Abstract. Gene therapy is an exciting novel approach for treating cancers resistant to currently available modalities. Treatment approaches are based on taking advantage of molecular differences between normal and tumor cells. Various strategies are currently in clinical development, with some promising early results reported with mutation compensation, molecular chemotherapy and replication competent viruses. Adenoviruses are among the most popular vehicles and there is a wealth of clinical data suggesting excellent safety for treatment of cancer patients. Current developments include improving targeting strategies for gene delivery to tumor cells with tumor specific promoters. Another rapidly developing field is replication competent agents, which allow improved tumor penetration and local amplification of the anti-tumor effect. Further, infectivity enhancement strategies can overcome variable expression of the primary adenovirus receptor on tumor cells, which may have reduced the clinical efficacy of previous strategies. Adenoviral cancer gene therapy approaches lack cross-resistance with other treatment options and frequently synergistic effects can be observed. Therefore, the first routine clinical applications are likely to be combination treatments.

Contents
1. Introduction
2. Vehicles for gene transfer
3. Cancer gene therapy approaches
4. Adenovirus for gene therapy
5. Cancer trials with adenoviral vectors
6. Transcriptional targeting
7. Transductional targeting
8. Conditionally replicating adenoviruses (CRAds)
9. Targeted conditionally replicating adenoviruses
10. Cancer trials with CRAds
11. Model systems - primary cells, spheroids
12. Future prospects
13. Conclusions

1. Introduction
In recent decades, an intense basic research effort has begun to reveal the nature of cancer as a disease of genes. Specifically, epigenetic and genetic alterations of tumor suppressor and oncogenes are the cause of cancer. A logical result of these findings is the idea of correcting the molecular defects. Alternatively, these differences could be used for targeting the anti-tumor effect to malignant cells. Thus, molecularly targeted therapies stem from our ability to detect molecular defects that set cancer cells apart from normal tissues (1). Cancer gene therapy includes a wide variety of heterogeneous approaches for which the common denominator is transfer of genes, which then code for the proteins that deliver the anti-tumor effect (2,3).

The principle of gene therapy is gene transfer in order to correct genetic defects or to express therapeutics within or near target cells. Gene transfer is performed by different vector systems with respective advantages and disadvantages. The specifics of these vector systems make them suitable for the treatment of either monogenic disorders or for cancer. Whereas the treatment of hereditary diseases typically benefits from long-term gene expression and thus a stable gene transfer method, gene therapy for cancer requires effective transduction and gene expression in target cells. Currently, the most common vector systems are adenoviruses (Ad), retroviruses, adeno-associated viruses (AAV) and non-viral gene transfer systems.

2. Vehicles for gene transfer
In addition to adenovirus, another traditional vehicle is the retrovirus, which integrates into the host cell genome and can
therefore achieve lasting gene expression. However, its major disadvantages are the possibility of insertion mutagenesis/oncogenesis, low transduction efficacy, difficulties in production of high titers and infection of only cycling cells (4-6). For these reasons, retroviruses may be more suitable for treatment of diseases where long-term gene expression is required. Adeno-associated viruses are non-pathogenic single-stranded DNA viruses which, when wild-type, may integrate into the human chromosome 19 and cause long-term gene expression, whereas recombinants stay mostly episomal (7). Early preclinical studies show encouraging results (8). However, recent findings suggest that adeno-associated viruses may cause deletions or changes in chromosome 19 (9). Finally, non-viral vectors are undergoing evaluation. Liposome vector systems are cationic complexes where transgenes are carried inside a lipid double-membrane, which can be modified to bind selectively to a specific target receptor. Interaction and fusion between the target cell membrane and the liposome causes release of the DNA into the cell. For treatment of cancer, a major problem has been low transduction efficiency, particularly prominent in vivo. Nevertheless, clinical trials have delivered promising preliminary results (10-14).

