{50}$/0.1 mL, intravenous, anterior facial vein<br>NB, *n* = 10<br>NB injected with $5 \times 10^6$ malaria parasite-infected mouse red blood cells (on d 90), *n* = 11<br>Controls: parasites only, *n* = 12<br>Adult, *n* = 31, some plus parasites | NB mice<br>Sarcomas of liver and/or spleen:<br>SV40 + parasites (100%)*; 9 mo<br>SV40 only (70%); 11 mo<br>Controls: parasites only (0%)<br>Adult (0%) | Murine malaria parasites: *Plasmodium berghei yoelii*.<br>*Four animals had lung metastases.<br>Tumours expressed SV40 LT. |

d, day; LT, large T-antigen; mo, month, NB, newborn; NR, not reported, RMKC, rhesus monkey kidney cells; SV40, simian virus 40; $TCID_{50}$, 50% tissue culture infective dose

beginning, SV40 LT expression has been directed to many different tissues and cell types by the use of tissue-specific promoters in transgenic mice. Those studies have established that SV40 LT is highly oncogenic. The transgenic models have contributed to fundamental principles related to SV40 LT transformation and tumour development (Lednicky & Butel, 1999; Butel, 2000a, 2012; Ahuja *et al.*, 2005; Pipas, 2009; Sáenz Robles & Pipas, 2009; Gjoerup & Chang, 2010). Only studies involving the use of SV40 with its natural promoter are reviewed here.

Brinster *et al.* (1984) created the first SV40 transgenic mice using an SV40 plasmid (pSV3) with the natural viral promoter. Of the transgenic founder pups ($n$ = 25), most developed brain tumours (choroid plexus papilloma or carcinoma). Some animals also displayed thymus and kidney lesions [not further specified]. The transgenic pups died within 1–5 months. Palmiter *et al.* (1985) followed up with a study of transgenic mice carrying not only WT SV40 but also SV40 plasmid constructs that expressed LT only (pSV11) or sT and truncated LT (pSV8). All viral constructs contained the natural viral promoter. The number of transgenic founder pups per group ranged from $n$ = 4 to $n$ = 9. With WT SV40, 7 (87%) of 8 transgenic pups developed choroid plexus papillomas, as did 5 (71%) of 7 pSV11 transgenic pups. Tumours expressed SV40 LT. In contrast, none of 9 pSV8 transgenic pups developed tumours, showing the requirement for LT in SV40 tumorigenicity. Thymic hyperplasia was present in some of the mice with brain tumours. Chen & Van Dyke (1991) extended the studies with pSV11 (the construct that expressed LT only). Transgenic founders ($n$ = 13) were created, and 9 (69%) developed choroid plexus tumours, with latency periods of 65–210 days. Some animals also exhibited thymic hyperplasia and polycystic kidneys. An SV11 transgenic line was derived; these transgenic mice developed brain tumours at approximately day 100. In SLT transgenic mice, the LT gene of lymphotropic papovavirus, under the control of the SV40 promoter, could substitute for SV40 LT to induce brain tumours (4/9).

The nuclear-transport-defective mutant of SV40, pSV40(*c*T), was examined in transgenic mice by Pinkert *et al.* (1987). Seven (88%) of 8 transgenic founder mice died with choroid plexus papillomas and/or with kidney and thymus lesions (including tumours). Mean time to death was 52 days. Non-transgenic pups ($n$ = 36) did not develop tumours. A transgenic line, 269, was established; those animals ($n$ = 68) developed brain tumours within a mean time of 81 days. SV40 LT was retained in the cytoplasm of the brain tumours.

### *3.3.2 LT with hybrid SV40 and JCV sequences*

See Table 3.9

Studies using the promoter of SV40 or JCV Mad-1 strain, driving expression of either SV40 LT or JCV LT, have revealed important information about the tropism of these viruses. Feigenbaum *et al.* (1992) reported that 3 of 5 founders carrying the SV40 early sequence under the control of the JCV Mad-1 promoter developed tumours, including neuroblastoma (60%), myenteric plexus tumours (60%), and choroid plexus carcinoma (40%), within 0.5–4 months, whereas 100% of 7 founders carrying the JCV early sequence under the control of the SV40 promoter developed tumours, including choroid plexus papilloma (86%), within 1–3 months. On a C57/CBA background, Ressetar *et al.* (1993) reported two founders with the JCV Mad-1 promoter driving SV40 LT gene expression that developed abdominal B-cell lymphoma, adrenal neuroblastoma, osteosarcoma, and astrocytoma within 1–2.5 months. In parallel, one founder with the SV40 776 promoter driving JCV LT gene expression developed choroid plexus papilloma and thymoma by 1.5 months. [Similar to the studies of JCV infection in hamsters and JCV LT transgenic mice, the animals frequently

## Table 3.6 Carcinogenicity studies of SV40 in other species

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: Tumours (%);<br>time to tumours | Comments |
|---|---|---|---|
| *Mastomys natalensis* (African rodent) (NB, < 1 d) (NR)<br>Up to 7.5 mo<br>Rabson *et al.* (1962) | SV40 strain 777, 0.05 mL ($10^7$ $TCID_{50}$), subcutaneous<br>*n* = 10<br>Controls: uninfected GMK cell fluid or medium<br>*n* = 29 | Brain tumours (80%): papillary ependymomas, maybe some choroid plexus tumours;<br>3.7–7.5 mo<br>Controls (0%) | First report that SV40 oncogenicity not limited to hamsters. Finding that subcutaneous inoculation of virus can produce brain tumours without any subcutaneous or visceral tumours. |
| Rat, Fischer (26 d, 7 wk),<br>rabbit (3 d),<br>kitten (1 d),<br>puppy (1 d) (sex NR),<br>rat (26 mo),<br>kitten (6 mo),<br>rabbit (10 mo),<br>puppy (1 yr)<br>Wilkins & Odom (1965) | SV40 from Dr A. Rabson, titre $10^7$ $TCID_{50}$/0.1 mL, intracerebral<br>Some rats and rabbits exposed to total body irradiation (41.15 mC/kg) before inoculation<br>Inoculation 0.1–0.2 mL<br>rats (*n* = 27),<br>rabbits (*n* = 8),<br>kittens (*n* = 5),<br>puppies (*n* = 22),<br>Uninoculated controls:<br>puppies only (*n* = 9) | Rats, rabbits, kittens, puppies: no lesions, except 1 rat had an incidental fibrosarcoma in the brain found at 18 mo. | Poor survival reduced the numbers of experimental animals for observation |
| Dog (adult) (F)<br>31–57 wk<br>Shah & Pond (1972) | SV40, $10^9$ $TCID_{50}$ in 5 mL, injected into submucosa of bladder,<br>*n* = 1<br>SV40, $10^{9.0}$ $TCID_{50}$ in 1 mL mixed with 1 mL Freund's complete adjuvant, submucosa of bladder,<br>*n* = 4 | No neoplastic changes in bladder; no virus detected in blood, urine, or kidney biopsies; no antibodies to LT. | |

[a] Hamsters are outbred unless otherwise indicated.

d, day; F, female; GMK/AGMK, African green monkey kidney; LT, large T-antigen; mo, month; NB, newborn; NR, not reported; SV40, simian virus 40; $TCID_{50}$, 50% tissue culture infective dose; ts, temperature-sensitive; wk, week; yr, year

