Ref. 45310.0117 updated September 2019

Position statement by the ZKBS on the classification of recombinant rabies- and vesicular stomatitis viruses

1. Introduction

The *Rhabdoviridae* family (*Mononegavirales order*) includes viruses with a wide host spectrum that, in part, can cause serious illnesses in humans and animals. Rhabdovirus prototypes are the *Indiana vesiculovirus* (VSV; previously: Vesicular stomatitis Indiana virus, Vesicular stomatitis virus) from the genus *Vesiculovirus* and *Rabies lyssavirus* (RABV) from the genus *Lyssavirus* [1].

Rhabdoviruses have a non-segmented, single-stranded, negatively oriented RNA genome at a total length of approx. 12 kb, composed as follows: 3‘ – *leader* – nucleoprotein (N) – phosphoprotein (P) – matrix protein (M) – glycoprotein (G) – large subunit of RNA-dependent RNA polymerase (L) – *trailer* – 5‘. The replication of the rhabdoviruses takes place entirely in the cytoplasm of the host cell [1].

VSV infects horses, cattle, pigs and a number of other mammals, among which also humans [1; 2]. Symptoms in animals include ulcerations on the mucous membranes of the mouth, on the udders and on the coronary band of the hooves and, in cattle, these greatly resemble Foot-andmouth disease. VSV outbreaks in animal husbandry lead to diminished production and significant economic losses. The disease usually subsides within two weeks. There is no medicinal therapy. Containment is based on hygienic and quarantine measures. Human VSV infections usually run their course asymptomatically, occasionally there are slight flu-like symptoms and infrequently oral blisters and swollen lymph nodes.

Outbreaks lead to the rapid spread of VSV through direct contact of animals among each other, for example through saliva and skin or mucous membrane injury. But insects also play a significant role in transmission, which is currently being studied more closely [3].

Because the VSV glycoprotein mediates a wide range of hosts and also broad cell tropism, VSV occupies a central role in molecular biology, for example in vaccine vectors, for the transmission of genetic material in cells or as an anti-tumour agent.

According to § 5 para. 6 GenTSV, VSV is assigned to **risk group 2** as a donor and recipient organism for genetic engineering operations.

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RABV is distributed worldwide and is distinguished by a broad host spectrum. Thus, in addition to dogs and foxes, racoons, skunks and bats also become infected. The transmission of RABV as a rule takes place via injuries from bites because there are high virus concentrations in the saliva of infected animals [4]. But the smallest injuries to the skin or mucous membranes can also make penetration of the virus possible per a contact infection. Moreover, individual cases are documented in which transmission of the virus via air can be assumed as the route of infection, because the patients were exposed to high concentrations of aerosolized virus before the illness [5]. RABV replicates in the salivary glands and in the central nervous system (CNS) of infected humans and animals and causes rabies in them. This is associated with an acute

encephalitis that is almost always deadly without exception, even with intensive medical care [6].

Further representatives of the genus *Lyssavirus* are the *Lagos bat lyssavirus* (LBV), *Mokola lyssavirus* (MOKV), *Duvenhage lyssavirus* (DUVV), *European bat 1 lyssavirus* (EBLV-1), *European bat 2 lyssavirus* (EBLV-2) and the *Australian bat lyssavirus* (ABLV). With the exception of MOKV, all of these viruses circulate in bats and also trigger a rabies-like neurological disease in humans and animals.

Various vaccines are available for the active immunisation of humans and animals. However, these confer no protection against an infection with LBV or MOKV [7, 8]. Post exposure prophylaxis can also be carried out with the vaccine that is approved for use in humans, and it features a highly protective effect if it is administered immediately after exposure [9]. Therapy with scientifically proven efficacy is not available.

According to § 5 para. 6 GenTSV, the RABV, DUVV, EBLV-1, EBLV-2 and ABLV are assigned to **risk group 3**** as donor and recipient organisms for genetic engineering operations. LBV and MOKV are assigned to **risk group 3**.

2. Classification of replication deficient VSV and lyssaviruses

Replication deficient lyssaviruses are used, for example, for research with neuronal networks or to develop vaccine vectors. Replication deficient VSV are also significant in research as vaccine vectors or vectors for tumour therapy [2].

An essential gene is deleted in the genome of these viruses (for example the gene for glycoprotein G); as a rule, it is replaced by an inserted transgene, such as a reporter gene or a gene for a heterologous, immunogenic protein. The production of the virus particles takes place in complementing cell lines. The resulting particles are infectious but are replication deficient. The genome of the defective virus particles is amplified in the cytoplasm of the transduced host cell and the transgene is expressed. There is no integration into the genome of the host cell, and no new viral particles are formed. Insofar as the transferred transgene possesses no intrinsic hazard potential or ability to complement the replication defect, no hazard potential from accidental infection is expected despite the amplification ability of the genome. Therefore, the ZKBS) has assigned the recombinant, replication deficient lyssaviruses (ref. 6790-01-1562 dated March 2007 and ref. 6790-01-1631 dated July 2009) and also replication deficient, possibly recombinant VSV (ref. 45110.1894 dated December 2015 and ref. 45110.1911 dated July 2016) to **risk group 1.**

If the transferred transgene has its own hazard potential or might possibly complement the replication defect, an individual case assessment of the genetic engineering operations by the ZKBS is then always required for lyssaviruses and, insofar as no comparison can be made to previous work, for VSV.