3. Cancer gene therapy approaches

Gene therapy for cancer can be divided into at least six different categories: a) mutation compensation; b) molecular chemotherapy; c) genetic immunopotentiation; d) genetic modulation of resistance/sensitivity; e) oncolytic agents and f) antiangiogenic gene therapy. The goal of mutation compensation is correction of a crucial molecular change within cancer cells. For example, mutations of tumor suppressor genes such as p53 or BRCA1 or overexpression of oncogenes such as erbB-2 are major targets for replacement or inactivation, respectively (15,16). Molecular chemotherapy, also known as suicide or prodrug gene therapy, is the selective delivery or expression of genes encoding a prodrug-activating enzyme for tumor cell eradication. Approaches tested in the clinic include herpes simplex virus thymidine kinase (HSV-TK) and Echerichia coli cytosine deaminase (E.coli CD), which convert non-toxic prodrugs (e.g. ganciclovir for HSV-TK or 5-fluorocytosine for E.coli CD) into potent cell poisons. Lateral diffusion of the activated drug into untransduced neighboring cells causes additional cell killing and is described as the ‘bystander effect’. This helps alleviate the daunting task of transduction of each tumor cell (17). In contrast, genetic immunopotentiation efforts involve the modification of either immune or tumor cells to augment immunological recognition of neoplastic cells (18). In addition, investigators have utilized a variety of strategies to modify resistance or sensitivity of cells for chemotherapy or radiation in order to enhance the therapeutic index (19,20). Oncolytic viruses, such as conditionally replicating adenoviruses (CRAds), take advantage of tumor specific changes, which allow preferential replication in and subsequent death of tumor cells. Finally, antiangiogenic gene therapy targets the development of new vessels in tumor tissue thus inhibiting tumor growth. The emphasis of this review will be on mutation compensation, molecular chemotherapy and oncolytic agents.

4. Adenovirus for gene therapy

Adenoviruses are double-stranded DNA viruses whose major capsid components are hexon, penton and fiber. Adenoviral infection is mediated by binding of the knob region, located at the carboxy terminus of the fiber, to its corresponding receptor, which is the coxsackie-adenovirus receptor (CAR) for most serotypes. Binding is followed by interaction between cellular integrins and an arginine-glycine-aspartic acid motif (RGD-motif) located at the penton base. This binding leads to formation of endosomes and viral internalization. Subsequently, the adenoviral DNA is transported to the nucleus and adenoviral protein synthesis, or in case of non-replicating Ads, transgene expression begins. Adenoviral DNA is not integrated into the host genome, thereby resulting in a low risk of mutagenesis. Nevertheless, the limited duration of gene expression may render Ads less desirable for the therapy of hereditary diseases, where long-term expression is needed, but is adequate for cancer gene therapy approaches, where the purpose typically is to kill the target cells. Infection is not dependent on cell cycle phase; therefore, both cycling and non-dividing cells are infected. Importantly, a most appealing feature of Ad for cancer therapy is its unparalleled capacity for gene transfer and expression in vivo. Further, production of high titers of cGMP Ad, necessary for clinical trials, is well established. Adenoviral infection of tissues is determined chiefly by the degree of CAR expression (21-34). The natural tropism of intravascular Ad results in accumulation mainly in the liver, spleen, heart, lung and kidneys (35-39). Tissue macrophages, such as Kupffer cells of the liver, have a major role in clearing Ad from blood. Although CAR is expressed ubiquitously on most normal epithelial tissues, lack or down-regulation of CAR has been reported for various tumor types and may be associated with tumor aggressiveness and could be a ubiquitous phenomenon (21-24,30-32,34,40-49) (Anders et al, Proc Am Assoc Cancer Res 42: 703, 2001). Also, recent findings suggest a connection between CAR function and cell adhesion, perhaps associated with a tumor suppressing effect (31,32), since CAR may be a transmembrane component of tight junctions (33). Furthermore, it has been suggested that CAR expression correlates inversely with the tumor stage (31), and that an over-activity of the RAS-MAPK pathway, found for many tumor types, may cause down-regulation of CAR (Anders et al, Proc Am Assoc Cancer Res 42: 703, 2001).

Due to the broad tropism of adenoviruses, targeting to tumors could be useful. Two principal means for achieving this goal exist: a) transcriptional targeting; and b) transductional targeting. Transcriptional targeting involves genetically limiting the expression of the introduced gene to specific tissues through the use of the promoter sequences of genes upregulated in these tissues (50-52). These regulatory sequences are referred to as tissue-specific promoters (TSPs) (53). Introducted genes, under the control of a TSP, are preferentially expressed in tissues that activate the TSP (54).

Transductional targeting involves the chemical or genetic modification of adenoviruses, redirecting its tropism from the native receptor, to a new one preferentially expressed on target cells. An ideal retargeting strategy would involve blocking binding to CAR while introducing a new tropism to
a tumor associated receptor or cell-surface marker. Recent advances in the understanding of adenovirus biology have led to many significant achievements in these areas.

5. Cancer trials with adenoviral vectors

Adenoviruses are currently the most common vector system for clinical gene therapy trials. By the end of the year 2001, more than 600 clinical trials were approved (55,56). Most were phase I or phase II, which means their primary goal is determining the safety of the agents (phase I) or their potential efficacy (phase II). Genetic immunotherapy has been the most commonly tested clinical approach. Adenoviruses may be ideal vaccination vectors (57-61), since they are strongly immunogenic and combine both safety and efficacy.

