ZKBS
The Central Committee on Biological Safety

Ref. 45246.0036 December 2019

Recommendation by the ZKBS on the risk assessment for *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* as donor or recipient organisms according to § 5 para. 1 GenTSV

General Information

The genus *Trypanosoma* (class of Kinetoplastida), includes single-celled flagellates that are present as parasites in the most varied vertebrates, from fish to mammals, whereby only a few species infect humans [1].

T. brucei includes three subspecies: The subspecies *Trypanosoma brucei brucei,* which is pathogenic to animals, the human pathogenic subspecies *Trypanosoma brucei gambiense* (group 1 and group 2) [2] and *Trypanosoma brucei rhodesiense*. *T. b. brucei* is a diploid organism with a 26 Mbp genome. In addition to 11 classic chromosomes, intermediate and mini-chromosomes are present, coding for *variable surface glycoproteins* (VSG) [3, 4].

Transmission occurs mainly from a bite by infected flies of the genus *Glossina*, known as tsetse flies. In addition to the main mode of transmission via the tsetse fly, infections through sexual contact or contaminated blood products, oral transmission and mechanical transmissions through blood-sucking organisms, needle stick injuries and medical instruments have been described [5, 14]. All three subspecies of *Trypanosoma brucei* exist naturally and exclusively in Africa, south of the Sahara in the range of tsetse flies, the so-called tsetse belt.

T. b. brucei triggers nagana disease in cattle, pigs and camels, but also in wild animals that serve as natural reservoirs; as a rule, humans are not infected by this trypanosome subspecies. In contrast, aside from animals, *T. b. gambiense* and *T. b. rhodesiense* can also infect humans and cause African sleeping sickness (*Human African Trypanosomosis*, HAT) [1, 5]. The infection is accompanied by excessive immune dysfunction and immune pathology that result from a persistent inflammatory immune reaction by the host [1]. Following an initial haemolytic phase with symptoms of strong headaches, lymph node swelling (Winterbottom sign), sleeplessness and rash, the parasite penetrates the central nervous system through the bloodstream and leads to advancing neurological disturbances in the meningoencephalitis phase. Without therapy, this leads to death in nearly 100% of cases [5]. With *T. b. gambiense,* illness can run for months or years; with *T. b. rhodesiense* the course is more acute (under nine months).

The parasites that live extracellularly feed by the endocytosis of components of the bodily fluids of the host. The life cycle is complex and includes various pleomorphic forms of life in the respective host animals: The bite of an infected tsetse fly leads to metacyclic, non-replicating, infectious trypanomastigote parasites in the saliva reaching the mammalian host tissue, where they transform and multiply in intermediate cells spaces into slender trypomastigotes. From there the cells also reach the bloodstream. In the further course of the infection the cells

transform into short, squat trypomastigotes, which are infectious for tsetse flies. The parasite cells are taken up with the blood when a tsetse fly bites an infected mammal. These transform into procyclic, replicating trypomastigotes in the midgut of the fly. The procyclic trypomastigotes multiply in the intestine of the fly, transform into epimastigotes and migrate into the salivary glands, where they multiply further. There, new metacyclic, trypomastogote cells ultimately arise and these are infectious for mammalian hosts. The reproduction cycle in the flies lasts about three weeks [1].

The interaction between host and parasite is very complex. With a frequent change in the expression of more than 100 different VSG genes, the parasite changes its cell surface periodically and thus escapes the specific immune response of the host immune system, which would lead to complement-mediated lysis of the parasite [5, 6].

Trypanosome lytic factors 1 and 2 (TLF1 and TLF2) of human serum, which, among others, contains the *haptoglobin related protein* (HPR) and apolipoprotein L1 (ApoL1), are responsible for the natural resistance of humans to exclusively animal-pathogenic trypanosomes [1]. The lipoprotein complex binds to the *haptoglobin/hemoglobin* receptor on the surface of the trypanosomes and is taken up by endocytosis. ApoL1 then forms pores in the lysosomal- and mitochrondrial membranes of the trypanosomes, which leads to their lysis [7].

