ZKBS
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für die Biologische Sicherheit

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Opinion of the ZKBS on the risk assessment of the recombinant adenovirus-based vaccine viruses rAd5-S-CoV2 and rAd26-S-CoV2 according to § 5 paragraph 1 GenTSV

General

rAd5-S-CoV2 and rAd26-S-CoV2 are replication-defective recombinant human adenoviruses of serotype 5 and 26, respectively, which are used as components of a vaccine against SARS-CoV-2. Taxonomically, adenovirus 5 (Ad5) is assigned to the species *human mastadenovirus C*, whereas adenovirus 26 (Ad26) is assigned to the species *human mastadenovirus D*. Members of the former species are primarily respiratory viruses that often cause mild illness in childhood. [1] . Viruses of the species *human mastadenovirus D* occur less frequently and are then mainly associated with gastrointestinal symptoms and conjunctivitis. [2] . Both species are assigned to risk group 2 as donor and recipient organisms for genetic engineering work.

Compared to the two wild-type viruses, the E1 and E3 gene regions were deleted in the genome of each of the vaccine viruses, with the absence of the E1 gene region causing the replication defect of the viral genomes. In addition, in the rAd26-S-CoV2 virus, the ORF6/7 within the E4 gene region was replaced by the homologous nucleic acid segment of Ad5. Finally, a nucleic acid segment encoding the complete spike protein (S) of SARS-CoV-2 was inserted into the virus genomes by homologous recombination in the *Escherichia coli* K12 derivative BJ5183. The nucleic acid segment was codon-optimized for expression in humans, but otherwise matches the unmodified sequence of a December 2019 isolate from Wuhan. Expression of the protein is under the control of the cytomegalovirus promoter. To produce rAd5-S-CoV2 and rAd26-S-CoV2 particles, the human cell line HEK293 is used, whose genome contains the first 4344 bp of the Ad5 genome including the E1 gene region [3] . The replication defect of the vaccine viruses is thus complemented. In addition, homologous recombination may occur between the DNA of rAd5-S-CoV2 and the nucleic acid segment of Ad5 integrated in the cellular genome. The recovery of replication competence during the manufacturing process of rAd5-S-CoV2 cannot be excluded.

(Preliminary) results from three clinical studies are currently available on the safety of rAd5-S-CoV2 and rAd26-S-CoV2. In the first two phase I/II studies, in which two formulations were tested, in one part of the study 9 subjects each aged 18 to 60 years were inoculated once intramuscularly (i. m.) with ^{1011} vector particles (vp) of rAd5-S-CoV2 or rAd26-S-CoV2. In the other part of each study, 20 subjects were initially vaccinated i. m. with ^{1011} vp of rAd26-S-CoV2 and after 21 days with ^{1011} vp of rAd5-S-CoV2. The side effects of each vaccination were recorded until day 21 after the last vaccination. Side effects were predominantly mild (grade 1) and moderate (grade 2) in a few cases. Severe and most severe side effects (grade 3 and 4)

did not occur. In subjects vaccinated only once, side effects tended to occur more frequently in the rAd26 group but were not more severe. In subjects who were vaccinated twice, most side effects occurred after the second vaccination. The joint analysis of both studies showed the most common systemic reactions to be fever (below 39 °C) (53%), headache (42%), fatigue (28%), and muscle and joint pain (25%). The most common local adverse reaction was pain at the injection site (58%) [4] .

Preliminary results are currently available from a phase III study in adults aged 18 years and older. The subjects were initially vaccinated i. m. with ^{1011} vp of rAd26-S-CoV2 and subsequently with the same dose of rAd5-S-CoV2 at intervals of 21 days. Thereby, 16427 subjects received at least the first vaccination, 14964 of which were vaccinated with both vaccine viruses. There were clinical complications of severity 4 in 45 of the 16427 subjects (0.3%), including 3 deaths, although these were not causally related to vaccination. The proportion of subjects with clinical complications of this severity in the placebo group, who were injected only with the vaccine buffer solution, was comparable at 0.4%. The remaining adverse events were predominantly mild to moderate [5]

The combination vaccine with the two recombinant adenoviruses is already licensed in more than 60 countries. Two observational studies in the context of national vaccination campaigns have appeared on a *preprint server.* In Argentina, 707 hospital employees were interviewed about their symptoms after initial vaccination with rAd26-S-CoV2. 683 of them provided information. Side effects occurred in 487 of the respondents (71%), and were mostly mild to moderate. Twenty-five individuals (5%) sought medical attention, of which one (0.2%) was hospitalized. Adverse reactions occurred more frequently in women and in persons less than 55 years of age [6] . In the second study, 2558 people aged 18 to 89 years were interviewed in San Marino about their symptoms after vaccination. 1288 of the individuals had already received both vaccine components. Overall, 53% of the subjects described side effects after the first vaccination and 67% after the second. Again, these were predominantly mild to moderate and occurred more frequently in younger people. Twenty (0.8%) and 17 (1.3%) individuals experienced severe side effects after the first and second vaccination, respectively. The most severe side effects occurred in 8 and 4 persons (0.3% each). [7] . The nature of the side effects in both studies was similar to those described in the phase I/II clinical trial.

A comparable Ad26-based vaccine against SARS-CoV-2 was recently approved by the EU Commission. The recombinant adenovirus Ad26.COVS1 contained in it was assigned to risk group 1 by the ZKBS (ref. 45242.0180, July 2020).

Recommendation

According to § 5 paragraph 1 GenTSV in conjunction with the criteria in Appendix 1 GenTSV, the recombinant adenoviruses rAd5-S-CoV2 and rAd26-S-CoV2 are assigned to **risk group 1** as genetically modified organisms.

Justification

The recombinant adenoviruses rAd5-S-CoV2 and rAd26-S-CoV2 are replication-defective viruses that carry a gene of SARS-CoV-2 without its own hazard potential and are licensed as components of a combination vaccine against SARS-CoV-2 outside the EU. The two vaccine viruses were well tolerated in clinical trials and mostly produced only mild or moderate side effects.

Note

Due to the possibility of homologous recombination and the resulting abrogation of the replication defect, the production of rAd5-S-CoV2 particles in HEK293 cells is a **safety level 2** genetic engineering operation. rAd5-S-CoV2 particles can only be handled under safety level 1 conditions once it has been demonstrated that (taking into account the detection limit permitted by the European production standards for medicinal products) no replication-competent adenoviruses are present .

Literature

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