ZKBS
The Central Committee on Biological Safety

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Recommendation by the ZKBS on the risk assessment for *Mycobacterium bovis* BCG strains as donor or recipient organisms according to § 5 para. 1 GenTSV

General Information

The *Mycobacterium bovis* BCG ("Bacillus Calmette Guérin“) strain that has been in use as a vaccine against tuberculosis worldwide for more than 90 years was generated at the start of the 20th century by Albert Calmette and Camille Guérin. They isolated *M. bovis* from the milk of a cow sickened with tuberculous mastitis and cultivated it on a medium of glycerine and potatoes. Under the administration of cattle gall and continuous subculturing (230 passages) they generated the attenuated strain *M. bovis* BCG [1]. This was initially used as a vaccination against *M. tuberculosis* in France in 1921 and worldwide as of 1924. The originally distributed cultures were further subcultured on location, so that various substrains developed from the original BCG strain over time. These can be distinguished both genotypically and phenotypically.

Even today, the BCG vaccine is still an important cornerstone in the *End TB* strategy by the *World Health Organization* (WHO) member states, the goal of which is to reduce the number of cases of death caused by tuberculosis by 95% by 2035 [2]. A vaccination with BCG thereby provides protection not only against tuberculosis, but also against leprosy [3].

According to a report to the WHO, the three BCG substrains that are most used worldwide for vaccination are the Russian strain Moscow-368, the Bulgarian substrain Sofia SL222, and the Tokyo 172-1 strain [2]. Other often used strains are the strains Pasteur 1173P2, Danish 1331 and Glaxo 1077, which is derived from the Danish strain, the Russian BCG-I and Moreau-RJ.

The genomes of all BCG strains feature the same attenuating mutations, which are traced back to the first *in vitro* passages of 1908 – 1921. Moreover, they contain strain-specific mutations that have arisen through individual culturing conditions after dispersion [5]. The region of difference 1 (RD1), which is conserved in pathogenic mycobacteria species, is deleted in the genome of all BCG strains. In *M. tuberculosis* this includes nine genes that, among others, code for a type-VII secretion system with its effecter proteins. The genome region is relevant for virulence because the deletion of the RD1 attenuates *M. tuberculosis* [6] and the reintroduction in BCG strains increases its virulence [7-9]. In the meantime, the genomes of most BCG substrains have been sequenced. A comparative analysis shows a high number of single nucleotide polymorphisms (SNPs) compared to *M. tuberculosis* and *M. bovis,* of which several are a common feature of all BCG substrains. On the other hand, individual differences become notable, being ascribed to the changed culture conditions after the dispersion [10-14]. Thus, the Pasteur 1173P2, Danish 1331 and Glaxo 1077 strains obtained after 1927 ("late") have the deletion of another region of difference (RD2) in common. This region codes, among others, for MPB64, a protein with antigenic properties. Furthermore, a mutation in the gene of the regulatory sigma-factor K leads to a significantly diminished expression of the antigens MPB70 and MPB83 [15]. A point mutation in the *mmaA3* gene, based on a defect in the synthesis of methoxymycolate, led to a change in the cell wall structure.

The ("earlier") strains obtained before 1927, of Russian BCG-I, Tokyo 172-1 and Moreau-RJ, have an insertion in the promoter area of the *phoP* gene in common. PhoP is a part of the two

component system PhoP-PhoR. Transferring a phosphate residue from histidine kinase PhoR to PhoP induces the expression of different genes. Inserting *phoP* into the promoter area leads to an increased expression of the target genes [16].

Furthermore, the Tokyo 172-1, Moreau-RJ and Glaxo 1077 strains are differentiated from all other BCG strains by mutations in biosynthesis genes for complex lipids with methyl-branched fatty acids. Thus, phthiocerol-dimycocerosate and phenolic glycolipids are no longer formed. In pathogenic mycobacteria these cell wall building blocks are ascribed a role in the immune modulation of the host. The causative mutations arose independently of one another. Thus, in the Moreau-RJ strain a deletion is described over 975 bp, which involves the biosynthesis genes *fadD26* and *ppsA* [17]. While in the Tokyo 172-1 strain a mutation was identified in the *ppsA* gene, [18], the cause of the deficiency in the Glaxo 1077 strain is not clear.

