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Enhancement of cancer gene therapy with modified viral vectors and fusion genes

Review Article

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Abbreviations: central polypurine tract, (cPPT); conditionally replicative adenoviruses, (CRAds); coxsackie- adenovirus receptor, (CAR); cyclo-oxygenase-2, (COX-2); early growth response 1, (EGR-1); glycoprotein from vesicular stomatitis virus, (VSV-G); herpes simplex virus type 1, (HSV-1); human telomerase reverse transcriptase, (hTERT); inverted terminal repeats, (ITRs); long terminal repeats, (LTR); posttranscriptional regulatory elements, (PRE); Protein transduction domains, (PTDs); self-inactivating type vectors, (SIN);

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Summary

The major obstacle in cancer gene therapy continues to be insufficient transduction of tumor cells and consequently poor therapeutic effect. However, several approaches have been developed to improve gene transfer rates. First, alternative viral vectors can be explored to find optimal gene transfer vehicles for each purpose. Secondly, viral vectors can be re-targeted to cancer cells, which can simultaneously enhance gene transfer to tumors and diminish undesired side effects in healthy tissue. In addition, it is possible to exploit viral replication *per se* to destroy cancer cells. To avoid side effects and increase the safety of these oncolytic agents, replication can be limited to tumor cells by partially deleting areas of the viral genome or by using tissue specific promoters to drive viral genes responsible for replication. Instead or in addition to modifying the gene transfer vector, one possibility is to modify the therapeutic gene so that the resulting therapeutic protein can spread to surrounding cells and thus compensate for low gene transfer efficiency and enhance therapeutic outcome.

I. Introduction

Cancer is a world wide health problem. In 2002, approximately 11 million people were estimated to be diagnosed with and almost 7 million people to die from cancer (Ferlay et al, 2004). The number of cancer patients has increased partly due to achievements in medical sciences, including reduction of deaths from infectious and cardiovascular causes, but also improved diagnostic methods for more sensitive detection. Further, many factors in the Western lifestyle may predispose to carcinogenesis.

Even though cancer can be considered a genetic disease, because it is caused by mutations and epigenetic changes in tumor suppressor and oncogenes, it's rarely caused by a defect of a single gene and the development of malignant tumors usually involves interaction between the environment and heredity (Hemminki and Hemminki, 2004; Tamura et al, 2004). It has been hypothesized that three to seven genetic changes are required for carcinogenesis (Vogelstein and Kinzler, 1998). They typically cause increased and irregular proliferation activity, lack of apoptosis, avoidance of immune defences, activation of telomerase and ability to metastasise and form vascularisation into tumor (Hanahan and Weinberg, 2000). When accumulated, these aberrant features result in cells which can proliferate unrestrictedly, form neoplastic lesions, invade local tissues and eventually establish distant metastasis (Zhang et al, 1995; Rieger, 2004). Even though the knowledge of molecular mechanisms, diagnostic methods and treatments of cancer have improved during the last decades, most cancer types still imply poor prognosis and high mortality, especially when metastatic. In fact, metastatic solid tumors can be cured only very rarely. Thus, more efficient approaches and novel tools are needed for treatment of cancer.

Since the first clinical gene therapy trial in the early 1990s, gene therapy has become a widely studied concept for treatment of various diseases. Even though gene therapy was initially thought to be more suitable for treatment of inherited monogenic diseases, it has been increasingly exploited for treatment of acquired and

complex diseases such as cancer. Actually, by the year 2004, a majority of clinical gene therapy trials (66%) have been focused on cancer diseases (http://www.wiley.co.uk/genetherapy/clinical/).

II. Challenges in gene therapy

Although the theoretical basis for gene therapy is rather simple and thus attractive, practical experience has demonstrated some obstacles to overcome. Nevertheless, perhaps the main finding thus far has been the generally very good safety data in clinical trials. Despite receiving high publicity, severe toxicity has been reported only rarely (Hacein-Bey-Abina et al, 2003; Raper et al, 2003). In particular, when compared to other experimental treatments (such as novel chemotherapeutics or eg. bone marrow transplantation) studied for life threatening illnesses; gene therapy has been well tolerated. While safety data has been promising, efficacy has been more variable. In a nutshell, trials have demonstrated that the key to safe and effective therapy is efficient and specific gene transfer, which is a sum of multiple factors. This review will focus on cytotoxic approaches, while immunomodulatory approaches are discussed elsewhere.

In order to deliver genes into target tissue, gene transfer vectors have to first negotiate the host immune system. It has been reported that 55% of adult humans may have some level of pre-existing circulating neutralizing antibodies against adenovirus serotype 5, which is one of the most used gene transfer vectors (Chirmule et al, 1999). In contrast, retroviruses, another group of viral vectors, rarely elicit neutralizing antibodies but they can be rapidly degraded by the complement system (Takeuchi et al, 1994). In addition, the route of viral vector administration plays an important role in gene delivery. It has been shown in several preclinical studies that regardless of the administration route adenoviral vectors almost invariably evoke neutralizing antibodies (Setoguchi et al, 1994; Van Ginkel et al, 1995; Smith et al, 1996; Gahery-Segard et al, 1997; Hemminki et al, 2002a). However, the proteins of the viral capsid have been reported to be differentially recognized depending on the route of administration (Gahery-Segard et al, 1997). In contrast, in humans adenoviral vectors can cause variable, administration route dependent humoral immune response (Harvey et al, 1999). Heretofore, viral vectors have been often administered directly into tumor tissue (intratumorally) to achieve therapeutically relevant gene transfer rates. In fact, the majority of published clinical studies have been carried out using intratumoral administration. However, the intratumoral route is conceivable only if the tumor mass is local and accessible. Some tumors are located primarily within specific body cavities which enables intracavitary administration. This has used in clinical trials for e.g. ovarian cancer (intraperitonenal) (Alvarez et al, 2000; Buller et al, 2002) and for malignant mesothelioma (intrapleural) (Sterman et al, 1998). Nevertheless, for treatment of most types of metastatic cancer systemic administration would be useful. Intravenous and intra-arterial administration have been used in clinical studies for e.g. metastatic osteosarcoma (Benjamin et al, 2001) and hepatic metastases for colorectal cancer (Reid et al, 2002), respectively.

The second challenge is to reach target cells and deliver the therapeutic genes into them. Because viral vector uptake usually requires binding to cellular receptors, one limiting factor is the expression level of viral receptors on target cells. For example, the expression of coxsackie-adenovirus receptor (CAR), which mediates adenoviral attachment to target cells, has been shown to be variable and often very low in tumor cells (Li et al, 1999a, b; Asaoka et al, 2000; Bauerschmitz et al, 2002a). Consequently, this receptor deficiency makes cancer cells rather refractory to adenoviral mediated gene transfer (Kanerva et al, 2002a). Further, viral receptors are often widely expressed in normal cells making healthy tissue susceptible to gene transfer (Kanerva et al, 2002b). This could cause side effects and decrease the total dose delivered to target cells.

The final step after viral uptake in cells is transgene expression. In order to get an adequate therapeutic response, transgene expression has to be at certain level for a sufficient time. When using non-integrating vectors such as adenoviruses, the transgene is maintained extrachromosomally in the nucleus, resulting in transient expression (Somia and Verma, 2000). While this is sufficient for cell killing, long term expression may be advantageous when attempting to correct genetic or acquired deficiencies responsible for disease phenotypes. Even though there are various viral vectors which integrate their payload into the host cell genome (including retroviral vectors) use of these vectors does not necessarily ensure successful long term gene expression (Kay et al, 2001). Because integration to host cell genome has random features, the therapeutic gene may integrate into an inactive part of the genome resulting in silenced transgene expression (Chen and Townes, 2000). Integrated transgenes may also inactivate host genes crucial for normal cellular function or activate harmful genes such as proto-oncogenes (Shiramizu et al, 1994; Bushman and Miller, 1997; Hacein-Bey-Abina et al, 2003) In addition, the host immune system may recognize transgene - encoded proteins as foreign and induce a response, which is likely to suppress the transgene expression (Tripathy et al, 1996).

III. Improvements in cancer gene therapy

A. Overview

Several approaches have been devised for overcoming some of the obstacles that have been identified in completed trials. One advance has been the characterisation of various different viruses and their utilization and further modification into safe and efficient gene transfer vehicles (Kootstra and Verma, 2003). Furthermore, various targeting techniques have been employed for modification of viral vectors to recognise cancer cells and thus cause reduced transduction of healthy, non-target tissues (Peng and Russell, 1999; Nettelbeck et al, 2000). Thus, more efficient and accurate gene transfer can be achieved with targeting. In addition,

the natural replication capability of various viruses including adenovirus, herpes simplex virus, alpha virus, Newcastle disease virus, measles virus and vesicular stomatitis virus has been exploited in cancer gene therapy (Alemany et al, 2000; Lundstrom, 2001; Russell, 2002; Post et al, 2003; Csatary et al, 2004). These oncolytic viruses can destroy tumor cells via replication which can be limited to target cells by genetic modification. Instead of viral vector modification, also transgenes can be modified so that the resulting therapeutic protein can spread to surrounding cells and thus help compensate for initially low gene transfer efficiency. Several so called translocatory proteins are currently known and their features have been characterized and evaluated for cancer gene therapy purposes (Leifert and Whitton, 2003).

B. Optimal gene transfer tools for each purpose: various viral vectors

Viruses need to transfer their genomes efficiently into host cells in order to replicate. Thus, viruses are gene transfer machines optimized by evolution. In order to use viruses as safe gene transfer vehicles, it can be advantageous to modify virulence genes and/or genes responsible for viral replication. While increasing safety of the viral vector, partial genome deletions also enable the insertion of foreign genetic material including transgenes.

Viral vectors can be divided into different categories based on their genome (DNA vs. RNA), structure (enveloped vs. non-enveloped) or integration (Fields et al, 1996). The most used viral vectors in human clinical gene therapy trials are based on adeno- and retroviruses and some of the less common vectors include adeno associated-, herpes simplex-, pox- and alphaviruses (http://www.wiley.co.uk/genetherapy/clinical/).

1. Adenoviral vectors

Human adenoviruses are a family of viruses (over 50 serotypes) that most commonly cause rather benign respiratory or gastrointestinal illness (Volpers and Kochanek, 2004). Adenoviruses are nonenveloped, double stranded DNA viruses, whose genome is surrounded by an icosohedral protein capsid comprising of three major proteins, hexon, penton base and knobbed fiber (Russell, 2000). The linear virus genome is about 36 bp in size and consists of immediate early (E1A), early (E1-E4), intermediate and late genes (L1-L5) (**Figure 1**). Transcription of these genes can be divided into early and late phase, respectively, occurring before or after DNA replication (Kootstra and Verma, 2003).

In order to get inside the host cell, adenoviruses first attached to their primary cellular receptor, which is the CAR for most serotypes (Bergelson et al, 1997) This is followed by interaction with cellular integrins, which results in internalization of the virus via receptor-mediated endocytosis (Wickham et al, 1993) (**Figure 2**). In the endosomes, the viral genome is released from the viral capsid and thereafter transported into the nucleus. The adenoviral replication cycle is initiated by transcription of E1A gene followed by transcription of other early genes (Volpers and Kochanek, 2004). Early gene products interfere with the host antiviral defence mechanism, alter the cell cycle and modulate cellular metabolism in favour of viral replication (Russell, 2000). The linear DNA is flanked by inverted terminal repeats (ITRs), which contain sequences required for DNA replication (Hay et al, 1995), mediated by E2 and E4 gene products. Next, intermediate genes are expressed at high levels followed by the expression of late genes driven by major late promoter (Russell, 2000; Kay et al, 2001). Late genes encode for structural viral proteins that assemble together with viral genomes in the nucleus followed by cell lysis and release of newly synthesized virions (Volpers and Kochanek, 2004).