## Table 3.7 Carcinogenicity studies of SV40 DNA in hamsters[a]

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: Tumours (%);<br>time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Hamster (NB) (NR)<br>6 mo<br>Sol & Noordaa (1977) | SV40 DNA, strain VA45-54,<br>$1.5 \times 10^6$ PFU/μg SV40 DNA, 1–2 μg in 70 μL/animal, subcutaneous<br>*n* = 33<br>*Eco*RI-linearized SV40 DNA<br>*n* = 8<br>DNase-treated SV40 DNA<br>*n* = 32 | SV40 DNA (33%), sarcoma<br>Linearized SV40 DNA (50%), sarcoma<br>DNase-treated SV40 DNA (0%) | NR | One tumour was cultured, and those cells expressed SV40 LT. Tumour type not further specified.<br>Addition of calf thymus DNA or poly-L-ornithine to injection mixture had no effect on oncogenic potential. |
| Hamster, strain LHC (NB, < 1 d) (NR)<br>1.5 yr<br>Topp *et al.* (1981) | WT SV40 DNA, 1 μg in 0.1 mL, ~$10^7$ PFU in 0.2 mL, subcutaneous<br>*n* = 9 | Fibrosarcomas, 8/9 (89%); 6.5 mo | NR | |
| Hamster (NB, 1 d) (NR)<br>Up to 12 mo<br>Bouchard *et al.* (1984) | SV40 DNA, strain 777<br>2 μg plasmid DNA ± 10 μg carrier DNA in 10–100 μL PBS (linearized by *Sal*I), subcutaneous<br>*n* = 7<br>dl mutant dl2005 DNA lacking sT gene<br>*n* = 22 | Tumours at site of inoculation, fibrosarcomas<br>WT SV40 DNA (57%); 6–7 mo<br>sT dl mutant dl2005 DNA (23%); 11–12 mo | NR | |

[a] Hamsters are outbred unless otherwise indicated.

d, day; dl, deletion; LT, large T-antigen; mo, month; NB, newborn; NR, not reported; PBS, phosphate-buffered saline; PFU, plaque-forming units; sT, small t-antigen; SV40, simian virus 40; WT, wild-type; yr, year

## Table 3.8 Carcinogenicity studies in transgenic mice involving SV40 with natural viral promoter[a]

| Species, strain (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: Tumours (%); time to tumours | Significance[b] | Comments |
|---|---|---|---|---|
| Mouse (C57BL6; C57 × SJL) (NR)<br>Up to 5 mo<br>Brinster *et al.* (1984) | SV40 plasmid pSV3, transgenic founder pups, *n* = 25 | Most transgenic animals developed brain tumours (choroid plexus papilloma or carcinoma) and occasional thymus or kidney lesions. Latency (death), 1–5 mo | NR | First SV40 transgenic mice. Thymus and kidney lesions not further specified. |
| Mouse (C57 × SJL) (NR)<br>Up to 12 mo<br>Palmiter *et al.* (1985) | WT SV40; SV40 plasmid pSV11 (LT only); SV40 plasmid pSV8 (sT + truncated LT)<br>Transgenic founder pups, *n* = 4–9/group | WT SV40, 7/8 (87%) CPP; pSV11, 5/7 (71%) CPP; pSV8, 0/9 (0%) CPP.<br>Thymic hyperplasia present in some mice with CPP. | NR | Tumours expressed SV40 LT. |
| Mouse (C57BL/6 × SJL) (NR)<br>Up to 18 mo<br>Pinkert *et al.* (1987) | pSV40(cT), cytoplasmic LT mutant.<br>Transgenic founder pups, *n* = 8; non-transgenic pups, *n* = 36<br>Transgenic line 269, *n* = 68 | 7/8 (88%) founders died, CPP and kidney and thymus lesions, including tumours.<br>Time to death: founders, 40–134 d (mean, 52 d); transgenic 269 line, 81 d.<br>No tumours in non-transgenic pups. | NR | SV40(cT): mutation in nuclear transport signal on LT results in retention of LT in the cytoplasm. SV40 LT was retained in the cytoplasm of CPP. |
| Mouse (B6D2F1) (NR)<br>Up to 18 mo<br>Chen & Van Dyke (1991) | SV40 plasmid pSV11<br>Founders, *n* = 13<br>Recombinant: SV40 regulatory region plus LT of lymphotropic papovavirus (SLT)<br>Founders, *n* = 9<br>SV11, SV40 transgenic line | SV40 founders, 9/13 (69%), choroid plexus tumours; some also had thymic hyperplasia and polycystic kidneys; latency, 65–210 d.<br>SLT founders, 8/9 (89%), total tumours; 4/9, choroid plexus tumours; some also had thymic hyperplasia and polycystic kidneys, same diseases as for WT SV40. Latency, 70–380 d.<br>SV11 line, ~100 d. | NR | LT of lymphotropic papovavirus could substitute for SV40 LT to induce brain tumours. |

[a] SV40 LT expressed under the control of tissue-specific foreign promoters in transgenic mice can induce pre-neoplastic/neoplastic changes in many different tissues and cell types. Those systems are not reviewed here.

[b] Statistical significance is listed if reported in the study.

CPP, choroid plexus papillomas; cT, cytoplasmic LT mutant; d, day; mo, month; LT, large T-antigen; NB, newborn; NR, not reported; sT, small t-antigen; SV40, simian virus 40; WT, wild-type

## Table 3.9 Carcinogenicity studies of transgenic mice involving hybrid SV40 and JCV sequences

| Species, strain (sex)<br>Duration<br>Reference | Dosing regimen,<br>Animals/group at start | For each target organ: Incidence (%);<br>time to tumours |
|---|---|---|
| Mouse, NR (NR)<br>Lifetime<br>Feigenbaum *et al.* (1992) | JCV Mad-1 promoter × SV40 LT gene<br>*n* = 5 | Tumours (60%), including neuroblastoma (60%), myenteric plexus tumours (60%), choroid plexus carcinoma (40%); 0.5–4 mo |
| | SV40 promoter × JCV LT gene<br>*n* = 7 | Tumours (100%), including choroid plexus papilloma (86%), renal hyperplasia (71%), thyroid hyperplasia (43%), thymic hyperplasia (29%); 1–3 mo |
| Mouse, C57BL/6 × CBA (M, F)<br>Up to 75 d<br>Ressetar *et al.* (1993) | JCV Mad-1 promoter × SV40 LT gene<br>*n* = 2 | Tumours (100%), including abdominal B-cell lymphoma (50%), adrenal neuroblastoma (50%), osteosarcoma (50%), astrocytoma (50%); 1–2.5 mo |
| | SV40 776 promoter × JCV LT gene<br>*n* = 1 | Tumours (100%), including choroid plexus papilloma (100%), thymic hyperplasia (100%), thymoma (100%); 1.5 mo |

d, day; F, female; JCV, JC polyomavirus; LT, large T-antigen; M, male; mo, month; NR, not reported; SV40, simian virus 40

## Table 3.10 Carcinogenicity studies of SV40 in combination with asbestos in experimental animals

| Species, strain (age) (sex)<br>Duration of observation<br>Reference | Virus strain, dose, route<br>Controls<br>Animals/group at start | For each target organ: tumours (%); time to tumours | Significance[a] | Comments |
|---|---|---|---|---|
| Hamster, Syrian golden (21 d) (F)<br>2 yr<br>Kroczynska *et al.* (2006) | SV40 dl883 (sT dl mutant), $10^{8.5}$ PFU, intracardiac, $n = 28$<br>– Crocidolite asbestos, 0.4 mg intrapleural space, plus 4 mg intraperitoneal (0.4 mg every 2 wk)<br>$n = 29$<br>Virus + asbestos, $n = 30$<br>Controls: medium only, $n = 30$ | Virus only, 0/28 (0%)<br>Asbestos alone (6/29, 20%), peritoneal mesotheliomas; 37.6 wk<br>Virus + asbestos (27/30, 90%), pleural and peritoneal mesotheliomas; 30.2 wk<br>Controls (0%) | Decrease in survival:<br>Asbestos + SV40 *vs* SV40, $P < 0.001$<br>Asbestos + SV40 *vs* asbestos, $P = 0.003$ | SV40 and asbestos acted as co-carcinogens to induce mesotheliomas. |
| Mouse (transgenic), C57/BL6 (NB) (NR)<br>24 mo<br>Robinson *et al.* (2006) | SV40 strain 776 LT under mesothelin promoter, generated high-copy-number (299 h, ~100 transgene copies) and single-copy (266 s) transgenic lines<br>Crocidolite asbestos, intraperitoneal, 0 (untreated) or 3 mg<br>Controls: WT mice<br>$n = 8$–15/group | Tumours (lifetime incidence)<br>Asbestos-treated: all developed mesotheliomas; latency: high-copy-number line, 35 wk; single-copy line, 63 wk; WT mice, 55 wk<br>Untreated mice: high-copy-number line, 5% sarcomas; single-copy line and WT mice, 0%. | Survival time after asbestos: high-copy-number line *vs* single-copy line or WT mice ($P < 0.0001$, decrease)<br>Direct relationship between SV40 transgene copy number and survival after asbestos, $r^2 = 0.89$ | *In vivo* demonstration in transgenic mice of co-carcinogenesis between SV40 and asbestos. |

d, day; dl, deletion; F, female; LT, large T-antigen; mo, month; NB, newborn; NR, not reported; PFU, plaque-forming units; sT, small T-antigen; SV40, simian virus 40; *vs*, *versus*; wk, week; WT, wild-type; yr, year

