ZKBS
Zentrale Kommission
für die **Biologische Sicherheit**

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Recommendation of the ZKBS for the risk assessment of the recombinant Adenovirus ChAdOx1 nCoV-19 according to § 5 paragraph 1 GenTSV

General

The species *Human mastadenovirus E* includes several serotypes, including human adenovirus (Ad) 4, and is assigned to risk group 2. The isolate Chimpanzee adenovirus Y25 (ChAdY25) was isolated from chimpanzees in 1969 and is closely related to human Ad4 [1, 2] .

To produce a vaccine vector, ChAdY25 adenovirus from chimpanzees was used and regions *E1* and *E3* were deleted. Due to these deletions, the virus is replication defective. In addition, in the E4 region, the open reading frames (ORF) *Orf4, Orf6* and *Orf6/7* were replaced with the corresponding nucleic acid segments of Ad5 (species *Human mastadenovirus C*). In reference to the place of production (Oxford, UK) this vector was named ChAdOx1 [2] . To produce a vaccine against SARS-CoV-2, a synthetically produced gene for the SARS-CoV-2 spike protein was introduced into ChAdOx1 (ChAdOx1 nCoV-19). The spike gene was codon-optimized for human expression and provided with the tissue plasminogen activator (tPA) leader sequence at its 5' end. The gene was introduced into the E1 gene locus using Gateway cloning. The vector was produced using a bacterial artificial chromosome (BAC) in HEK293 cells, which provide the adenoviral *E1* gene region necessary for replication [3]

ChAdOx1 is being used as a platform to produce several potential vaccines, most of which are currently in preclinical development. ChAdOx1 containing the glycoprotein genes of *Rift valley fever virus* (RVFV) has been tested in sheep, goats, cattle and dromedaries [4, 5] . The ChAdOx1 vector with immunogenic hepatitis C virus nucleic acid segments elicited an immune response in mice. [6] . ChAdOx1 with membrane and envelope protein genes of *Zika virus* protected mice from infection. [7] . As a drug against prostate cancer, ChAdOx1 with tumor antigen 5T4 was tested in a mouse tumor model . [8] . The ChAdOx1 vector with nucleic acid segments for epitopes against papillomaviruses was tested in mice (in combination with a Modified vacciniavirus Ankara (MVA) vector with the same nucleic acid segments). [9] . ChAdOx1 containing the *Middle east respiratory syndrome-related coronavirus* (MERS-CoV) spike protein gene was tested in mice, dromedaries and rhesus macaques. [10–13] .

Results are available from clinical trials for ChAdOx1-based vaccines against influenza A virus (FLUAV), *Mycobacterium tuberculosis,* and MERS-CoV. ChAdOx1 containing the nucleoprotein (NP) and matrix protein 1 (M1) of FLUAV subtype H3N2 (A/Panama/2007/99) was initially tested in 15 subjects at doses ranging from 5×10^8 to 5×10^{10} viral particles (vp), with some subjects also receiving boost vaccination with an MVA-based NP + M1 vaccine. This was given sufficiently far apart from the administration of ChAdOx1 NP + M1 (seven and 14 weeks, respectively) [14] . In a follow-up study, the highest well-tolerated dose of 2.5×10^{10} vp was administered to 73 additional subjects, either alone or in combination with MVA NP +

M1, with eight and 52 weeks between the first and second vaccinations, respectively [15] . ChAdOx1 85A, containing mycobacterial antigen 85A, was administered to 42 subjects at doses ranging from 5 x 10^9 to 2.5 x 10^{10} vp. The highest dose was administered twice four weeks apart and, if necessary, subjects received a boost vaccination with an 85A-expressing MVA [16] . ChAdOx1 MERS was tested in 24 subjects at doses ranging from 5 x 10^9 to 5 x 10^{10} vp [17] . In all of these clinical trials, the ChAdOx1 vaccines were well tolerated and no serious adverse events (SAEs) occurred. However, in the trial of the FLUAV vaccine ChAdOx1 NP + M1 described above, serious adverse events (SAEs) were observed in the highest dose group (5 x 10^{10} vp) in three of six subjects, and were considered unacceptable for prophylactic vaccination in two subjects. Therefore, a dose of 2.5 x 10^{10} vp was established for further clinical trials [14] . However, most adverse events associated with ChAdOx1-based vaccines were mild to moderate and resolved spontaneously. These were local reactions such as pain, redness, swelling, itching, and warmth at the injection site, and systemic reactions such as fever, joint and muscle pain, fatigue, malaise, nausea, headache [14–17] or hematologic events such as leukopenia, neutropenia, or thrombocytopenia. [16] .

