Gene therapy of HIV-positive patients by the expression of membrane-anchored gp41 peptides

The invention relates to the gene therapy of HIV infection by the expression of membrane-anchored gp41 peptides. With this treatment vectors are made available for the first time which code for a fusion protein that contains a peptide derived from gp41 of HIV and a carboxy terminal trans-membrane anchor connected by a flexible linker.

A very wide variety of therapeutic approaches have been proposed for the treatment of HIV infections. However, the active substances available have proved to be poorly tolerated by many patients. To improve AIDS therapy, new points of attack and active substances with different toxicity profiles are constantly being sought. In this context, various gene therapeutical approaches have already been proposed, to inhibit the different steps in HIV replication (cf. Sorg T. & Methali, M. *Transfus. Sci.* 18, 277-289 (1977)).

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Wild et al. (Wild, C.T. Shugars, D.C. Greenwell, T.K. McDanal, C.B. & Matthews, T.J. *Proc. Natl. Acad. Sci U.S.A.* 91, 9770-9774 (1994)) have proposed a therapeutic approach based on the observation that peptides which are derived from the trans-membrane protein gp41 (cf. SEQ ID NO: 4 corresponding to the numeric code <400> in accordance with WIPO Standard ST. 25: 4) of HIV, such as, for example, the peptide T-20, formerly known as DP178, can effectively inhibit HIV fusion and entry into the cell. T-20 is a peptide which overlaps with the C-terminal heptad repeat, one of two domains (cf. positions 539-589 and 622-662 of SEQ ID NO: 4) in the ectodomain of the gp41, and can effectively inhibit the HIV infection in vitro (Weissenhborn, W., Dessen., A., Harrison, S.C. Skehel, J.J & Wiley, D.C. *Nature* 387, 426-430 (1997); Chan, D.C. Fass, D., Berger, J.M. & Kim, P.S. *Cell* 89. 263-273 (1997); Furuta, R.A., Wild, C.T., Weng Y & Weiss, C.D. *Nat. Struct. Biol.* 5, 276-279 (1998)).

Within the context of a clinical study (Kilby, J.M., Hopkins S., Venetta, T.M. et al. *Nat. Med.* 4, 1302-1307 (1998)), in the case of short-term administration T-20 proved to be safe and effectively inhibit HIV replication. As, however, very large quantities of the peptide are needed, to achieve an antiviral effect and as the peptides are not bio-available if administered orally, possess a very short half-life, and production on a large scale is still extremely expensive, the object of the invention is to make possible intracellular immunisation with peptides which are derived from gp41, via a gene therapeutical approach.

The inventors therefore first cloned the sequences coding for T-20 5′ of the IRES-NEO-Cassette (IRES: Internal ribosomal entry site; NEO: Neomycin-resistance gene) of the retroviral vector MPIN (cf. Figure 1, Hildinger et al., *Hum. Gene Ther. 9* (1998) 33-42). To achieve the secretion of the T-20,

on the one hand the peptide was expressed directly behind the signal peptide of the human low affinity nerve growth factor receptor (LNGFR, Fehse et al., *Human Gene Therapy* 8 (1997) 1815) (Constructs M85 and M86), and on the other hand a construct was chosen, which contains the coding sequences for a membrane-translocation signal (mts) derived from Kaposi fibroblast growth factor (amino acid positions 43-58 in mts protein; Rojas, M., Donahue, J.P., Tan, Z., Lin, Y.Z. *Nat. Biotechnol.* 16, 370-375 (1998)) in frame with T20 (cf. constructs M89 and M90 in Figure 1). Using these constructs, retroviral vectors were produced by transfection of Phoenix packaging cells (Grignani, F., Kinsella, T., Mencarelli, A. et al., *Cancer Res.* 58, 14-19 (1998)), and the supernatants were used to infect the T-helper cell line PM-1 (Bou-Habib, D.C. et al., *J. Virol.* 68 6006-6013 (1994)). As a control, the MPIN vector was used, which exclusively contains the neomycin resistance gene as a foreign gene. After G418 selection, the mass cultures were infected with HIV-1, which was produced from the proviral clones NL4-3 (Adachi, A., Gendelman H.E., Koenig, S., Folks, T., Willey, R., Rabson, A. & Martin, M.A., *J. Virol.* 59 284-291 (1986)) or NL4-3/GFP (Welker, R., Harris, M., Cardel, B. & Krausslich, H.G. *J., Virol.* 72, 8833-8840 (1998)). These two clones differ in that, in the case of NL4-3/GFP, the green fluorescent protein (GFP) is expressed instead of the nef protein, so that the HIV-1 replication can be analyzed on the basis of the p24 antigen production and/or by flow cytometry. Contrary to all expectations, the expression of T-20 peptide using the abovementioned retroviral vector constructs however surprisingly led to no antiviral activity of any kind.

