was transfected into HEK293 cells and 11 days *post transfectionem* the ablating cells were removed together with the medium in order to release the adenoviruses through repeated freeze-thaw cycles.

It is within the present invention and feasible for the one skilled in the art with regard to the technical teaching provided herein, that other systems such as the system AdEasy of QBIOGENE and Microbix may be used for the manufacture of the adenoviruses according to the present invention, preferably the recombinant adenovirus, in particular those which contain, individually and/or together, the cassettes E4orf6-IRES-E1B55k and YB-1-IRES-E1A12S. Additionally, individual transgenes may be exchanged between the cassettes. It is within the present invention that also such adenoviruses can be manufactured and used in accordance with the present invention, where the cassette has the following design: E1B55k-IRES-E4orf6 and E1A12S-IRES-YB1.

In connection with the present invention a so called E1/E3 deleted recombinant Adenovirus was used which contains the cassette E4orf6-IRES-E1B55k. It is, however, within an embodiment that the virus comprises only an E1-deletion, which means that the E3-region remains intact. Optionally, the E4-region may be partially and/or completely deleted.

In the manufacture of the vector using different systems it was proceeded as follows.

Manufacture of the adenovirus Ad-Xvir 3'UTR having an intact E3-region with the vector system according to Graham (company Microbix).

Cloning of the vector CMV-E4ORF6-IRES-E1B55k 3'UTR-polyA in pDelta E1sp1A

For the plasmid E1B55k 3'UTR-pShuttle (Clontech) the open reading frame having the 3'-UTR was prepared by amplification from the DNA of adenovirus type 5 (E1B55k forward primer = 5'-ATGGAGCGAAGAAACCC-3'(SEQ ID No. 4) and E1B55k 3'UTR backward primer = 5'-CACGTCCTGGAAAAAATACAC-3'(SEQ ID No. 5)) and introduced in the blunt ended *Nhe*I restriction site, which was provided with T-ends (TA-cloning) and cloned into the pShuttle plasmid of the company Clontech. Thus, the transgene was provided with a hCMV-promoter at the 5'end and with the bovine growth hormone polyadenylation signal at the 3'end. However, it is also within the present invention that E1B55k is used from the plasmid pCGNE1B from Dobbelstein (Dobbelstein, M. et al., EMBO Journal, 16, 4276-4284, 1997) by means of Bam HI and blunt

ending and TA-cloning, respectively. The E1B55k-3'UTR which has been cloned, is, among others, described in more detail in Figs. 23 and 24.

Cloning of the vector E4ORF6-IRES-pcDNA3.1(+)

The amplificates E4orf6 using the adenovirus type 5 DNA as template (E4orf6 forward primer 5'-CTTCAGGATCCATGACTACGTCCGGCG-3'(SEQ ID No. 6) and E4orf6 backward primer 5'-GAAGTGAATTCCTACATGGGGGTAGAGTCATAATCGT-3'(SEQ ID No. 7)) and from the plasmid pCMVE4-34kD which has been cut with Bam HI (Dobbelstein et al., EMBO, 16, 4276-4284,1997), and the IRES element having the pCITE-4a(+) of the company Novagen as template (IRES forward primer = 5'-TCCGGTTATTTTCCACCATATTGC-3'(SEQ ID No. 8) and IRES backward primer = 5'-TTATCATCGTGTTTTTCAAAGG-3'SEQ ID No. 9)) were subsequently cloned into the multiple cloning site of the pcDNA3.1(+)-vector. For such purpose, primers were used for the E4orf6 transgene which create a *Bam*HI cleavage site at the 5'-end and a *Eco*RI cleavage site at the 3'-end of the open reading frame. The amplificate was digested with the respective restriction enzymes and the ends thereof were made compatible for the directed cloning into the vector which has been opened using *Bam*HI and *Eco*RI. Subsequently, plasmid E4orf6 in pcDNA3.1(+) was linearized with *Eco*RV, the T-ends added and the amplificate cloned into the IRES element. After checking the correct orientation of the IRES element, the vector was used for further cloning.

The linkage of both transgenes with the IRES element resulted from a cloning of the E4orf6-IRES cassette into the previously generated plasmid CMV-E1B55k 3'UTR-polyA-pShuttle (Clontech) which was linearized with *Not*I, blunt ended and subsequently cut with *Xba*I. E4orf6-IRES in pcDNA3.1 (+) was linearized with *Not*I, the ends made blunt ended and further digested with *Nhe*I. By ligating the E4orf6-IRES insert with the CMV-E1B55k 3'UTR-polyA-pShuttle (Clontech) XVIR-3'UTR was generated in pShuttle (Clontech).

Generation of the used adenoviral shuttle vector

As the shuttle vector pΔE1sp1A, now used for the adenoviral generation system of the company Microbix, did neither contain a CMV promoter nor a bovine growth hormone polyadenylation signal, these elements were cloned into pΔE1sp1A. For such purpose, pΔE1sp1A was linearized with *Cla*I, made blunt ended and cut with *Eco*RI. The element CMV-MCS (multiple cloning site)-poly-A was linearized from pShuttle (Clontech) with MfeI, the ends made blunt ended and

For increasing the infectivity the HI Loop of the fibre knob domain was modified following Dmitriev et al. 1998 (An Adenovirus Vector with Genetically Modified Fibers Demonstrates Expanded Tropism via Utilization of a Coxsackievirus and Adenovirus Receptor-Independent Cell Entry Mechanism): The respective region was amplified using the primers RGD-Hpa fw (5'-GAGgttaacCTAAGCACTGCCAAG-3'(SEQ ID No. 10)), RGD-EcoRV rev (5'-CATAGAGTATGCAGATATCGTTAGTGTTACAGGTTTAGTTTTG-3'(SEQ ID No. 11)) and RGD-EcoRV fw (5'-GTAACACTAACGATATCTGCATACTCTATGTCATTTTCATGG-3'(SEQ ID No. 12)) and RGD-Bfr rev (5'-CAGCGACATGAActtaagTGAGCTGC-3'(SEQ ID No. 13)) and thus an EcoRV restriction site generated. In this restriction site the paired oligonucleotides were cloned which code for an Arg-Gly-Asp (RGD)-peptide: RGD-oligo 1 (5'-CACACTAAACGGTACACAGGAAACAGGAGACACAACTTGTGACTGCCGCGGAGACTG TTTCTGCCC-3'(Seq ID No. 14)) and RGD-oligo 2 (5'-GGGCAGAAACAGTCTCCGCGGCAGTCACAAGTTGTGTCTCCTGTTTCCTGTGTACCGT TTAGTGTG-3'(SEQ ID No. 15)). Thus, the RGD motif is present in the HI Loop of the fibre knob domain.

The vector described above is in principle suitable as are the other viruses described herein for use in accordance with the present invention. In particular the afore-described vector is suitable to replicate and trigger lysis insofar, in cells which are YB-1 nucleus-positive cells as well as in cells where YB-1 is deregulated, i. e. is overexpressed compared to normal cells and non-tumor cells, respectively. The use of this vector particularly applies to those diseases and groups of patients or collectives of patients which are disclosed in connection with the other adenoviruses which are described herein to be used in accordance with the present invention and the other adenoviruses of the present invention disclosed herein.