3. Classification of the RABV strain SAD B19 and recombinant rabies viruses derived therefrom

SAD B19 is an attenuated RABV strain that was produced through *in vivo* and *in vitro* passaging of an isolate obtained from a dog in 1935 in North America [10, 11]. It is approved in Germany under the name of Fuchsoral® for the immunisation of wild foxes. It is assumed that the attenuation is attributable primarily to mutations in the gene for the glycoprotein G [12]. SAD B19 has residual pathogenicity for rodents under experimental conditions [13]. In the context of post vaccination monitoring, individual SAD B19-associated rabies cases were additionally described in foxes (3 cases after distributing approx. 97 million vaccine baits) [11]. Although the vaccine strain also proved safe for primates [15, 16], a residual risk to humans cannot be ruled out. According to § 5 para. 6 GenTSV, the RABV strain SAD B19 is assigned

to **risk group 2** as a donor and recipient organism for genetic engineering operations. The World Health Organisation and the responsible vaccine committee of the Robert Koch Institute recommend the implementation of post exposure prophylaxis in case of accidental contact with the vaccine virus as of degree of exposure II (contact of vaccine liquid from damaged vaccine bait with non-intact skin).
The recombinant rabies virus **ORA DPC** is derived from the RABV strain SAD L16, which is a cDNA-clone of SAD B19 [17]. To produce ORA DPC, three genetic engineering changes were undertaken in the genome of SAD L16. A mutation was introduced in the gene for glycoprotein G, leading to the amino acid exchange Arg333 → Asp333. This amino acid exchange already resulted in a further attenuation of SAD L16 [18]. The codons for amino acids 143 - 149 were additionally deleted in the gene for phosphoprotein P. This part of the P protein includes a motif for interaction with the cellular protein *dynein light chain* LC8, which possibly participates in the retrograde transport of RABV to the CNS [19]. Additionally, the gene for glycoprotein G from the RABV-wild type strain CVS was inserted. This gene was also modified such that the resulting glycoprotein featured the amino acid exchange Arg333→Asp333. ORA DPC is genetically stable and apathogenic for immune-suppressed mice and dogs. The residual risk for humans in case of accidental contact with the virus, however, cannot be assessed. The ZKBS has thus assigned the recombinant rabies virus ORA DPC to **risk group 2** (ref. 45110.1774 dated March 2013).

The **recombinant rabies virus SPBN GASGAS** is derived from the RABV strain SPBN, which in turn is a derivative of the vaccine strain SAD B19. SPBN is distinguished from SAD B19 by the deletion of pseudogene ψ and the introduction of four recognition sequences for restriction enzymes. In the construction of SPBN GASGAS, the gene for glycoprotein G is exchanged with the corresponding gene of the SAD B19 derivative SN10-333. The G protein of SN10-333 contains the amino acid exchange Arg333→Glu333, on which the attenuation of this strain is based. Moreover, a second mutation was introduced into the gene, which led to the amino acid exchange Asn194→Ser194 and stabilizes the mutation at position 333 [20]. The glycoprotein that was changed in this manner was duplicated. The duplication of the gene led to the reduced probability of a *reversion to pathogenicity* because the attenuated variant of the glycoprotein is phenotypically dominant [21]. It was also shown that the recombinant rabies virus is genetically stable. SPBN GASGAS is apathogenic under natural conditions of (vaccine) uptake for immunocompetent target- and non-target animals, including non-human primates. Because oral administration led anaesthetized mice to become diseased with rabies, even though sporadically, the ZKBS in 2016 decided to leave SPBN GASGAS in risk group 2 for the time being (ref. 45242.0129 dated October 2016). In an application for vaccine approval for wild foxes at the end of 2017, comprehensive additional data on the safety of SPBN GASGAS were submitted to the *European Medicines Agency* (EMA). On the recommendation of the EMA, this took place through a decision by the European Commission, and SPBN GASGAS was approved in Europe under the name of Rabitec. Under the precondition that the manufacturing conditions described in the application are adhered to (number of passages, cell cultures used for virus replication), the *Rabies virus*-vaccine SPBN GASGAS was downgraded to **risk group 1** according to § 5 para. 1 in conjunction with the criteria in attachment I GenTSV as a genetically modified organism.

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4. Literatur

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