Since their first description in the 1950s, adenoviruses have been increasingly studied and they have become one of the most used gene transfer tools in human gene therapy. From a gene therapy standpoint, adenoviruses have numerous advantages: 1) reasonable characterization and understanding of their biology, 2) relatively low pathogenicity in humans, 3) capability to infect both dividing and quiescent cells, 4) capacity to accommodate relatively large transgenes, 5) low risk for insertional mutagenesis due to inability to integrate into host cell genome and 6) relatively easy manipulation and high-titer production (Danthinne and Imperiale, 2000).

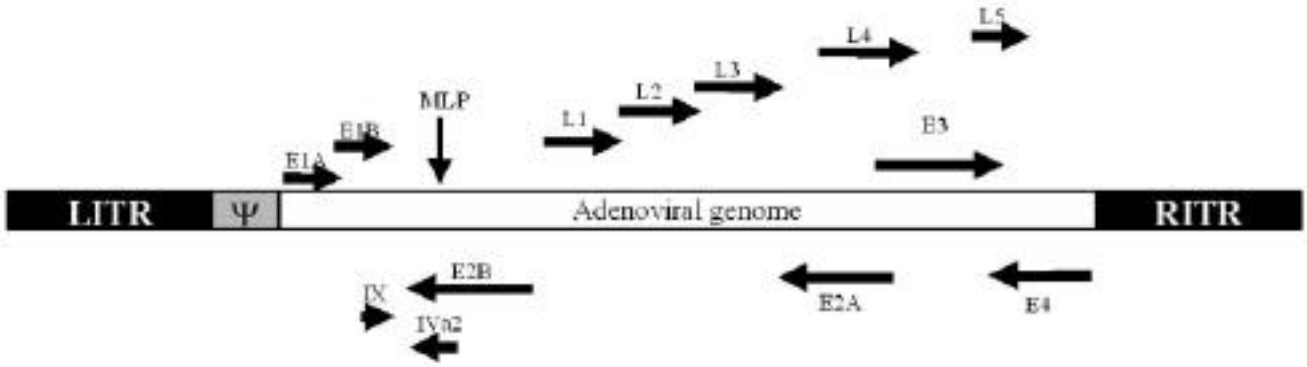

Figure 1. Adenoviral genome. The genome contains early (E1-4), intermediate (IX and IVa2) and late (L1-5) genes flanked by left and right inverted terminal repeats (LITR and RITR, respectively) MLP: major late promoter, Ψ packaging signal.

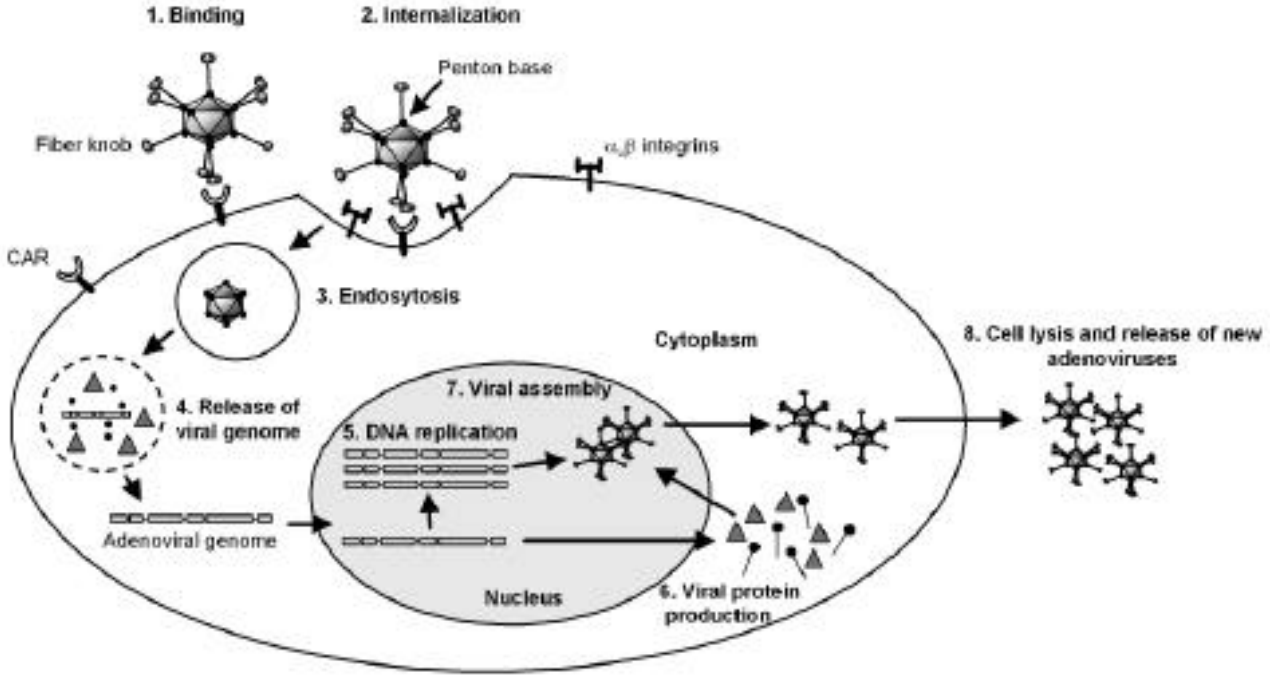

Figure 2. Adenoviral replication cycle. Viruses first attach to the coxsackie- adenovirus receptor (CAR) followed by interaction with cellular integrins resulting in internalization of the virus via receptor-mediated endocytosis. In the endosomes, the viral genome is released from the viral capsid and thereafter transported into the nucleus for DNA replication. Structural viral proteins assemble together with viral genomes in the nucleus followed by cell lysis and release of newly synthesized virions.

The most frequently used adenoviral vectors are based on serotype 5. By deleting partially the viral genome adenoviruses can be converted into viral gene transfer vehicles. First generation adenoviral vectors are made by deleting the E3 and E1 regions, the latter responsible for initiation of viral replication. These deletions enable inserting of ~8 kbp of foreign DNA (Danthinne and Imperiale, 2000). To increase the safety and transgene capacity, additional deletions have been engineered into second generation vectors (E1-4 regions deleted) (Armentano et al, 1995; Gorziglia et al, 1996) and so called gutless vectors (all viral genes deleted) (Kochanek et al, 1996). Although deletion of various viral genes can decrease immunogenicity and toxicity and prolonge persistence of transgene expression (Engelhardt et al, 1994; Kochanek et al, 1996; Wang et al, 1997), viruses must nevertheless be packaged into virions and capsid proteins continue to elicit an immune response, which might hinder repeated administration (Somia and Verma, 2000). One possibility to circumvent the immune system would be the use of vectors based on different human adenovirus serotypes (Mack et al, 1997; Barouch et al, 2004) or animal adenoviruses (Moffatt et al, 2000; Rasmussen et al, 1999) for readministration. A disadvantageous feature of adenoviral vectors is their propensity to accumulate into liver and cause hepatotoxicity. However, this problem can be partially circumvented by targeting the viral vectors to cancer cells.

2. Retro- and lentiviral vectors

Retroviruses are lipid-enveloped, single stranded RNA viruses, which can be divided into oncoretro-, lenti- and spumaviruses (Fields et al, 1996). Enveloped viral particles contain the viral genome which consists of two copies of 8-12 kilobase-sized RNA strands surrounded by the nucleocapsid (Kootstra and Verma, 2003). The genome is flanked by long terminal repeats (LTR) and contains three essential genes: *gag*, which encodes viral structural protein, *pol* encodes reverse transcriptase and integrase and *env* encodes viral envelope glycoprotein, which mediates virus entry (**Figure 3**) In the lentiviral genome, there are additional accessory genes: for example HIV-1 has *vif*, *vpr*, *vpu*, *tat*, *rev* and *nef* genes that encode proteins necessary for efficient viral replication and persistence of infection in the natural target cells of this virus (Kootstra and Verma, 2003) .

Early in the retroviral replication cycle (**Figure 4**), the virus binds to its receptor, which is followed by membrane fusion and release of the RNA genome from the viral capsid (Fields et al, 1996). In the cytosol, the viral genome is copied into double-stranded DNA by the viral reverse transcriptase (Jolly, 1994). The viral DNA is then translocated to the nucleus (retroviruses with passive migration and lentiviruses with active transport), where it becomes integrated into host cell genome by its own integrase enzyme to yield a provirus. Cellular machinery is then utilized to make viral RNA, using the provirus as a template. The viral RNA also serves as mRNA, which is translated into viral proteins (Kootstra and Verma, 2003). For viral particle formation, translated viral proteins or their precursors assemble together with two viral RNA strands followed by budding from the plasma membrane. During the budding process virus attains the lipid-coated envelope with incorporated env-glycoproteins from the host cell membrane (Jolly, 1994).

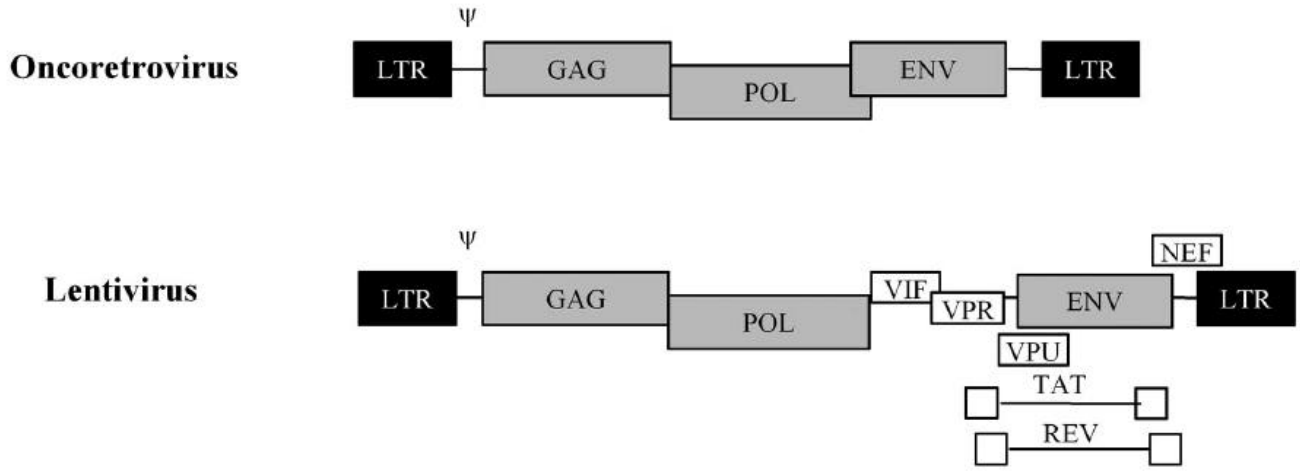

Figure 3. Genome structure of oncoretrovirus and HIV-1 lentivirus. Both genomes contain *gag*, *pol* and *env* genes flanked by long terminal repeats, LTRs. The HIV-1 genome contains six additional genes encoding *vif*, *vpr*, *vpu*, *tat*, *rev* and *nef.* Ψ Packaging signal.