Example 12: Structural design of the adenoviral vector Xvir03/01

As may be taken from Fig. 17, Xvir03/01 is a further development of Xvir03. Therapeutic genes such as, for example, the genes described herein and the transgene can be cloned into the E3 region. Additionally, a deletion was introduced into the E4 region so as to avoid homologous recombination with the E4orf6 from the expression cassette of Xvir03. This allows that larger transgenes can be cloned in this construct. The deleted E3 region contains SacI, NdeI and NheI restriction sites for introducing a cassette, into which, for example, the therapeutic transgenes

CAR-expression and thus significantly improves infectability of the cells. On the other hand, Irinotecan translates YB-1 into the cell nucleus and thus induces an improved adenoviral replication. Additionally, the cellular YB-1 is assisting adenoviral replication after infection with dl520 and is no longer available for DNA-repair processes. Depending on the point of view, this results in an improved efficacy of dl520 on the one hand and an increased efficacy of the cytostatics on the other hand.

Example 18: Northern blot analysis of the E2 gene expression of adenovirus Ad312

In each case 1 million A549 and U2OS cells were plated in 10 cm Petri dishes. At the next day the cells were infected with Ad312 (50 pfu/cell) and Adwt (which served as control, 5 pfu/cell). The high virus titer of Ad312 which was used resulted in an E1-independent replication in tumor cells. The infection was done in 1-2 ml serum-free DMEM medium for 1 h at 37°C. Subsequently, the infection medium was removed and replaced by 10 ml complete medium (10 % FCS/DMEM). After 3 days the RNA was isolated. Subsequently, the concentration of the isolated RNA was measured in a photometer at 260 nm. Then the RNA samples were electrophoretically separated in a 0.8 % formaldehyde agarose gel. Subsequently, the RNA was blotted on a nylon membrane (conducted according to the system of Schleicher & Schuell). The RNA blotted on the membrane is blotted against an "early probe" E2 and a "late probe" E2. The 1501 bp "late probe" specifically binds behind the E2-late promoter. The probe was prepared prior to that by PCR (primer: 5'- GTC GGA GAT CAG ATC CGC GT (SEQ. ID. NO. 42), 5'- GAT CCT CGT CGT CTT CGC TT (SEQ. ID. NO. ~~5~~3)) and radioactively labelled using ^{32}P. In contrast, the early probe binds between the E2-early promoter and the E2-late promoter (position: 226791-227002) and was also generated by means of PCR (primer: 5'- AGCTGATCTTCGCTTTTG (SEQ. ID. NO. ~~6~~55), 5'- GGATAGCAAGACTCTGAC AAAG (SEQ. ID. NO. ~~7~~56)). Subsequently, the membrane was washed and exposed to a film.

The result is depicted in Fig. 25. Both the early as well as the late probe provided specific signals in the control infection with wildtype adenovirus, whereas tumor cells infected with Ad312 only provided a specific signal when the late probe was used. This confirms the finding underlying the present invention that the expression of E4orf6 and E1B55K and the absence of E1A transports overexpressed and deregulated YB-1, respectively, into the nucleus and thus induces E2 gene expression as a prerequisite for efficient adenoviral replication.

Example 19: Northern blot analysis of the E2 gene expression of adenovirus Addelta 24

In each 1 million U2OS cells were plated in 10 cm Petri dishes. At the next day the cells were infected with adenovirus delta 24 (Addelta24) (10 pfu/cell) and wildtype adenovirus (Adwt) (served as a control, 10 pfu/cell). The used recombinant adenovirus Addelta24 (Fueyo, J. et al., Oncogene 19, 2-12, 2000) has a specific deletion in the CR2 region of the E1A protein and is thus only capable of replicating in Rb-negative tumors. Additionally, the virus expresses the genes E1B55k and E4orf6 comparable to the wildtype adenovirus. The infection occurred in 1-2 ml serum-free DMEM medium for 1 h at 37°C. Subsequently, the infection medium was removed and replaced by 10 ml complete medium (10 % FCS/DMEM). The RNA was isolated after 12 h and 24 h. Subsequently, the concentration of the isolated RNA was determined in a photometer at 260 nm. Then the RNA samples were electrophoretically separated in a 0.8 % formaldehyde agarose gel. Subsequently, the RNA was blotted on a nylon membrane (conducted according to the system of Schleicher & Schuell). The RNA blotted onto the membrane is hybridised against the "early probe" and against the "late probe". The "late probe" comprising 1501 bp, binds specifically behind the E2-late promoter. The probe was prepared prior to that by PCR (primer: 5'- GTC GGA GAT CAG ATC CGC GT (SEQ. ID. NO. 42), 5'- GAT CCT CGT CGT CTT CGC TT (SEQ. ID. NO. 53)) and radioactively labelled using ^{32}P. The early probe, however, binds between the E2-early promoter and the E2-late promoter and was also prepared by PCR (primer: 5'- AGCTGATCTTCGCTTTTG (SEQ. ID. NO. 655), 5'- GGATAGCAAGACTCTGACAAAG (SEQ. ID. NO. 756)). Subsequently, the membrane was washed and exposed to a film.

The result is shown in Fig. 26.

After 12 h only the late probe provided for a specific signal. Only after 24 h also the early probe provided a signal in cells infected with Addelta24. Compared to wildtype adenoviruses, however, the signal is significantly weaker. Also this result confirms the finding underlying the present invention that the expression of E4orf6 and E1B-55K transports overexpressed and deregulated YB-1, respectively, into the nucleus which subsequently binds to the E2-late promoter and induces E2 gene expression.

Generation of the adenovirus AdPSJL-E2-late promoter 12S-AdEASY with E1A12S in the deleted E3-region with the AdEASY-system (company Microbix).

Cloning of PSJL 12S

First, the E2-late promoter was cloned into the *Hind*III and *Bgl*II cleavage site of the pGL3-enhancer plasmid (pGL3-E2-late) as paired oligonucleotides (upper primer 5'-TCGAGCTCCGCATTTGGCGGGCGGGATTGGTCTTCGTAGAACCTAATCTCGTGGG CGTGGTAGTCCTCAGGTACAAAT-3'(SEQ ID NO. 16) and lower primer 5'-AGCTTATTTGTACCTGAGGACTACCACGCCCACGAGATTAGGTTCTACGAAGACC AATCCCGCCCGCCAAATGCGGAGC-3'(SEQ ID NO. 17)).

Subsequently, the luciferase gene was excised using *Nco*I and *Xba*I, the ends made blunt ended and T-ends added. The transgene E1A 12S which was amplified by the primers E1A 12S forward primer 5'-ATGGCCGCCAGTCTTTTG-3'(SEQ ID no. 18) and E1A 12S backward primer 5'-TTATGGCCTGGGGCGTTTAC-3'(SEQ ID No. 19), was introduced by TA-cloning into the thus opened site.

This cassette was excised using *Pvu*I and *Cla*I, the ends made blunt ended and cloned into the blunt ended and dephosphorylated *Nhe*I-cleavage site in the E3-region of E3E4-pShuttle (-*Nde*I)-AdEASY. The cassette thus contains the E2-late promoter, the open reading frame E1a-12S and the SV-40 late polyadenylation signal. The resulting construct is E2-late-E1a-12S-E3E4-pShuttle(-*Nde*I)-AdEASY.

Subsequently the E2-late-E1a 12S-E3E4 was excised from the E2-late-E1a 12S-E3E4-pShuttle (-*Nde*I)-AdEASY using *Spe*I and *Pac*I and cloned into the *Spe*I and *Pac*I cut pAdEASY. The thus resulting construct was referred to as E2-late-E1a 12S-E3E4-pAdEASY.

AdPSJL-12S-AdEASY was generated by homologous recombination upon transforming BJ5183 (EC) bacteria with the plasmids Xvir-3'UTR in pShuttle AdEASY and E2-late-E1a 12S-E3E4-pAdEASY.