In addition to genetic immunotherapy, cancer trials involving adenoviral vectors can be divided into three main groups: a) suicide gene therapy; b) gene replacement; and c) receptor targeting. Suicide gene therapy typically features introduction of a prodrug-converting enzyme. Phase I trials have been performed for the treatment of glioma (62-64), ovarian cancer (65-67), prostate cancer (68,69) and mesothelioma (70). Interestingly, when glioma patients were resected and randomized into Ad-HSVtk/ganciclovir, retrovirus-HSVtk/ganciclovir or control groups, overall survival was significantly improved in the Ad-HSVtk/ganciclovir group (62). Several trials combine suicide gene therapy with common treatment options. Ten patients with recurrent ovarian cancer underwent secondary debulking followed first by intraperitoneal suicide gene therapy with an Ad coding for HSVtk and then intravenous ganciclovir administration and topotecan chemotherapy. When the study was published, 3 out of 10 patients were still alive with a follow-up between 30 and 31 months (71). In another phase I/II study, adenoviral suicide gene therapy with or without hormonal treatment was combined with radiotherapy for prostate cancer. The aim of this study was the expansion of the therapeutic index of radiotherapy (72). No additional toxicity was noted and safety of the approach could be demonstrated. Two years after treatment, no significant long-term toxicity was detected. In the group with low-risk patients, negative biopsies and lack of metastases was seen at 21 months (Aguilar et al, Proc ASCO 21: 7, 2002).

With regard to gene replacement strategies, p53 has been a major target in phase I and II trials (73-78) (Pisters et al, Proc ASCO 20: 699, 2001; Hao et al, Proc ASCO 20: 1045, 2001; Muller et al, Proc ASCO 20: 257, 2001; Pagliaro et al, Proc ASCO 20: 799, 2001; Buller et al, Cancer Gene Ther 9: 553-566, 2002). Perhaps the most encouraging results were seen when Ad was given intra-tumorally in combination with chemotherapy (79). Another tumor suppressor gene used in a phase I study is mdm-7. Ten patients with solid tumors were injected intratumorally followed by excision of the lesions, which allowed demonstration of transgene expression (Cunningham et al, Proc ASCO 21: 23, 2002). Receptor targeting has been endeavored mainly for ovarian cancer with anti-erbB2 as the target (80,81). None of these studies showed dose-limiting side effects, even with a viral dose of 7.5x10^11 VP daily for 5 days (Buller et al, Cancer Gene Ther 9: 553-566, 2002). Therefore, it can be concluded that cancer gene therapy with replication deficient adenoviral vectors is safe and although evidence of gene transfer in general has been variable, in some cases there is evidence of efficacy. A comprehensive analysis of adenovector trial trials has been published (49).

6. Transcriptional targeting

A promoter is the component of a gene that is involved in binding of the RNA polymerase, required for initiation of mRNA transcription. Further, the promoter is activated by transcription factors presented under tissue-specific control. Therefore, in order for a promoter to be activated in a particular tissue type, that tissue must express specific factors that recognize the promoter. A number of TSPs have been studied for cancer gene therapy, but some promoters lack sufficient activity, specificity, or both. Therefore, recent research has focused on rigorously evaluating candidate promoters with regard to these attributes.

Tumor-specific promoters. One of the earliest tumor-specific promoters explored for cancer was the carcinoembryonic antigen (CEA) promoter, expressed in most gastric, pancreatic, and lung cancers (82). This promoter was used to drive HSVtk expression and CEA-negative cell lines were resistant to ganciclovir therapy while CEA-positive cells were 1000 times more sensitive (83). Upon intraperitoneal injection into mice bearing CEA-expressing tumors, significant regression could be noted (83). Importantly, a significant bystander effect was reported (82,84). An Ad carrying either lacZ or CD under the CEA promoter showed specific expression in tumor xenografts and was able to increase survival time (85). Also, intravascular administration of an Ad employing the CEA promoter showed little toxicity in the normal liver (86). For possible treatment of hepatomas, the promoter of the α-fetoprotein (AFP) has been investigated. When an Ad employing this promoter was injected subcutaneously into hepatomas in vivo, tumor regression was noted (87).