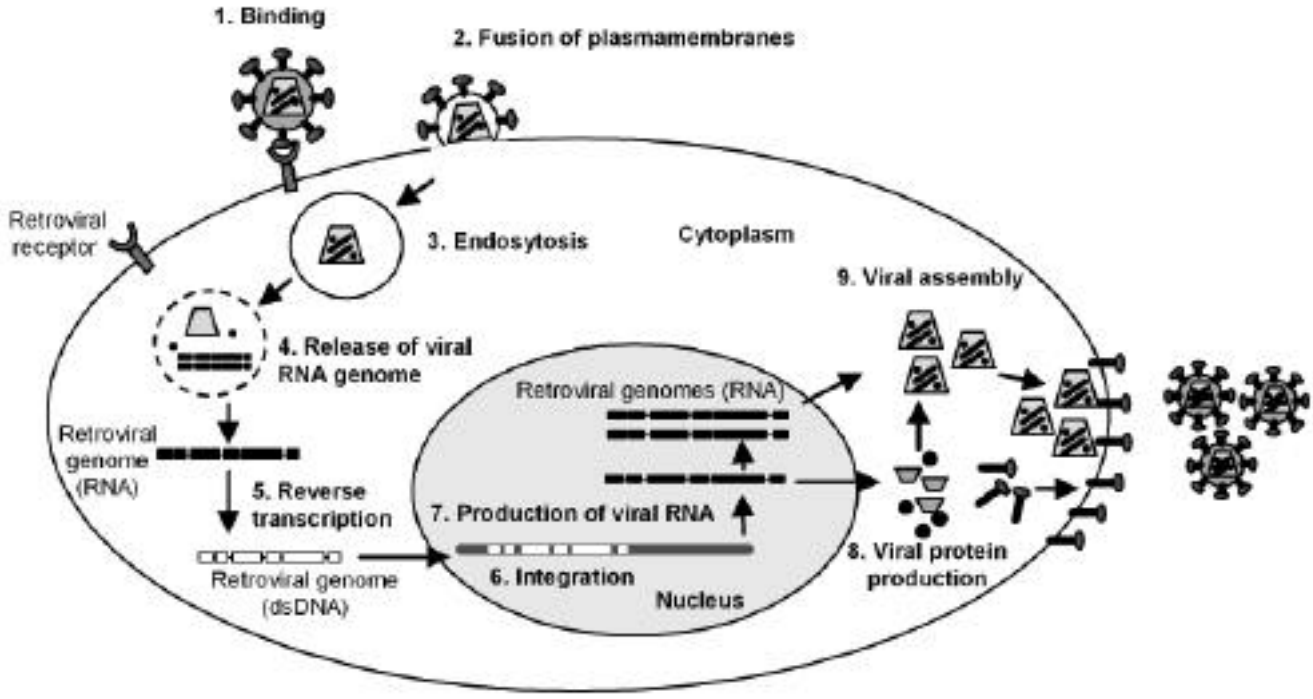

Figure 4. Retroviral replication cycle. Retrovirus binds to its receptor followed by membrane fusion and release of the RNA genome from the viral capsid. In the cytosol, the viral genome is copied into double-stranded DNA by viral reverse transcriptase. The viral DNA is then translocated to the nucleus where it integrates into the host cell genome. Cellular machinery is then utilized to make new viral genomes and proteins. Translated viral proteins assemble together with two viral RNA strands followed by budding from the plasma membrane. During the budding process virus attains a lipid-coated envelope (with incorporated env–glycoproteins) from the host cell membrane.

Retroviruses were the first viral vectors used in clinical studies (Blaese et al, 1995). Several features have lead to their wide use. First, their genome is rather simple, making the genetic modification required for vector production relatively straightforward. Second, they are able to integrate into the host cell genome, enabling long-term expression of transgene in target cells. However, integration does not necessarily ensure stable transgene expression, but it may increase the risk for insertional mutagenesis (Shiramizu et al, 1994; Bushman and Miller, 1997; Kay et al, 2001; Hacein-Bey-Abina et al, 2003). Third, retroviral vectors do not elicit a strong immune response, which reduces their cytotoxicity and allows readministration. On the other hand, retroviral vectors are susceptible to rapid degradation by the complement (Takeuchi et al, 1994). The main limiting factor for most retroviral vectors (including the frequently used Moloney murine leukaemia virus based vectors) is their inability to transduce non-dividing cells (Barquinero et al, 2004). In contrast, vectors based on lentiviruses are capable of

transduction of both dividing and quiescent cells (Delenda, 2004) Another limiting factor for retroviral vectors is inefficient production in high titers (Romano et al, 1999) However, by replacing the region responsible for initiation of transcription (U3 –region) from the 5' LTR with a CMV promoter, higher vector titers were obtained (Finer et al, 1994) In addition, modification of viral glycoproteins has generated more stable viral particles allowing their concentration for higher titers (Burns et al, 1993). Nevertheless, retroviral titers still lag behind adenoviral titers - adenoviruses can be routinely produced in titers 2-3 orders of magnitude higher than the current best retroviral-/lentiviral titers.

Due to the relatively small size of the retroviral genome, their transgene capacity can be up to 8 kbp (McCormick, 2001). When turning retroviruses into gene transfer vectors, the viral genes are completely replaced by the desired transgene and in many cases also an internal promoter. The viral proteins required for functionality of the viral vector are produced from separate packaging constructs, which minimize the probability for generation of replication competent viruses and thus increases the safety of these vectors (Romano et al, 1999). Instead of using packaging cell lines, transient transfection can be used to deliver the required constructs into producer cells to obtain efficient virus production (Pear et al, 1993; Soneoka et al, 1995). To further increase the safety of retroviral vectors, self-inactivating type vectors (SIN) have been developed (Yu et al, 1986). These vectors contain a deletion on the 3' LTR, which inactivates the enhancer and promoter. When the vector genome is reversely transcribed, this deletion is transferred to the 5'LTR abolishing transcriptional activity of the integrated provirus. In the context of HIV-1 based lentiviral vectors, safety aspects have been considered even more carefully. Consequently, all HIV-1 accessory genes have been abolished and virus production components are divided into 3-4 separate parts (Zufferey et al, 1997) and self-inactivating deletions have been introduced into vector backbones (Zufferey et al, 1998).

Various approaches have also been developed to enhance the transduction rates of retroviral vectors. To broaden host cell tropism, retroviral particles have been pseudotyped. For instance, the *env*-glycoprotein has been replaced by other viral proteins such as glycoprotein from vesicular stomatitis virus (VSV-G), which has also been shown to stabilize the vector particles (Yee et al, 1994). Furthermore, oncoretroviral vectors have been retargeted by fusing polypeptides into envelope glycoproteins (Peng et al, 1999, 2001). Furthermore, transduction efficiency and transgene expression with lentivirus vectors has been enhanced by incorporating central polypurine tract (cPPT) and posttranscriptional regulatory elements (PRE) into vector constructs. The cPPT has been reported to act by increasing nuclear transport of the viral preintegration complex (Follenzi et al, 2000; Zennou et al, 2000) and has also been suggested to facilitate nuclear import of viral RNA species, thereby increasing lentiviral transduction efficiency (Van Maele et al, 2003). Also, PREs of human or woodchuck hepatitis viral origin have been shown to stabilize viral vector RNA improving transgene expression (Patzel and Sczakiel, 1997; Zufferey et al, 1999).

Although use of retroviral vectors is mainly focused on inherited genetic disease where stable, long-term transgene expression is required, also several clinical studies have been reported for cancer diseases. Due to the inability of retroviral vectors to transduce non-proliferating cells (e.g. neurons), retrovirally mediated suicide gene therapy has studied for treatment of malignant brain (Culver et al, 1994) or lung tumors (Roth et al, 1996). In preclinical studies, lentiviral vectors have been evaluated for several cancer types including ovarian (Indraccolo et al, 2002), prostate (Bastide et al, 2003; Zheng et al, 2003) and bladder cancer (Kikuchi et al, 2004). In addition, cancer gene therapy approach based on lentiviral vector targeting to tumor endothelium has been introduced (De Palma et al, 2003). However, safety concerns have prevented clinical cancer trials with lentiviruses thus far.

C. Enhanced gene transfer and increased specificity: adenoviral targeting

Adverse side effects caused by unspesific gene transfer to non-target organs can be avoided by targeting viral vectors and/or transgenes to cancer cells (Glasgow et al, 2004). Additionally, such manoeuvres allow enhancement of gene transfer rates in tumor tissue resulting in enhanced therapeutic outcome. Especially with regard to adenoviral gene transfer, a big obstacle is the variable and often low expression level of CAR in tumor cells, making these cells rather refractory to adenoviral gene transfer. Further, it would be also important to minimize the adverse side effects by detargeting the vectors from the liver. Targeting strategies can be based on transductional or transcriptional approaches.

Transductional targeting is based on altered viral tropism via modification of viral proteins mediating receptor binding. In adenoviral vectors, re-targeting moieties allowing CAR- independent delivery can be linked physically to fiber knob or introduced genetically by incorporating necessary changes to the viral genome (**Figure 5**). Simultaneously, the binding to the primary viral receptor can be blocked. Alternative receptors are typically expressed at high levels in cancer cells but to a lesser extent in normal cells, which improves the tumor cell specificity of the gene transfer. Heretofore, several reports indicate successful adenoviral targeting *in vitro* and in animal models to a considerable number of alternative cellular receptors including α_v integrins, CD3, CD40, adenovirus serotype 3 receptor, prostate specific membrane antigen and epidermal growth factor receptor (Wickham et al, 1996, 1997; Tillman et al, 1999; Hemminki et al, 2001b; Kanerva et al, 2002a; Kraaij et al, 2005). Further, some studies have shown that adenoviral vector mediated liver toxicity can be reduced by targeting the vectors to cancer cells (Einfeld et al, 2001; Printz et al, 2000). However, liver uptake is mostly a non-receptor mediated process resulting in virus clearance by Kuppfer cells (Schiedner et al, 2003).

In addition to re-routing viral vectors to alternative receptors, expression of therapeutic genes can be limited into tumor tissue. The expression of transcriptionally

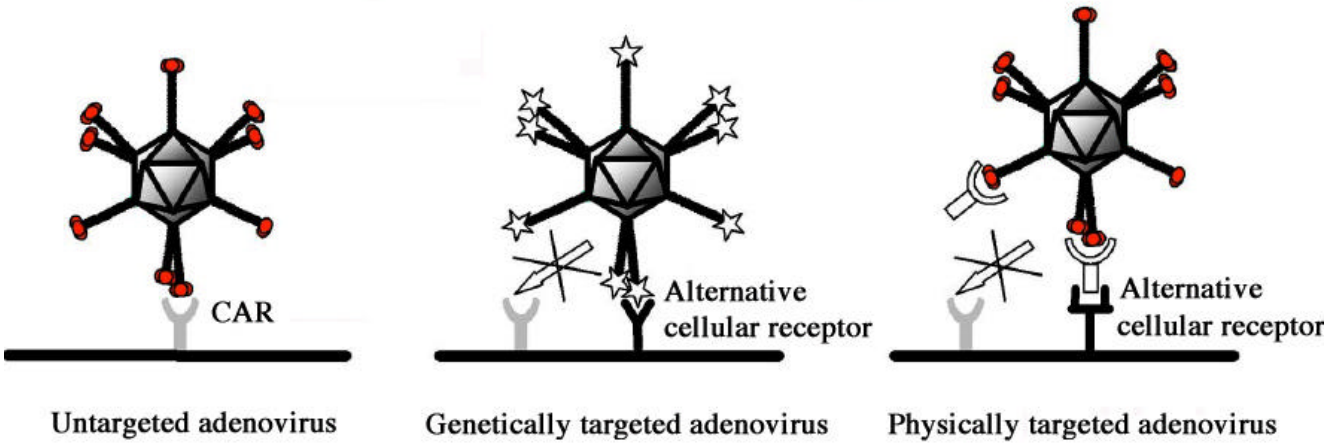

Figure 5. Transductional targeting of adenoviruses. Via transductional targeting, adenoviral vector can re-routed to alternative cellular receptors instead of its primary coxsackie-adenovirus receptor (CAR) Targeting can be based on genetically modified knobs or bi-specific ligands. The latter bind both to the viral knob and an alternative receptor.

targeted genes is driven by tissue specific promoters, which are activated in target cells by tissue specific transcription factors (reviewed in Bauerschmitz et al, 2002a; Saukkonen and Hemminki, 2004). In recent studies, several tissue specific promoters have been characterized and studied for cancer gene therapy purposes. These include α-fetoprotein for hepatomas (Kanai et al, 1997), the cyclo-oxygenase-2 (COX-2) for ovarian and gastric cancer (Casado et al, 2001; Yamamoto et al, 2001) and osteocalcin for metastatic prostate cancer (Koeneman et al, 2000). Additionally, radiation or drug inducible promoters such as early growth response gene 1 (EGR-1), have been exploited for transcriptionl targeting (Manome et al, 1998).