Cloning of the cassette E2-late promoter-E1A-12S and/or E2-late promoter-E1A-12S-IRES-YB-1 in the E4-region

After manipulation and deletion, respectively, of the E4 region using PstI 634 bp were removed. The cassettes E2-late promoter-E1A-12S and/or E2-late promoter-E1A-12S-IRES-YB-1 can be introduced into the E4-region. Alternatively, the E2-region may remain intact under such conditions.

Cloning of the RGD-motive

For an improved infectivity the HI loop of the fibre knob domain was modified according to Dmitriev et al. 1998 (An Adenovirus Vector with Genetically Modified Fibers Demonstrates Expanded Tropism via Utilization of a Coxsackievirus and Adenovirus Receptor-Independent Cell Entry Mechanism): The respective region was amplified using the primes RGD-Hpa fw (5'-GAGgttaacCTAAGCACTGCCAAG-3'(SEQ ID No. 20)), RGD-EcoRV rev (5'-CATAGAGTATGCAGATATCGTTAGTGTTACAGGTTTAGTTTTG-3'(SEQ ID No. 21)), as well as RGD-EcoRV fw (5'-GTAACACTAACGATATCTGCATACTCTATGTCATTTT CATGG-3'(SEQ ID No. 22)) and RGD-Bfr rev (5'-CAGCGACATGAActtaagTGAGCTGC-3'(SEQ ID No. 23)) and an EcoRV-cleavage site thus generated. Paired oligonucleotides were cloned into this cleavage site which code for an Arg-Gly-Asp (RGD)- peptide with RGD oligo 1 (5'-CACACTAAACGGTACACAGGAAACAGGAGACACAACTTGTGACTGCCGCGGAGA CTGTTTCTGCCC-3'(SEQ ID No. 24)) and RGD oligo 2 (5'-GGGCAGAAACAGTCTCCGCGGCAGTCACAAGTTGTGTCTCCTGTTTCCTGTGTACC GTTTAGTGTG-3'(SEQ ID No. 25)). Thus the RGD motif is contained in the HI loop of the fibre knob domain.

Example 21: Infection of HeLa cells with adenovirus dl520

100.000 HeLa cells were plated per dish. At the next day the cells were infected with various titers (pfu/ml) of adenovirus dl520. The infection was done in 500 µl serum-free DMEM medium for 1 h at 37°C. Subsequently, the infection medium was removed and replaced by 2 ml complete medium (10 % FCS/DMEM). After 3-5 days an analysis was performed using crystal violet staining.

4. comprising no Y-box as acting as the blank reading

Part of the adenoviral E2 region (taken from Virology 1992, 186, 280-285)
(The YB-1 binding sites are printed in bold):

25561 aggaactttatcctagagcgctcaggaatcttgcccgccacctgctgtgcacttcctagc
25621 gactttgtgcccattaagtaccgcgaatgccctccgccgctttggggccactgctaccttt
25681 ctgcagctagccaactaccttgcctaccactctgacataatggaagacgtgagcggtgac
25741 ggtctactggagtgtcactgtcgctgcaacctatgcaccccgcaccgctccctggtttgc
25801 aattcgcagctgcttaacgaaagtcaaattatcggtacctttgagctgcagggtccctcg
25861 cctgacgaaaagtccgcggctccggggttgaaactcactccggggctgtggacgtcggct
25921 taccttcgcaaatttgtacctgaggactaccacgcccacgagattaggttctacgaagac
25981 caatcccgcccgccaaatgcggagcttaccgcctgcgtcattacccagggccacattctt
26041 ggccaattgcaagccatcaacaaagcccgccaagagtttctgctacgaaagggacggggg
26101 gtttacttggacccccagtccggcgaggagctcaacccaatcccccccgccgccgcagccc
26161 tatcagcagcagccgcgggcccttgcttcccaggatggcacccaaaaagaagctgcagct
26221 gccgccgccacccacggacgaggaggaatactgggacagtcaggcagaggaggttttgga
26281 cgaggaggaggaggacatgatggaagactgggagagcctagacgaggaagcttccgaggt
26341 cgaagaggtgtcagacgaaacaccgtcaccctcggtcgcattcccctcgccggcgcccca
26401 gaaatcggcaaccggttccagcatggctacaacctccgctcctcaggcgccgccggcact
26461 gcccgttcgccgacccaaccgtagatgggacaccactggaaccagggccggtaagtccaa
26521 gcagccgccgccgttagcccaagagcaacaacagcgccaaggctaccgctcatggcgcgg
26581 gcacaagaacgccatagttgcttgcttgcaagactgtgggggcaacatctccttcgcccg
26641 ccgctttcttctctaccatcacggcgtggccttcccccgtaacatcctgcattactaccg
26701 tcatctctacagcccatactgcaccggcggcagcggcagcggcagcaacagcagcggcca
26761 cacagaagcaaaggcgaccggatagcaagactctgacaaagcccaagaaatccacagcgg

(SEQ. ID. No. ~~8)~~ 26)

The results presented in Fig. 30 confirm in an impressive manner that the individual promoter fragments which contain different E2-late/Y-boxes, are suitable for the expression of therapeutic

Example 25: Detection of protein IX expression

This experiment was performed in order to confirm the importance of the expression of protein IX for an effective particle formation in YB-1 mediated replication. Therefor the oncolytic YB-1 dependent replicating adenovirus Xvir 03-3´UTR has been used which is described in the prior art and is depicted in Fig. 50.

The experiment was performed as follows: For each 10 cm dish 10^6 293 and 257 RDB cells were plated. The next day the cells were, as depicted in Fig. 10, either not infected (K), infected with wildtype adenovirus or with Xvir03. The infection occurred in 1.5 ml serum-free DMEM medium for 1 h at 37 °C. Subsequently, the infection medium was removed and replaced by 10 ml complete medium (10 % FKS/DMEM). After 24 – 48 h the RNA was isolated. Subsequently, a Northern blot analysis was performed. For such purpose each 10 µg RNA were separated electrophoretically in an agarose gel with 3 % formaldehyde, subsequently blotted onto a nylon membrane and hybridised against a specific 386 bp probe. A P32 labelled probe directed against protein IX was used as a probe and generated using PCR. The following primers were used for the PCR: 5'-TATTTGACAACGCG(SEQ ID No. 27); 5'-TTTTAAACCGCATTGGG(SEQ ID No. 28). The position of the probe in wildtype adenovirus genome is between position 3648 and 4033. The virus which is used, is Xvir 03 which does not expression of protein IX.

The results of the experiment are depicted in Fig. 42.

As may be taken from Fig. 42, the virus Xvir03-3´UTR shows a decreased expression in tumor cells 257RDB compared to wildtype adenovirus. In 293 cells which express E1A and E1B proteins, among others also the E1B19K protein, sufficient protein IX is expressed.

Example 26: Recombination analysis of vector Xvir03-3´UTR

Per each 10 cm dish 10^6 293 cells were plated. The next day the cells were infected with the various adenoviruses as depicted in Fig. 43. The infection was performed in 1.5 ml serum-free DMEM medium for 1 h at 37 °C. Subsequently, the infection medium was removed and replaced by 10 ml complete medium (10 % FKS/DMEM). After 48 h the DNA was released by alkaline

lysis and purified over a column. Subsequently, 2 µg DNA were cut with Hind III. The samples were separated electrophoretically in a 1 – 2 % agarose gel and subsequently blotted onto a nylon membrane. The DNA blotted onto the membrane was hybridised against a specific 386 bp probe. A P32 labelled probe targeting the protein IX was used as a probe and generated by means of PCR. The following primers were used for the PCR: 5'-TATTTGACAACGCG(SEQ ID No. 29); 5'-TTTTAAACCGCATTGGG(SEQ ID No. 30). The position of the probe in the wildtype adenovirus genome is between position 3468 and 4033. The result shows that adenovirus Xvir03 does not recombine after infection in 293 cells. The sizes of the cleavage product are represented in the figure.