The WHO recommends that, in areas with a high prevalence of *M. tuberculosis* and/or *Mycobacterium leprae, the* BCG vaccine be administered to all neonates with an intact immune system. According to this recommendation, approx. 100 million neonates worldwide are vaccinated with the indicated BCG strains annually. This corresponds to a total coverage of approx. 90% of the concerned population. The corresponding reference strains with recommendations for licensing are filed at the WHO, respectively the *European Pharmacopeia* [19]. *M. bovis* BCG is currently the only internationally available vaccine for tuberculosis. Since 1998, the permanent vaccine commission (STIKO) in Germany has no longer recommended vaccination with BCG strains, based on the lower risk of infection and limited efficacy in association with non-rare adverse reactions [20; 21]. Not carrying out any BCG vaccination is in harmony with the recommendation of the WHO in populations in which their infection rate for tuberculosis is below 0.1%. The WHO, however, also recommends the BCG vaccination in low-prevalence areas for tuberculin- or *interferon gamma release assay*-negative persons, who might come into contact with tuberculosis patients as part of their profession (e. g. medical personnel or social workers) [2].

The vaccine strains recommended by the WHO are administered once intradermally. The live cell number in the ampoules after reconstituting the freeze dried bacteria thereby varies between $5 \times 10^5 - 3 \times 10^6$ colony forming units, depending on the strain. Adverse side effects are described for the use of the vaccine depending on the route of administration, the strain used, and the administered dose [2; 22; 23]. Side effects of a milder nature thereby are papule- or ulcer formations that arise in nearly all vaccinated persons at the site of the injection. Serious side effects at a frequency of 0.01% – 0.1% include the formation of abscesses or scars outside of the area of the injection site, or purulent lymph adenitis. As a rule, these usually heal without medical treatment. Quast *et al*. thereby described that the frequency of side effects such as lymphadenitis was associated with the administered quantity of live vaccine. A reduction in the number of viable bacteria in the Danish 1331 strain by a quarter was thus associated with a significant decrease in all side effects [22]. In very rare cases there are reports of systemic BCG infections (1.6 – 4.3 cases / 10^6 vaccinations) or inflammation of the bones (osteitis) or of the bone marrow (osteomyelitis; 0.01 – 30 / 10^6 vaccinations) [23]. Purulent lymphadenitis and systemic BCG infections in most cases (> 99%) can be associated with suppression of the immune system of the vaccinated person [24]. The osteitis cases are associated with the use of specific vaccine batches [10; 24]. It is noticable that the Tokyo 172-1, Glaxo 1077 and Moreau-RJ strains cause fewer overall side effects than the Pasteur 1173P2, Russian BCG-I and Danish 1331 strains. Phenotypic differences, in particular in the cell wall structure, are discussed as the cause of this. [25]. For immune suppressed or HIV infected person, the WHO advises against a vaccination with BCB strains unless HIV-infected children from high-prevalence areas are involved, who are immunologically stable and healthy and who receive antiretroviral therapy [2]. Studies from South Africa, among others, support a direct association between immunodeficiency and the outbreak of a disseminated BCG infection after the vaccination. In immunosuppressed, small children at an age of less than one year, a disseminated BCG infection progresses with a probability of 85% mortality [26].

Despite the intense attenuation of the BCG strains, there have been infections in medical personnel in the past. Two nurses developed hand infections after they pricked themselves during the preparation of a BCG vaccine, respectively a bladder instillation with BCG strains with a syringe, respectively a cannula [27; 28]. Another nurse developed arthritis of the index finger after she stabbed herself in the finger before administering the BCG vaccine [29]. A fourth nurse became ill with a persistent abscess of the thigh after she let the used syringe fall on the thigh after the administration of BCG vaccine [30]. Surgical interventions in addition to medicinal treatment were required for the successful treatment of the infections [27-30]. The nurses were described as immunocompetent.

For genetic engineering operations, the German Central Committee on Biological Safety (ZKBS) so far has assigned *M. bovis* BCG as a donor and recipient organism, independent of any allocation to a substrain, to risk group 1. This was justified by the world-wide use of the strains as a vaccine against tuberculosis.

The ABAS has assigned the strain *M. bovis* BCG Pasteur 1173P2 to risk group 2 in accord with the Biomaterial Regulation in a ruling of 2013 and based on the described side effects [31].

Recommendation

According to § 5 para. 1 GenTSV in conjunction with the criteria in annex I GenTSV and according to § 7 para. 3 (1) GenTSV, the *M. bovis* BCG derived strains are assigned **risk group 2** as donor and recipient organisms for genetic engineering operations.

Reasoning

The *M. bovis* BCG strains that have been used as a vaccine for almost 100 years are phenotypically and genotypically well-characterized bacteria. Their attenuation was demonstrated in an animal model before use as a vaccine against tuberculosis in humans. Serious vaccination side effects are very rare in immunocompetent individuals and are related mainly to the erroneous administration of the vaccine and to the dose of the applied live bacteria. In the case of accidental exposure in the lab, for example during the course of a needle stick injury, it cannot be ruled out that lab personnel will become ill with a possibly protracted infection that is difficult to treat.

Literature

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