D. Improved therapeutic outcome by increased spreading of the therapeutic element: oncolytic viruses and protein transduction domains

One possibility to circumvent initially low gene transfer efficiency is to facilitate spreading of the therapeutic element inside the tumor tissue. One approach is based on oncolytic adenoviruses, which replicate in tumor cells killing the host cell and spreading to the neighboring cells and eventually throughout the tumor. Another possibility is to exploit protein transduction domains (PTD), which can spread the fused therapeutic protein from one cell to another.

1. Conditionally replicative adenoviruses (CRAds)

Utilization of conditionally replicative adenoviruses (CRAds) is based on their theoretical ability to spread throughout the tumor as long as tumor cells persist, by virtue of viral replication and concomitant cell lysis and viral progeny dissemination (Alemany et al, 2000; Post et al, 2003).

To minimize adverse side effects and increase the safety of these anti-cancer agents, replication can be limited to tumor tissue by genetically modifying the CRAd genome. Replication can be limited either with partial deletions in the E1 region or by using tissue specific promoters to drive genes responsible for viral replication (Alemany et al, 2000). Partial viral genome deletions allow virus to replicate selectively in cells with defective p53/p14ARF (Bischoff et al, 1996) or Rb-p16 pathway (Fueyo et al, 2000) that are hallmarks of many cancer cells. Replication can also be limited by using tissue specific promoters such as human telomerase reverse transcriptase (hTERT) (Irving et al, 2004), α-fetoprotein (Hallenbeck et al, 1999) or tyrosinase (Nettelbeck et al, 2002) to drive E1A expression.

To increase the specificity and antitumoral activity of oncolytic agents, several targeting approaches have been developed. CRAds have been successfully targeted to alternative cellular receptors, e.g. the adenovirus serotype 3 receptor and integrins (Suzuki et al, 2001; Bauerschmitz et al, 2002b; Kanerva et al, 2003, 2005). In addition, the antitumoral activity of CRAds is affected by the presence or absence of native E3 region viral genes, frequently deleted in earlier generation vectors. It has been reported that retaining the adenovirus E3 region (Suzuki et al, 2002) and overexpression of the adenovirus death protein (ADP) (Yun et al, 2004) can increase the oncolytic activity of CRAds. In contrast, deletion of the apoptose inhibitor E1B-19 kDa protein might enhance oncolysis (Liu et al, 2004).

The most widely studied and first clinically tested CRAd is ONYX-015 (dl1520), in which the E1B-55 kDa gene is mutated (Bischoff et al, 1996). This protein is responsible for p53 binding and inactivation for effective replication and thus it was hypothesized that ONYX-015 could only replicate in cells with a deficient p53-p14ARF pathway, thus including most cancer cells. In initial preclinical studies, ONYX-015 was reported to replicate selectively in p53-pathway-deficient tumor cells (Bischoff et al, 1996) and in addition, it was suggested not to replicate in normal epithelial and endothelial cells (Heise et al, 1997). However, However, later studies questioned

the correlation between viral replication and p53-status in tumor cells and suggested that the mechanism of selectivity was more complex than initially suggested (Goodrum and Ornelles, 1998; Rothmann et al, 1998).

ONYX-015 has been tested extensively in humans; over 10 clinical trials (phase I-II) have enrolled more than 300 patients with head and neck cancer (Ganly et al, 2000; Nemunaitis et al, 2000, 2001b), metastatic colorectal cancer (Reid et al, 2002), pancreatic cancer (Mulvihill et al, 2001) and ovarian cancer (Vasey et al, 2002). ONYX-015 has also studied in patients with lung metastasis (Nemunaitis et al, 2001a). The results from these studies show that although ONYX-015 has been a very safe, well tolerated vector, administerable by various routes (intratumoral, intraperitoneal, intra-arterial and intravenous), the majority of treated tumors did not respond to the therapy and significant antitumoral effects were rare (reviewed in Hemminki and Alvarez, 2002; Kirn, 2001). Of note, most of these patients had advanced disease already refractory to all available treatments. Further, most patients were enrolled in Phase I trials, which by definition do not attempt to assess efficacy as a primary endpoint.

A PSA -selective CRAd (CV706) has been studied in humans (DeWeese et al, 2001). The results were in parallel with results obtained from ONYX-trials: the virus was well tolerated and evidence of its replication was obtained and although PSA responses were frequently seen, the achieved antitumoral effect was relatively modest.

Taken together, these results suggest that early generation CRAds are safe anticancer agents, but their ability to completely destroy tumors as a single agent is limited. However, improved antitumoral activity has been obtained by combining CRAds with e.g. conventional therapy and suicide gene therapy. Unfortunately, no Phase III trials have yet been completed and therefore conclusive evidence with regard to efficacy is still lacking. Nevertheless, recent news with regard to ONYX-015 provides some optimism that Phase III trials may soon be initiated (Pollack, 2005).

2. Protein transduction domains (PTDs)

There are several so called translocatory proteins which are reported to be secreted from cells via a non-classical Golgi-independent route and to move intercellularly in a receptor- and transport –independent manner (Schwarze and Dowdy, 2000) The best known translocatory proteins are herpes simplex virus type 1 (HSV-1) tegument protein VP22 (Elliott and O'Hare, 1997), HIV-1 tat (Frankel and Pabo, 1988; Green and Loewenstein, 1988) and *Drosophila* antennapediae (Joliot et al, 1991). These intercellularly trafficking proteins share several features: all of them appear to localize in the nucleus and each of them has a highly basic region (Leifert and Whitton, 2003). Although the exact mechanism of intercellular spreading is still unknown, domains responsible for protein transduction have been identified. These small PTDs contain several basic amino acid residues, which have been suggested to mediate cellular binding and penetration (Gratton et al, 2003; Lundberg et al, 2003).

The most studied PTD is VP22, a 38 kDa sized major protein of HSV-1 tegument encoded by the UL49 gene (Elliott and Meredith, 1992). Several reports suggest that VP22 is able to retain its trafficking capacity even when fused to other proteins. VP22 has been reported to spread intercellularly *in vitro* when fused to GFP (Elliott and O'Hare, 1997, 1999; Aints et al, 1999; Wybranietz et al, 1999; Brewis et al, 2000) and enhance the antitumoral effect *in vivo* when fused to HSV-1 thymidine kinase and tumor suppressors p53 and p27 (Dilber et al, 1999; Wills et al, 2001; Zavaglia et al, 2003). On the other hand, however, there are also reports suggesting that VP22 mediated intercellular trafficking might be an artifact, related to the fixation process (Fang et al, 1998; Lundberg et al, 2003), thus making the exploitation of protein transduction approach for gene therapy purposes rather debatable. According to recent findings by Lundberg and co-workers, positively charged PTDs e.g. VP22 only mediate cell surface adherence via electrostatic interactions and observed translocation across the cell membrane is due to the fixation procedure (Lundberg et al, 2003). However, PTDs have been successfully employed to enhance both lenti- and adenoviral transduction *in vitro* and *in vivo* (Gratton et al, 2003; Kretz et al, 2003). In addition, PTDs have been demonstrated to improve the viral uptake and replication of tumor-specific oncolytic adenoviruses *in vitro* (Kuhnel et al, 2004).

IV. Future prospects: Combination therapies

Although several gene therapy approaches have been demonstrated to destroy tumor cells, clinical trials suggest that the ability of early generation agents to completely eradicate advanced tumor masses is limited. A main reason for the not unexpected discrepancy between humans and preclinical models is the complex nature of advanced solid tumors; a large tumor mass will have areas of necrosis, hypoxia, variable stromal contents and other infiltrating normal tissue, variable vasculature, etc. There is also preclinical evidence that intratumoral complexity can compromise the efficacy of gene therapeutic agents (Pipiya et al, 2005; Sauthoff et al, 2003). Thus, for improved antitumoral effect, the following strategies can be identified (with some references as examples):

1. Transductional targeting of agents to receptors expressed preferentially on tumor cells in comparison to normal cells (Hemminki et al, 2001a, 2002b)
2. Transcriptional targeting for tumor specific activity (Barker et al, 2003a)
3. Combination of transductional and transcriptional targeting (Barker et al, 2003b)
4. Develop viruses that can be tracked *in vivo* to obtain maximum correlative data (Kanerva et al, 2005)
5. Combine gene therapeutics with more conventional approaches (Raki et al, 2005)
6. Arm vectors and CRAds with transgenes that can help overcome intratumoral barriers (Conrad et al, 2005)
7. Improve the intratumoral dissemination of agents (Ahmed et al, 2003; Liu et al, 2004)

8. Develop agents that allow multiple administration without inactivation by the immune system (Mastrangeli et al, 1996; Mok et al, 2005).

Conventional therapies have been efficiently exploited in cancer gene therapy and for metastatic disease, chemotherapy, radiation therapy and hormonal therapies remain front fine options. However, in most cases, metastatic solid tumors remain incurable and thus new therapeutic options are needed (DeVita et al, 2001). In this regard, there are some exciting recent results which demonstrate the utility of gene therapy in advanced cancer patients. In a Phase II randomized trial, patients with malignant glioma were resected and half received adenovirus coding for HSV-TK into the resection cavity margins, followed by i.v. ganciclovir. The survival of the patients was doubled in comparison to randomized controls (Immonen et al, 2004). In another clinical trial, patients with advanced high-grade glioma were treated with oncolytic Newcastle disease virus. This treatment resulted in survival rates of 5-9 years whereas typical prognosis for this aggressive disease ranges averagely from six months to a year. In addition, these patients were regularly treated with virotherapy for several years without interruption (Csatary et al, 2004).

