Ref. No. 6790-10-88 May 2006

General position statement of the ZKBS

on the risk assessment of the expression of Tat-fusion proteins

The transcription transactivating protein Tat of the human immunodeficiency virus 1 is able to penetrate cellular membranes independently of receptors (1, 2). In Tat, as with other proteins also able to penetrate membranes, such as Antennapedia from *Drosophila* or VP22 of Herpes simplex virus type 1 (3, 4, 5), the protein transduction domain (PTD) can be narrowed down to a small cationic area of 10 to 16 amino acids (4, 5). Fusion of heterologous proteins to PTD provides an experimental means of delivering these proteins into eukaryotic cells.

There are both prokaryotic and eukaryotic expression systems for producing Tat-fusion proteins. Generally, the prokaryotic system developed in the research group of Dowdy is used (6). This involves inserting the heterologous gene in a pBR-derived vector, pTAT, which contains an expression cassette with the T7 promoter, an N-terminal His_6-tag, the Tat PTD with 11 amino acids that are flanked by glycine residues, a hemagglutinin-tag and a multi-cloning site. To synthesize the fusion protein the recombinant vector is introduced into *E. coli* BL21(DE3) pLysS, an *E. coli* B derivative that expresses the T7 polymerase under the control of the inducible lacUV5 promoter. However, functional Tat-fusion protein can also be expressed in mammalian cells using a vector that contains a eukaryotic expression cassette with the CMV promoter, the SV40 origin of replication and the Tat PTD with 11 amino acids (7).

Assessment and recommendations

Tat-fusion proteins have the potential to penetrate membranes of various cell types and to carry out its functions in the cell, although no genetic material is transferred to the target cell. The fusion protein is broken down in the cell so that the transferred protein only functions transiently. Therefore, the Tat-fusion protein is not expected to be a potential hazard, if the protein delivered causes no cell damage.

a. If Tat-fusion proteins are expressed using *E. coli* K12 or *E. coli* B derivatives and prokaryotic pBR-derived expression vectors, or using established cell lines of risk group 1 and eukaryotic expression vectors, and if it involves proteins fused to Tat-PTD other than those proteins listed in section b, then the GMOs are to be allocated to **risk group 1**. Genetic operations with these GMOs are assigned to **containment level 1** according to § 7 para. 3 of the GenTSV.

b. If Tat-fusion proteins are expressed using *E. coli* K12 or *E. coli* B derivatives and prokaryotic pBR-derived expression vectors, or using established cell lines of risk group 1 and eukaryotic expression vectors, and if it involves proteins fused to Tat-PTD that are viral oncoproteins with a “hit and run” mechanism, prions or prion proteins from humans or cows, apoptosis-inducing proteins or toxic proteins, then the GMOs are to be allocated to **risk group 2** Genetic operations with these GMOs are assigned to **containment level 2** according to § 7 para. 3 of the GenTSV.

It is recommended for both a and b to wear protective gloves and to protect against too high levels of aerosols when performing genetic engineering operations with bacterial or cell cultures expressing the fusion protein.

References:

1. Frankel AD & Pabo CO (1988). Cellular uptake of the tat protein from human immunodeficiency virus. Cell 55:1189-1193.
2. Green M & Loewenstein PM (1988). Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. Cell 55: 1179-1188.
3. Elliott G & O'Hare P (1997). Intercellular trafficking and protein delivery by a herpesvirus structural protein. Cell 88: 223-233.
4. Kaplan IM, Wadia JS & Dowdy SF (2005). Cationic TAT peptide transduction domain enters cells by macropinocytosis. J Control Release 102: 247-253.
5. Schwarze SR, Hruska KA, Dowdy SF (2000). Protein transduction: unrestricted delivery into all cells? Trends in Cell Biology 10: 290-295.
6. Nagahara H, Vocero-Akbani A M, Snyder EL, Ho A, Latham DG, Lissy NA, Becker-Hapak M, Ezhevsky SA & Dowdy SF (1998). Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration. Nat Med. 4(12):1449-52.
7. Barka T, Gresik ES, Henderson SC (2004). J Histochem Cytochem. Production of cell lines secreting TAT fusion proteins. J Histochem Cytochem 52: 469-477.