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Recommendation of the ZKBS on the risk assessment of *Betapolyomavirus macacae* as donor or recipient organism according to § 5 (1) GenTSV

General

Betapolyomavirus macacae (synonyms: Macaca mulatta polyomavirus 1 (MmPV1), Simian virus 40 (SV40)) belongs to the genus *Betapolyomavirus* of the family *Polyomaviridae.* In addition to *B. macacae*, the betapolyomaviruses also include the human polyomaviruses BK virus (*Betapolyomavirus hominis)*, JC virus (*Betapolyomavirus secuhominis)* and the viruses KI virus (*Betapolyomavirus tertihominis)* and WU virus (*Betapolyomavirus quartihominis),* which were identified in 2007 [1–3].

B. macacae was first discovered in 1960 as a contaminant in kidney cell cultures from rhesus macaques (*Macaca mulatta*) used to produce polio vaccines [4, 5].

The icosahedral non-enveloped virion contains a circular, double-stranded DNA genome of 5243 bp with a bidirectional replication origin (ori). It encodes nine proteins and is basically divided into three functional regions: the early coding region, the late coding region and the non-coding control region. Transcription of the two coding regions begins near the replication origin and proceeds divergently. The early region is transcribed at the very beginning of infection, before DNA replication. It encodes four proteins: the ELP (early leader protein*)*, the 17KT protein, the small tumour antigen (t-Ag) and the large tumour antigen (T-Ag), whose coding regions partially overlap and are produced by alternative splicing. Under certain conditions, 17KT, t-Ag and T-Ag contribute to the malignant transformation of the host cell [6]. The T-Ag is a multifunctional phosphoprotein with ATPase and DNA helicase activity. In the nucleus of the infected host cell, it binds to specific DNA sequences in the ori of the viral genome and to a number of cellular proteins. In permissive cells, it is involved in viral DNA replication as a complex with the host cell DNA polymerase. In semi-permissive and non-permissive cells for viral replication, the T-Ag can cause uncontrolled cell proliferation for example by binding and inactivating the tumour suppressor proteins Rb and p53. In addition, by binding these and other factors, it induces the transition of the host cell from the G_1 phase to the S phase and thus the proliferation of the infected cell. In rare cases, immortalisation occurs [7–9]. The T antigen alone is sufficient for the transformation of many cell types (e.g. rodent cells [10]) in cell culture, but both the T-Ag and the t-Ag are required for the transformation of human cells [11, 12]. The t antigen mainly acts by binding and inhibiting protein phosphatase 2 (PP2A). This triggers cell proliferation and cell transformation. The 17KT protein also contributes to cell cycle progression, e.g. of resting primary fibroblasts [11]. The function of ELP is unknown. The late region is transcribed after DNA replication. It encodes the capsid proteins VP1, VP2, VP3, VP4 and the agnoprotein. The main function of the agnoprotein is the assembly of the virion [13, 7, 14].

The natural hosts of *B. macacae* are monkeys, especially Asian macaques and rhesus macaques. Almost all adult rhesus macaques are infected with *B. macacae*. In monkeys, the virus generally does not cause any clinical symptoms, but persists in the kidneys throughout their lifetime and can be detected in urine [15, 16]. In specific cases, also severe disease progression can occur. Minor *et al.* [17] investigated groups of cynomolgus macaques (*Macaca fascicularis)* with regard to the transmission and kinetics of conversion to seropositivity against *B. macacae* and reported the death of four out of 43 animals. Transmission is assumed to occur either indirectly via contamination of the environment or through direct contact between animals. Vertical or perinatal transmission is unlikely.

Rodent cells are non-permissive for viral replication of *B. macacae*, while human cells can be permissive (e.g. epithelial cells), semi-permissive (e.g. fibroblasts, mesothelial cells) or non-permissive (lymphocytes) depending on the cell type [9, 18, 19]. The immortalising potential for human cells has been documented *in vitro* [7, 9, 20]. A neoplastic transforming potential of *B. macacae* was demonstrated after injection into newborn hamsters, which developed tumours [21].

It was already demonstrated in the 1960s that humans exposed to *B. macacae* can develop an infection [22]. Replication-competent virions of *B. macacae* were found as contamination in an experimental vaccine against *Human orthopneumovirus* (synonym: Human respiratory syncytial virus (RSV)). After intranasal inoculation, 60% of study participants developed neutralising antibodies at a low titre. Eight subjects had received replication-competent *B. macacae* virions. Seven to eleven days after inoculation, small amounts of B*. macacae* virions were obtained from throat swabs of several individuals. In the months following inoculation, they showed no signs of disease.

