

Position statement of the ZKBS on the risk assessment of recombinant measles virus vaccine strains

1. Introduction

Measles viruses (MeV) are enveloped, negative-stranded RNA viruses (genome ~ 16 kb) belonging to the genus *Morbillivirus* in the family *Paramyxoviridae* [overview in Ref. 1 and 2]. The virus is distributed by aerosols and highly contagious (contagion index ~ 95 %). Humans are the only natural hosts known. The viruses undergo lytic replication in the cytoplasm and spread from the respiratory tract to various other organs. The infection causes fever, symptoms of a common cold as well as a generalized maculopapular rash, and is associated with an impairment of memory B and T cells lasting for months, thus favoring secondary infections. Serious complications and fatal outcomes occur particularly in developing countries. A rare albeit lethal long-term consequence is a subacute sclerotizing panencephalitis. A lifelong immunity remains after the full recovery. According to Art. 5 Paragraph 6 of the German Genetic Engineering Safety Regulation (GenTSV), MeV as a donor or recipient organism for genetic engineering operations is assigned to **risk group 2**.

Vaccination strains derived from measles viruses belong to the safest and most efficacious vaccines worldwide [overview in Ref. 3]. They are highly attenuated, elicit a strong immune response and infect various tumor cells selectively. This, and the fact that foreign genes of up to 6 kb can be easily inserted and expressed [4], makes these viruses attractive candidates for oncolytic virotherapy and for recombinant vaccines against various viral infections.

The attenuation is the result of an adaptation to non-permissive cell cultures, especially avian cell lines [overview in Ref. 3 and 5]. Some of the vaccine strains originate from MeV wildtype isolates which were independently isolated in Russia (Leningrad-4), Japan (CAM-70) and China (Shanghai-191). Other vaccine strains were derived from the MeV strain **Edmonston** [5]. This strain was isolated in 1954 from an infant with measles, propagated on primary human kidney cells and finally adapted to various cell cultures, thus creating the attenuated Edmonston seed strains A and B [6; 7]. By way of further passages at reduced temperatures on chicken embryo fibroblasts (CEF) the following attenuated strains were established:

- (1) MeV strain **Edmonston B**, whose genome sequence differs from that of the MeV isolate Edmonston in 36 nucleotides. It was approved as a vaccine under the name of Rubeovax® in the USA in 1963 [8].
- (2) MeV strains **Schwarz** and **Moraten**, whose genome sequences are identical although they were created by divergent passages from Edmonston seed strain A and B, respectively [5]. They differ in 42 nucleotides from the MeV Edmonston strain, and in 16 nucleotides from the strain Edmonston B. Both strains were approved in the 1970s and are used for vaccination until today.

Like the wildtype, these attenuated strains use the CD150 receptor to enter the host cell, which is predominately located on immune cells. The strains have additionally acquired the ability to utilize Nectin 4 and the complement regulator CD46. These two receptors are particularly found on tumor cells which are thus preferentially infected and destroyed [9]. Additional passages also produced the following vaccine strains: the Edmonston-Zagreb strain [10], i.e. the measles vaccine strain which is most frequently used in the scope of the *Expanded Program on Immunization* of the WHO and whose genome sequence differs in 33 nucleotides from that of the MeV Edmonston strain and the strains AIK-C [11] and MVbv [12].

The genome sequences of the strongly attenuated MeV strains accordingly differ from the wildtype MeV in at least 33 nucleotide positions [5]. The mutations are distributed over the entire genome. The cause of the attenuation has not been identified on the molecular level; however, it seems that mutations in almost all genes contribute cumulatively to the attenuated phenotype [overview in Ref. 5]. The vaccine strains have been applied all over the world for decades as a well tolerable standard vaccine for immunocompetent persons [2]. According to the Position Statement of the ZKBS on "Handling animal viruses of risk group 1" from 2011 (Ref. no. 6790-05-02-0075), officially approved, replication-competent **MeV vaccine strains** pursuant to Art. 5 Paragraph 1 of the of the German Genetic Engineering Safety Regulation (GenTSV) in conjunction with the criteria of Annex I of the GenTSV can be assigned to **risk group 1** as donor and recipient organisms for genetic engineering operations under the provision that the number of passages does not exceed those that have been permitted by the regulatory authority, and that no other cell cultures or host systems are applied for replication than those used in vaccine production.

