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Recommendation of the ZKBS on the risk assessment of Talimogen laherparepvec (Imlygic ®) and JS1/34.5-/47-/mGM-CSF as donor or recipient organisms according to § 5 paragraph 1 GenTSV

General

Human alphaherpesvirus 1 (also Herpes simplex virus 1, HSV-1) is a member of the family *Herpesviridae* and a globally distributed obligate pathogen of humans. However, other species such as hares and rodents can also be infected experimentally.

The virus has a double-stranded DNA genome 152 kbp long with a long and a short unique region (U_L and U_S), each flanked by inverted units of repeated sequences [1] .

The virus is usually transmitted in childhood or through sexual contact in young adults. The seroprevalence for HSV-1 in Germany is about 80 % [2] . Infection occurs through direct contact [1], transmission via droplets is also possible [3] . In pregnancy, transmission can occur through the placental barrier or during birth. The virus remains latent in the dorsal posterior ganglia after the initial infection and can be reactivated. In rare cases, systemic spread can occur, with replication in the central nervous system resulting in severe neurological damage, such as the rare HSV encephalitis. [1] . More common clinical symptoms of HSV-1 infection are infections of the mucous membranes, other skin areas (e.g. fingers), eyes and genitals. In newborns, disseminated herpes infection or herpes infection of the central nervous system may occur. These conditions are associated with a mortality rate of 29 % and 4 %, respectively, even when treated with high-dose aciclovir [4] . According to Section 5 (6) in conjunction with Annex I GenTSV, HSV-1 is classified in **risk group 2** as a donor and recipient organism for genetic engineering work.

Talimogene laherparepvec (JS1/ICP34.5-/ICP47-/hGM-CSF, formerly known as OncoVEX^{GM-CSF}) is based on HSV-1 strain JS1 (ECACC No. 01010209) isolated from a cold sore swab of an otherwise healthy individual. The genome of JS1 has not yet been sequenced, but its restriction pattern largely matches that of the wild-type HSV-1 strain 17*syn*+ [5] .

Talimogen laherparepvec is attenuated by deletions of the neurovirulence factor ICP34.5 and the immune evasion protein ICP47 [5] . Talimogen laherparepvec replicates almost exclusively in tumor cells. In these cells, the function of the cellular proteins eIF2α and Beclin-1 is impaired, so ICP34.5 is not required to compensate for their antiviral functions. In nondegenerate cells, replication of talimogen laherparepvec is reduced by the absence of ICP34.5 [6-9] . Tumor-specific replication of ICP34.5-deficient HSV has been demonstrated in cell lines of various tumor types (including hepatoma, pancreatic, breast, prostate, and colorectal carcinoma) [10-12] . ICP34.5-deleted viruses show a low establishment of latency and correspondingly a lower reactivation rate [13] .

As a further modification, the gene for human granulocyte-macrophage colony-stimulating factor (hGM-CSF) was inserted into the genome of talimogen laherparepvec. hGM-CSF is involved in

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the proliferation and differentiation of granulocyte and macrophage progenitor cells (summarised in [14]) and is approved as a drug in the USA under the name Leukine®. hGM-CSF in the form of Leukine® can be administered in much higher doses than would be expected in a Talimogen laherparepvec infection. The gene is not classified as a nucleic acid with neoplastic transforming potential.

Talimogen laherparepvec is genetically stable, occurring variations compared to the HSV-1 wild-type strains 17*syn*+, H129, KOS, McKrae and Strain F are mainly naturally occurring variations or mutations in non-coding regions of the genome. Reversion to the more virulent wild type, which can only occur when a cell is simultaneously infected with Talimogen laherparepvec and a wild-type virus, is considered very unlikely.

HSV-1 is taken up into the host cell via binding of the viral glycoproteins to cellular receptors. The changes in Talimogen laherparepvec do not alter the expression and structure of the viral glycoproteins and therefore cannot be assumed to alter host or cell tropism. Consistent with this, Talimogen laherparepvec did not replicate in the cell lines CHO and U937, which are not permissive for HSV-1. In permissive cell lines such as the human squamous cell carcinoma cell line FaDu, the wild-type virus and Talimogen laherparepvec replicate.

JS1/34.5-/47-/mGM-CSF is a variant of Talimogene laherparepvec that contains the murine variant of this gene instead of human GM-CSF (mGM-CSF). This virus is also known as OncoVEX^{mGM-}CSF. JS1/34.5-/47-/mGM-CSF was developed to study the effect of Talimogen laherparepvec *in vivo* in mice [15] . Human and murine GM-CSF have a nucleic acid sequence identity of 70 % and an amino acid sequence identity of 54 %, and are each species-specific in that they cannot bind to the GM-CSF receptor of the other species.