There is also evidence that humans can become infected through direct contact with infected monkeys. Engels *et al.* [15] reported in 2004 on an increased seroprevalence of virus-specific antibodies against *B. macacae* in zoo workers with occupational exposure to non-human primates. Seropositivity to *B. macacae* was higher in the group of zoo workers with frequent, prolonged exposure (animal keepers, veterinarians, laboratory technicians) than in the group of zoo workers without such exposure (23% versus 10%). In general, however, antibody titres were very low compared with the titres found in zoo workers against BK virus and JC virus. The data were adjusted for false-positive seroreactivity against *B. macacae,* which proved to result from cross-reactivity against BK virus and JC virus. The prevalence of neutralising antibodies against the capsid proteins increased with increasing length of service. However, it could not be clearly established what exactly caused the specific human seroreactivity to *B. macacae*.

For a long time, it was suspected that people worldwide may have been infected with *B. macacae* between 1955 and 1963 through contamination in viral vaccines (polio vaccine, adenovirus vaccine) and would develop various tumour diseases due to its neoplastic transforming potential. In the 1990s, several researchers detected viral DNA from *B. macacae* in biopsy samples from patients with certain types of cancer, such as mesothelioma, osteosarcoma and non-Hodgkin's lymphoma, using PCR and DNA sequencing (including the sequence of the T-Ag, sequences of regulatory elements and the sequence of VP1) [23–25] – regardless of whether they had received contaminated vaccinations or not. However, other researchers were unable to detect viral DNA from B*. macacae* in these human tumours [26]. To date, no causal link between infection with B*. macacae* and the development of cancer in humans has been established [27–29, 16]. Several long-term follow-up studies have also failed to find an increased risk of cancer in recipients of SV40-contaminated polio vaccines [30].

The seroprevalence of *B. macacae* in the general US and European population is estimated to be at 5-10%. The actual prevalence of *B. macacae* in the human population and its transmission routes are unknown [15, 16]. It is likely that the low antibody titres against *B. macacae* frequently detected in seropositive individuals were not caused by contact with contaminated vaccines, but by cross-reactivity with the widespread, closely related human polyomaviruses BK virus and JC virus [31]. There is currently no evidence of human-to-human transmission [27].

Recommendation

According to § 5 paragraph 1 GenTSV in conjunction with the criteria in Annex 1 GenTSV, *Betapolyomavirus macacae* is assigned to **risk group 2** as donor and recipient organism for genetic engineering operations.

Reason

B. macacae has a broad host range and can infect humans in addition to macaques, such as rhesus macaques and cynomolgus macaques. Infections in the natural host typically proceed without clinical symptoms, but in rare cases, severe courses can occur. Infections in humans have so far been described to be asymptomatic. In addition, long-term follow-up studies have not yet established a causal link between infection of humans with *B. macacae* and cancer. Nevertheless, a low human pathogenic potential cannot be ruled out.

Literature

1. **Moens U, Calvignac-Spencer S, Lauber C, Ramqvist T, Feltkamp MCW, Daugherty MD, Verschoor EJ, Ehlers B, Ictv RC** (2017). *J Gen Virol* **98** (6):1159–60. E-Pub ahead of print 22 June 2017.
2. **Caldeira DB, Souza Luna LK de, Watanabe A, Perosa AH, Granato C, Bellei N** (2019). The occurrence of polyomaviruses WUPyV and KIPyV among patients with severe respiratory infections. *Braz J Microbiol* **50** (1):133–7. E-Pub ahead of print 30.11.2018.
3. Ref. 45242.0187 - Statement by the ZKBS on the risk assessment of polyomaviruses as donor or recipient organisms in accordance with Section 5 (1) GenTSV (February 2021)
4. **Shah K, Nathanson N** (1976). Human exposure to SV40: review and comment. *Am J Epidemiol* **103**(1):1–12.
5. **Sweet BH, Hilleman MR** (1960). The vacuolating virus, S.V. 40. *Proc Soc Exp Biol Med* **105**.
6. **Cheng J, DeCaprio JA, Fluck MM, Schaffhausen BS** (2009). Cellular transformation by Simian Virus 40 and Murine Polyoma Virus T antigens. *Semin Cancer Biol* **1919**(4):218–28. E-Pub ahead of print 31.03.2009.
7. **Howley PM, Knipe DM, Cohen JL, Damania BA** (eds.) (2021). Fields Virology: DNA Viruses, 7th edition. Wolters Kluwer Health.
8. **Fiers W, Contreras R, Haegemann G, Rogiers R, van de Voorde A, van Heuverswyn H, van Herreweghe J, Volckaert G, Ysebaert M** (1978). Complete nucleotide sequence of SV40 DNA. Nature **273**(5658):113–20.
9. **Gazdar AF, Butel JS, Carbone M** (2002). SV40 and human tumours: myth, association or causal ity? *Nat Rev Cancer* **2**(12):957–64.