Example 27: MRP expression analysis in 257RDB cells

Per 10 cm dish 10^6 257RDB cells were plated. The next day the cells were infected with the various adenoviruses as depicted in Fig. 44. The infection occurred in 1.5 ml serum-free DMEM medium for 1 h at 37 °C. Subsequently, the infection medium was removed and replaced by 10 ml complete medium (10 % FKS/DMEM). After 3 – 4 days the RNA was isolated. Subsequently, a Northern blot analysis was performed. For such purpose each 10 µg RNA were electrophoretically separated in an agarose gel with 3 % formaldehyde, subsequently blotted onto a nylon membrane and hybridised against the specific P32 labelled MRP probe. The probe is generated by restriction EcoRI from plasmid pCRII-MRP. The result shows that the adenovirus Xvir03 is capable of inhibiting the expression of the ABC transporter MRP.

Example 28: MDR expression analysis in 257RDB cells

Per 10 cm dish 10^6 257RDB cells were plated. The next day the cells were infected with the various adenoviruses as depicted in Fig. 45. The infection was performed in 1.5 ml serum-free DMEM medium for 1 h at 37 °C. Subsequently, the infection medium was removed and replaced by 10 ml complete medium (10 % FKS/DMEM). After 3 – 4 days the RNA was isolated. Subsequently, a Northern blot analysis was performed. For such purpose each 10 µg RNA were electrophoretically separated in an agarose gel containing 3 % formaldehyde, subsequently blotted onto a nylon membrane and hybridised against a specific P32 labelled MDR probe. (Holm et al., British J. Cancer, 1994, 70, 239-243). The result shows that the adenovirus Xvir03 is capable of inhibiting the expression of the ABC transporter MDR1.

For manipulating the E3 and the E4 region the ΔE3E4 region of plasmid pAdEASY was cloned with *Spe*I and *Pac*I into plasmid CMV-MCS-PolyA-pShuttle-AdEASY and thus the plasmid ΔE3E4-pShuttle-AdEASY generated. By restriction with *Nde*I and religation one of the two *Nde*I restriction sites was deleted and thus also a multiple cloning site from the plasmid. By this procedure plasmid ΔE3E4-pShuttle (-*Nde*I)-AdEASY was generated.

E4 manipulation

In order to provide space for potential therapeutic transgenes and in order to avoid an undesired homologous recombination, the E4 region in plasmid ΔE3E4-pShuttle (-*Nde*I)-AdEASY was specifically deleted. When doing so, the E4orf6 region is shortened by about 634 bp by excising with *Pst*I and religation = ΔE3E4ΔORF6-pShuttle (-*Nde*I)-AdEASY. Respective deletions may be performed in different systems for the generation of recombinant adenoviruses by the one skilled in the art.

Cloning of the RGD motif in ΔE3E4ΔORF6-pShuttle (-*Nde*I)-AdEASY

For the improved infectivity the HI loop of the fibre knob domain was modified in accordance with Dmitriev et al. 1998 (An Adenovirus Vector with Genetically Modified Fibers Demonstrates Expanded Tropism via Utilization of a Coxsackievirus and Adenovirus Receptor-Independent Cell Entry Mechanism): The respective region was amplified using the primers RGD-Hpa fw (5'-GAGgttaacCTAAGCACTGCCAAG-3'(SEQ ID No. 31)), RGD-*Eco*RV rev (5'-CATAGAGTATGCAGATATCGTTAGTGTTACAGGTTTAGTTTTG-3'(SEQ ID No. 32)) as well as RGD-*Eco*RV fw (5'-GTAACACTAACGATATCTGCATACTCTATGTCATTTTCATGG-3'(SEQ ID No. 33)) and RGD-*Bfr*I rev (5'-CAGCGACATGAActtaagTGAGCTGC-3'(SEQ ID No. 34)) and thereby an *Eco*RV-cleavage site generated. Paired oligonucleotides were cloned into this cleavage site coding for an Arg-Gly-Asp (RGD) peptide: RGD-Oligo 1 (5'-CACACTAAACGGTACACAGGAAACAGGAGACACAACTTGTGACTGCCGCGGAGA CTGTTTCTGCCC-3'(SEQ ID No. 35)) and RGD-Oligo 2 (5'-GGGCAGAAACAG TCTCCGCGGCAGTCACAAGTTGTGTCTCCTGTTTCCTGTGTACCGTTTAGTGTG-3'(SEQ ID No. 36)). By cloning using the HpaI and BfrI cleavage sites in ΔE3E4ΔORF6-pShuttle (-*Nde*I)-AdEASY the ΔE3-RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY was generated. The RGD motif is present in the HI loop of the fibre knob domain.

Cloning of the E3a region in ΔE3 region of ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY.

For such purpose the vector pcDNA3.1(+) of the company Invitrogen was cleaved with *Bgl*II and *Bam*HI, whereby the CMV promoter was removed and the vector religated (pcDNA3.1(+) without CMV = oCMV). Using these *Spe*I and *Xho*I restriction sites of the pcDNA3.1(+) oCMV vector the 2709 bp fragment was cloned which was excised with *Spe*I (27083 bp) and *Xho*I (29792 bp) from wildtype virus DNA (pcDNA3.1(+) oCMV/E3a*Xho*I). Alternatively, one can cleave with *Hpa*I (30570 bp) rather than *Xho*I at the 3′ end. For such purpose the vector pcDNA3.1(+) oCMV is then cleaved with *Spe*I and *Eco*RV and the adenoviral *Spe*I-HpaI fragment is cloned therein (pcDNA3.1(+) oCMV/E3a*Hpa*I). A further option is the 2718 bp *Eco*RI fragment of adenovirus wildtype DNA (positions 27332 bp and 30050 bp) which is cloned into the pcDNA3.1(+) oCMV which has been opened using EcoRI (pcDNA3.1(+) oCMV/E3a*EcoRI*).

Using the von pcDNA3.1(+) oCMV/E3a the E3a region could be cloned into the vector ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY: The shuttle vector ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY was cleaved for such purpose with *Nhe*I, the ens made blunt-ended and further cleaved with *Spe*I. The insert from pcDNA3.1(+) oCMV/E3a*Xho*I was cloned into this site. For such purpose the plasmid was cleaved with *Xho*I, the ends made blunt-ended and further cleaved with *Spe*I. The fragment thus cut out was cloned into the previously cut plasmid ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY.

The fragments *Spe*I – *Hpa*I (position 27083 bp to 30570 bp) and *Eco*RI (position 27332 bp to 30050 bp) may be excised the same way from the respective pcDNA3.1(+) oCMV/E3a constructs and transferred by cloning.

Alternatively, the E3a region may be amplified by PCR using the primers E3a *forward (SpeI)* 5'-CTTAAGGACTAGTTTCGCGC -3'(SEQ ID No. 37) and E3a *reverse* (*Xho*I, *Nhe*I) 5'-CAAGCTAGCTCGAGGAATCATG -3'(SEQ ID No. 38) with the adenovirus type 5 wildtype DNA as template. Using the E3a *reverse* primer an *Nhe*I cleavage site is generated. The amplificate is restricted

with *Spe*I and *Nhe*I and cloned into the similarly *Spe*I and *Nhe*I cleaved vector ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY.