[a] Statistical significance is listed if reported in the study.

developed multiple tumours or lesions in a single animal. These studies suggest that promoter tropism is linked to tumorigenesis since JCV LT sequences under the control of the SV40 promoter demonstrated a similar tumour profile to transgenic mice expressing SV40 LT under the control of its own promoter, i.e. choroid plexus papilloma. The JCV promoter driving SV40 LT expression exhibited the tropism seen in JCV experimental animals, including neuroblastoma and astrocytoma. However, those animals also developed tumour types induced by SV40, including osteosarcoma and a much higher frequency of tumours of the choroid plexus, suggesting that the LT sequences may also play a role in viral tropism.]

## 3.4 Carcinogenicity of SV40 in combination with asbestos

See Table 3.10

Studies in hamsters and transgenic mice have revealed co-carcinogenic effects of SV40 and asbestos.

Kroczynska *et al.* (2006) inoculated SV40 dl883 at $10^{8.5}$ PFU by the intracardiac route into female hamsters ($n$ = 28; age, 21 days), which resulted in no mesotheliomas (0%). Crocidolite asbestos given as 0.4 mg in the intrapleural space plus 4 mg intraperitoneally ($n$ = 29) produced peritoneal mesotheliomas in 6 recipients (20%), with a mean latency of 37.6 weeks. When given together, virus and asbestos produced pleural and/or peritoneal mesotheliomas in 27 (90%) of 30 animals, with a mean latency of 30.2 weeks. Controls inoculated with medium only ($n$ = 30) developed no tumours. The following significant differences in survival between groups were observed: asbestos + SV40 *versus* SV40, $P < 0.001$; and asbestos *versus* asbestos + SV40, $P = 0.003$. [This study demonstrated that SV40 and asbestos act as co-carcinogens *in vivo* in hamsters for induction of mesotheliomas.]

Robinson *et al.* (2006) produced transgenic mice, using SV40 strain 776 LT under control of the mesothelin promoter, and generated high-copy-number (about 100 transgene copies) and single-copy SV40 transgenic mouse lines. Non-transgenic (WT) mice served as controls. Crocidolite asbestos (3 mg) was injected intraperitoneally. Group sizes ranged from $n$ = 8 to $n$ = 15. The lifetime tumour incidence was 0% for the untreated WT mice and the single-copy line and 5% (sarcomas) for the high-copy-number line. After treatment with asbestos, all mice developed mesotheliomas, with latency periods of 55 weeks for the WT mice, 63 weeks for the single-copy line, and 35 weeks for the high-copy-number line. There was a significant difference in survival time after asbestos treatment when the high-copy-number line was compared with either the single-copy line or the WT mice ($P < 0.0001$). There was a direct relationship between SV40 transgene copy number and survival after asbestos ($r^2 = 0.89$). [This study showed that SV40 and asbestos act as co-carcinogens *in vivo* in transgenic mice.]

# 4. Mechanistic and Other Relevant Data

## 4.1 Transforming capacity of SV40

The transforming capacity of SV40 is reflected by the induction of tumours in NB hamsters (Eddy *et al.*, 1961; Ashkenazi & Melnick, 1963; Black & Rowe, 1964). SV40 induces, for example, undifferentiated sarcomas or ependymomas in NB hamsters upon inoculation of SV40 (Eddy *et al.*, 1961; Gerber & Kirschstein, 1962). Depending on the site of injection, hamsters developed leukaemias, lymphomas, or osteosarcomas after intravenous injection (Diamandopoulos, 1972), mesotheliomas after intrapleural injection (Cicala *et al.*, 1993), and

diverse brain tumours after intracranial injection (Gerber & Kirschstein, 1962). The presence of SV40 LT in the tumour cells was proven by indirect immunofluorescence staining in lymphosarcoma cells (Diamandopoulos, 1972), or the presence of SV40 itself was assessed by testing the effect of brain tumour homogenates on fresh kidney cell cultures (Gerber & Kirschstein, 1962). Besides SV40 LT expression as tested by immunofluorescence and immunohistochemistry in SV40-induced mesotheliomas, Southern blotting revealed the presence and integration of SV40 sequences in these tumours (Cicala *et al.*, 1993). However, it is noteworthy that the SV40 virus itself appears to be relatively harmLess to whole animals since only NB hamsters were reported to be susceptible to tumour formation (see Section 3). SV40 has been shown to transform hamster kidney cells *in vitro* (Black & Rowe, 1963; Black *et al.*, 1963). SV40 also transformed other rodent cell lines, for example, mice, rats, guinea-pigs, and cattle, which are non-permissive for viral replication (reviewed in Arrington & Butel, 2001; Atkin *et al.*, 2009; Pipas, 2009; Gjoerup & Chang, 2010). These transformed cell lines demonstrated cytological, chromosomal, and growth abnormalities. One of the earlier SV40 transforming studies was conducted in an established immortalized mouse fibroblast cell line, NIH3T3 (Todaro *et al.*, 1964).

Human tissues were also shown to be transformed by SV40: primary human renal cells (Shein & Enders, 1962b), primary human epithelial cells derived from buccal mucosa and skin cells (Koprowski *et al.*, 1962; Ponten *et al.*, 1963; Jensen *et al.*, 1964), and human fetal lung tissue (Moyer *et al.*, 1964).

The transforming capacity of SV40 resides in the early region of the viral genome, which encodes two oncogenes, LT and sT. Expression of LT, alone or in combination with sT, will oncogenically transform most rodent cell types. Expression of LT alone often allows cells to become immortalized and to grow in reduced serum and to high saturation density. Moreover, these transformed cells escape contact inhibition, as shown in focus formation assays. LT alone, or more frequently together with sT, induces anchorage – independent growth in soft-agar and tumours in nude mice (reviewed in Manfredi & Prives, 1994; Pipas, 2009; Gjoerup & Chang, 2010).

In human cells, SV40 DNA was shown to induce a morphologically transformed phenotype (focus formation); however, the transformed cells are not tumorigenic in nude mice (Sager *et al.*, 1983). The tumorigenic transformation of normal human fibroblast, kidney epithelial, and mammary epithelial cells was demonstrated to require co-expression of the SV40 early region with the gene encoding the telomerase catalytic subunit (*hTERT*), and an oncogenic allele of the *H-ras* gene (Hahn *et al.*, 2002).

Interestingly, it has been demonstrated that transformation of primary human fibroblasts by transfection of origin-defective SV40 is significantly enhanced, indicating that viral replication might hamper the transformation (Small *et al.*, 1982; Gjoerup & Chang, 2010).

Mice transgenic for SV40 LT developed characteristic brain tumours in which SV40 LT copy numbers and transcripts were significantly elevated, indicating in this animal model a role for SV40 LT in the tumorigenesis process (Brinster *et al.*, 1984; Messing *et al.*, 1985).

## 4.2 Biological properties and regulation of SV40 viral proteins relevant to carcinogenesis

The SV40 LTs are some of the most intensively studied and best understood oncoproteins. Mutants of SV40 with temperature-sensitive mutations in LT showed that this oncoprotein was required for both the initiation and the maintenance of the transformed state (Brugge & Butel, 1975; Martin & Chou, 1975; Tegtmeyer, 1975).

This finding established that a single viral protein was capable of mediating cell transformation.

LT is a 708 amino acid nuclear phosphoprotein with biochemically separable functions important in promoting viral DNA replication in the natural life-cycle of the virus. LT is produced early in infection and initiates replication by binding to the viral origin and recruiting cellular replication factors (reviewed in Cheng *et al.*, 2009; Fanning & Zhao, 2009).