Further clinical trials are currently being conducted with investigational products based on ChAdOx1. These are a study with the potential ChAdOx1 5T4 vaccine against prostate cancer, which is being tested together with an antibody against the checkpoint inhibitor PD-L1 (EudraCT number 2017-001992-22), as well as studies with ChAdOx1-based vaccines for the treatment of HIV infection and for vaccination against malaria (EudraCT numbers 2018-002125-30 and 2017-001049-28). However, data from these clinical trials are not yet available.

For ChAdOx1 nCoV-19 itself, only preliminary preclinical data for mice and primates are available so far, published on the preprint server bioRxiv. According to these data, mice developed a robust humoral and cellular immune response after administration of ChAdOx1 nCoV-19. Rhesus macaques that received a dose of 2.5 x 10^{10} vp formed neutralizing antibodies and a T cell-mediated immune response against the spike protein compared to the control group treated with a recombinant ChAdOx1 vector containing a gfp gene (ChAdOx1 GFP). After challenge with 2.6 x 10^6 TCID50 (tissue culture infection dose 50) SARS-CoV-2 via the upper and lower respiratory tract, all animals inoculated with ChAdOx1 nCoV-19 had good clinical general condition, while those treated with ChAdOx1 GFP had an increased respiratory rate. The amount of viral RNA was determined in lung fluid (obtained by bronchoalveolar lavage), in lung tissue after necropsy, and in nasal swabs. Both genomic RNA (gRNA) and subgenomic RNA (sgRNA), indicative of viral RNA synthesis, were detected. In the lungs, gRNA and sgRNA were detected less frequently in the vaccinated animals than in the control animals; sgRNA was not detectable at all in the lung fluid of the vaccinated animals. However, in nasal swabs on days 0, 1, 3, 5, and 7 postinfection, gRNA was detected in all animals, with the greatest amount detected on day 3. Accordingly, excretion of viral particles can be expected even after vaccination. The lungs of the vaccinated animals showed no signs of viral pneumonia at necropsy, which is why an antibody-mediated increase in pathogenicity caused by vaccination cannot be assumed [3] . Based on these data, the first Phase 1/2 clinical trial of ChAdOx1 nCoV-19 was initiated in the United Kingdom on April 23, 2020, and is expected to enroll 1112 adult subjects (EudraCT number 2020-001072-15). A further Phase 2/3 study is investigating ChAdOx1 nCoV-19 in a further 10560 UK examination subjects and will include older subjects (> 65 years) and children (two to eleven years) (EudraCT number 2020-001228-32).

Recommendation

According to § 5 paragraph 1 GenTSV in conjunction with the criteria in Annex I GenTSV, the recombinant adenovirus ChAdOx1 nCoV-19 is assigned to **risk group 1** as a genetically modified organism.

Justification

The recombinant adenovirus ChAdOx1 nCoV-19 is a replication-deficient virus that carries a gene from SARS-CoV-2 without its own hazard potential and is to be tested as a vaccine against SARS-CoV-2. Vaccines based on the vector ChAdOx1 are already well researched preclinically. There are also clinical data of ChAdOx1-based vaccines against FLUAV, *Mycobacterium tuberculosis* and MERS-CoV from currently 154 subjects. The ChAdOx1-based vaccines were well tolerated, with mostly mild or moderate adverse events.

Literature

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