For the *Spe*I-*Hpa*I fragment

Alternatively, the E3a region can be amplified using the primers E3a *forward (SpeI)* 5'-CTTAAGGACTAGTTTCGCGC -3'(SEQ ID No. 39) and E3a *reverse* (*HpaI*, *Nhe*I) 5'-CACGCTAGCAAGTTAACCATGTCTTGG-3'(SEQ ID No. 40) using adenovirus type 5 wildtype DNA as template. Using the E3a *reverse* primer an *Nhe*I cleavage site is generated. The amplificate is restricted with *Spe*I and *Nhe*I and is cloned into the vector ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY which is also cleaved by *Spe*I and *Nhe*I.

For the *Eco*RI fragment

Alternatively, the E3a region can be amplified by PCR using the primers E3a *forward (EcoRI)* 5'-GAAACCGAATTCTCTTGGAAC -3'(SEQ ID No. 41) and E3a *reverse* (*Nhe*I, *Eco*RI) 5'-GAATTCTAGCTAGCTCAGCTATAG -3'(SEQ ID No. 42) with adenovirus type 5 wildtype DNA as template. Using the E3a *reverse* primer an *Nhe*I cleavage site is generated. The amplificate is restricted with *Eco*RI and *Nhe*I and cloned into the vector ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY which is also cleaved by *Eco*RI and *Nhe*I.

By transferring the E3a region through cloning from pcDNA3.1(+) oCMV/E3a in ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY E3aΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY was generated.

The thus cloned region comprises the E3 region until the open reading frame for the E3 ADP (position 29772 bp) and thus the E3 promoter, the complete E3A region with polyadenylation signal, the transcription start and the open reading frame for 12.5 K, E3 6.7 K, E3 gp19 K and E3 ADP.

The E3 region is, compared to the adenovirus type 5 DNA sequence, in case of *Spe*I-*Xho*I cloning deleted from position 29796 to 31509 bp (= 1713 bp).

Further deletions are possible between the E3 promoter and the open reading frame for the ADP in plasmid pcDNA3.1(+) oCMV/E3a: By further restrictions between positions 27596 bp and

29355 bp, for example with *Eco*RII, *Bsi*WI, *Dra*I, *Mun*I, the open reading frames for 6.7 K and gp19 K positioned in between, may be removed and thus provided 1.8 kb more space for incorporating further transgenes. The above mentioned E3a amplificates can also be truncated by a corresponding restriction and as previously described transferred by cloning.

Cloning of the second expression cassette E1a 12S under the control of the E2Late promoter

First the E2Late promoter was cloned as paired oligonucleotide (Upper Primer 5'-TCGAGCTCCGCATTTGGCGGGCGGGATTGGTCTTCGTAGAACCTAATCTCGTGGGCGT GGTAGTCCTCAGGTACAAAT-3'(SEQ ID NO. 43) and Lower Primer 5'-AGCTTATTTGTACCTGAGGACTACCACGCCCACGAGATTAGGTTCTACGAAGACCAAT CCCGCCCGCCAAATGCGGAGC-3'(SEQ ID NO. 44)) into the *Hind*III and *Bgl*II cleavage site of the pGL3 enhancer plasmid of the company Promega (pGL3-E2Late).
Subsequently, the luciferase gene was excised with *Nco*I and *Xba*I, the ends made blunt-ended and T ends added. At the thus opened site the transgene E1A12S which was amplified by RT-PCR using the primers E1a 12S *forward* 5'-ATGGCCGCCAGTCTTTTG-3'(SEQ ID No. 45) and E1a 12S *reverse* 5'-TTATGGCCTGGGGCGTTTAC-3'(SEQ ID No. 46), was introduced by TA cloning.

The cassette thus contains the E2Late promoter, the open reading frame E1a-12S and the SV-40 Late polyadenylation signal of the vector pGL3.

This cassette was excised with *Pvu*I and *Cla*I, the ends made blunt-ended and can now be cloned optionally into the *Eco*RII, *Bsi*WI, *Dra*I, *Mun*I deleted E3a region (after removal of the open reading frames for E3 6.7 K and gp19 K, see above) or in deletion of E4ORF6 cloned, for example into the blunt-ended and dephosphorylated *Bfr*I cleavage site.

The resulting construct is E3a/E2Late-E1a-12S/ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY or E3aΔE3RGD-E4ΔORF6/E2Late-E1a-12S -pShuttle (-*Nde*I)-AdEASY.

Cloning of the second expression cassette E1a 12S with YB-1 under the control of the E2Late promoter

The amplificates E1a 12S (see above) and the IRES element (pCITE-4a(+) of the company Novagen as template, IRES *forward* = 5'-TCCGGTTATTTTCCACCATATTGC-3' (SEQ ID No. 47) and IRES

reverse = 5'-TTATCATCGTGTTTTTCAAAGG-3'(SEQ ID No. 48)) were subsequently cloned into the multiple cloning site of the pcDNA3.1(+) vector (Invitrogen). For such purpose the E1a-12S amplificate was introduced into the blunt-ended *Bam*HI restriction site through TA cloning. Subsequently the plasmid E1a-12S in pcDNA3.1(+) was linearised with *Eco*RV, the T ends added and the amplificate for the IRES element cloned. The thus generated construct E1a-12S-IRES-pcDNA3.1(+) was linearised using *Not*I and the ends blunt-ended, also the YB-1 *Eco*RI cleavage product of plasmid pHVad2c CMV/S40 + Yb-1 s (Stephan Bergmann) blunt-ended and introduced into the dephosphorylated vector E1A-12S-IRES-pcDNA3.1(+).Alternatively, the PCR amplificate for the open reading frame of protein IX can be introduced into the blunt-ended *Not*I cleavage site of the vector E1a-12S-IRES-pcDNA3.1(+) after adding T ends, specifically with the primers IX *forward* 5'-ATGAGCACCAACTCGTTTG-3'(SEQ ID No. 49) and IX *reverse* 5'-GTTTTAAACCGCATTGGGAGG-3'(SEQ ID NO. 50).

The cassette E1A-12S-IRES-YB-1 or E1A-12S-IRES protein IX were excised with *Pme*I and cloned into the above described plasmid pGL3-E2Late after removal of the luciferase gene with *Nco*I and *Xba*I and blunt-ending and dephosphorylation.

This cassette E2late-E1A-12S-IRES-YB-1 was excised with *Pvu*I and *Cla*I, the ends blunt-ended and can now optionally be cloned into the *Eco*RII, *Bsi*WI, *Dra*I, *Mun*I deleted E3a region (after removal of the open reading frames for E3 6.7 K and gp19 K, see above) or in deletion of the E4ORF6, for example in the blunt-ended and dephosphorylated *Bfr*I cleavage site.

The resulting construct is E3a/E2Late-E1a-12S-IRES-YB-1/ΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY or E3aΔE3RGD-E4ΔORF6/E2Late-E1a-12S-IRES-YB-1 -pShuttle (-*Nde*I)-AdEASY.

Generation of the rescue plasmid E3a/E2Late-E1a-12S/ΔE3RGD-E4ΔORF6-pAdEASY or E3aΔE3RGD-E4ΔORF6/E2Late-E1a-12S -pAdEASY and E3a/E2Late-E1a-12S-IRES-YB-1/ΔE3RGD-E4ΔORF6-pAdEASY, respectively, or E3aΔE3RGD-E4ΔORF6/E2Late-E1a-12S-IRES-YB-1 -pAdEASY

The E3aΔE3RGD-E4ΔORF6 region with the second expression cassette E2Late-E1a-12S or E2Late-E1a-12S-IRES-YB-1 in E3a or E4ΔORF6 were excised using *Spe*I and *Pac*I from the

corresponding pShuttle plasmid E3aΔE3RGD-E4ΔORF6-pShuttle (-*Nde*I)-AdEASY and cloned into the accordingly opened vector pAdEASY, whereby the new *rescue* vector E3a/E2Late-E1a-12S/ΔE3RGD-E4ΔORF6-pAdEASY or E3aΔE3RGD-E4ΔORF6/E2Late-E1a-12S -pAdEASY and E3a/E2Late-E1a-12S-IRES-YB-1/ΔE3RGD-E4ΔORF6-pAdEASY, respectively, or E3aΔE3RGD-E4ΔORF6/E2Late-E1a-12S-IRES-YB-1 -pAdEASY were generated.

