ZKBS
The Central Committee on Biological Safety

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Recommendation of the ZKBS on the risk assessment of

***Chikungunya virus* strain 181/clone 25**

as donor or recipient organism according to Article 5 paragraph 1 GenTSV

General

The *Chikungunya virus* (CHIKV) belongs to the family *Togaviridae* (genus *Alphavirus*). Like all alphaviruses, it is enveloped and has a linear, single-stranded RNA genome of positive polarity. CHIKV belongs to the group of arboviruses and is transmitted by mosquitoes of the genus *Aedes*. Its natural hosts include humans, monkeys, rodents and birds. Characteristic symptoms of CHIKV infection in humans are abrupt onset of fever, rash and joint pain, of which the latter may last for months. In rare cases, a CHIKV infection can lead to death, especially in newborns and persons with pre-existing medical conditions (case fatality rate 0.1%). 5 - 15% of infections are asymptomatic [1; 2]. In the list of risk-assessed donor and recipient organisms for genetic engineering operations CHIKV is assigned to **risk group 3****.

The strain 181/clone 25 (181/25) was developed in 1985 by the US military as a vaccine candidate. For this purpose, the field isolate AF15561 (Thailand, 1962) was first passaged ten times in primary grivet kidney cells. This was followed by 18 additional plaque passages in human embryonic lung cells (MRC-5). One of the resulting virus clones was characterized by the formation of highly reduced plaques sizes and a complete loss of lethality in 1-3 days old mice after intracranial infection. Likewise, rhesus monkeys showed no or only a slight, transient viremia after an intramuscular administration of $10^{3.5}$ - $10^{5.5}$ *plaque forming units* (pfu) of the vaccine candidate, which was 100 times lower than that of viruses following passage of the starting isolate. With regard to the stability of the attenuation, no change in the plaque morphology in cell culture and lethality in mice was found even after a further four passages in MRC-5 cells. Similarly, the virus population isolated from the rhesus monkeys was not distinguishable from that of viruses used to infect the rhesus monkeys in terms of their plaque morphology and homogeneity [3].

The results of two phase I / II immunization efficacy studies with 181/25 (name of the test product TSI-GSD-218) have been published so far. A total of 114, predominantly alphavirus-naive volunteers, were given a vaccine dose of $10^{4.4}$ or 10^5 pfu subcutaneously. As a result of the vaccination, six subjects (5%) developed the joint pain typical of CHIKV infection, which, however, only lasted a day at most and was described as moderate. Further local or systemic reactions, such as fever, rash, headache and muscle pain, did not significantly differ from the placebo groups [4;5]

In a study to investigate the basis of attenuation, ten point-mutations were identified based on a sequence comparison of 181/25 with its starting isolate AF15561, all of which are located

within coding regions. Five of these mutations lead to an amino acid exchange, with one mutation occurring in the non-structural protein nsP1 (T301I) and in the structural proteins E1 (A404V) and 6k (C42F). The other two mutations concern the structural protein E2 (T12I and G82R). After the targeted introduction of these five mutations, individually and in combination, only the mutations in E2 affected the viremia and survival of subcutaneously or intradermally infected mice. In these, the average virus titer in the blood, was as in 181/25 100 to 1000 times below the titer of AF15561. The separate introduction of each of the two mutations resulted in a reduction and the simultaneous introduction of both mutations to a complete loss of lethality in mice. The attenuation of 181/25 can therefore be completely attributed to the mutations E2/T12I and E2/G82R. The acquisition of additional positive charges of the glycoproteins is often observed in the culture of alphaviruses in cell culture and often correlates with attenuation as it may inhibit virus spread by causing an increased binding of the viruses to negatively charged extracellular components. E2/G82R is also responsible for the reduced plaque size of 181/25 [6].

To study the genetic stability of the two mutations in E2 viral RNA from 181/25 was isolated from the blood of infected mice three days after infection. The corresponding cDNA showed the expected mutations in all eight animals infected with the double mutant. In the case of the single mutants, however, a mixed population consisting of the expected mutant and revertants was detected in two out of eight (E2/T12I) or in nine out of nine (E2/G82R) animals [6]. Similarly, in another study, after a five-time intracranial passage of 181/25 in mice, reversion to E2/R82G was found or mutations occurred, resulting in the loss of positive charges in E2. These mutations were associated with enlargement of the plaques and with a recaptured 100% lethality in mice [7]. E2/R82G reversion was also demonstrated in one of the subjects in the Phase I / II studies [8].

Recommendation

According to Article 5 paragraph 1 GenTSV in conjunction with the criteria in Annex I GenTSV *Chikungunya virus* strain 181/clone 25 is assigned to **risk group 3**** as donor and recipient organism for genetic engineering operations**.**

Reasoning

Due to the limited extent of the mutations responsible for attenuation and their tendency to reversion, it can be assumed that the strain 181/clone 25 has a similarly high hazard potential as other *Chikungunya virus* strains and thus must also be assigned to the risk group 3**.

References

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