10. **Zhu JY, Abate M, Rice PW, Cole CN** (1991). The ability of simian virus 40 large T antigen to immortalise primary mouse embryo fibroblasts cosegregates with its ability to bind to p53. *J Virol* **65**(12):6872–80.
11. **Boyapati A, Wilson M, Yu J, Rundell K** (2003). SV40 17KT antigen complements *dnaj* mutations in large T antigen to restore transformation of primary human fibroblasts. *Virology* **315**(1):148–58.
12. **Ahuja D, Sáenz-Robles MT, Pipas JM** (2005). SV40 large T antigen targets multiple cellular path ways to elicit cellular transformation. *Oncogene* **24**(52):7729–45.
13. **Gerits N, Moens U** (2012). Agnoprotein of mammalian polyomaviruses. *Virology* **432** (2):316–26. E-Pub ahead of print 21 June 2012.
14. **Rotondo JC, Mazzoni E, Bononi I, Tognon M, Martini F** (2019). Association Between Simian Virus 40 and Human Tumours. *Front Oncol* **9** :670. E-Pub ahead of print 25.07.2019.
15. **Engels EA, Switzer WM, Heneine W, Viscidi RP** (2004). Serologic evidence for exposure to simian virus 40 in North American zoo workers. *J Infect Dis* **190** (12):2065–9. E-Pub ahead of print 11/16/2004.
16. **Stratton KR, Alamario DA, McCormick MC** (2003). Immunisation safety review. SV40 contamination of polio vaccine and cancer. National Academies Press, Washington D.C.
17. **Minor P, Pipkin PA, Cutler K, Dunn G** (2003). Natural infection and transmission of SV40. *Virology* **314**(1):403–9.
18. **Bocchetta M, Di Resta I, Powers A, Fresco R, Tosolini A, Testa JR, Pass HI, Rizzo P, Carbone M** (2000). Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. *Proc Natl Acad Sci U S* A **97**(18):10214–9.
19. **Shaikh S, Skoczylas C, Longnecker R, Rundell K** (2004). Inability of simian virus 40 to establish productive infection of lymphoblastic cell lines. *J* Virol **78**(9):4917–20.
20. **Manfredi JJ, Prives C** (1994). The transforming activity of simian virus 40 large tumour antigen. *Biochim Biophys* Acta **1198**(1):65–83.
21. **Cicala C, Pompetti F, Carbone M** (1993). SV40 induces mesotheliomas in hamsters. *Am J Pathol* **142**(5):1524–33.
22. **Morris JA, Johnson KM, Aulisio CG, Chanock RM, Knight V** (1961). Clinical and serologic responses in volunteers given vacuolating virus (SV-40) by respiratory route. *Proc Soc Exp Biol* Med **108**.
23. **Bergsagel DJ, Finegold MJ, Butel JS, Kupsky WJ, Garcea RL** (1992). DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumours of childhood. *New Engl J Med* **326**:988-93.
24. **Lednicky JA, Garcea RL, Bergsagel DJ, Butel JS** (1995). Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumours. *Virology* **212**(2):710–7.
25. **Pepper C, Jasani B, Navabi H, Wynford-Thomas D, Gibbs AR** (1996). Simian virus 40 large T antigen (SV40LTAg) primer-specific DNA amplification in human pleural mesothelioma tissue. *Thorax* **51**(11):1074–6.
26. **Manfredi JJ, Dong J, Liu W-j, Resnick-Silverman L, Qiao R, Chahinian P, Saric M, Gibbs AR, Phillips JI, Murray J, Axten CW, Nolan RP, Aaronson SA** (2005). Evidence against a role for SV40 in human mesothelioma. *Cancer Res* **65**(7):2602–9.
27. *Shah* **KV** (2004). Simian virus 40 and human disease. *J Infect Dis* **190**(12):2061–4. E-Pub ahead of print 11/16/2004.
28. **Destefano F, Offit PA, Fisher A** (2018). Vaccine Safety. *Plotkin's Vaccines*:1584-1600.e10.
29. **Thu GO, Hem LY, Hansen S, Møller B, Norstein J, Nøkleby H, Grotmol T** (2006). Is there an association between SV40 contaminated polio vaccine and lymphoproliferative disorders? An age-period-cohort analysis on Norwegian data from 1953 to 1997. *Int J Cancer* **118**(8):2035–9.
30. **Engels EA** (2005). Cancer risk associated with receipt of vaccines contaminated with simian virus 40: epidemiologic research. *Expert Rev Vaccines* **4**(2):197–206.
31. **Carter JJ, Madeleine MM, Wipf GC, Garcea RL, Pipkin PA, Minor PD, Galloway DA** (2003). Lack of serologic evidence for prevalent simian virus 40 infection in humans. *J Natl Cancer Inst* **95**(20):1522–30.