Introduction of suicide, tumor suppressor or immunotherapeutic genes to the patients receiving chemotherapy has been studied in ovarian and lung cancer and multiple myeloma (Hasenburg et al, 2001; Schuler et al, 2001; Trudel et al, 2001). However, in these studies, only a minor additional benefit was evident with combination therapy and the lack of randomization obscures the magnitude of the benefit. Furthermore, transfer of MDR-1 gene into hematopoietic stem cells to reduce the toxic effects of cancer chemotherapy has also been evaluated (Hesdorffer et al, 1998). Combining radiotherapy with therapeutic genes encoding e.g. thymidine kinase and p53 has been studied in prostate and lung cancer (Swisher et al, 2003; Teh et al, 2004). Furthermore, utilizing chemotherapy and/or radiotherapy in conjunction with CRAds has been suggested to augment antitumor activity in pre-clinical studies with colon cancer (Rogulski et al, 2000) and malignant glioma (Lamfers et al, 2002) and in clinical studies with head and neck cancer (Khuri et al, 2000; Young, 2005) and metastatic gastrointestinal carcinoma (Reid et al, 2002).

In pre-clinical studies, immunotherapy combined with suicide genes has been demonstrated to induce antitumoral immunity and enhance the tumor regression in lung, colon and metastatic breast cancer (Jones et al, 2000; Majumdar et al, 2000; Park et al, 2003).

Another approach to enhance the antineoplastic activity of oncolytic adenoviruses is to utilize their ability to multiply and amplify inserted therapeutic genes during replication. Several pre-clinical and clinical studies suggest that combining therapeutic genes, such as p53, cytosine deaminase and thymidine kinase with replicative viruses might improve their oncolytic potency and increase the anti-tumoral effect (Wildner et al, 1999; Freytag et al, 2002, 2003; van Beusechem et al, 2002; Fuerer and Iggo, 2004).

In summary, several clinical trials in cancer patients have shown that gene therapy is relatively safe and the heretofore utilized gene transfer vectors are well tolerated. Results from clinical trials have also been promising in demonstrating preliminary efficacy, especially when combined with conventional treatments. Also, the first randomized and therefore unequivocal evidence of gene therapy giving clinical benefit was recently published (Immonen et al, 2004). In addition, several strategies to improve the therapeutic outcome are constantly being developed and evaluated in pre-clinical studies in order to create more powerful tools for gene therapy purposes.

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References

Ahmed A, Jevremovic D, Suzuki K, Kottke T, Thompson J, Emery S, Harrington K, Bateman A and Vile R **(2003)** Intratumoral expression of a fusogenic membrane glycoprotein enhances the efficacy of replicating adenovirus therapy. **Gene Ther** 10, 1663-1671.

Aints A, Dilber MS and Smith CI **(1999)** Intercellular spread of GFP-VP22. **J Gene Med** 1, 275-279.

Alemany R, Balague C and Curiel DT **(2000)** Replicative adenoviruses for cancer therapy. **Nat Biotechnol** 18, 723-727.

Alvarez RD, Gomez-Navarro J, Wang M, Barnes MN, Strong TV, Arani RB, Arafat W, Hughes JV, Siegal GP and Curiel DT **(2000)** Adenoviral-mediated suicide gene therapy for ovarian cancer. **Mol Ther** 2, 524-530.

Armentano D, Sookdeo CC, Hehir KM, Gregory RJ, St George JA, Prince GA, Wadsworth SC and Smith AE **(1995)** Characterization of an adenovirus gene transfer vector containing an E4 deletion. **Hum Gene Ther** 6, 1343-1353.

Asaoka K, Tada M, Sawamura Y, Ikeda J and Abe H **(2000)** Dependence of efficient adenoviral gene delivery in malignant glioma cells on the expression levels of the Coxsackievirus and adenovirus receptor. **J Neurosurg** 92, 1002-1008.

Barker SD, Coolidge CJ, Kanerva A, Hakkarainen T, Yamamoto M, Liu B, Rivera AA, Bhoola SM, Barnes MN, Alvarez RD, Curiel DT and Hemminki A **(2003a)** The secretory leukoprotease inhibitor (SLPI) promoter for ovarian cancer gene therapy. **J. Gene Med.** 5, 300-310.

Barker SD, Dmitriev IP, Nettelbeck DM, Liu B, Rivera AA, Alvarez RD, Curiel DT and Hemminki A (**2003b)** Combined transcriptional and transductional targeting improves the specificity and efficacy of adenoviral gene delivery to ovarian carcinoma. **Gene Ther.** 10, 1198-1204.

Barouch DH, Pau MG, Custers JH, Koudstaal W, Kostense S, Havenga MJ, Truitt DM, Sumida SM, Kishko MG, Arthur JC, Korioth-Schmitz B, Newberg MH, Gorgone DA, Lifton MA, Panicali DL, Nabel GJ, Letvin NL and Goudsmit J (**2004)** Immunogenicity of recombinant adenovirus serotype 35 vaccine in the presence of pre-existing anti-Ad5 immunity. **J. Immunol.** 172, 6290-6297.

Barquinero J, Eixarch H and Perez-Melgosa M (**2004)** Retroviral vectors: new applications for an old tool. **Gene Ther.** 11 Suppl 1, S3-9.

Bastide C, Maroc N, Bladou F, Hassoun J, Maitland N, Mannoni P and Bagnis C (**2003)** Expression of a model gene in prostate cancer cells lentivirally transduced in vitro and in vivo. **Prostate Cancer. Prostatic Dis.** 6, 228-234.

Bauerschmitz GJ, Barker SD and Hemminki A (**2002a)** Adenoviral gene therapy for cancer: from vectors to targeted and replication competent agents (review) **Int. J. Oncol.** 21, 1161-1174.

Bauerschmitz GJ, Lam JT, Kanerva A, Suzuki K, Nettelbeck DM, Dmitriev I, Krasnykh V, Mikheeva GV, Barnes MN, Alvarez RD, Dall P, Alemany R, Curiel DT and Hemminki A (**2002b)** Treatment of ovarian cancer with a tropism modified oncolytic adenovirus. **Cancer Res.** 62, 1266-70.

Benjamin R, Helman L, Meyers P and Reaman G (**2001)** A phase I/II dose escalation and activity study of intravenous injections of OCaP1 for subjects with refractory osteosarcoma metastatic to lung. **Hum Gene Ther** 12, 1591-1593.

Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL and Finberg RW (**1997)** Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. **Science** 275, 1320-1323.

Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fattaey A and McCormick F (**1996)** An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. **Science** 274, 373-6.

Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, Shearer G, Chang L, Chiang Y and Tolstoshev P (**1995)** T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. **Science** 270, 475-480.

Brewis N, Phelan A, Webb J, Drew J, Elliott G and O'Hare P (**2000)** Evaluation of VP22 spread in tissue culture. **J Virol** 74, 1051-1056.

Buller RE, Runnebaum IB, Karlan BY, Horowitz JA, Shahin M, Buekers T, Petrauskas S, Kreienberg R, Slamon D and Pegram M (**2002)** A phase I/II trial of rAd/p53 (SCH 58500) gene replacement in recurrent ovarian cancer. **Cancer Gene Ther** 9, 553-566.

Burns JC, Friedmann T, Driever W, Burrascano M and Yee JK (**1993)** Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. **Proc Natl Acad Sci USA** 90, 8033-8037.

Bushman FD and Miller MD (**1997)** Tethering human immunodeficiency virus type 1 preintegration complexes to target DNA promotes integration at nearby sites. **J Virol** 71, 458-464.

Casado E, Gomez-Navarro J, Yamamoto M, Adachi Y, Coolidge CJ, Arafat WO, Barker SD, Wang MH, Mahasreshti PJ, Hemminki A, Gonzalez-Baron M, Barnes MN, Pustilnik TB, Siegal GP, Alvarez RD and Curiel DT (**2001)** Strategies to accomplish targeted expression of transgenes in ovarian cancer for molecular therapeutic applications. **Clin Cancer Res** 7, 2496-2504.

Chen WY and Townes TM (**2000)** Molecular mechanism for silencing virally transduced genes involves histone deacetylation and chromatin condensation. **Proc Natl Acad Sci USA** 97, 377-382.

Chirmule N, Propert K, Magosin S, Qian Y, Qian R and Wilson J (**1999)** Immune responses to adenovirus and adeno-associated virus in humans. **Gene Ther** 6, 1574-1583.

Conrad C, Miller CR, Ji Y, Gomez-Manzano C, Bharara S, McMurray JS, Lang FF, Wong F, Sawaya R, Yung WK and Fueyo J (**2005)** Delta24-hyCD adenovirus suppresses glioma growth in vivo by combining oncolysis and chemosensitization. **Cancer Gene Ther** 12, 284-294.

Csatary LK, Gosztonyi G, Szeberenyi J, Fabian Z, Liszka V, Bodey B and Csatary CM (**2004)** MTH-68/H oncolytic viral treatment in human high-grade gliomas. **J Neurooncol** 67, 83-93.

Culver KW, Van Gilder J, Link CJ, Carlstrom T, Buroker T, Yuh W, Koch K, Schabold K, Doornbas S and Wetjen B (**1994)** Gene therapy for the treatment of malignant brain tumors with in vivo tumor transduction with the herpes simplex thymidine kinase gene/ganciclovir system. **Hum Gene Ther** 5, 343-379.

Danthinne X and Imperiale MJ (**2000)** Production of first generation adenovirus vectors: a review. **Gene Ther** 7, 1707-14.

De Palma M, Venneri MA and Naldini L (**2003)** In vivo targeting of tumor endothelial cells by systemic delivery of lentiviral vectors. **Hum Gene Ther** 14, 1193-1206.

Delenda C (**2004)** Lentiviral vectors: optimization of packaging, transduction and gene expression. **J Gene Med** 6 Suppl 1, S125-38.

DeVita, VT Jr., Hellman S and Rosenberg SA (**2001)** Cancer: Principles & Practise of Oncology, 6th Edition (Philadelphia: Lippincott/Williams & Wilkins).

DeWeese TL, van der Poel H, Li S, Mikhak B, Drew R, Goemann M, Hamper U, DeJong R, Detorie N, Rodriguez R, Haulk T, DeMarzo AM, Piantadosi S, Yu DC, Chen Y, Henderson DR, Carducci MA, Nelson WG and Simons JW (**2001)** A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. **Cancer Res** 61, 7464-7472.

Dilber MS, Phelan A, Aints A, Mohamed AJ, Elliott G, Smith CI and O'Hare P (**1999)** Intercellular delivery of thymidine kinase prodrug activating enzyme by the herpes simplex virus protein, VP22. **Gene Ther** 6, 12-21.

Einfeld DA, Schroeder R, Roelvink PW, Lizonova A, King CR, Kovesdi I and Wickham TJ (**2001)** Reducing the native tropism of adenovirus vectors requires removal of both CAR and integrin interactions. **J Virol** 75, 11284-11291.

Elliott G and O'Hare P (**1997)** Intercellular trafficking and protein delivery by a herpesvirus structural protein. **Cell** 88, 223-233.

Elliott G and O'Hare P (**1999)** Intercellular trafficking of VP22-GFP fusion proteins. **Gene Ther.** 6, 149-151.

Elliott GD and Meredith DM (**1992)** The herpes simplex virus type 1 tegument protein VP22 is encoded by gene UL49. **J Gen Virol** 73 (Pt 3), 723-726.

Engelhardt JF, Ye X, Doranz B and Wilson JM (**1994)** Ablation of E2A in recombinant adenoviruses improves transgene persistence and decreases inflammatory response in mouse liver. **Proc Natl Acad Sci USA** 91, 6196-6200.

Fang B, Xu B, Koch P and Roth JA (**1998)** Intercellular trafficking of VP22-GFP fusion proteins is not observed in cultured mammalian cells. **Gene Ther** 5, 1420-1424.

Ferlay J, Bray, F, Pisani P and Parkin DM (**2004)** GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence WorldwideIARC CancerBase (Lyon: IARCPress).