Into these constructs, a sequence coding for the integrin-binding RGD peptide in frame with the region coding for T-20 was cloned. The production of these constructs was based on the consideration that the RGD motif could hold the secreting peptides on the cell membrane. However, even with the RGD-

containing secreted T-20 peptide (cf. Figure 1, M86) a reproducible HIV-replication was still observed.

Within the context of the present invention, it has now surprisingly been ascertained that the production of p24 and the spread of NL4-3/GFP can be greatly reduced, if a fusion protein is expressed, which, in addition to an amino-terminal gp41 peptide, contains a trans-membrane anchor tagged thereto carboxyterminally via a flexible linker. In this context, the term "gp41 peptide" means a fragment of the gp41 protein of the HIV or a fragment, variant or mutant thereof.

It has, for example, been ascertained, that the p24 production can be reduced by more than 2 powers of ten, if PM-1 is transduced with a retroviral vector, which expresses a fusion protein in which T-20 is connected C-terminally via a flexible peptide linker with a trans-membrane peptide (membrane spanning domain, MSD) (cf. Figure 1, M87), wherein the fusion protein has the sequence indicated in SEQ ID NO: 2.

The subject of the present invention is therefore a nucleic acid sequence of the general formula

5' - SP - FI - Hinge - MSD - 3',

in which

"5'" denotes the 5'end of the nucleic acid sequence,

"3'" denotes the 3'end of the nucleic acid sequence,

"SP" codes for a signal peptide, which mediates the transfer of an expressed peptide into the endoplasmatic reticulum.

"FI" codes for a fragment of the gp41 protein of HIV (preferably HIV-1), which contains a section from a heptad repeat region,

"MSD" codes for a trans-membrane-anchor of a type-1 membrane protein and

"Hinge" codes for a protein sequence, which, as a flexible linker, connects the peptides encoded by "FI" and "MSD".

The sequence coding for the signal peptide is derived from human, non-immunogenic proteins, preferably selected from the group consisting of sequences which code for signal peptides of cellular membrane proteins, such as, for example of the (human) low affinity nerve growth factor receptor (LNGFR), of the interleukin-2-receptor (IL-2R) and of the granulocyte macrophage colony stimulating factor receptor (GM-CSFR).

As trans-membrane anchors of a type-1 membrane protein (i.e. of a membrane protein, whose N-terminus is located outside and whose C-terminus is located inside the cell), proteins are used, whose cytoplasmatic domain should be deleted, to avoid any undesired signal transduction through the protein. In preliminary tests, it can be clarified that no signal transduction effects emanate from the expressed regions, and they do not oligomerise with other, similar membrane proteins and in this way exert an indirect effect on the cell functions. Peptides from the group consisting of the trans-membrane region of the LNGFR or the CD34 are preferably considered. The nucleic acid sequence therefore preferably contains as MSD, a nucleic acid sequence coding for these trans-membrane anchors and/or a nucleic acid sequence coding for fragments with a deleted cytoplasmatic domain.

