ZKBS
The Central Committee on Biological Safety

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Position statement of the ZKBS
on the risk assessment of the recombinant vaccinia virus MVA

The modified vaccinia virus Ankara, MVA, was produced in 570 passages of the wild-type vaccinia virus Ankara (CVA, **risk group 2**) on primary chicken embryo fibroblasts (CEF). During these passages, numerous mutations in the genome of MVA occured and six large deletions totalling 31 kbp were produced (CVA genome: 208 kbp).

The functions of the deleted genes are not fully understood yet. The deletion of the corresponding six genomic regions in the genome of CVA did not cause the host range to be restricted or CVA to be significantly attenuated. The attenuation of MVA is therefore not based exclusively on the deletion of the six genome regions. The host range of MVA is clearly limited compared to that of CVA: To date, productive replication of MVA has only been demonstrated in CEF and other embryonic chicken, duck or quail cell lines, in the hamster cell line BHK-21, and in fruit bat cells. After infection of human cells, both early and late vaccinia virus genes are expressed. However, there is a block in the morphogenesis of the virus particles, so that no virus progeny is formed. Moreover, MVA exhibits a highly attenuated phenotype. In a series of animal experiments, including immunosuppressed animals, MVA turned out to be avirulent. The clinical use of MVA as a vaccine in more than 150,000 people also showed no significant side effects. MVA was therefore assigned to **risk group 1**.

Marker rescue experiments with wild-type DNA fragments only partially extended the host range of MVA. Responsible mutations are mainly located at the left end of the genome. At the same time, these studies indicate that multiple viral functions must be restored to achieve MVA replication capability that is comparable to wild-type vaccinia virus. The viral functions, whose loss contributes to attenuation, as well as the cellular functions that enable virus replication in the few permissive cells are only partially known. Therefore, during production of recombinant MVA (rMVA), it must be checked whether the expression of the inserted gene affects the attenuation. An indication for this is the study of the ability to replicate in human cell cultures. If the rMVA continues to be replication-defective after the insertion of a transgene, it cannot be assumed that the hazard potential of the recombinant MVA has increased beyond **risk group 1**. In certain nucleic acid segments, however, it is generally not to be expected that they will influence the replication capability of MVA or increase the hazard potential of MVA.

Therefore, the ZKBS recommends carrying out the risk assessment of recombinant MVA as follows:

1. rMVA with nucleic acid segments that do not increase the hazard potential of MVA, must be assigned to **risk group 1**.
   This includes especially
   - reporter genes,
   - genes of RNA polymerases

- genes of viral proteins which lack their own hazard potential (exception: proteins of orthopoxviruses)
- as well as the nucleic acid segments listed under 2, for which it has been shown that their insertion into the genome of MVA does not lead to the replication ability of the rMVA being restored

2. rMVA with

   - a nucleic acid segment of an orthopoxvirus, such as vaccinia virus, ectromelia virus, cowpox virus, elephant pox virus, catpox virus or other cowpox-like viruses, or
   - a nucleic acid segment without its own hazard potential, but for which it cannot be ruled out that the replication ability of MVA in human cells is restored,

   must be assigned to **risk group 2**. It cannot be ruled out that the attenuation of MVA is reduced by the transfer of such nucleic acid segments. If it is indicated that virus production does not occur after infection of human cells, the rMVA can be downgraded to **risk group 1**.

   HeLa or HaCat cell lines are suitable to test the replication of recombinant MVA. Information about the tests will be forwarded to the office of the ZKBS and made available to the state authorities, so that they can also rely on test results from other federal states.

3. rMVA with

   - a nucleic acid segment of an orthopoxvirus (except variola virus[1]), such as monkeypox virus or camelpox virus, or
   - a nucleic acid segment that encodes a toxin or prion, or another nucleic acid segment that is believed to have its own hazard potential,

   must be subjected to a risk assessment by the ZKBS on a case-by-case basis.

Notes

A vaccine prophylaxis using a replication-deficient MVA strain is possible.

Please consider the following position statements of the ZKBS:

- Position statement of the ZKBS on working with recombinant vaccinia viruses (updated version as of April 2014, ref_ 6790-10-04)
- Position statement of the ZKBS on classifying genetic engineering operations where cytokine and apoptosis-regulating genes are integrated into replication-competent microorganisms (July 2002, ref_ 6790-03-05)

References

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[1] Based on the recommendation of the *World Health Organization*, handling of variola DNA is prohibited in laboratory facilities where orthopoxic viruses are used (*WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA*, revised in 2016).

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