However, the three *T. brucei* subspecies are not distinguished morphologically but rather in their configuration with virulence-associated factors that determine the host-specificity and the course of the infection. In contrast to *T. b. brucei, T. b. gambiense* and *T. b. rhodesiense* express proteins that inhibit TLF1 and TLF2. In *T. b. rhodesiense* there is a *serum resistance associated* (SRA) gene that codes for a shortened VSG. This binds ApoL1 and, through its neutralisation, prevents the lysis [8]. There is no SRA gene in the genome of *T. b. gambiense. T. b. gambiense* uses various mechanisms that convey resistance to lysis. [2]. The resistance of Group 1 *T. b. gambiense* is based on a point mutation in the TLF1 receptor and a diminished expression of the receptor, so that the uptake of TLF1 is reduced. Additionally, there is a *T. b. gambiense-specific glycoprotein* (TgsGP), a receptor-like, shortened VSG that reduces membrane fluidity and thus prevents the lysis. Increased activity of cysteine proteases in the digestive vacuoles of parasites additionally accelerates the degradation of ApoL [9–11]. The resistance of Group 2 *T. b. gambiense* can be attributed to various mechanisms that have still not been extensively researched. However, genes similar to TgsGP have been identified [2].

T. b. rhodesiense as a rule leads to an acutely progressing HAT, as a result of which patients often die within a few months [12]. Cattle and wild animals such as antelopes are the main reservoir. The nagana disease is triggered by *T. b. rhodesiense* in these animals [13]. Infections with *T. b. gambiense*, by contrast, are characterized by long latency and a chronic progression over a number of years [14]. *T. b. gambiense* is responsible for more than 97% of human trypanosome infections. The human is the main reservoir. If untreated, the infection ends in death in most cases [15]. The existing therapies are unsatisfactory based on their toxicity or deficiency of CNS (central nervous system) access [16]. There is neither an effective vaccination nor a medicinal prophylaxis for *T. b. rhodesiense* or *T. b. gambiense.*

According to annex II of guideline 2000/54/EG on protecting an employee from hazard from substances while working, dated September 18, 2000, *T. b. rhodesiense* is assigned to risk group 3** and *T. b. gambiense* to risk group 2. In the technical regulations for biological substances 464 classification of parasites, *T. b. gambiense* as well as *T. b. rhodesiense* are assigned to risk group 3**, with the recommendation that "when working with epimastigote forms in culture and trypomastigote forms in a vertebrate host [...] the protective measures of protection class 2 together with the measures additionally listed in annex 1 of the TRBA 100 are to be applied“ [17].

Recommendation

According to § 5 para. 1 GenTSV in conjunction with the criteria in annex I GenTSV, *Trypanosoma brucei gambiense*is and *Trypanosoma brucei rhodesiense* as a donor and recipient organism for genetic engineering operations are assigned **risk group 3****.

Genetic engineering operations with *T. b. gambiense* and *T. b. rhodesiense* as donor and/or recipient organisms are to be carried out in protection class 3 insofar as work is being done with vectors, in this case blood sucking insects, or infectious stages of the parasite. With genetic engineering operations without vectors or without infectious stages of the parasite, the classification can take place in protection class 2, insofar as there is no transfer of nucleic acid sections that increase the hazard potential of the above named recipient organisms.

Reasoning

If untreated, infections with *T. b. gambiense* and *T. b. rhodesiense* have a deadly progression in nearly all cases for humans and for susceptible animals. A prophylaxis or vaccination is not available. Transmission is also possible by mechanical means, for example through accidental self-inoculation. *T. b. gambiense* and *T. b. rhodesiense* are differentiated only by the type of resistance to human serum and by the time frame in which the infection leads to death.

The parasites feature a complex life cycle with various parasite stages. Analogous to the position statement by the German Central Committee on Biological Safety (ZKBS) in the "Classification of Parasites *Leishmania brasiliensis, Leishmania donovani*, *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* as recipient organisms in genetic engineering operations“ (Ref. 6790-10-70, June 2001), the classification can therefore be differentiated. Determinative thereby is whether an infectious stage of the parasite is present or not.