According to the invention, it is possible to use, as flexible linkers, all flexible peptides which enable a flexible connection between the gp41 peptide and the trans-membrane anchor, such as, for example, the hinge of immunoglobin G (IgG), the linker of the human P-Glycoprotein (C.A. Hrycyna et al., *Biochemistry* 37 13660-13673 (1998)), the C-terminal linker of the

Human Replication Protein A (hsRPA; cf. D.M. Jacobs et al., *J. Biomol. NMR* 14, 321-331 (1999)) and the linker of the parathyroid hormone-related protein (M. Weidler et al. *FEBS Lett.* 444, 239-244 (1999)). The linker preferably has a length of up to 30 amino acids. The nucleic acid sequence of the above formula according to the invention therefore contains a nucleic acid sequence section coding for such a linker, preferably the hinge of IgG corresponding to nucleotides 1636 to 1683 of SEQ ID NO: 1.

As already mentioned, FI codes for a peptide (designated as gp41 peptide) which corresponds to a sequence section of the HIV-gp41-protein (cf. SEQ ID NO: 4, including the gp41 protein of the HIV quasi-species (clades)), which is selected from a region which includes the two heptad repeat regions (cf. amino acid positions 539-589 and 622-662 in SEQ ID NO: 3 and 4). In this context "HIV" includes all HIV types, especially HIV-1. In this context it is, for example, to be expected that use of an HIV-2 gp41 peptide will result in cross-reactivity with HIV-1 and vice-versa.

The gp41 peptides encoded by FI preferably have a minimum length of 28 amino acids, so that FI includes at least 84 nucleotides. Within the context of the present invention, in particular a nucleic acid sequence coding for the amino acid sequence represented in SEQ ID NO: 2 from Position 31 to 66 (corresponds to peptide T-20) is used as FI, preferably the sequence represented in SEQ ID NO: 1 from nucleotide 1528 to nucleotide 1635. According to a particular embodiment, the peptide encoded by FI has a maximum length of 40 amino acids, corresponding to a maximum length of 120 bases for the coding region FI.

According to a particular embodiment of the invention, the nucleic acid sequence of the general formula "SP-FI-Hinge-MSD" has the sequence represented in SEQ ID NO: 1 from nucleotide 1438 to nucleotide 1773, which codes for the fusion protein represented in SEQ ID NO: 2.

A further subject of the invention is the fusion protein encoded in each case by the abovementioned vectors/nucleic acid sequences, of the general formula

NH_2 - sp - fi - hinge - msd -COOH,

in which

"NH_2" denotes the amino-terminal end of the protein

"COOH" denotes the carboxy-terminal end of the protein

"sp" is a signal peptide, which enables the transfer of an expressed peptide into the endoplasmatic reticulum,

"fi" is a fragment of the gp41 protein of HIV, which contains a section from a heptad repeat region,

"msd" is a trans-membrane anchor of a type I membrane protein and

"hinge" is a protein sequence which connects the "fi" and "msd" peptides as a flexible linker.

With regard to the definitions of "sp", "fi", "hinge" and "msd", reference should be made to the above definitions of the sequence elements "SP", "FI, "Hinge" and "MSD", where the structural and functional characteristics of the fusion protein and/or the individual partial regions of the protein have already been mentioned.

According to a preferred embodiment, the fusion protein has the amino acid sequence represented in SEQ ID NO: 2. The fusion protein ("fi-hinge-msd") obtained therefrom after the cleaving off of the signal peptide accordingly has the sequence shown in SEQ ID NO: 2 from Position 31 to Position 111.

Also included according to the invention are homologues and/or variants and fragments of the abovementioned fusion protein, which essentially have the same pharmacological and biological effectiveness and/or immunogenicity and

the nucleic acid sequences coding for these homologues and fragments. In this context, the terms "homologues" and "variants" mean sequences which differ from the natural sequences or sequence sections known hitherto, by the exchange, deletion or insertion of individual amino acids. In this context in particular homologues, variants and fragments of the protein represented in SEQ ID NO: 2 and the nucleic acid sequences encoding the same are included.

The invention further relates to a vector which contains one abovementioned nucleic acid sequence. The vector is preferably a retroviral vector.

According to a preferred embodiment of the invention, the vector has the structure represented in Figure 1. The vector-insert of the general formula "SP-FI-Hinge-MSD", encoding the fusion protein according to the invention, preferably has the nucleic acid sequence represented in SEQ ID NO: 1. A sample of the last-mentioned vector was deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ), Mascheroder Weg 1b, 38124 Brunswick, Germany, on 11.11.1999, under number DSM 13139 under the Budapest Treaty.