Fields BN., Knipe D.M and Howley P.M (**1996)** Fundamental virology, 3rd edition (Philadelphia: Lippincott/Williams & Wilkins).

Finer MH, Dull TJ, Qin L, Farson D and Roberts MR (**1994)** kat: a high-efficiency retroviral transduction system for primary human T lymphocytes. **Blood** 83, 43-50.

Follenzi A, Ailles LE, Bakovic S, Geuna M and Naldini L (**2000)** Gene transfer by lentiviral vectors is limited by nuclear

translocation and rescued by HIV-1 pol sequences. **Nat Genet** 25, 217-222.

Frankel AD and Pabo CO (**1988)** Cellular uptake of the tat protein from human immunodeficiency virus. **Cell** 55, 1189-1193.

Freytag SO, Khil M, Stricker H, Peabody J, Menon M, DePeralta-Venturina M, Nafziger D, Pegg J, Paielli D, Brown S, Barton K, Lu M, Aguilar-Cordova E and Kim JH (**2002)** Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. **Cancer Res** 62, 4968-4976.

Freytag SO, Stricker H, Pegg J, Paielli D, Pradhan DG, Peabody J, DePeralta-Venturina M, Xia X, Brown S, Lu M and Kim JH (**2003)** Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer. **Cancer Res** 63, 7497-7506.

Fuerer C and Iggo R (**2004)** 5-Fluorocytosine increases the toxicity of Wnt-targeting replicating adenoviruses that express cytosine deaminase as a late gene. **Gene Ther** 11, 142-151.

Fueyo J, Gomez-Manzano C, Alemany R, Lee PS, McDonnell TJ, Mitlianga P, Shi YX, Levin VA, Yung WK and Kyritsis AP (**2000)** A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. **Oncogene** 19, 2-12.

Gahery-Segard H, Juillard V, Gaston J, Lengagne R, Pavirani A, Boulanger P and Guillet JG (**1997)** Humoral immune response to the capsid components of recombinant adenoviruses: routes of immunization modulate virus-induced Ig subclass shifts. **Eur J Immunol** 27, 653-659.

Ganly I, Kirn D, Eckhardt G, Rodriguez GI, Soutar DS, Otto R, Robertson AG, Park O, Gulley ML, Heise C, Von Hoff DD and Kaye SB (**2000)** A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. **Clin Cancer Res** 6, 798-806.

Glasgow JN, Bauerschmitz GJ, Curiel DT and Hemminki A (**2004)** Transductional and transcriptional targeting of adenovirus for clinical applications. **Curr Gene Ther** 4, 1-14.

Goodrum FD and Ornelles DA (**1998)** p53 status does not determine outcome of E1B 55-kilodalton mutant adenovirus lytic infection. **J Virol** 72, 9479-9490.

Gorziglia MI, Kadan MJ, Yei S, Lim J, Lee GM, Luthra R and Trapnell BC (**1996)** Elimination of both E1 and E2 from adenovirus vectors further improves prospects for in vivo human gene therapy. **J Virol** 70, 4173-4178.

Gratton JP, Yu J, Griffith JW, Babbitt RW, Scotland RS, Hickey R, Giordano FJ and Sessa WC (**2003)** Cell-permeable peptides improve cellular uptake and therapeutic gene delivery of replication-deficient viruses in cells and in vivo. **Nat Med** 9, 357-362.

Green M and Loewenstein PM (**1988)** Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. **Cell** 55, 1179-1188.

Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JI, de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A and Cavazzana-Calvo M (**2003)** LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. **Science** 302, 415-419.

Hallenbeck PL, Chang YN, Hay C, Golightly D, Stewart D, Lin J, Phipps S and Chiang YL (**1999)** A novel tumor-specific replication-restricted adenoviral vector for gene therapy of hepatocellular carcinoma. **Hum Gene Ther** 10, 1721-33.

Hanahan D and Weinberg RA (**2000)** The hallmarks of cancer. **Cell** 100, 57-70.

Harvey BG, Hackett NR, El-Sawy T, Rosengart TK, Hirschowitz EA, Lieberman MD, Lesser ML and Crystal RG (**1999)** Variability of human systemic humoral immune responses to adenovirus gene transfer vectors administered to different organs. **J Virol** 73, 6729-6742.

Hasenburg A, Tong XW, Fischer DC, Rojas-Martinez A, Nyberg-Hoffman C, Kaplan AL, Kaufman RH, Ramzy I, Aguilar-Cordova E and Kieback DG (**2001)** Adenovirus-mediated thymidine kinase gene therapy in combination with topotecan for patients with recurrent ovarian cancer: 2.5-year follow-up. **Gynecol Oncol** 83, 549-554.

Hay RT, Freeman A, Leith I, Monaghan A and Webster A (**1995)** Molecular interactions during adenovirus DNA replication. **Curr Top Microbiol Immunol** 199 (Pt 2), 31-48.

Heise C, Sampson-Johannes A, Williams A, McCormick F, Von Hoff DD and Kirn DH (**1997)** ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. **Nat Med** 3, 639-645.

Hemminki A and Alvarez RD (**2002)** Adenoviruses in oncology: a viable option? **BioDrugs** 16, 77-87.

Hemminki A, Belousova N, Zinn KR, Liu B, Wang M, Chaudhuri TR, Rogers BE, Buchsbaum DJ, Siegal GP, Barnes MN, Gomez-Navarro J, Curiel DT and Alvarez RD (**2001a)** An adenovirus with enhanced infectivity mediates molecular chemotherapy of ovarian cancer cells and allows imaging of gene expression. **Mol Ther** 4, 223-231.

Hemminki A, Dmitriev I, Liu B, Desmond RA, Alemany R and Curiel DT (**2001b)** Targeting oncolytic adenoviral agents to the epidermal growth factor pathway with a secretory fusion molecule. **Cancer Res** 61, 6377-81.

Hemminki A, Wang M, Desmond RA, Strong TV, Alvarez RD and Curiel DT (**2002a)** Serum and ascites neutralizing antibodies in ovarian cancer patients treated with intraperitoneal adenoviral gene therapy. **Hum. Gene Ther** 13, 1505-1514.

Hemminki A, Zinn KR, Liu B, Chaudhuri TR, Desmond RA, Rogers BE, Barnes MN, Alvarez RD and Curiel DT (**2002b)** In vivo molecular chemotherapy and noninvasive imaging with an infectivity-enhanced adenovirus. **J Natl Cancer Inst** 94, 741-749.

Hemminki, A. and Hemminki, K (**2004)** The Genetic Basis of Cancer, in: *Cancer Gene Therapy*. Curiel, D.,T. and Douglas, J. eds., (Totowa, NJ: Humana Press) .

Hesdorffer C, Ayello J, Ward M, Kaubisch A, Vahdat L, Balmaceda C, Garrett T, Fetell M, Reiss R, Bank A and Antman K (**1998)** Phase I trial of retroviral-mediated transfer of the human MDR1 gene as marrow chemoprotection in patients undergoing high-dose chemotherapy and autologous stem-cell transplantation. **J Clin Oncol** 16, 165-172.

Immonen A, Vapalahti M, Tyynela K, Hurskainen H, Sandmair A, Vanninen R, Langford G, Murray N and Yla-Herttuala S (**2004)** AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. **Mol Ther** 10, 967-972.

Indraccolo S, Habeler W, Tisato V, Stievano L, Piovan E, Tosello V, Esposito G, Wagner R, Uberla K, Chieco-Bianchi L and Amadori A (**2002)** Gene transfer in ovarian cancer cells: a comparison between retroviral and lentiviral vectors. **Cancer Res** 62, 6099-6107.

Irving J, Wang Z, Powell S, O'Sullivan C, Mok M, Murphy B, Cardoza L, Lebkowski JS and Majumdar AS (**2004)** Conditionally replicative adenovirus driven by the human telomerase promoter provides broad-spectrum antitumor activity without liver toxicity. **Cancer Gene Ther** 11, 174-185.

Joliot A, Pernelle C, Deagostini-Bazin H and Prochiantz A (**1991)** Antennapedia homeobox peptide regulates neural morphogenesis. **Proc Natl Acad Sci USA** 88, 1864-1868.

Jolly D (**1994)** Viral vector systems for gene therapy. **Cancer Gene Ther** 1, 51-64.

Jones RK, Pope IM, Kinsella AR, Watson AJ and Christmas SE (**2000)** Combined suicide and granulocyte-macrophage colony-stimulating factor gene therapy induces complete tumor regression and generates antitumor immunity. **Cancer Gene Ther** 7, 1519-1528.

Kanai F, Lan KH, Shiratori Y, Tanaka T, Ohashi M, Okudaira T, Yoshida Y, Wakimoto H, Hamada H, Nakabayashi H, Tamaoki T and Omata M (**1997)** In vivo gene therapy for alpha-fetoprotein-producing hepatocellular carcinoma by adenovirus-mediated transfer of cytosine deaminase gene. **Cancer Res** 57, 461-465.

Kanerva A, Mikheeva GV, Krasnykh V, Coolidge CJ, Lam JT, Mahasreshti PJ, Barker SD, Straughn M, Barnes MN, Alvarez RD, Hemminki A and Curiel DT (**2002a)** Targeting adenovirus to the serotype 3 receptor increases gene transfer efficiency to ovarian cancer cells. **Clin Cancer Res** 8, 275-280.

Kanerva A, Wang M, Bauerschmitz GJ, Lam JT, Desmond RA, Bhoola SM, Barnes MN, Alvarez RD, Siegal GP, Curiel DT and Hemminki A (**2002b)** Gene transfer to ovarian cancer versus normal tissues with fiber-modified adenoviruses. **Mol Ther** 5, 695-704.

Kanerva A, Zinn KR, Chaudhuri TR, Lam JT, Suzuki K, Uil TG, Hakkarainen T, Bauerschmitz GJ, Wang M, Liu B, Cao Z, Alvarez RD, Curiel DT and Hemminki A (**2003)** Enhanced therapeutic efficacy for ovarian cancer with a serotype 3 receptor-targeted oncolytic adenovirus. **Mol Ther** 8, 449-458.

Kanerva A, Zinn KR, Peng KW, Ranki T, Kangasniemi L, Chaudhuri TR, Desmond RA, Wang M, Takayama K, Hakkarainen T, Alfthan H, Stenman UH, Curiel DT and Hemminki A (**2005)** Noninvasive dual modality in vivo monitoring of the persistence and potency of a tumor targeted conditionally replicating adenovirus. **Gene Ther** 12, 87-94.

Kay MA, Glorioso JC and Naldini L (**2001)** Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. **Nat Med** 7, 33-40.

Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C, Randlev B, Gillenwater AM, Bruso P, Kaye SB, Hong WK and Kirn DH (**2000)** a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. **Nat Med** 6, 879-885.

Kikuchi E, Menendez S, Ohori M, Cordon-Cardo C, Kasahara N and Bochner BH (**2004)** Inhibition of orthotopic human bladder tumor growth by lentiviral gene transfer of endostatin. **Clin Cancer Res** 10, 1835-1842.

Kirn D (**2001)** Clinical research results with dl1520 (Onyx-015), a replication-selective adenovirus for the treatment of cancer: what have we learned? **Gene Ther** 8, 89-98.

Kochanek S, Clemens PR, Mitani K, Chen HH, Chan S and Caskey CT (**1996)** A new adenoviral vector: Replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. **Proc Natl Acad Sci USA** 93, 5731-5736.