Pathogenicity

Talimogene laherparepvec was approved in the EU on 16 December 2015 (IMLYGIC®, marketing authorisation number: EU/1/15/1064/001-002) as a medicinal product for the treatment of melanoma in adults and has previously undergone multiple preclinical and clinical trials.

In the preclinical setting, Talimogene laherparepvec was shown to be safe when administered at up to 60 times the human dose to mice, rats and dogs [16] . Some of the preclinical data were obtained using JS1/34.5-/47-/mGM-CSF in mice. In repeated-dose toxicity studies, Talimogene laherparepvec and JS1/34.5-/47-/mGM-CSF behaved similarly. Mild transient effects such as injection site inflammation, increased lymphocyte and neutrophil counts, and immune activation (splenic enlargement, lymphoid hyperplasia in spleen and bone marrow) were observed. In efficacy studies, JS1/34.5-/47-/mGM-CSF, like Talimogene laherparepvec, resulted in partial or complete regression in immunocompetent mice that had syngeneic A20 tumors [15] .

In clinical trials, Talimogene laherparepvec was tested with a total of 430 subjects at doses ranging from 10^4 to 10^8 plaque forming units (PFU)/ml, with up to 4 ml administered per tumor [17-19] . The most common side effects consisted of nausea, vomiting, diarrhea, fever, fatigue, influenza-like symptoms, pain or reaction at the injection site, and muscle pain, some of which were more common in subjects previously seronegative for HSV-1. Herpetic lesions occurred in some subjects (14 of 292 in a phase 3 study). However, it was not investigated whether these were caused by Talimogen laherparepvec or a wild-type virus. None of these trial subjects developed encephalitis and replication in non-tumor tissue was not detected.

In the study with the most subjects, a phase 3 trial with 292 melanoma patients, the following serious side effects were identified: 6.2 % developed cellulitis caused by the entry of bacteria via the injection site. 5.5 % of those treated showed slowed wound healing at the tumor tissue injection site, and 2 % of subjects developed immune reactions such as glomerulonephritis, vasculitis, pneumonitis, or worsening psoriasis. These immune-associated conditions cannot be clearly linked to Talimogene laherparepvec because of other factors such as pre-existing immune-associated disease, concurrent treatment with other drugs, or other intervening conditions. White spot disease, a presumed autoimmune-induced pigmentary disorder,

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occurred in 5.1 % of subjects treated with Talimogene laherparepvec. 2.1 % of subjects developed deep vein thrombosis, but this generally occurs in cancer patients with an incidence of 2-20 %. One subject was diagnosed with plasmacytoma adjacent to the injection site; however, underlying disease (multiple myeloma) was diagnosed prior to Talimogene laherparepvec administration. In another subject, obstructive airway disease occurred during treatment for melanoma adjacent to the airway.

Theoretically, there is a risk of antibody formation to endogenous GM-CSF, which has been associated with cryptococcal meningitis and pulmonary alveolar proteinosis in case reports [20] . This was not observed in clinical trials, and it is not clear whether the amount of hGM-CSF expressed by Talimogene laherparepvec could trigger formation of anti-GM-CSF antibodies. In addition, cryptococcal meningitis and pulmonary alveolar proteinosis have not occurred as side effects during treatment with Leukine®, the hGM-CSF approved as a drug in the United States [21] .

Infectious viral particles were detected in clinical trial subjects only at the injection site; no transmission of Talimogene laherparepvec to subjects' contacts was observed. However, four accidental exposures to Talimogene laherparepvec (three needlestick injuries, one splash to the eye) have been reported in healthcare workers. All but one needlestick injury were treated with antiviral therapy. One of the needlesticks resulted in a lesion that was Talimogene laherparepvec-positive and resolved after antiviral treatment with aciclovir.

Talimogene laherparepvec is not indicated in melanoma patients with severe immunosuppression or immunodeficiency because disseminated HSV infection may occur in these patients. Clinical data are not available in this regard. Data from preclinical studies in immunodeficient mice show inconclusive results. There are also no clinical data for fetuses or neonates. However, no effects on embryo-fetal development were observed in preclinical studies in BALB/cAnNCrl mice.

Recommendation

According to Section 5(1) GenTSV in conjunction with the criteria in Annex I GenTSV, HSV-1 Talimogen laherparepvec and JS1/34.5-/47-/mGM-CSF are assigned to **risk group 1** as donor and recipient organisms for genetic engineering operations.

Justification

In immunocompetent individuals, based on the data obtained in the clinical and preclinical studies as well as data from further studies, no hazard is assumed for ICP34.5-deficient HSV-1. In particular, viral replication and the resulting mediated hGM-CSF expression are significantly lower in healthy individuals than in tumor patients. The neurovirulence of the virus is strongly attenuated. Severe side effects depend on the immune status of the individual. When the virus is handled by trained personnel in the laboratory, no hazard is to be assumed.

Literature

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