The transforming capability of SV40 LT has been mapped, in part, to its ability to directly interact with the family of retinoblastoma proteins, comprising pRb, p107, and p130 (Goodrich *et al.*, 1991). This family of transcriptional cofactors can bind to and either antagonize or potentiate the function of numerous transcription factors (Burkhart & Sage, 2008). The pRb family members are also referred to as pocket proteins, based on the structural pocket formed from conserved A and B domains necessary for their tumour suppression functions. *RB* mutations in human malignancies map to this pocket region, and several viral oncoproteins (e.g. LT, EIA, HPV E7) interact with this same region via an LXCXE motif. Through an N-terminally positioned LXCXE motif (residues 103–107), SV40 LT interacts with all three pRb family members to disrupt their repressive complexes with E2F family members (Zalvide *et al.*, 1998, 2001; Sullivan *et al.*, 2000, 2004). As a consequence, many E2F target genes are activated or de-repressed. These downstream targets of E2F are diverse, including genes involved in DNA replication, mitosis, DNA repair, differentiation, development, and apoptosis (Burkhart & Sage, 2008).

For LT to functionally inactivate pRb and affect transformation, the N-terminal DnaJ region containing the HPDK residues is also required. Deletion of the DnaJ domain does not prevent physical binding of LT to pRb family members; however, many functions attributed to the inactivation of pRb by LT are lost. DnaJ proteins are molecular co-chaperones that recruit members of the family heat shock proteins for the regulation of various cellular processes, including protein folding, protein transport, and remodelling of protein complexes. The LT DnaJ domain binds cellular hsc70 and brings this powerful regulatory system in proximity to LT–pRb complexes. The energy released from hsc70 ATPase activity is postulated to be necessary for the dissociation of pRb family proteins from E2F transcription factors (Sullivan *et al.*, 2000; Sullivan & Pipas, 2001).

SV40 LT interacts with a second tumour suppressor protein, p53, to affect transformation. The p53 protein was first discovered as an SV40 LT binding protein in 1979 (Lane & Crawford, 1979; Kress *et al.*, 1979; Linzer & Levine, 1979). Subsequent investigations have revealed that expression of viral genes capable of inactivating p53 is a common feature of known tumour viruses. LT binds p53 within its core DNA binding domain, thus impairing the ability of p53 to activate the transcription of its target genes. The region of LT required for p53 binding is bipartite and comprises residues 351–450 and 533–626. The p53 binding site is critical for rodent embryo fibroblast immortalization and for lifespan extension in human diploid fibroblasts (Lin & Simmons, 1991; Zhu *et al.*, 1992; Kierstead & Tevethia, 1993). However, in transformation assays using established cell lines, p53 binding is often not strictly required (Manfredi & Prives, 1994). It is also noteworthy that in transgenic mice expressing mutant LT, p53 binding is not required for *in vivo* dysplasia or tumour formation in some organs, including the choroid plexus (Chen *et al.*, 1992), pancreatic acinar cells (Tevethia *et al.*, 1997), and intestinal enterocytes (Markovics *et al.*, 2005). Details of the functional effects and mechanistic consequences of the interaction between LT and p53 are still not fully defined, but it is known that LT stabilizes p53 by preventing Mdm2-mediated proteasomal degradation of p53 (Henning *et al.*, 1997). LT has

not been found to interact with the p53 family members p63 or p73 (Marin *et al.*, 1998).

Whereas inhibition of the tumour suppressors pRb and p53 by LT is critical for LT-mediated transformation, recent work has revealed other LT targets that may also contribute to transformation. Investigations are in progress to understand the role of LT interaction with Cul7, Bub1, IRS1, NBS1, Fbw7, p300/CBP, and p400 (Cotsiki *et al.*, 2004; Poulin *et al.*, 2004; Wu *et al.*, 2004; Kasper *et al.*, 2005; Welcker & Clurman, 2005; DeAngelis *et al.*, 2006; and reviewed in Gjoerup & Chang, 2010).

LT is required for initiation of viral DNA replication, and much is known through crystallographic and biophysical studies about the temporal and spatial events that occur. Currently, much more is known about the functions of SV40 LT in replication than about those of other polyomavirus LT. This *Monograph* does not deal with the specifics of LT loading, architecture, and cellular interactions at the viral origin (for reviews, see Fanning & Knippers, 1992; Fanning & Zhao, 2009).

All polyomaviruses have sT. SV40 sT alone cannot transform cells; however, it can potentiate the transforming activity of LT (Noda *et al.*, 1986; Hahn *et al.*, 2002). Two major motifs have been identified on sT: the DnaJ HPDK interaction domain, shared with LT, and a PP2A binding domain. PP2A is a family of heterotrimeric phosphatases abundantly expressed in cells. These phosphatases have diverse cellular functions dictated by modular combinations of various A scaffold, B regulatory, and C catalytic subunits. It was shown that sT binds to PP2A A and C complexes and displaces specific B subunits or prevents them from binding to the AC core complex. This interaction inhibits PP2A enzyme activity (Pallas *et al.*, 1990; Yang *et al.*, 1991); however, there are also examples where sT delivers PP2A to the substrate, thus mediating dephosphorylation rather than inhibiting it (e.g. histone $H_1$, 4E-BP1) (for a review, see Gjoerup & Chang, 2010). A microarray analysis has identified many genes involved in cell proliferation, apoptosis, integrin signalling, and immune responses whose expression is altered by sT. The altered expression of many of these genes occurs via interaction of sT with PP2A, but for several other genes the alteration appears to be independent of sT binding to PP2A. This suggests that other cellular targets of sT remain to be identified (Moreno *et al.*, 2004).

MicroRNAs (miRNAs) are a class of ~22-nucleotide-long non-coding RNAs that function in the post-transcriptional regulation of gene expression. In eukaryotes, they are involved in processes including cell development, cell-cycle control, immunity, and oncogenesis (Garzon *et al.*, 2009). Viral miRNAs have also been found to be encoded by the genomes of nuclear double-stranded DNA viruses, specifically viruses of the herpesvirus, adenovirus, and polyomavirus families (Gottwein & Cullen, 2008; Grundhoff & Sullivan, 2011). The first miRNAs derived from polyomaviruses were identified in SV40 (Sullivan *et al.*, 2005). Two miRNAs from a single miRNA stem-loop precursor are expressed from the late viral transcript and accumulate at late times in infection. Both miRNAs have exact binding site sequences in the early region and have been validated to direct the cleavage of early LT transcripts, resulting in decreased expression of LT and sT. Thus, these miRNA products of late gene expression exert a negative regulatory role on early gene expression. Surprisingly, this does not appear to affect the replicative fitness of SV40 in cultured cells. Although molecular clones of the virus with mutations that disrupt the pre-miRNA stem-loop structure express higher levels of LT and sT, they do not differ from WT virus in the generation of infectious virus. Cells infected with miRNA mutant SV40 were more susceptible to cytotoxic T-cell-mediated lysis *in vitro*, suggesting that the miRNA may play a role in immune evasion *in vivo* (Sullivan *et al.*, 2005).

## 4.3 *In vivo* and *in vitro* evidence for a role for SV40 in human malignancies

### 4.3.1 *Major concerns about the methods used for the detection of SV40*

Studies suggesting a role for SV40 in human cancers include a large number of case series reporting detection of SV40 DNA by PCR in cancer tissue. However, the contribution of these studies to mechanistic evidence for the carcinogenic role of SV40 in human cancers is problematic because major concerns have been raised about the methods used for the detection of SV40 in human malignancies (see also Section 1.2 and Section 2.4 in this *Monograph*).