The abovementioned vector can be used for the *in vitro* transfection of T-lymphocytes and haematopoietic stem cells, whereby these transfected cells can be administered to HIV-infected patients for therapeutic treatment. The vectors according to the invention are especially suitable for use (i.e. for direct (*in vivo*) application) in gene therapeutical treatment of patients infected with HIV, in which case the vectors are preferably vectors with targeted tropism, i.e. with specificity towards HIV target cells (CD4+ cells). The invention further relates to a gene therapeutical medicament, which contains an abovementioned vector.

The invention is further explained below, with reference to examples.

Examples

Cells and Viruses

HeLa-, 293- and Phoenix-Ampho cells were cultivated in Dulbecco's Medium (Gibco, Paisley, Great Britain), which was supplemented with 10% foetal calf serum (FCS; Sigma, Deisenhofen, Germany). PM-1 was cultivated in RPMI with 10% FCS. The viral clones NL4-3/GFP, NL4-3env-GFP and pNL4-3 have already been described above. (NL4-3: Welker R., Harris, M., Cardel, B. & Krausslich, H.G. *J. Virol.* 72, 8833-8840 (1998); NL4-2env-GFP: He, J. Chen, Y., Farzan, M., et al. *Nature* 385, 645-649) (1997)). To pseudotype the env-deficient clone NL4-3env-GFP, the plasmids pSNJG, pSVIIIenvJRFL, pSVIIIenvYU2 and pSVIIIenvHXB2 (Welker, R., Harris, M., Cardel, B. & Krausslich, H.G. *J. Virol.* 72, 8833-8840 (1998); He, J. Chen, Y., Farzan, M., et al. *Nature* 385, 645-649) (1997); von Laer, D., Thomson, S., Vogt, B., et al., *J. Virol 72, 1424-1430* (1998)) were used. These plasmids contain the VSV G-cDNA or the cDNA of the HIV envelopes JR-FL, YU2 and HXB2.

Infectious replication-competent viruses were produced by transfection of NL4-3 or NL4-3/GFP-DNA in HeLa cells. Pseudotyped replication-incompetent HIV were produced by co-transfection of pNL4-3env-GFP and one of the envelope-expression plasmids in 293-cells. The retroviral vectors were packaged in Phoenix packaging cells as described above (Grignani, F., Kinsella, T., Mencarelli, A., et al., *Cancer Res.* 58, 14-19 (1998)).

Cloning of retroviral vectors

M85 and M86: The sequence coding for the signal peptide of the human low affinity nerve growth factor receptor (LNGFR) was amplified by polymerase chain reaction (PCR) starting from the vector dLN using the primers SPNot+ (Sequence ID NO: 5) and SPBgl2 (SEQ ID NO: 6), whereby a NotI cleavage site was inserted at the 5' end and a BglII cleavage site was inserted at the 3' end (dLN: Fehse et al., Human Gene Therapy 8 (1997) 1815). The sequence coding for the fusion-inhibiting peptide was amplified from NL4-3 using the primer T20Bgl+ (M85; SEQ ID NO: 7) or T20Bgl-RGD+ (M86; SEQ ID NO: 8) and T20Hind- (SEQ NO ID: 9), whereby a BglII cleavage site was inserted at the 5' end and a HindIII cleavage site was inserted at the 3' end. The fragments were ligated into the vector pBluescriptKS digested with NotI and HindIII.

M87 and M88: The trans-membrane domain of dLNGFR was amplified from dLN using a 5' primer, which also contains that for the hinge-region of the murine IgG heavy chain and a BglII cleavage site (hingeTMBgl+; SEQ ID NO: 10), and a 3' primer of the retroviral vector dLN (U3-; sequence ID NO: 11). This PCR product was inserted, together with the signal peptide PCR product (SPNot+/SPBgl-) into the vector pBluescriptKS (after digestion with NotI and HindIII), and the sequence for T20 (starting from NL4-3) was inserted subsequently as PCR product, which contains flanking BglII cleavage sites (by using the PCR primer T20Bgl+ corresponding to SEQ ID NO: 7 (see above) and T20Bgl- corresponding to SEQ ID NO: 12).