E3aΔE3RGD-E4ΔORF6-pAdEASY contains the E3a region, an RGD motif and a deleted E4ORF6, as a second expression cassette either the E2Late-E1a-12S or the E2Late-E1a-12S-IRES-YB-1 are present in E3a or E4ΔORF6. This construct is the *rescue* plasmid for introducing further transgenes into the E1 region through a shuttle plasmid.

Generating the transgene cassette for the E1 region

Cloning of the E1B region

The adenogenome was restricted with *Xba*I (position 1340 bp) and *Mun*I (position 3925 bp) for the E1B region and the 2585 bp fragment cloned into the pShuttle of AdEASY into the *Xba*I and *Mun*I cleavage sites which thus contains the complete E1B region (pShuttle/E1B).

Alternatively, the E1B region can be amplified by PCR using the primers E1B *forward* 5'-GTGTCTAGAGAATGCAATAGTAG-3'(SEQ ID No. 51) and E1B *reverse* 5'-GTCAAAGAATCCAATTGTGC-3'(SEQ ID No. 52) using the adenovirus type 5 wildtype DNA as template, can be restricted with *Xba*I and *Mun*I and cloned into the *Xba*I and *Mun*I restriction sites of the pShuttle of AdEASY.

Thus the pShuttle/E1B comprises the E1B promoter, the open reading frames for E1B19K, E1B55K and the protein IX and the natural poly-A portion. The E1B promoter was removed using *Xba*I and *Hpa*I, the ends of the vectors blunt-ended and replaced by the CMV promoter from the pcDNA3.1(+) of the company Invitrogen, which was cut with *Mlu*I and *Xho*I and the ends of which have also been blunt-ended. Alternatively, an RSV promoter used instead of the CMV promoter or a tumor specific and viral promoter, respectively, can control the expression of the E1B region, for example the promoters recited in the patent.

Preparing the RSV plasmid for the preparation of the cassette RSV-E4ORF6-polyA.

The plasmid pRc/RSV of the company Invitrogen was cleaved with *Xho*I, *Spe*I and *Xba*I. The thus generated 2810 bp and 278 bp fragments were again ligated so that the F1 origin and the neomycine resistance gene (oNeo) were removed.

The thus generated vector pRc/RSV (oNeo) contains one *Bam*HI cleavage site only into which the open reading frame of E4ORF6 from the plasmid CGN from Dobbelstein was cloned. Alternatively, the amplificate of a PCR using the primers E4ORF6-*forward* 5'–ATGACTACGTCCGGCGTTCC-3'(SEQ ID No. 53) and E4ORF6-*reverse* 5'–CTACATGGGGGTAGAGTC-3'(SEQ ID No. 54) can be introduced into the *Eco*RV cleavage site of the vector pRc/RSV (oNeo) after adding the T ends (TA cloning). Alternatively, a CMV promoter (taken from pcDNA3.1(+) with *Mlu*I and *Hind*III) instead of the RSV promoter (by removal with *Mlu*I and *Hind*III) or a tumor-specific and viral promoters, respectively, direct the expression of the E4orf6, for example the promoters recited in the patent.

The cassette RSV-E4ORF6-polyA (the *Bovine Growth Hormone* polyadenylation signal is derived from plasmid pRC/RSV) was cleaved with *Mun*I, the ends made blunt-ended and further retrieved from the plasmid with *Xho*I. The expression cassette was subsequently cloned into the vector pShuttle/E1B which had been cleaved with *Not*I, made blunt-ended and subsequently cleaved with *Xho*I. By doing so, the vector RSV-E4ORF6-polyA/E1B-pShuttle-AdEASY was generated.

Introducing the transgene cassette into the *rescue* vector

The vector RSV-E4ORF6-polyA/E1B-pShuttle-AdEASY for the E1 region was linearised using *Bst*1107I and *Mro*I and introduced into BJ5183 (EC) bacteria together with the *rescue* plasmid (see above) by means of electroporation. The adenoviral plasmid RSV-E4ORF6-polyA/E1B-E3a/E2Late-E1a-12S/ΔE3RGD-E4ΔORF6–pAdEASY generated by homologous recombinant (or correspondingly with the other above recited rescue vector variants) which resulted in virus production after transfection in HEK293 cells.

It is within the present invention and feasible for the one skilled in the art in the light of the present invention that the generation of the adenoviruses in accordance with the present

Claims

1. In vitro use of an adenovirus for reversing drug resistance in a tumor cell, wherein the adenovirus is replicating in cells having YB-1 in the nucleus, but is replication-deficient in cells not having YB-1 in the nucleus;
wherein the resistance is mediated by a gene the transcription of which is activated by YB-1;
wherein the adenovirus encodes E1A which transactivates an E1B55kDa gene and an E4orf6 gene or
wherein the adenovirus expresses a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A protein.

2. Use of an adenovirus for the manufacture of a medicament for reversing drug resistance in a tumor cell, wherein the adenovirus is replicating in cells having YB-1 in the nucleus, but is replication-deficient in cells not having YB-1 in the nucleus;
wherein the resistance is mediated by a gene the transcription of which is activated by YB-1;
wherein the adenovirus encodes E1A which transactivates an E1B55kDa gene and an E4orf6 gene or
wherein the adenovirus expresses a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A protein.

3. In vitro use of an adenovirus for restoring drug sensitivity of a drug resistant tumor cell, wherein the adenovirus is replicating in cells having YB-1 in the nucleus, but is replication-deficient in cells not having YB-1 in the nucleus;
wherein the resistance is mediated by a gene the transcription of which is activated by YB-1;
wherein the adenovirus encodes E1A which transactivates an E1B55kDa gene and an E4orf6 gene or
wherein the adenovirus expresses a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A protein.

4. Use of an adenovirus for the manufacture of a medicament for restoring drug sensitivity of a drug resistant tumor cell, wherein the adenovirus is replicating in cells having YB-1 in the nucleus, but is replication-deficient in cells not having YB-1 in the nucleus;
wherein the resistance is mediated by a gene the transcription of which is activated by YB-1;
wherein the adenovirus encodes E1A which transactivates an E1B55kDa gene and an E4orf6 gene or
wherein the adenovirus expresses a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A protein.

5. Use according to any of claims 1 to 4, whereby the cell is an animal cell, preferably a mammalian cell and more preferably a human cell.

6. Use according to any of claims 1 to 5, whereby the cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

7 Use according to claim 6, whereby the pharmaceutically active agent is a cytostatic.

8. Use according to any of claims 1 to 7, characterised in that the resistance is mediated by an ABC transporter.

9. Use according to any of claims 1 to 8, characterised in that the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against cytostatics and/or radiation.

10. Use according to claim 8 or 9, whereby the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

11. Use according to any of claims 1 to 10, characterised in that the E1A does not induce the localisation of YB-1 into the nucleus.