#### *(a) Detection of viral DNA by PCR*

It has been compellingly demonstrated that it is possible for commercial plasmids containing SV40 sequences to contaminate tissue samples (López-Ríos *et al.*, 2004). López-Ríos *et al.* initially found SV40 DNA in 62% of mesothelioma tissue samples using a widely used PCR targeting the LT gene. However, they observed occasional positive results in the no-DNA negative control reactions, which led them to explore the reasons for this observation. Analysis of the expected amplicon sequence showed that it was within a region of the SV40 genome included in many common laboratory plasmids. Using primers designed to amplify an intronic fragment of the SV40 genome not encompassed by the SV40 genomic region found in commercial plasmids, they failed to detect SV40 in the majority of samples and in a minority of instances (6%) observed only weak positive bands that could not be consistently amplified in repeated experiments. Control samples were consistently negative using the newly designed PCR. Although this represents the experience of a single laboratory, the source of the contamination they documented could affect results from many other laboratories. In addition, it is worth noting that some primer sets designed to amplify the regulatory region of SV40 can also amplify BKV and JCV (Lednicky & Butel, 1997). The use of these primer sets could again lead to false-positive results for SV40 if appropriate controls are not performed. Only a few published studies after that of López-Ríos *et al.* (2004) have forthrightly addressed these concerns in the methodological design of their studies (Manfredi *et al.*, 2005; Schüler *et al.*, 2006). The value of PCR data as evidence for a mechanistic role of SV40 in human cancers is also problematic because of the considerable inconsistency among studies in detection of viral DNA and the lack of a clear-cut and widely accepted explanation for the discrepancies across laboratories.

#### *(b) Detection of viral protein by immunohistochemistry*

The most common method used to demonstrate the presence of viral protein in cells has been immunohistochemistry. Immunohistochemistry is potentially very sensitive and specific, but it depends on the quality of the immunological reagents that are used. Of note, the specificity of the Pab101 monoclonal antibody raised against SV40 transformed cells of mouse origin and the Ab-1 (or Pab419) monoclonal antibody raised against full-length SV40 LT, which have been widely used for studies of the association of SV40 with tumours, has been called into question by Pilatte *et al.* (2000), who found that the antibody preparations are contaminated with a protein of similar size (90 kD) to SV40 LT and that the contaminating protein reacts with various secondary horseradish peroxidase-conjugated anti-mouse immunoglobulin G commercial reagents. The 90 kD protein was detected in different lots of the Ab-1 and Pab101 reagents, reacted with secondary antibodies from several commercial suppliers, and was found in murine monoclonal antibodies directed against other antigens but of the same isotype. Although the researchers showed that the problem was not

restricted to a single lot, no firm conclusions can be drawn about the implications of this finding for other studies. No studies published after the appearance of this report in 2000 have explicitly addressed this potential concern in the description of the methods used. In addition, the interpretation of immunohistochemical staining is inherently subjective, but most of the findings described below for particular cell lines and tumour tissue samples have not been confirmed by multiple independent laboratories and by the use of multiple validated immunological reagents.

In light of the above considerations, the Working Group elected to review only those studies of human cancer cell lines and human cancer tissues that included detection of SV40 proteins and associated changes in proto-oncogenes and host chromosomal structure as the evidential basis for establishing a mechanistic role for the virus. Although demonstration of the presence of viral protein in tumour tissue is not definitive evidence for an etiological role of the virus in carcinogenesis, such evidence indicates that the viral genome is transcriptionally active and supports the possibility that the viral oncogene is functional. Since all published studies of detection of viral protein also sought to detect viral DNA, these studies are inherently more robust than those relying solely on DNA detection. A potential loss in sensitivity is inevitable, but specificity is judged to be the more important criterion for addressing mechanistic questions.

### *4.3.2 Tumours of the central nervous system*

Bergsagel *et al.* (1992) reported the detection of SV40 viral DNA in 10 of 20 choroid plexus tumours and 10 of 11 ependymomas. Immunohistochemical analysis using a polyclonal antiserum against SV40 LT revealed intense staining of 5–15% of the nuclei of tumour cells from 4 of 5 choroid plexus tumours and 3 of 6 ependymomas.

Zhen *et al.* (1999) examined 65 frozen tumour specimens of diverse histological type for SV40 LT by immunoprecipitation with the Pab101 monoclonal antibody directed against the *C* terminus of SV40 LT. SV40 LT was detected in 43 (66%) of 65 specimens, whereas 8 normal brain tissue samples were negative for SV40 LT. SV40 LT-positive tumours included ependymoma, choroid plexus papilloma, pituitary adenoma, astrocytoma, meningioma, glioblastoma multiforme, and medulloblastoma, with frequencies of LT-positive tumours ranging from 33% to 100%. In a subset of SV40-positive tumours, complexes between LT and p53 (18 of 18 tumours) and LT and pRb (15 of 15 tumours) were also detected. [The Working Group noted that the highly positive results reported in this study were based only on the detection of SV40 LT with the Pab101 monoclonal antibody, the specificity of which was called into question (Pilatte *et al.*, 2000; see also 4.3.1).]

Weggen *et al.* (2000) failed to detect SV40 in most brain tumour samples. Using a PCR assay targeting LT gene sequences, they found SV40 DNA in only 1 of 131 meningiomas, 2 of 116 medulloblastomas, 1 of 25 ependymomas, and 1 of 2 subependymomas. Only a single case of ependymoma contained SV40 VP1 gene sequences. Sufficient tissue was available for immunohistochemical studies of one medulloblastoma and one meningioma, and both were negative for LT protein staining using Pab101.

There are only a few studies reported about the detection of SV40 protein in human brain cancer cell lines.

Weiss *et al.* (1975) detected LT in 2 of 7 meningiomas by indirect immunofluorescence using polyclonal hamster anti-SV40 LT antiserum. Fusion of the LT-positive tumour cells with the SV40 permissive African green monkey kidney cell line, MA 134, gave rise to a very small proportion (0.01–0.05%) of cells that stained for SV40 capsid antigen using a rabbit antiserum against SV40 viral capsid.

Martini *et al.* (1996) detected SV40 LT by indirect immunohistochemical analysis with the Pab101 monoclonal antibody in 3 of 7 glioblastoma cell lines. Positive cells in the immunofluorescence assay, estimated to be 1 out of 1000, showed nuclear staining typical of LT.

Kim *et al.* (2002) detected SV40 LT in the nuclei of the D283 medulloblastoma cell line by immunohistochemistry using the Pab419 antibody directed against the N-terminal domain of SV40 LT. The Daoy medulloblastoma cell line and 4 primary medulloblastoma tumours were negative for SV40 LT staining.

Lednicky *et al.* (1995) performed studies to establish whether the entire SV40 genome was present in SV40-positive paediatric brain tumours. By introducing tumour DNA into permissive monkey kidney cells by lipofection, they were able to rescue infectious SV40 virus from one SV40 DNA-positive tumour. Neither the state nor the site of integration of the viral DNA could be determined.

### *4.3.3 Mesothelioma*

Carbone *et al.* (1994) first reported the detection of SV40 DNA sequences in human mesotheliomas. They found SV40 DNA in 29 (60%) of 48 mesotheliomas and demonstrated SV40 LT nuclear staining in 11 of 14 specimens using the Pab419 monoclonal antibody.

Results from the multi-institutional study reported by Testa *et al.* (1998) included immunohistochemical analysis of 12 mesothelioma tissues by a single laboratory. Two anti-LT monoclonal antibodies were used (Pab101 and Pab419); in this laboratory, both antibodies yielded similar results. Ten of the 12 mesotheliomas contained malignant cells positive for LT staining, which was localized to the nucleus.

De Luca *et al.* (1997) demonstrated SV40 LT in 4 mesothelioma tissues by immunoprecipitation followed by western blotting.

In contrast to these findings, there are several publications reporting failure to detect expression of LT in mesothelioma tissues.

Galateau-Salle *et al.* (1998) were able to detect SV40 DNA sequences in mesotheliomas and bronchopulmonary carcinomas, as well as in non-malignant lung samples, but immunohistochemistry performed on 15 mesotheliomas and 16 bronchopulmonary carcinomas did not show nuclear staining for SV40 LT, although nonspecific cytoplasmic staining was observed.

Jin *et al.* (2004) detected SV40 DNA sequences in 8 (44%) of 18 mesothelioma samples collected from patients in Japan. However, no immunohistochemical staining for SV40 LT was found in the 18 tumour samples. Analyses were done with both Pab101 and Pab416 (Ab-2) monoclonal antibodies, directed against the *C*-terminal and the N-terminal domain of SV40 TL, respectively.