Koeneman KS, Kao C, Ko SC, Yang L, Wada Y, Kallmes DF, Gillenwater JY, Zhau HE, Chung LW and Gardner TA (**2000)** Osteocalcin-directed gene therapy for prostate-cancer bone metastasis. **World J Urol** 18, 102-110.

Kootstra NA and Verma IM (**2003)** Gene therapy with viral vectors. **Annu Rev Pharmacol Toxicol** 43, 413-439.

Kraaij R, van Rijswijk AL, Oomen MH, Haisma HJ and Bangma CH (**2005)** Prostate specific membrane antigen (PSMA) is a tissue-specific target for adenoviral transduction of prostate cancer in vitro. **Prostate** 62, 253-259.

Kretz A, Wybranietz WA, Hermening S, Lauer UM and Isenmann S (**2003)** HSV-1 VP22 augments adenoviral gene transfer to CNS neurons in the retina and striatum in vivo. **Mol. Ther.** 7, 659-669.

Kuhnel F, Schulte B, Wirth T, Woller N, Schafers S, Zender L, Manns M and Kubicka S (**2004)** Protein transduction domains fused to virus receptors improve cellular virus uptake and enhance oncolysis by tumor-specific replicating vectors. **J Virol** 78, 13743-13754.

Lamfers ML, Grill J, Dirven CM, Van Beusechem VW, Geoerger B, Van Den Berg J, Alemany R, Fueyo J, Curiel DT, Vassal G, Pinedo HM, Vandertop WP and Gerritsen WR (**2002)** Potential of the conditionally replicative adenovirus Ad5-Delta24RGD in the treatment of malignant gliomas and its enhanced effect with radiotherapy. **Cancer Res** 62, 5736-5742.

Leifert JA and Whitton JL (**2003)** "Translocatory proteins" and "protein transduction domains": a critical analysis of their biological effects and the underlying mechanisms. **Mol Ther** 8, 13-20.

Li D, Duan L, Freimuth P and O'Malley BW, Jr (**1999a)** Variability of adenovirus receptor density influences gene transfer efficiency and therapeutic response in head and neck cancer. **Clin Cancer Res** 5, 4175-4181.

Li Y, Pong RC, Bergelson JM, Hall MC, Sagalowsky AI, Tseng CP, Wang Z and Hsieh JT (**1999b)** Loss of adenoviral receptor expression in human bladder cancer cells: a potential impact on the efficacy of gene therapy. **Cancer Res** 59, 325-330.

Liu TC, Hallden G, Wang Y, Brooks G, Francis J, Lemoine N and Kirn D (**2004)** An E1B-19 kDa gene deletion mutant adenovirus demonstrates tumor necrosis factor-enhanced cancer selectivity and enhanced oncolytic potency. **Mol Ther** 9, 786-803.

Lundberg M, Wikstrom S and Johansson M (**2003)** Cell surface adherence and endocytosis of protein transduction domains. **Mol Ther** 8, 143-150.

Lundstrom K (**2001)** Alphavirus vectors for gene therapy applications. **Curr Gene Ther** 1, 19-29.

Mack CA, Song WR, Carpenter H, Wickham TJ, Kovesdi I, Harvey BG, Magovern CJ, Isom OW, Rosengart T, Falck-Pedersen E, Hackett NR, Crystal RG and Mastrangeli A (**1997)** Circumvention of anti-adenovirus neutralizing immunity by administration of an adenoviral vector of an alternate serotype. **Hum Gene Ther** 8, 99-109.

Majumdar AS, Zolotorev A, Samuel S, Tran K, Vertin B, Hall-Meier M, Antoni BA, Adeline E, Philip M and Philip R (**2000)** Efficacy of herpes simplex virus thymidine kinase in combination with cytokine gene therapy in an experimental metastatic breast cancer model. **Cancer Gene Ther.** 7, 1086-1099.

Manome Y, Kunieda T, Wen PY, Koga T, Kufe DW and Ohno T (**1998)** Transgene expression in malignant glioma using a replication-defective adenoviral vector containing the Egr-1 promoter: activation by ionizing radiation or uptake of radioactive iododeoxyuridine. **Hum Gene Ther** 9, 1409-1417.

Mastrangeli A, Harvey BG, Yao J, Wolff G, Kovesdi I, Crystal RG and Falck-Pedersen E (**1996)** "Sero-switch" adenovirus-mediated in vivo gene transfer: circumvention of anti-adenovirus humoral immune defenses against repeat adenovirus vector administration by changing the adenovirus serotype. **Hum Gene Ther** 7, 79-87.

McCormick F (**2001)** Cancer gene therapy: fringe or cutting edge? **Nat Rev Cancer** 1, 130-141.

Moffatt S, Hays J, HogenEsch H and Mittal SK (**2000)** Circumvention of vector-specific neutralizing antibody response by alternating use of human and non-human adenoviruses: implications in gene therapy. **Virology** 272, 159-167.

Mok H, Palmer DJ, Ng P and Barry MA (**2005)** Evaluation of polyethylene glycol modification of first-generation and helper-dependent adenoviral vectors to reduce innate immune responses. **Mol Ther** 11, 66-79.

Mulvihill S, Warren R, Venook A, Adler A, Randlev B, Heise C and Kirn D (**2001)** Safety and feasibility of injection with an E1B-55 kDa gene-deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: a phase I trial. **Gene Ther** 8, 308-315.

Nemunaitis J, Cunningham C, Buchanan A, Blackburn A, Edelman G, Maples P, Netto G, Tong A, Randlev B, Olson S and Kirn D (**2001a)** Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: safety, feasibility and biological activity. **Gene Ther** 8, 746-759.

Nemunaitis J, Ganly I, Khuri F, Arseneau J, Kuhn J, McCarty T, Landers S, Maples P, Romel L, Randlev B, Reid T, Kaye S and Kirn D (**2000)** Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. **Cancer Res** 60, 6359-6366.

Nemunaitis J, Khuri F, Ganly I, Arseneau J, Posner M, Vokes E, Kuhn J, McCarty T, Landers S, Blackburn A, Romel L, Randlev B, Kaye S and Kirn D (**2001b)** Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. **J Clin Oncol** 19, 289-298.

Nettelbeck DM, Jerome V and Muller R (**2000)** Gene therapy: designer promoters for tumour targeting. **Trends Genet** 16, 174-181.

Nettelbeck DM, Rivera AA, Balague C, Alemany R and Curiel DT (**2002)** Novel oncolytic adenoviruses targeted to melanoma: specific viral replication and cytolysis by expression of E1A mutants from the tyrosinase enhancer/promoter. **Cancer Res** 62, 4663-70.

Park KH, Kim G, Jang SH, Kim CH, Kwon SY, Yoo CG, Kim YW, Kwon HC, Kim CM, Han SK, Shim YS and Lee CT (**2003)** Gene therapy with GM-CSF, interleukin-4 and herpes simplex virus thymidine kinase shows strong antitumor effect on lung cancer. **Anticancer Res** 23, 1559-1564.

Patzel V and Sczakiel G (**1997)** The hepatitis B virus posttranscriptional regulatory element contains a highly stable RNA secondary structure. **Biochem Biophys Res Commun** 231, 864-867.

Pear WS, Nolan GP, Scott ML and Baltimore D (**1993)** Production of high-titer helper-free retroviruses by transient transfection. **Proc Natl Acad Sci USA** 90, 8392-8396.

Peng KW and Russell SJ (**1999)** Viral vector targeting. **Curr Opin Biotechnol** 10, 454-457.

Peng KW, Pham L, Ye H, Zufferey R, Trono D, Cosset FL and Russell SJ (**2001)** Organ distribution of gene expression after intravenous infusion of targeted and untargeted lentiviral vectors. **Gene Ther** 8, 1456-63.

Peng KW, Vile R, Cosset FL and Russell S (**1999)** Selective transduction of protease-rich tumors by matrix-metalloproteinase-targeted retroviral vectors. **Gene Ther** 6, 1552-1557.

Pipiya T, Sauthoff H, Huang YQ, Chang B, Cheng J, Heitner S, Chen S, Rom WN and Hay JG (**2005)** Hypoxia reduces adenoviral replication in cancer cells by downregulation of viral protein expression. **Gene Ther** 12, 911-917.

Pollack, A (**2005)** TECHNOLOGY; Cancer Therapy Dropped In U.S. Is Revived in China. **New York Times,** Business/Financial Desk.

Post DE, Khuri FR, Simons JW and Van Meir EG (**2003)** Replicative oncolytic adenoviruses in multimodal cancer regimens. **Hum Gene Ther** 14, 933-946.

Printz MA, Gonzalez AM, Cunningham M, Gu DL, Ong M, Pierce GF and Aukerman SL (**2000)** Fibroblast growth factor 2-retargeted adenoviral vectors exhibit a modified biolocalization pattern and display reduced toxicity relative to native adenoviral vectors. **Hum Gene Ther** 11, 191-204.

Raki M, Kanerva A, Ristimäki A, Desmond RA, Chen DT, Ranki T, Särkioja M, Kangasniemi L and Hemminki A (**2005)** Combination of gemcitabine and Ad5/3¢24, a tropism modified conditionally replicating adenovirus, for the treatment of ovarian cancer. **Gene Ther**, in press.

Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, Wilson JM and Batshaw ML (**2003)** Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. **Mol Genet Metab** 80, 148-158.

Rasmussen UB, Benchaibi M, Meyer V, Schlesinger Y and Schughart K (**1999)** Novel human gene transfer vectors: evaluation of wild-type and recombinant animal adenoviruses in human-derived cells. **Hum Gene Ther** 10, 2587-2599.

Reid T, Galanis E, Abbruzzese J, Sze D, Wein LM andrews J, Randlev B, Heise C, Uprichard M, Hatfield M, Rome L, Rubin J and Kirn D (**2002)** Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520) : phase II viral, immunologic and clinical endpoints. **Cancer Res** 62, 6070-6079.

Rieger PT (**2004)** The biology of cancer genetics. **Semin Oncol Nurs** 20, 145-154.

Rogulski KR, Wing MS, Paielli DL, Gilbert JD, Kim JH and Freytag SO (**2000)** Double suicide gene therapy augments the antitumor activity of a replication-competent lytic adenovirus through enhanced cytotoxicity and radiosensitization. **Hum Gene Ther** 11, 67-76.

Romano G, Pacilio C and Giordano A (**1999)** Gene transfer technology in therapy: current applications and future goals. **Stem Cells** 17, 191-202.

Roth JA, Nguyen D, Lawrence DD, Kemp BL, Carrasco CH, Ferson DZ, Hong WK, Komaki R, Lee JJ, Nesbitt JC, Pisters KM, Putnam JB, Schea R, Shin DM, Walsh GL, Dolormente MM, Han CI, Martin FD, Yen N, Xu K, Stephens LC, McDonnell TJ, Mukhopadhyay T and Cai D (**1996)** Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. **Nat Med** 2, 985-991.

Rothmann T, Hengstermann A, Whitaker NJ, Scheffner M and zur Hausen H (**1998)** Replication of ONYX-015, a potential anticancer adenovirus, is independent of p53 status in tumor cells. **J Virol** 72, 9470-9478.

Russell SJ (**2002)** RNA viruses as virotherapy agents. **Cancer Gene Ther** 9, 961-966.

Russell WC (**2000)** Update on adenovirus and its vectors. **J Gen Virol** 81, 2573-604.

Saukkonen K and Hemminki A (**2004)** Tissue-specific promoters for cancer gene therapy. **Expert Opin Biol Ther** 4, 683-696.