M89 and M90: The sequence coding for T20 with a membrane translocation signal (mts) was amplified from NL4-3 using the primer RGD-T20Not+ (M89; SEQ ID NO: 13) or T20Not+ (M90; SEQ ID NO: 14) and T20mtsHind- (SEQ ID NO: 15). In the 5' primer, a NotI cleavage site is present, and in the 3' primer there is a HindIII cleavage site. The membrane translocation signal (mts) was introduced

with the 3' primer. The product was inserted into the vector pBluescriptKS digested with NotI and HindIII.

The genes encoding the different T20-fusion proteins were then transferred as NotI x HindIII fragments together with the polio-IRES from SF1 MIN into the vector MP1N (Hildinger et al., Hum. Gene Ther. 9 (1998) 33-42).

Infection with HIV

PM-1-cells ($5x10^4$ in 0.5 ml) were infected with 3000 to 6000 TCID50 (tissue culture infectious dose 50%) replication-competent HIV. For analysis of the p24 production the medium was changed on day 5, the cells were incubated overnight, and the cell-free residues were examined using a p24 ELISA as described above (Konvalinka, J., Litterst., M.A., Welker, R., et al. *J. Virol.* 69, 7180-7186 (1995)). The results are represented in Fig. 2.

Infection with NL4-3/GFP was further monitored at the moments indicated by means of flow cytometry analysis with a FACScalibur (Beckton Dickinson, Heidelberg, Germany). For this purpose, PM-1 (with and without the vectors MP1N and M85 to M87) were infected with a moi of 0.01 (moi: multiplicity of infection; describes the number of virus particles with which a cell is infected) with NL-4/GFP and analysed by flow cytometry on days 4, 7 and 10. The percentage of EGFP-positive and thus HIV-infected cells is shown in Fig. 3 in course for the various cultures. The detection threshold is approx. 0.1% positive cells.

Examination of the mechanism of action

To determine at which stage the HIV replication is inhibited, "single round" infections were carried out with the clone NL4-3env-GFP. The clone NL4-3env-GFP, due to a mutation in the env-gene is replication-defective and expresses GFP instead of nef. To produce infectious virions, the vector was pseudotyped with the envelopes of three different HIV clones (HXB, YU2 and JR-FL) and with the G protein of the vesicular stomatitis virus (VSV G). HXB is classified as T-tropic, with use of the co-receptor CXCR4, whilst YU2 and JR-FL are M-tropic and use CCR-5. The env-genes of the last two clones mentioned were cloned directly from primary HIV isolates (He, J., Chen, Y., Farzan, M. et al. *Nature* 385, 645-649 (1997). These HIV pseudotypes are capable of "single round" infections, but do not spread throughout the whole culture. In PM1/M87 cells (i.e. in cells which are transfected with the vector according to the invention, encoding the membrane-anchored T-20 fusion protein) the infection was more strongly inhibited via the three different HIV envelopes, by a factor of 15 to 30, than in the case of the VSV-G pseudotypes, in which no significant inhibition was observed (cf. Figure 4). Just like the free T-20 peptide (cf. Wild C.T. et al. *Proc. Natl. Acad. Sci. USA* 91, 9770-9774), the genetically expressed membrane-anchored T-20 fusion protein also inhibits entry mediated via the envelopes of the different HIV variants. These results clearly show that the virus is inhibited at the stage of virus entry mediated via HIV-env, in which it is very probably a matter of membrane fusion. All HIV-replication steps following the virus entry were not influenced.