Dhaene *et al.* (1999) detected SV40 DNA sequences in 13 (46%) of 28 mesotheliomas. Cytoplasmic staining but no nuclear staining was found in 10 of 13 cases by immunohistochemical analysis using Pab101 and Pab419.

### *4.3.4 Lymphoma and other human tumours*

There are isolated reports of detection of SV40 LT expression in lymphoma and other human tumours. Vilchez *et al.* (2005) examined 45 NHLs from HIV-infected patients hospitalized in Houston, Texas, and 10 lymphomas obtained from a HIV/AIDS programme in New Jersey for the presence of SV40 DNA and LT oncoprotein expression. SV40 DNA sequences were identified in 12 (22%) of the 55 cases. Immunohistochemical analyses were carried out with the monoclonal antibodies Pab416 and Pab101. Pab101 revealed positive staining in all 12 SV40 DNA-positive samples, whereas Pab416 gave detectable staining in only 5 of those samples. None of the 68 SV40 DNA-negative samples reacted with either monoclonal antibody. [The Working Group noted that unlike Pab101, the Pab416 monoclonal antibody

was not shown to be contaminated with the 90 kD protein reported to give false-positive reactions.]

Meneses *et al.* (2005) detected SV40 virus DNA in 28 (26%) of 106 NHLs and 2 (10%) of 19 Hodgkin lymphomas obtained from HIV-negative, chemotherapy-naive patients in Costa Rica. None of 51 reactive lymph nodes and tonsil samples or 40 gastric and hepatic carcinoma samples were positive for SV40 DNA. Expression of SV40 LT was detected by immunohistochemistry using the monoclonal antibodies Pab416 and Pab101 in 18 (64%) of 28 NHLs that contained SV40 virus DNA. None of the lymphomas or control samples that tested negative for viral DNA was positive by immunohistochemical analysis for SV40 LT. In general, few malignant cells were immunopositive, and staining was of low intensity.

Martini *et al.* (1998), in a study of NHL and Hodgkin lymphoma in HIV-positive and HIV-negative subjects, observed no significant difference in the detection rate for SV40 DNA in the two populations: 3 (10.7%) of 28 *versus* 18 (14.7%) of 122, respectively. Using semiquantitative PCR, the investigators estimated that SV40 DNA was present at $10^{-4}$ to $10^{-2}$ genome equivalents (geq) per cell, which is not consistent with the presence of the virus within all lymphoma cells. Expression of LT was recorded in 5 of 18 SV40 DNA-positive samples, and < 1% of tumour cells displayed positivity by immunohistochemical analysis with the Pab419 reagent.

Went *et al.* (2008) failed to detect expression of SV40 LT in tissue microarrays constructed with archived samples from 655 NHLs and 337 Hodgkin lymphomas diagnosed between 1974 and 2001 in patients in Italy, Switzerland, and Austria. [Of note, these investigators used the Ab-2 (Pab416) reagent, which has not been shown to potentially give false-positive reactions.]

Martinelli *et al.* (2002) detected SV40 DNA in 28 (62%) of 45 parotid gland adenomas and none of 11 normal salivary gland tissue samples. SV40 LT expression was detected by immunohistochemistry with the Pab101 monoclonal antibody in 26 (93%) of 28 SV40 DNA-positive tumour specimens.

Vivaldi *et al.* (2003) investigated human thyroid tumours for SV40 DNA sequences by PCR and a subset of the tumours for LT expression by reverse transcriptase PCR (RT-PCR) of total tumour RNA and immunohistochemistry. LT gene transcripts were found in 9 of 13 SV40 DNA-positive papillary thyroid cancers and 8 of 11 anaplastic thyroid cancers. Transcripts were not detected in 30 SV40 DNA-negative tumour specimens. RT-PCR positive samples were further analysed by immunohistochemistry using the Pab101 monoclonal antibody. Strong cytoplasmic staining but no nuclear staining was detected in 3 of 8 papillary thyroid tumours and 8 of 8 anaplastic thyroid tumours.

Animals with SV40-induced tumours frequently produce high-level antibodies against LT oncoprotein. This observation prompted Engels *et al.* (2005) to assess whether SV40 LT antibodies are associated with NHL in humans. LT antibodies were measured by enzyme immunoassay. Hamsters with SV40-induced tumours all produced robust SV40 LT antibody levels, whereas SV40-uninfected hamsters and macaques, as well as macaques naturally infected with SV40, had much lower levels. Low levels of LT antibody response were observed in NHL cases and controls. Overall, only 5 cases (6%) and 5 controls (5%) had LT antibody responses classified as seropositive, demonstrating the lack of association between the presence of SV40 LT antibody and NHL.

### *4.3.5 Mechanistic studies of SV40 LT expression in human mesothelioma cells*

Several publications from the Carbone laboratory have explored potential mechanisms for enhanced susceptibility of mesothelial cells to the oncogenic effects of SV40 virus. Carbone *et*

*al.* (1997) reported that expression of SV40 LT was associated with high levels of p53 in mesotheliomas. They also showed that LT and p53 were co-precipitated by either the Pab419 anti-SV40 LT monoclonal antibody or a monoclonal antibody to p53, suggesting that LT is physically associated with p53. SV40 LT from lysates of mesothelioma cells was also able to co-precipitate *in vitro* translated p53 protein. No p21 was detected by immunohistochemistry in mesothelioma cells co-expressing SV40 LT and p53, suggesting that the p53 was functionally inactive. The LT precipitate also contained proteins of the expected size of SV40 sT and 17K T viral proteins. In another report from the Carbone laboratory, De Luca *et al.* (1997) showed that SV40 LT in mesothelioma cells could co-precipitate retinoblastoma family proteins, pRb, p107, and pRb2/p130. Bocchetta *et al.* (2000) reported that primary human mesothelial cells infected with SV40 exhibit a unique semi-permissive infection phenotype. The cells were not lysed by infection; all expressed LT and high levels of p53 and generated transformed foci. Foddis *et al.* (2002) found that mesothelioma tissues and mesothelioma cell lines were telomerase-positive and that *in vitro* infection with SV40 induced telomerase activity of cultured human mesothelial cells.

Waheed *et al.* (1999) detected SV40 DNA and LT and sT mRNA in 3 of 5 mesothelioma cell lines derived from primary tumour specimens. LT protein could not be detected by western blotting using a monoclonal antibody to the $NH_2$ terminus of LT and sT (Pab 108), indicating that the levels of LT and sT expression were quite low. Transduction of one SV40-positive cell line with an adenoviral vector expressing antisense RNA to the first 550 bp of the LT and sT coding sequence resulted in marked inhibition of cell proliferation; this suggests that LT would have a significant contribution to the ability of these cells to grow in culture. [The Working Group noted, however, that the specificity of this inhibition is unclear because long double-stranded RNAs can also activate interferon pathways that would have the same effect. No *in vitro* molecular studies on other putatively SV40-associated cancers were described because it has not been possible to propagate a SV40-positive tumour cell line from these other cancers.]

## 4.4 Interaction between SV40 and potential cofactors

Several reports have suggested a role of SV40 in mesothelioma, a cancer associated with asbestos exposure (see Section 2). As described above, SV40 LT has been reported to promote a transformed phenotype in primary human mesothelial cells (Bocchetta *et al.*, 2000). Bocchetta *et al.* (2000) found that when mesothelial cells surviving exposure to asbestos were transfected with SV40 LT and sT, they were more prone to cell transformation, but the effect could not be quantified because these cells are highly susceptible to asbestos (crocidolite)-induced cell death.

## 4.5 Transgenic models for cancers associated with SV40 infection

The literature on transgenic animal models of SV40 is reviewed in detail in Section 3.