12. Use according to any of claims 1 to 11, characterised in that the adenovirus, is selected from the group comprising AdΔ24, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, dl520 and

viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

13. Use according to claim 12, wherein the adenovirus is dl520.

14. Use according to claim 11 or 12 , wherein the adenovirus is E1B19 kDa deficient.

15. Use according to any of claims 1 to 14, wherein the first protein is an E1B55kDa protein.

16. Use according to any of claims 1 to 15 , wherein the first protein is an E4orf6 protein.

17. Use according to any of claims 1 to 16, wherein the first protein is a combination of E1B55kDa protein and E4orf6 protein, preferably the first protein is a complex of E1B55kDa protein and E4orf6 protein.

18. Use according to any of claims 1 to 17, wherein the E1A protein is E1A12S protein.

19. Use according to any of claims 1 to 18, wherein the adenovirus expresses an E1B55kDa protein and an E4orf6 protein prior to an E1A12S protein.

20. Use according to any of claims 1 to 19, wherein characterised in that the virus, particularly the adenovirus, codes for YB-1.

21. Use according to claim 20, wherein characterised in that YB-1 is under the control of a tissue-specific and/or tumor-specific promoter.

22. Use according to any of claims 1 to 21, wherein the tumor cell has YB-1 in the nucleus, preferably the cell has YB-1 in the nucleus independent from the cell cycle.

23. Use according to any of claims 1 to 22, wherein the tumor cells comprises YB-1 in the nucleus after induction of the transport of YB-1 into the nucleus.

24. Use according to claim 23, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

25. Use according to any of claims 1 to 24, whereby the adenovirus comprises a nucleic acid coding for a transgene.

26. Use according to any of claims 1 to 25 , whereby the virus comprises the translation and/or transcription product of a transgene.

27. Use according to claim 25 or 26 , whereby the transgene is selected from the group comprising prodrug genes, cytokines and genes for cytokines, apoptosis-inducing genes, tumor suppressor genes, genes for metalloproteinase inhibitors and genes for angiogenesis inhibitors.

28. Use according to any of claims 25 or 26, whereby the transgene is selected from the group comprising nucleic acids for siRNA, for aptamers, for antisense molecules and for ribozymes, whereby the siRNA, the aptamer, the antisense molecule and/or the ribozyme are targeting a target molecule.

29. Use according to claim 28, whereby the target molecule is selected from the group comprising resistance relevant factors, anti-apoptosis factors, oncogenes, angiogenesis factors, DNA synthesis enzymes, DNA repair enzymes, growth factors, receptors for growth factors, transcription factors, metalloproteinases, preferably matrix metalloproteinase kinases, and plasminogen activator of the urokinase type.

30. Use according to any of claims 1 to 29, wherein the medicament is for the treatment of cancer or tumors.

31. Use according to any of claims 1 to 30, whereby the medicament further comprises at least one pharmaceutically active agent.

32. Use according to claim 31, whereby the pharmaceutically active agent is selected from the group comprising cytokines, metalloproteinase inhibitors, angiogenesis inhibitors, cytostatics, cell cycle inhibitors, proteosome inhibitors, recombinant antibodies, inhibitors of the signal transduction pathway and inhibitors of protein kinases.

Claims

1. Use of an adenovirus for the manufacture of a medicament for reversing resistance in human or mammalian tumor cells, whereby the adenovirus is replication deficient in cells which lack YB-1 in the nucleus but replicates in human or mammalian tumor cells which have YB-1 in the nucleus or which comprise deregulated YB-1 and whereby the resistance is a resistance to one or several pharmaceutically active agents and/or radiation, whereby the resistance to one or several pharmaceutically active agents is mediated by an ABC transporter and whereby resistance against radiation is resistance against radiation as used in the radiation of tumor diseases, whereby

the adenovirus encodes an oncogene protein which transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group consisting of E1B55kDa, E4orf6, E4orf3 and E3ADP and whereby the oncogene protein is E1A and wherein the adenovirus is selected from the group consisting of dl520, AdΔ24, dl922-947, E1Ad/01/07, dl1119/1131 and CB 016,

or

the adenovirus is expressing a first protein which is a combination of ElB55kDa protein and E4orf6 protein, prior to a second protein which is selected from the group consisting of an E1A-protein, wherein the adenovirus comprises at least one nucleic acid coding for an E1A12S protein, whereby the expression of the E1A protein is under the control of a promoter which is different from the E1A promoter.

2. Use of an adenovirus for the manufacture of a medicament for reversing resistance in human or mammalian tumor cells, whereby the adenovirus is replication deficient in cells which lack YB-1 in the nucleus but replicates in human or mammalian tumor cells which have YB-1 in the nucleus or which comprise deregulated YB-1 and whereby the resistance is a resistance to one or several pharmaceutically active agents, whereby the resistance to one or several pharmaceutically active agents is mediated by an ABC transporter, whereby

the adenovirus encodes an oncogene protein which transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group consisting of E1B55kDa, E4orf6, E4orf3 and E3ADP and whereby the oncogene protein is E1A and wherein the adenovirus is selected from the group consisting of dl520, AdΔ24, dl922-947, E1Ad/01/07, dl1119/1131 and CB 016,

or

the adenovirus is expressing a first protein which is a combination of ElB55kDa protein and E4orf6 protein, prior to a second protein which is selected from the group consisting of an E1A-protein, wherein the adenovirus comprises at least one nucleic acid coding for an E1A12S protein, whereby the expression of the E1A protein is under the control of a promoter which is different from the E1A promoter.

3. Use of an adenovirus for the manufacture of a medicament for restoring drug sensitivity of human or mammalian tumor cells,
whereby the adenovirus is replication deficient in cells which lack YB-1 in the nucleus but replicates in human or mammalian tumor cells which have YB-1 in the nucleus or which comprise deregulated YB-1 and whereby the sensitivity is a sensitivity to one or several pharmaceutically active agents and/or a sensitivity to radiation, whereby the sensitivity to one or several pharmaceutically active agents is mediated by an ABC transporter and sensitivity to radiation is sensitivity to radiation as used in the radiation of tumor diseases, whereby

the adenovirus encodes an oncogene protein which transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group consisting of E1B55kDa, E4orf6, E4orf3 and E3ADP and whereby the oncogene protein is E1A and wherein the adenovirus is selected from the group consisting of dl520, AdΔ24, dl922-947, E1Ad/01/07, dl1119/1131 and CB 016,

or

the adenovirus is expressing a first protein which is a combination of ElB55kD protein and E4orf6 protein, prior to a second protein which is selected from the group consisting of an E1A-protein, wherein the adenovirus comprises at least one nucleic acid coding for an E1A12

protein, whereby the expression of the E1A protein is under the control of a promoter which is different from the E1A promoter.

4. Use of an adenovirus for the manufacture of a medicament for restoring drug sensitivity of human or mammalian tumor cells, whereby the adenovirus is replication deficient in cells which lack YB-1 in the nucleus but replicates in human or mammalian tumor cells which have YB-1 in the nucleus or which comprise deregulated YB-1 and whereby the sensitivity is a sensitivity to one or several pharmaceutically active agents, whereby the sensitivity to one or several pharmaceutically active agents is mediated by an ABC transporter, whereby

the adenovirus encodes an oncogene protein which transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group consisting of E1B55kDa, E4orf6, E4orf3 and E3ADP and whereby the oncogene protein is E1A and wherein the adenovirus is selected from the group consisting of dl520, AdΔ24, dl922-947, E1Ad/01/07, dl1119/1131 and CB 016,

or

the adenovirus is expressing a first protein which is a combination of E1B55kDa protein and E4orf6 protein, prior to a second protein which is selected from the group consisting of an E1A-protein, wherein the adenovirus comprises at least one nucleic acid coding for an E1A12S protein, whereby the expression of the E1A protein is under the control of a promoter which is different from the E1A promoter.