Recombinant vaccine strains of the MeV are generated by means of reverse genetics [13]. To this end, heterologous genes (e.g. encoding a viral structural protein or glycoprotein) are introduced as additional reading frames into the MeV genome. It was shown that measles viruses stably express foreign genes for more than 12 passages [3]. Numerous recombinant MeV are currently being tested as oncolytic measles viruses against various tumors or as vaccine strains against various viral infections (e.g. *Human immunodeficiency*

virus 1 [14; 15] and the *Chikungunya virus* [16; 17]) in (pre)clinical studies [overview in Ref. 3]. They are mostly based on the MeV strains Edmonston B and Schwarz. In general, the hitherto available data confirm the good tolerance and safety of the recombinant vaccine strains.

2. Evaluation of recombinant measles vaccine strains expressing heterologous antigens

Cell tropism

An extension of cell tropism must not be anticipated if a foreign glycoprotein is additionally built into the envelope of the MeV particles, as the hemagglutinin of the MeV vaccine strains uses the CD46 receptor for infection. This receptor is expressed on all human cells with the exception of erythrocytes.

Host range

MeV have a very narrow host range which under natural conditions is limited to humans. Experimentally, an infection of non-human primates can also be accomplished by natural transmission pathways. In principle, it could be that the additional expression of a foreign envelope protein will result in an extension of the host range of the recombinant MeV particles. However, the host range does not exclusively depend on the envelope proteins, instead, cellular factors play a crucial role (e.g. specific host factors which are essential for replication). An extension of the host range can often not be ruled out entirely.

Genetic stability

MeV are viruses possessing an RNA genome whose mutation rate during replication is higher than that of DNA genomes. Compared with other RNA viruses, however, MeV are genetically very stable [18], even after the repeated replication of an MeV vaccine strain in humans [19]. To date, no reversions to the pathogenic form have been described [3; 20] and there is no evidence for recombination events between MeV wildtype and vaccine strains in people who were co-infected with both strains [9].

Shedding

Only little data are available on the shedding of MeV vaccine strains [overview in Ref. 2 and 21]. It cannot be ruled out that recombinant MeV are shed by experimental animals or humans. A transmission from human to human has not yet been observed among the approved vaccine strains [3; 22].

Effect on a preexisting immunization

It must be assumed that an existing MeV immunization would also be effective against recombinant viral particles. As the introduced foreign antigens are expressed in addition to

the paramyxoviral proteins, the glycoproteins (hemagglutinin and the fusion protein) of MeV are still on the surface of the virus besides the foreign glycoproteins. They induce both the cellular and the antibody-mediated immunity to the MeV particles. Therefore, it has to be assumed that MeV-specific antibodies neutralize recombinant MeV particles with heterologous glycoproteins in the viral envelope just as effectively as wildtype MeV particles.

3. Recommendation:

Genetic engineering operations conducted with approved vaccine strains that are assigned to **risk group 1** can be performed in **class 1** under the provision that the officially certified and approved conditions of vaccine production are fulfilled.

In case of deviations from the conditions of vaccine production the genetic engineering operations are assigned at least to **class 2**.

Such deviations are the application of different cell culture conditions, e. g. virus propagation in a different cell culture system, an extended number of passages (compared to the number that has been permitted) or modifications in the genome, e. g. the introduction of mutations or insertions. This comprises the introduction of a reporter gene as well.

In principle, recombinant MeV can be assigned to **risk group 2** in the context of comparability according to Art. 12 Paragraph 4 of the German Genetic Engineering Act (GenTG). However, this does not apply, if the introduced nucleic acid segment fulfills one of the following criteria:

- it possesses a hazard potential on its own, for example, by coding for a toxin or a prion,
- it gives reason to expect a decrease in the attenuation of the recombinant MeV or
- it possesses any immunomodulatory properties.

If one or more criteria are fulfilled and no experimental data are available that confirm that the introduced nucleic acid segment does not lead to an increase of hazard potential, an individual risk assessment has to be performed by the ZKBS.

If data are provided proving the attenuation, and especially the stability of the recombinant MeV, a downgrading to **risk group 1** may be envisaged.

Information

Further information is to be found in the position statements of the ZKBS below:

- Statement of the ZKBS on the classification of genetic engineering operations, in which genes for immunomodulating proteins are inserted into the genome of replication-competent microorganisms, updated April 2019 (Ref. no. 6790-03-05)
- Information of the ZKBS on handling animal viruses of risk group 1 in genetic engineering operations, dated November 2011 (Ref. no. 6790-05-02-0075)

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