These investigations show that membrane-anchored gp41 peptide effectively inhibits HIV replication, whilst secreted pure gp41 peptide is not effective.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Heinrich-Pette-Institut
Martinistr. 52

20251 Hamburg

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
M87	DSM 13139

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 1999-11-11 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss
Address: Mascheroder Weg 1b, D-38124 Braunschweig	Date: 1999-11-15

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Heinrich-Pette-Institut
Martinistr. 52

20251 Hamburg

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Heinrich-Pette-Institut Martinistr. 52 Address: 20251 Hamburg	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 13139 Date of the deposit or the transfer[1]: 1999-11-11

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 1999-11-12 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Mascheroder Weg 1b D-38124 Braunschweig	U. Weiss Date: 1999-11-15

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit, date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 0196

Claims

1. Nucleic acid sequence, characterised by the general formula

SP - FI - hinge - MSD,

in which

"SP" codes for a signal peptide, which mediates the transfer of an expressed peptide into the endoplasmatic reticulum.

"FI" codes for a fragment of the gp41 protein of HIV, which contains a section from a heptad repeat region,

"MSD" codes for a trans-membrane-anchor of a type-1 membrane protein and

"Hinge" codes for a protein sequence, which connects the peptides encoded by "T20" and "MSD" as a flexible linker.

2. Nucleic acid sequence according to Claim 1, characterised in that SP is a sequence, which encodes a signal peptide of the low affinity nerve growth factor receptor (LNGFR), of the interleukin-2-receptor (IL-2R) or of the granulocyte macrophage colony stimulating factor receptor (GM-CSFR).

3. Nucleic acid sequence according to Claim 1 or 2, characterised in that MSD is a sequence which encodes a trans-membrane anchor of the LNGFR or of the CD34 or a fragment thereof.

4. Nucleic acid sequence according to Claims 1 to 3, characterised in that hinge is a sequence which encodes the linker of immunoglobulin G (IgG), of the human P-glycoprotein, of the human replication protein A and of parathyroid hormone-related protein.

5. Nucleic acid sequence according to Claims 1 to 4, characterised in that FI encodes the amino acid sequence represented in SEQ ID NO: 2 from position 31 to position 66.

6. Nucleic acid sequence according to Claim 5, characterised in that it has the sequence represented in SEQ ID NO: 1 from nucleotide 1528 to nucleotide 1635.

7. Nucleic acid sequence according to Claims 1 to 6, characterised in that it has the sequence represented in SEQ ID NO: 1 from nucleotide 1528 to nucleotide 1773.

8. Vector, characterised in that it contains a nucleic acid sequence according to Claims 1 to 7.

9. Vector according to Claim 8, characterised in that it is a retroviral vector.

10. Vector according to Claim 9, characterised in that it has the structure represented in Figure 1.

11. Vector according to Claim 10, characterised in that it contains the nucleic acid sequence represented in SEQ ID NO: 1.

12. Vector according to Claim 11 with the deposit number DSM 13139.

13. Use of a vector according to Claims 8 to 12 for *in vitro* transfection of T-lymphocytes and haematopoietic stem cells.

14. Use of a vector according to Claims 8 to 12 for gene therapeutical treatment of patients infected with HIV.

15. Use of T-lymphocytes or haematopoietic stem cells which are transfected with a vector according to Claims 8 to 12 for gene therapy treatment of patients infected with HIV.

16. Gene therapeutical medicament containing a vector according to Claims 8 to 12.

17. Gene therapeutical medicament containing T-lymphocytes or haematopoietic stem cells which are transfected with a vector according to Claims 8 to 12.

18. Protein of the general formula "sp-fi-hinge-msd" which is encoded by a nucleic acid sequence according to Claims 1 to 7, in which "sp", "fi", "hinge" and "msd" have the meaning indicated in Claim 1.

19. Protein according to Claim 18, characterised in that it has the sequence represented in SEQ ID NO: 2.

20. Protein of the general formula "fi-hinge-msd", which is encoded by a nucleic acid sequence according to Claims 1 to 7, in which "fi", "hinge" and "msd" have the meaning indicated in Claim 1.

21. Protein according to Claim 20, characterised in that it has the sequence represented in SEQ ID NO: 2 from position 31 to position 111.

22. Protein characterised in that it is a mutant, variant or derivative of the protein according to Claims 18 to 21.

23. Nucleic acid sequence characterised in that it encodes a protein according to Claim 22.