Literature

1. **Radwanska M, Vereecke N, Deleeuw V, Pinto J, Magez S** (2018). Salivarian Trypanosomosis: A Review of Parasites Involved, Their Global Distribution and Their Interaction With the Innate and Adaptive Mammalian Host Immune System. *Front Immunol* **9**:2253.
2. **Jamonneau V, Truc P, Grébaut P, Herder S, Ravel S, Solano P, Meeus T de** (in press). *Trypanosoma brucei gambiense* Group 2: The Unusual Suspect. *Trends Parasitol*. E-Pub ahead of print 24.10.2019.
3. **Achcar F, Kerkhoven EJ, Barrett MP** (2014). *Trypanosoma brucei*: meet the system. *Curr Opin Microbiol* **20**:162–9.
4. **Rudenko G** (2011). African trypanosomes: the genome and adaptations for immune evasion. *Essays Biochem* **51**:47–62.
5. **Ponte-Sucre A** (2016). An Overview of *Trypanosoma brucei* Infections: An Intense Host-Parasite Interaction. *Front Microbiol* **7**:2126.
6. **Glover L, Hutchinson S, Alsford S, McCulloch R, Field MC, Horn D** (2013). Antigenic variation in African trypanosomes: the importance of chromosomal and nuclear context in VSG expression control. *Cell Microbiol* **15**(12):1984–93.
7. **Vanwalleghem G, Fontaine F, Lecordier L, Tebabi P, Klewe K, Nolan DP, Yamaryo-Botté Y, Botté C, Kremer A, Burkard GS, Rassow J, Roditi I, Pérez-Morga D, Pays E** (2015). Coupling of lysosomal and mitochondrial membrane permeabilization in trypanolysis by APOL1. *Nat Commun* **6**:8078.
8. **Stephens NA, Kieft R, Macleod A, Hajduk SL** (2012). Trypanosome resistance to human innate immunity: targeting Achilles' heel. *Trends Parasitol* **28**(12):539–45.

9. **Capewell P, Clucas C, DeJesus E, Kieft R, Hajduk S, Veitch N, Steketee PC, Cooper A, Weir W, Macleod A** (2013). The TgsGP Gene Is Essential for Resistance to Human Serum in *Trypanosoma brucei gambiense*. *PLoS Pathog* **9**(10).
10. **Capewell P, Cooper A, Clucas C, Weir W, Macleod A** (2015). A co-evolutionary arms race: trypanosomes shaping the human genome, humans shaping the trypanosome genome. *Parasitology* **142 Suppl 1**:S108-19.
11. **Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homblé F, Grélard A, Zhendre V, Nolan DP, Lins L, Crowet J-M, Pays A, Felu C, Poelvoorde P, Vanhollebeke B, Moestrup SK, Lyngsø J, Pedersen JS, Mottram JC, Dufourc EJ, Pérez-Morga D, Pays E** (2013). Mechanism of *Trypanosoma brucei gambiense* resistance to human serum. *Nature* **501**(7467):430–4.
12. **Barrett MP, Burchmore RJS, Stich A, Lazzari JO, Frasch AC, Cazzulo JJ, Krishna S** (2003). The trypanosomiases. *The Lancet* **362**(9394):1469–80.
13. **Cooper C, Clode PL, Peacock C, Thompson RCA** (2017). Chapter Two - Host–Parasite Relationships and Life Histories of Trypanosomes in Australia . p. 47–109. *In* Rollinson D, Stothard JR (ed), Advances in Parasitology, vol. 97. Academic Press, London.
14. **Franco JR, Simarro PP, Diarra A, Jannin JG** (2014). Epidemiology of human African trypanosomiasis. *Clin Epidemiol* **6**:257–75.
15. **WHO** (2019). Trypanosomiasis, human African (sleeping sickness). https://www.who.int/newsroom/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness). Besucht am 18. November 2019.
16. **Gehrig S, Efferth T** (2008). Development of drug resistance in *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Treatment of human African trypanosomiasis with natural products (Review). *Int J Mol Med* **22**(4):411–9.
17. **TRBA** (2013). Einstufung von Parasiten in Risikogruppen (TRBA 464) https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRBA/TRBA-464.html. Besucht am 5. September 2019.