5. Use according to any one of claims 1 to 4, whereby the pharmaceutically active agent is a cytostatic.

6. Use according to any of claims 1 to 2, characterised in that the resistance is a multiple resistance or polyresistance and/or a resistance against radiation.

7. Use according to any one of claims 1 to 2, characterized in that the resistance is a multiple or polyresistance against cytostatics.

8. Use according to any of claims 1 to 7, whereby the ABC transporter is selected from the group consisting of MRP, MDR, and MDR-1.

9. Use according to any one of claims 1 to 8, wherein the adenovirus is selected from the group consisting of dl520, AdΔ24, dl922-947, E1Ad/01/07, dl1119/1131 and CB 016.

10. Use according to claim 9, wherein the adenovirus is selected from the group consisting of dl520 and dl1119/1131.

11. Use according to any one of claims 1 to 9, wherein the adenovirus is dl520.

12. Use according to anyone of claims 1 to 11, wherein the adenovirus selected from the group consisting of AdΔ 24, dl922-947, E1Ad/01/07, dl1119/1131, CB 016 and dl520 has further E1B 19 kDa-deficiency.

13. Use according to claim 12, wherein the adenovirus is dl520 having further E1B 19 kDa-deficiency.

14. Use according to anyone of claim 1 to 13, wherein E1A is E1A12S.

15. Use according to anyone of claims 1 to 14, wherein the cells have YB-1 in the nucleus independent of the cell cycle.

16. Use according to anyone of claims 1 to 15, characterized in that the adenoviral oncogene protein E1A does not induce nucleus localization of YB-1.

17. Use according to any of claims 1 to 16, characterized in that the cells are Rb negative and the cells are YB-1 nucleus positive.

18. Use according to any of claims 1 to 17, characterized in that the cells are Rb negative and the cells are YB-1 nucleus positive independent from the cell cycle.

19. Use according to any of claims 1 to 18, characterized in that the adenoviral oncogene protein is under the control of a tissue and/or tumor specific promoter.

20. Use according to any of claims 1 to 16, characterized in that the adenovirus codes for YB-1, wherein YB-1 is under the control of a tissue specific and/or tumor specific promoter.

21. Use according to any of claims 1 to 20, characterized in that the adenovirus codes for at least one protein, whereby the protein is selected from the group consisting of E4orf6, E4orf3, E1B55kDa and adenoviral E3ADP protein.

22. Use according to any one of claims 1 to 8, wherein the adenovirus is expressing a first protein which is a combination of E1B55kD protein and E4orf6 protein, prior to a second protein which is selected from the group consisting of an E1A-protein, wherein the adenovirus comprises at least one nucleic acid coding for an E1A12 protein, whereby the expression of the E1A protein is under the control of a promoter which is different from the E1A promoter.

23. Use according to claim 22, characterised in that the E1A protein is an E1A12S protein.

24. Use according to any of claims 22 to 23, characterised in that the adenovirus comprises a nucleic acid coding for E1B55kD protein under the control of a promoter which is different from the promoter controlling the expression of the protein in a wildtype adenovirus.

25 Use according to any of claims 22 to 24, characterised in that the adenovirus comprises a nucleic acid coding for E4orf6 protein under the control of a promoter which is different from the promoter controlling the expression of the protein in a wildtype adenovirus.

26. Use according to any of claims 22 to 24, characterised in that the adenovirus comprises a nucleic acid coding for a combination of E1B55kDa protein and E4orf6 protein under the control of a promoter which is different from the promoter controlling the expression of the protein in a wildtype adenovirus.

27. Use according to any of claims 22 to 26, characterised in that the expression of the E1B55kD protein is controlled by a promoter, whereby the promoter is selected from the group consisting of tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E1B promoter.

28. Use according to any of claims 22 to 27, characterised in that the expression of the E4orf6 protein is controlled by a promoter, whereby the promoter is selected from the group consisting of tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter.

29. Use according to claims 27 or 28, whereby the adenoviral promoter is the E1A promoter.

30. Use according to any of claims 22 to 29, characterised in that the expression of the E1A protein is under the control of a promoter selected from the group consisting of tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters.

31. Use according to any of claims 27 to 30, characterised in that the promoter controlling the expression of the ElA protein is YB-1 controlled or can be regulated by YB-1.

32. Use according to any of claims 27 to 31, characterised in that the promoter controlling the expression of the E1A protein is the adenoviral E2 late promoter.

33. Use according to any of claims 22 to 33, characterised in that the E4orf6 protein and the ElB55kDa protein are under the control of the same or a common promoter.

34. Use according to any of claims 22 to 33, characterised in that the adenovirus provides YB-1 in the nucleus through at least one adenoviral protein or that the provision of YB-1 in the nucleus is mediated through at least one adenoviral protein, whereby the adenoviral protein is different from E1A.

35. Use according to any of claims 22 to 34, characterised in that the adenovirus provides YB-1 for adenoviral replication through at least one adenoviral protein or mediates the provision of YB-1 for adenoviral replication through at least one adenoviral protein, whereby the adenoviral protein is different from E1A.

36. Use according to claim 34 or 35, characterised in that the adenoviral protein is a complex of E4orf6 and E1B55kDa.

37. Use according to any of claims 22 to 36, characterised in that the nucleic acid of the adenovirus comprises at least one functionally inactive adenoviral region, whereby the region is selected from the group consisting of the El region, the E3 region, the E4 region and combinations thereof.

38. Use according to claim 37, characterised in that the at least one functionally inactive adenoviral region is the El region.

39. Use according to claim 37 or 38, characterised in that the at least one functionally inactive adenoviral region is the E3 region.

40. Use according to any of claims 37 to 39, characterised in that the at least one functionally inactive adenoviral region is the E4 region.

41. Use according to any of claims 37 to 40, characterised in that the at least one functionally inactive adenoviral region comprises the El region, the E3 region and the E4 region.

42. Use according to any of claims 22 to 41, characterised in that the adenovirus comprises a nucleic acid, whereby the nucleic acid codes for YB-1.

43. Use according to claim 42, characterised in that the nucleic acid coding for YB-1 is under the control of a promoter, whereby the promoter is the E2 late promoter.

44. Use according to claim 42 or 43, characterised in that the nucleic acid coding for YB-1 is under the control of a promoter, whereby the promoter is YB-1 dependent and YB-1 controlled, respectively.

45. Use according to any of claims 22 to 44, characterised in that the adenovirus comprises an expression cassette comprising a promoter and a nucleic acid sequence, whereby

the nucleic acid sequence is selected from the group consisting of aptamers, ribozymes, aptazymes, antisense molecules and siRNA.

46. Use according to any of claims 22 to 44, characterised in that the adenovirus comprises an expression cassette comprising a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is a coding nucleic acid, whereby the nucleic acid codes for a molecule which is selected from the group consisting of peptides, polypeptides, proteins, anticalines, antibodies and antibody fragments.

47. Use according to any of claims 22 to 44, characterised in that the adenovirus comprises an expression cassette, whereby the expression cassette comprises a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is selected from the group consisting of apoptosis inducing genes, prodrug genes, nucleic acids coding for protease inhibitors, tumor suppressor genes, nucleic acids coding for cytokines and nucleic acids coding for angiogenesis inhibitors.

48. Use according to any of claims 22 to 47, characterised in that the adenovirus is an adenovirus mutant.

49. Use according to any one of claims 1 to 48, wherein the expression of the ABC transporter is controlled by YB-1.