## 4.6 Susceptible human populations

There is no evidence to support an association between SV40 infection and immunocompromised status or between SV40 infection and birth cohorts at risk for exposure to SV40 due to vaccination with potentially contaminated vaccines. Jafar *et al.* (1998) reported no difference in SV40 seroprevalence by plaque reduction neutralization assay in HIV-positive patients (16.1%) compared with HIV-negative subjects (12.0%). Furthermore, there was no significant difference in seroprevalence when subjects were stratified by year of birth as born before 1941,

between 1941 and 1962, and after 1962. In a study of lymphomas diagnosed in HIV-positive and HIV-negative patients, Martini *et al.* (1998) observed no significant difference in the prevalence of SV40 DNA in tumour tissue between the two populations (10.7% *versus* 14.8%). In a meta-analysis of cancer incidence in HIV/AIDS patients and immunosuppressed transplant recipients, brain cancers did not occur at an increased rate (Grulich *et al.*, 2007). No data were reported for mesothelioma, most likely because this is a rare cancer. The incidence of lymphomas was significantly increased, but the known association of Epstein–Barr virus and these cancers may explain the entire effect.

## 4.7 Mechanistic considerations in relation to carcinogenesis

SV40 is the best-studied example for experimental transformation of a small DNA tumour virus in cell culture and experimental animals. There is broad evidence from many research groups and experimental systems that in specific experimental environments SV40 has transforming capacity. SV40 in these experimental systems follows a transformation mechanism in principle similar to that identified for oncogenic human papillomaviruses (HPV) (IARC, 2007, 2012) in human cells. In its natural host, rhesus macaques, even under conditions of immune suppression, SV40 does not appear to induce tumours (for a review, see Butel & Lednicky, 1999). In humans the molecular evidence for presence and transforming activity of SV40 is scarce and controversial (see review in Garcea & Imperiale, 2003).

The prevailing model for polyomavirus- and papillomavirus-induced carcinogenesis is that at least one viral genome is persistently present and biologically active in each transformed cell and the lytic viral life-cycle is interrupted. Viral persistence can be mediated through integration or through maintenance as viral episome. The presence of one integration site per tumour present in different parts of the tumour and in primary tumour as well as in tumour metastasis indicates that viral integration occurred before clonal tumour expansion. Viral genes encoding regulatory proteins, i.e. the LTs in polyomaviruses and the viral oncoproteins E6 and E7 in HPV, are consistently expressed through transcription and translation. Viral oncoproteins, among other functions, interact directly or indirectly with cellular tumour suppressor proteins such as pRb and p53, leading to cell-cycle and apoptosis deregulation. The presence of viral oncoproteins is nearly always necessary to maintain the transformed phenotype and in the tumour-bearing animal or human host can lead to the induction of antibodies to the viral oncoproteins. Such antibodies are rarely induced during the natural course of infection. An additional essential feature of virally induced cell transformation is the interruption of the lytic viral life-cycle. This can be due to lack of host factors essential for viral replication or lack of viral protein functions or *cis* elements on the viral genome necessary for viral replication (IARC, 2007; Butel, 2012).

SV40, like other small DNA viruses, needs the host-cell DNA replication machinery to drive viral replication. As described above in detail, SV40 LT protein can directly interact with the family of retinoblastoma proteins (Goodrich *et al.*, 1991) and disrupt their repressive complexes with E2F family members (Zalvide *et al.*, 1998, 2001; Sullivan *et al.*, 2000, 2004).

SV40 LT also interacts with p53 and impairs the ability of p53 to activate the transcription of its target genes (Kress *et al.*, 1979; Lane & Crawford, 1979; Linzer & Levine, 1979).

SV40 sT contains the DnaJ HPDK interaction domain, in common with LT, and a PP2A binding domain. SV40 sT alone cannot transform cells; however, it can potentiate the cell proliferation activity of LT (Noda *et al.*, 1986; Hahn *et al.*, 2002). The transforming ability of

SV40 LT appears to be much stronger than that of the human polyomaviruses BKV and JCV (Bollag *et al.*, 1989).

### *4.7.1* In vitro *studies*

As described in detail above, stable expression of the experimentally introduced SV40 early genome region encoding sT and LT can lead to transformation of rodent cells and also primary human cells (Noda *et al.*, 1986; Hahn *et al.*, 2002). Viral DNA usually persists through integration into the host chromosome. However, episomal polyomavirus DNA has also been detected in various types of tumours and transformed cells. Whether integration is required for the transformed state is unclear. Virtually all stably SV40-transformed cells have detectable nuclear LT expression (Butel & Lednicky, 1999).

Bocchetta *et al.* (2000) reported that human mesothelial cells infected with SV40 exhibit a unique semi-permissive infection phenotype. The cells were not lysed by infection, showed stabilization of p53, and generated transformed foci. Human fetal lung fibroblasts can be immortalized by persistent SV40 infection and can also continuously produce low amounts of infectious SV40 virions (Morelli *et al.*, 2004; Mazzoni *et al.*, 2012).

### *4.7.2 Studies in experimental animals*

Inoculation of SV40 virions but also DNA alone by various routes into rodents, especially hamsters, can result in a broad variety of tumours (described in Section 3). In the tumours, the SV40 genome stably persists and viral LT is consistently expressed, and tumour-bearing animals frequently develop high-titre antibodies against SV40 LT.

Studies of transgenic mice stably containing and expressing the SV40 complete early gene region under the control of the native SV40 early promoter/enhancer element have contributed to the understanding of fundamental principles related to SV40 LT transformation and tumour development. These studies have demonstrated that continuous expression of SV40 LT in the context of transformation-permissive cells is a potent oncoprotein able to induce neoplasms in a variety of tissues, can stimulate resting cells to enter the cell cycle and proliferate, and can inhibit the pRb and p53 tumour suppressor pathways. However, in some examples inhibition of the p53 pathway was found to not be necessary for tumour formation. The transgenic SV40 mouse models further demonstrated that additional genetic changes beyond viral oncogene expression are necessary for tumour formation (Lednicky & Butel, 1999; Butel, 2000b, 2012; Ahuja *et al.*, 2005; Pipas, 2009; Sáenz Robles & Pipas, 2009; Gjoerup & Chang, 2010).

### *4.7.3 Human tumour studies*

SV40 DNA by PCR analysis has been found in a broad variety of human tumours, including central nervous system tumours, mesothelioma, lymphoma, parotid gland tumours, and papillary and anaplastic thyroid cancers (see above for detailed study descriptions). In addition, several studies reported also the detection of SV40 LT in the nuclei of tumour cells by immunohistochemistry, or in the tumour tissues by immunoprecipitation or western blot analysis. Frequently, in immunofluorescence-positive tumour tissues only a few tumour cells stained positive, and with weak signals. The presence of LT in complex with p53 or pRb has been reported, and in a few cases also the presence of SV40 early region RNA. However, several groups did not detect SV40 DNA sequences in the same tumour types, and others reported that they could not detect LT in mesothelioma that they had identified as positive for SV40 DNA or found the SV40 LT monoclonal antibodies to stain in the cytoplasm.

Detailed studies in substantial numbers of SV40 PCR DNA-positive human tumour tissues

analysing viral load, status of the viral DNA, mutational status of the LT encoding region, involvement of the pRb pathway, and status of the pRb and p53 gene sequences are completely missing. In analogy to the occurrence of antibodies to the viral oncoproteins specifically in patients with HPV- or Merkel cell polyomavirus (MCV)-induced tumours or in experimental animals bearing SV40-induced tumours, similar antibody response to SV40 LT could be expected in patients with SV40-induced tumours. However, no such antibody data have been reported for any patient with SV40 DNA PCR positive tumours because no such studies have been done yet.

## 5. Summary of Data Reported

### 5.1 Exposure data

The natural host of SV40 is the rhesus macaque, in which the virus is maintained as a silent infection. Human exposure to SV40 is considered a rare event and is restricted to people in contact with monkeys. In the past, millions of people have been exposed through vaccination with SV40-contaminated vaccines, primarily poliovirus vaccine. It is unclear whether human-to-human transmission occurs. Exposure to SV40 is established both by detection of DNA sequences by PCR techniques and by detection of specific antibodies by ELISA or neutralization tests. Substantial doubts have been raised that laboratory contamination from plasmids or cell lines commonly used may have contributed to positive PCR results. Half of the published studies since 1995 have failed to detect SV40 DNA in healthy individuals. Similarly, serological cross-reactivity between SV40 and BKV and JCV can explain many of the discrepancies observed between the serological studies. In contrast to human polyomaviruses, well-controlled serological tests with pre-incubation of sera with BKV and JCV capsids provide little or no evidence that SV40 does infect human populations.