Sauthoff H, Hu J, Maca C, Goldman M, Heitner S, Yee H, Pipiya T, Rom WN and Hay JG (**2003)** Intratumoral spread of wild-type adenovirus is limited after local injection of human

xenograft tumors: virus persists and spreads systemically at late time points. **Hum Gene Ther** 14, 425-433.

Schiedner G, Bloch W, Hertel S, Johnston M, Molojavyi A, Dries V, Varga G, Van Rooijen N and Kochanek S (**2003)** A hemodynamic response to intravenous adenovirus vector particles is caused by systemic Kupffer cell-mediated activation of endothelial cells. **Hum Gene Ther** 14, 1631-1641.

Schuler M, Herrmann R, De Greve JL, Stewart AK, Gatzemeier U, Stewart DJ, Laufman L, Gralla R, Kuball J, Buhl R, Heussel CP, Kommoss F, Perruchoud AP, Shepherd FA, Fritz MA, Horowitz JA, Huber C and Rochlitz C (**2001)** Adenovirus-mediated wild-type p53 gene transfer in patients receiving chemotherapy for advanced non-small-cell lung cancer: results of a multicenter phase II study. **J Clin Oncol** 19, 1750-1758.

Schwarze SR and Dowdy SF (**2000)** In vivo protein transduction: intracellular delivery of biologically active proteins, compounds and DNA. **Trends Pharmacol Sci** 21, 45-48.

Setoguchi Y, Jaffe HA, Chu CS and Crystal RG (**1994)** Intraperitoneal in vivo gene therapy to deliver alpha 1-antitrypsin to the systemic circulation. **Am J Respir Cell Mol Biol** 10, 369-377.

Shiramizu B, Herndier BG and McGrath MS (**1994)** Identification of a common clonal human immunodeficiency virus integration site in human immunodeficiency virus-associated lymphomas. **Cancer Res** 54, 2069-2072.

Smith TA, White BD, Gardner JM, Kaleko M and McClelland A (**1996)** Transient immunosuppression permits successful repetitive intravenous administration of an adenovirus vector. **Gene Ther** 3, 496-502.

Somia N and Verma IM (**2000)** Gene therapy: trials and tribulations. **Nat Rev Genet** 1, 91-99.

Soneoka Y, Cannon PM, Ramsdale EE, Griffiths JC, Romano G, Kingsman SM and Kingsman AJ (**1995)** A transient three-plasmid expression system for the production of high titer retroviral vectors. **Nucleic Acids Res** 23, 628-633.

Sterman DH, Treat J, Litzky LA, Amin KM, Coonrod L, Molnar-Kimber K, Recio A, Knox L, Wilson JM, Albelda SM and Kaiser LR (**1998)** Adenovirus-mediated herpes simplex virus thymidine kinase/ganciclovir gene therapy in patients with localized malignancy: results of a phase I clinical trial in malignant mesothelioma. **Hum Gene Ther** 9, 1083-1092.

Suzuki K, Alemany R, Yamamoto M and Curiel DT (**2002)** The presence of the adenovirus E3 region improves the oncolytic potency of conditionally replicative adenoviruses. **Clin Cancer Res** 8, 3348-59.

Suzuki K, Fueyo J, Krasnykh V, Reynolds PN, Curiel DT and Alemany R (**2001)** A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. **Clin Cancer Res** 7, 120-126.

Swisher SG, Roth JA, Komaki R, Gu J, Lee JJ, Hicks M, Ro JY, Hong WK, Merritt JA, Ahrar K, Atkinson NE, Correa AM, Dolormente M, Dreiling L, El-Naggar AK, Fossella F, Francisco R, Glisson B, Grammer S, Herbst R, Huaringa A, Kemp B, Khuri FR, Kurie JM, Liao Z, McDonnell TJ, Morice R, Morello F, Munden R, Papadimitrakopoulou V, Pisters KM, Putnam JB,Jr, Sarabia AJ, Shelton T, Stevens C, Shin DM, Smythe WR, Vaporciyan AA, Walsh GL and Yin M (**2003)** Induction of p53-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral p53 (INGN 201) and radiation therapy. **Clin Cancer Res** 9, 93-101.

Takeuchi Y, Cosset FL, Lachmann PJ, Okada H, Weiss RA and Collins MK (**1994)** Type C retrovirus inactivation by human complement is determined by both the viral genome and the producer cell. **J Virol** 68, 8001-8007.

Tamura K, Utsunomiya J, Iwama T, Furuyama J, Takagawa T, Takeda N, Fukuda Y, Matsumoto T, Nishigami T, Kusuhara K, Sagayama K, Nakagawa K and Yamamura T (**2004)** Mechanism of carcinogenesis in familial tumors. **Int J Clin Onco**9, 232-245.

Teh BS, Ayala G, Aguilar L, Mai WY, Timme TL, Vlachaki MT, Miles B, Kadmon D, Wheeler T, Caillouet J, Davis M, Carpenter LS, Lu HH, Chiu JK, Woo SY, Thompson T, Aguilar-Cordova E and Butler EB (**2004)** Phase I-II trial evaluating combined intensity-modulated radiotherapy and in situ gene therapy with or without hormonal therapy in treatment of prostate cancer-interim report on PSA response and biopsy data. **Int J Radiat Oncol Biol Phys** 58, 1520-1529.

Tillman BW, de Gruijl TD, Luykx-de Bakker SA, Scheper RJ, Pinedo HM, Curiel TJ, Gerritsen WR and Curiel DT (**1999)** Maturation of dendritic cells accompanies high-efficiency gene transfer by a CD40-targeted adenoviral vector. **J Immunol** 162, 6378-6383.

Tripathy SK, Black HB, Goldwasser E and Leiden JM (**1996)** Immune responses to transgene-encoded proteins limit the stability of gene expression after injection of replication-defective adenovirus vectors. **Nat Med** 2, 545-550.

Trudel S, Li Z, Dodgson C, Nanji S, Wan Y, Voralia M, Hitt M, Gauldie J, Graham FL and Stewart AK (**2001)** Adenovector engineered interleukin-2 expressing autologous plasma cell vaccination after high-dose chemotherapy for multiple myeloma--a phase 1 study. **Leukemia** 15, 846-854.

van Beusechem VW, van den Doel PB, Grill J, Pinedo HM and Gerritsen WR (**2002)** Conditionally replicative adenovirus expressing p53 exhibits enhanced oncolytic potency. **Cancer Res** 62, 6165-6171.

Van Ginkel FW, Liu C, Simecka JW, Dong JY, Greenway T, Frizzell RA, Kiyono H, McGhee JR and Pascual DW (**1995)** Intratracheal gene delivery with adenoviral vector induces elevated systemic IgG and mucosal IgA antibodies to adenovirus and beta-galactosidase. **Hum Gene Ther** 6, 895-903.

Van Maele B, De Rijck J, De Clercq E and Debyser Z (**2003)** Impact of the central polypurine tract on the kinetics of human immunodeficiency virus type 1 vector transduction. **J Virol** 77, 4685-4694.

Vasey PA, Shulman LN, Campos S, Davis J, Gore M, Johnston S, Kirn DH, O'Neill V, Siddiqui N, Seiden MV and Kaye SB (**2002)** Phase I trial of intraperitoneal injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. **J Clin Oncol** 20, 1562-1569.

Vogelstein B and Kinzler, KW (**1998)** The Genetic Basis of Human Cancer, 2nd edition (The McGraw-Hill Companies Inc.)

Volpers C and Kochanek S (**2004)** Adenoviral vectors for gene transfer and therapy. **J Gene Med** 6 Suppl 1, S164-71.

Wang Q, Greenburg G, Bunch D, Farson D and Finer MH (**1997)** Persistent transgene expression in mouse liver following in vivo gene transfer with a delta E1/delta E4 adenovirus vector. **Gene Ther** 4, 393-400.

Wickham TJ, Lee GM, Titus JA, Sconocchia G, Bakacs T, Kovesdi I and Segal DM (**1997)** Targeted adenovirus-mediated gene delivery to T cells via CD3. **J Virol** 71, 7663-7669.

Wickham TJ, Mathias P, Cheresh DA and Nemerow GR (**1993)** Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. **Cell** 73, 309-319.

Wickham TJ, Segal DM, Roelvink PW, Carrion ME, Lizonova A, Lee GM and Kovesdi I (**1996)** Targeted adenovirus gene

transfer to endothelial and smooth muscle cells by using bispecific antibodies. **J Virol** 70, 6831-6838.

Wildner O, Blaese RM and Morris JC (**1999)** Therapy of colon cancer with oncolytic adenovirus is enhanced by the addition of herpes simplex virus-thymidine kinase. **Cancer Res** 59, 410-413.

Wills KN, Atencio IA, Avanzini JB, Neuteboom S, Phelan A, Philopena J, Sutjipto S, Vaillancourt MT, Wen SF, Ralston RO and Johnson DE (**2001)** Intratumoral spread and increased efficacy of a p53-VP22 fusion protein expressed by a recombinant adenovirus. **J Virol** 75, 8733-8741.

Wybranietz WA, Prinz F, Spiegel M, Schenk A, Bitzer M, Gregor M and Lauer UM (**1999)** Quantification of VP22-GFP spread by direct fluorescence in 15 commonly used cell lines. **J Gene Med** 1, 265-274.

Yamamoto M, Alemany R, Adachi Y, Grizzle WE and Curiel DT (**2001)** Characterization of the cyclooxygenase-2 promoter in an adenoviral vector and its application for the mitigation of toxicity in suicide gene therapy of gastrointestinal cancers. **Mol Ther** 3, 385-394.

Yee JK, Friedmann T and Burns JC (**1994)** Generation of high-titer pseudotyped retroviral vectors with very broad host range. **Methods Cell Biol** 43 Pt A, 99-112.

Young B (**2005)** Clinical study on the oncolytic virus induced personalized in vivo tumor vaccine. Oncolytic Viruses as Cancer Therapeutics, Banff, Alberta, 9-13 March.

Yu SF, von Ruden T, Kantoff PW, Garber C, Seiberg M, Ruther U anderson WF, Wagner EF and Gilboa E (**1986)** Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. **Proc Natl Acad Sci USA** 83, 3194-3198.

Yun CO, Kim E, Koo T, Kim H, Lee YS and Kim JH (**2005)** ADP-overexpressing adenovirus elicits enhanced cytopathic effect by induction of apoptosis. **Cancer Gene Ther** 12, 61-71.

Zavaglia D, Favrot MC, Eymin B, Tenaud C and Coll JL (**2003)** Intercellular trafficking and enhanced in vivo antitumour activity of a non-virally delivered P27-VP22 fusion protein. **Gene Ther** 10, 314-325.

Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L and Charneau P (**2000)** HIV-1 genome nuclear import is mediated by a central DNA flap. **Cell** 101, 173-185.

Zhang WW, Fujiwara T, Grimm EA and Roth JA (**1995)** Advances in cancer gene therapy. **Adv Pharmacol** 32, 289-341.

Zheng JY, Chen D, Chan J, Yu D, Ko E and Pang S (**2003)** Regression of prostate cancer xenografts by a lentiviral vector specifically expressing diphtheria toxin A. **Cancer Gene Ther** 10, 764-770.

Zufferey R, Donello JE, Trono D and Hope TJ (**1999)** Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. **J Virol** 73, 2886-2892.

Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L and Trono D (**1998)** Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. **J Virol** 72, 9873-9880.

Zufferey R, Nagy D, Mandel RJ, Naldini L and Trono D (**1997)** Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. **Nat Biotechnol** 15, 871-875.

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