ZKBS
The Central Committee on Biological Safety

Ref_ 6790-10-65 updated version as of December 2018

# Position statement by the ZKBS on the risk assessment of porcine endogenous retroviruses as donor or recipient organisms according to § 5 (1) GenTSV

## General Information

Depending on breed and variety, porcine genomes contain different numbers of copies of proviral genomes of *Porcine type C oncovirus* (previously: porcine endogenous retrovirus, PERV) [1; 2]. Only a few of the proviral sequences encode replication-competent viruses [3]. PERV belong to the genus *Gammaretrovirus* within the *Retroviridae* family. They have a simply constructed (+)ssRNA genome with a total length of about 8 - 9 kb [3]. The genome contains the genes *gag*, *pol* and *env*, which are flanked by the *long terminal repeats*. So far, three different PERV subtypes (PERV-A, PERV-B and PERV-C) are described which are widely distributed. While PERV-A and PERV-B are found in all pigs, the phylogenetically younger PERV-C is present in about 90 % of all pigs [4; 5]. The three subtypes differ mainly in their envelope proteins so that they bind to different cellular receptors [1; 6].

The PERV-A and PERV-B subtypes have been shown to have a broad host range *in vitro*. This also includes human cell lines that can be productively infected by PERV-A and PERV-B [2; 7 - 11]. The virus particles produced by the human cells are resistant to inactivation by the human complement system [7]. This leads to the potential for PERV to replicate and spread in humans. In clinical trials, more than 200 patients have already received a porcine xenotransplant (tissue or cells). However, no transmission of PERV was detected in any of the patients [4].

Experiments were also carried out for the subtype PERV-C to determine the host range. In this case, vector particles derived from *murine leukaemia virus* that transfer a reporter gene were pseudotyped with the envelope protein of PERV-C. Porcine cell lines as well as a single lot of the human fibrosarcoma cell line HT1080 could be transduced with these particles [9]. This result could not be reproduced with other batches of the same cell line. However, in the presence of the receptor-binding domain of the envelope protein of the *gibbon ape leukaemia virus*, replication-deficient particles pseudotyped with the PERV-C envelope protein could transduce the human cell line HeLa [12]. In the literature, therefore, the question is discussed whether an envelope protein of a human endogenous retrovirus could have a similar transactivating effect [4]. However, in all the *in vitro* infection studies so far, PERV-C proved to be ecotropic.

In addition to the three subtypes, natural recombinants between PERV-A and PERV-C are also described in somatic porcine cells [4; 13]. They possess the receptor binding domain of PERV-A and are therefore also polytropic.

In pigs and humans, no diseases causally related to PERV have been reported. However, in the course of an infection, the viral genome integrates randomly into the genome of the host cell. In this insertion mutagenesis, cellular oncogenes can be activated or cellular tumour suppressor genes can be inactivated. In case of high viral loads, PERVs can also have an immunosuppressive effect, since at high concentrations the envelope protein has a similar inhibiting effect on the proliferation of human mitogen-stimulated lymphocytes as the envelope proteins of the *human immunodeficiency virus 1* and the *baboon endogenous virus* [14].

## Recommendation

According to § 5 (1) GenTSV in conjunction with the criteria in Annex I GenTSV, the polytropic subtypes PERV-A and PERV-B as well as recombinant PERV-A/C are assigned to **risk group 2** as donor and recipient organisms for genetic engineering operations. The ecotropic subtype PERV-C is assigned to **risk group 1**.

## Justification

The polytropic retroviruses PERV-A, PERV-B and PERV-A/C have a broad host range that also includes human cell lines. A transfer to humans and a resulting insertion mutagenesis can therefore not be ruled out for these viruses. The host range of the ecotropic subtype PERV-C, however, is limited to pigs.

## References


1. Akiyoshi DE, Denaro M, Zhu H, Greenstein JL, Banerjee P, and Fishman JA. (1998). Identification of a full-length cDNA for an endogenous retrovirus of miniature swine. *J Virol* **72**(5):4503-7.

2. Yang L, Güell M, Niu D, George H, Lesha E, Grishin D, Aach J, Shrock E, Xu W, Poci J, Cortazio R, Wilkinson RA, Fishman JA, and Church G. (2015). Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science* **350**(6264):1101-4.

3. Czauderna F, Fischer N, Boller K, Kurth R, and Tönjes RR. (2000). Establishment and characterization of molecular clones of porcine endogenous retroviruses replicating on human cells. *J Virol* **74**(9):4028-38.

4. Denner J, and Tönjes RR. (2012). Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. *Clin Microbiol Rev* **25**(2):318-43.

5. Niebert M, and Tönjes RR. (2005). Evolutionary spread and recombination of porcine endogenous retroviruses in the *Suiformes*. *J Virol* **79**(1):649-54.

6. Le Tissier P, Stoye JP, Takeuchi Y, Patience C, and Weiss RA. (1997). Two sets of human-tropic pig retroviruses. *Nature* **389**:681-2.

7. Patience C, Takeuchi Y, and Weiss RA. (1997). Infection of human cells by an endogenous retrovirus of pigs. *Nat Med* **3**(3):282-6.

8. Niu D, Wei HJ, Lin L, George H, Wang T, Lee IH, Zhao HY, Wang Y, Kan Y, Shrock E, Lesha E, Wang G, Luo Y, Qing Y, Jiao D, Zhao H, Zhou X, Wang S, Wei H, Güell M, Church GM, and Yang L. (2017). Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science* **357**(6357):1303-7.

9. Takeuchi Y, Patience C, Magre S, Weiss RA, Banerjee PT, Le Tissier P, and Stoye J. (1998). Host range and interference studies of three classes of pig endogenous retrovirus. *J Virol* **72**(12):9986-91.

10. Martin U, Kiessig V, Blusch JH, Haverich A, von der Helm K, Herden T, and Steinhoff G. (1998). Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. *Lancet* **352**:692-4.

11. Wilson CA, Wong S, Muller J, Davidson CE, Rose TM, and Burd P. (1998). Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. *J Virol* **72**(4):3082-7.

12. Lavillette D, and Kabat D. (2004). Porcine endogenous retroviruses infect cells lacking cognate receptors by an alternative pathway: implications for retrovirus evolution and xenotransplantation. *J Virol* **78**(16):8868-77.

13. Karlas A, Irgang M, Votteler J, Specke V, Özel M, Kurth R, and Denner J. (2010). Characterisation of a human cell-adapted porcine endogenous retrovirus PERV-A/C. *Ann Transplant* 15(2):45-54.

14. Denner J. (1998). Immunosuppression by retroviruses: implications for xenotransplantation. *Ann N Y Acad Sci* 862:75-86.