### 5.2 Human carcinogenicity data

Research on the oncogenic effects of SV40 in humans has focused on mesothelioma, non-Hodgkin lymphoma (NHL), and brain tumours. Cohort studies have followed people who received SV40-contaminated inactivated poliovirus vaccine in the 1950s and early 1960s. Although these studies have limitations, including lack of individual exposure assessment and adjustment for potential confounding factors, the majority have not demonstrated an elevated risk of cancer related to this exposure. In addition, several case–control studies have evaluated associations of serological markers of SV40 infection with mesothelioma, NHL, brain tumours, or other cancers. Most of these studies have demonstrated a similarly low prevalence of antibodies to SV40 in cancer cases and cancer-free control subjects.

In the context of these largely negative studies, several other studies have suggested an association between SV40 and cancer. For example, in a retrospective cohort study in the United Kingdom, some birth cohorts exposed to SV40-contaminated inactivated poliovirus vaccine had an elevated incidence of mesothelioma. In a case–control study of NHL, a higher prevalence of serum SV40 antibodies was observed in cases than in controls, although the majority of SV40 antibodies appeared to be cross-reactive with BKV or JCV. Finally, an elevated incidence of haematological malignancies and neural tumours was observed in a cohort of children whose mothers received potentially contaminated poliovirus vaccines during pregnancy. However, few of the mothers developed antibodies to SV40, so it was not possible to link the cancers in those children to SV40. Limitations in these studies include uncertainties in the assessment of SV40 infection status, residual confounding by other causes

of cancer (e.g. asbestos), and small numbers of cancer cases.

In addition, many case series have reported detection of SV40 DNA in a high proportion of human tumours, notably from mesothelioma, NHL, and brain tumours, whereas others have not. The reasons for discrepancies among case series are uncertain, but they may relate to differences in patient populations or laboratory methods. Contamination of tumour specimens by low levels of SV40 DNA (present in plasmids, cell lines, or other sources) is of special concern for SV40.

## 5.3 Animal carcinogenicity data

The tumorigenic potential of SV40 in experimental animals has been studied extensively. Different routes of inoculation, different viral strains, and variation in host animals and experimental conditions have been used.

In 13 studies involving newborn (NB) outbred hamsters, the virus was inoculated subcutaneously. Fibrosarcomas were reproducibly induced at very high incidence (> 60%). Tumour induction was dose-dependent, with an increased tumour incidence related to higher levels of infectious SV40. Host age was found to be a factor, with the incidence of tumours decreasing with increasing age of the recipient animal at time of exposure.

In four studies, intracerebral inoculation of SV40 into NB outbred hamsters resulted in a high incidence of brain tumours classified as either ependymomas or choroid plexus tumours. One study showed a virus dose dependence for brain tumour induction.

Weanling hamsters were exposed to SV40 via the intravenous or intracardiac route in six studies. A variety of tumour types were reproducibly observed; the most frequent were lymphomas, osteosarcomas, and mesotheliomas. It was demonstrated that tumour incidence decreased with decreasing virus dose and with increasing age of the recipient animal. It was also observed that SV40 small T-antigen (sT) deletion mutants preferentially gave rise to lymphomas.

When weanling hamsters were inoculated with SV40 by the intraperitoneal or intrapleural routes in four studies, the predominant tumour types induced were again mesothelioma, lymphoma, and osteosarcoma. In one study, intrapleural inoculation of SV40 yielded mesotheliomas in all recipients. Another study found that after SV40 inoculation of pregnant hamsters, tumours developed in the offspring. Other observations from these studies included a statistically significant difference in tumour incidence between SV40 virus strains.

In three studies, SV40 DNA was inoculated subcutaneously into NB hamsters. The viral DNA was tumorigenic; tumours were induced with similar tumour incidences and tumour types to those observed after exposure to infectious virus.

In two studies, SV40 was inoculated into neonatal mice. One study reported the development of sarcomas of the liver and/or spleen, the incidence of which was increased by co-infection of mice with murine malaria parasites. The other study was negative.

A single study in the rodent *Mastomys natalensis* reported a high incidence of brain tumours (ependymomas and maybe some choroid plexus tumours) after subcutaneous inoculation of SV40.

Four studies in transgenic mice involved the use of SV40 with its natural viral promoter. In all studies, choroid plexus tumours were observed at a high incidence. Thymic hyperplasia and renal lesions were also sometimes seen. In one study, the large T-antigen (LT) of lymphotropic papovavirus could substitute for SV40 LT to induce brain tumours.

In two studies comparing SV40 and JCV promoter and the LT gene in transgenic mice, the tumour types observed (e.g. choroid plexus papilloma) strongly correlated with the SV40 promoter tropism. Taken together, the evidence suggested that LT may contribute to tropism.

Co-injection of weanling hamsters with SV40 and crocidolite asbestos revealed a co-carcinogenic effect since virus plus asbestos yielded a significantly higher incidence of mesotheliomas. Synergy of SV40 with asbestos was also observed when transgenic mice carrying a high copy number of a transgene encoding only SV40 LT were exposed to asbestos: mesotheliomas developed with a shorter latent period than in the single-copy line or wild-type mice.

## 5.4 Mechanistic and other relevant data

There is strong and consistent evidence from animal and cell-culture studies that SV40 can be directly oncogenic and transforming through its oncoproteins, i.e. the LTs encoded in the early region of its genome. The mechanisms involve immortalization, transformation, and inhibition of apoptosis. There is only weak and controversial evidence for such mechanisms being active in human tumours.

- The presence of SV40 DNA, based on analysis by PCR, has been reported in a broad variety of human tumours by several groups but not by others. Experimentally substantiated doubts have been raised that laboratory contaminations with commercial plasmids may have contributed to some positive PCR results. Other differences in experimental methods may also contribute to discrepancies across laboratories.
- In the few studies that have quantified the amount of SV40 DNA, SV40 DNA, if found in human tumours, appears to be present mostly in low copy numbers. This indicates that the majority of cells within a tumour potentially associated with SV40 do not contain the viral genome. Thus, novel mechanisms for SV40-induced carcinogenesis would need to be invoked, for which there is currently little evidence.
- Studies that demonstrate viral oncogene expression in cells of SV40 DNA-positive human tumours are few, and the specificity of the two most widely used monoclonal antibodies has been questioned, based on experimental results from one study.
- Studies that demonstrate evidence for cell-cycle and apoptosis regulation by SV40 through pathways involving pRb family proteins and p53 in human tumours are few, and the evidence is weak.

# 6. Evaluation

## 6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of SV40.

## 6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of SV40.

## 6.3 Overall evaluation

SV40 is *not classifiable as to its carcinogenicity to humans (Group 3).*

## 6.4 Rationale

- Compared with human polyomaviruses, seroresponsiveness to SV40 is very low.
- Well-conducted sero-epidemiological studies, using SV40 antibody tests that exclude antibodies that react to BKV and JCV, do not provide evidence that SV40 infects humans.
- Studies of sewage using different techniques in several developing and developed countries did not reveal the presence of SV40,

while the presence of other polyomaviruses and papillomaviruses was shown.

- Millions of people were exposed to SV40-contaminated vaccines, but multiple follow-up studies of recipients of contaminated inactivated poliovirus vaccines have not revealed them to be at increased risk of cancer compared with unexposed cohorts.
- Studies of cancer patients have not revealed increased prevalence of antibodies to SV40 compared with control populations.
- Case series that evaluated human tumours for SV40 DNA produced conflicting results, and there is concern that PCR contamination is responsible for some positive results.
- The mechanism of transformation in rodents has been very well established, but despite extensive studies, there is no persuasive evidence that this mechanism is operative in humans. (In addition, some members of the Working Group considered that the effects of viruses can be species-specific.)

In view of the strong evidence presented in Sections 1, 2, and 4, the Working Group decided to give less weight to the data from experimental animals when considering cancer in humans.

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