For treating gynecological cancers, a number of promoters have been explored. The L-plastin promoter (LP-P) was used to transcriptionally control the expression of lacZ in ovarian and breast cancer cell lines, and was compared to the ubiquitously expressed cytomegalovirus (CMV) promoter (88). Expression was observed in tumor cell lines and ascites samples with both promoters, but little activity was seen in normal human skin fibroblasts and normal peritoneum with the LP-P. Another report on LP-P showed specific expression of lacZ and CD in ovarian and bladder cancer cell lines when compared to the CMV promoter (89). Over three-quarters of human epithelial ovarian carcinomas express the DF3 protein, while normal peritoneal mesothelium does not (90). The DF3 promoter showed ovarian cancer-specific activity when driving the expression of BAX in vitro (91). Upon intraperitoneal injection into ovarian tumor-bearing nude mice, BAX expression was most prominent in tumor tissue and greater than 99% eradication of tumor explants was reported. The cyclooxygenase-2 (Cox-2) promoter has also been investigated in ovarian tumor cell lines, along with the midkine (MK) promoter. Both promoters were activated in a panel of ovarian cancer cell lines, as well as, ovarian primary
Figure 1. (A), Suicide gene therapy: after infection of cells with a vector coding for HSVtk, administration of ganciclovir causes cell death in infected cells followed by cell death in surrounding cells due to the bystander effect. (B), Mutation compensation: expression of a wild-type p53 gene in cells with mutated version of this gene causes cell death selectively in cells with former mutated gene. (C), Immunopotentiation: the patient's own cells are extracted and infected in vitro with an interleukin encoding vector, followed by retransfusion of the transfected cells back into patient, causing an immune response to the tumor.
tumor cells, with a reduced level of activity in normal primary mesothelium and liver (92).

The Cox-2 promoter has also been explored in the context of gastric carcinomas. The activity profile of the promoter correlated to the Cox-2 RNA status of gastric carcinoma cell lines, and upon intravascular injection, liver expression with the Cox-2 promoter was lower than with the CMV promoter. The promoter was sufficiently active to cause tumor cell killing when driving HSVtk in Cox-2 positive cell lines but not negative lines. In vivo administration resulted in less liver toxicity with the Cox-2 promoter versus the CMV promoter (93).

TSPs have been studied for their capability of controlling gene expression in metastatic tumors. Osteocalcin (OC) is a bone protein expressed in osteotropic tumors and differentiated osteoblasts, as well as, numerous solid tumors, including osteosarcoma and prostate cancer (94). An Ad utilizing the OC promoter to drive HSVtk expression in prostate cancer cells resulted in destruction of tumor cells in vitro and in subcutaneous or bone tumor xenografts. Interestingly, tissue-specific toxicity was seen in prostate bone metastases perhaps due to the disruption of the cellular communication between the bone stroma and the prostate cancer, possibly due to cotargeting of the regenerating bone and tumor. A phase I clinical trial is ongoing (68).

The secretory leukoprotease inhibitor (SLPI) gene is expressed in several different carcinomas, including ovarian cancer. Its expression in normal organs, such as the liver, is low (95). Therefore, the SLPI promoter was utilized to drive transgene expression in ovarian cancer cell lines and primary tumor cells isolated from patient samples (96). The promoter was activated in both cell lines and primary tumor cells in an Ad context in vitro. A murine orthotopic model of peritoneally disseminated ovarian cancer was used to demonstrate high tumor gene expression versus low liver expression with the SLPI promoter, and that Ad-delivered HSVtk under the control of the SLPI promoter is able to increase survival in combination with ganciclovir (96). Further TSPs have been studied with promising preclinical results (97-101).

**Tumor vasculature-specific promoters.** Targeting the endothelium of tumors may be amenable to gene therapy. This tissue is commonly independent of tumor type and is more easily accessible to intravascular vector administration. Also, endothelial cells (EC) are not malignant and thus are less sensitive to selection pressure and rarely gain resistance to treatment (102). E-selectin expression is minimal in normal blood vessels but high in the capillaries of tumors and the promoter was used for driving gene expression in an Ad. Upon infection, EC cell lines expressed high levels of reporter gene expression, while non-EC cell lines showed low expression. The addition of TNF-α, an inducer of the promoter, further increased the E-selectin’s activity (103). The murine preproendothelin-1 (PPE-1) promoter was also used as a TSP for adenoviral-mediated delivery to EC cells. Systemic administration to lung tumor-bearing mice resulted in gene expression in the new vasculature of primary tumors (104).

**Treatment responsive promoters.** Another strategy for cancer gene therapy involves restricting gene expression with a conventional form of treatment, such as chemotherapy or radiation. For example, the early growth response gene 1 (EGR-1) promoter, which is radiation inducible, has been used as a TSP for the specific expression of lacZ and HSVtk in glioma and hepatocellular carcinoma cells. Radiation-induced transcription of EGR-1 in these cells was accomplished with relatively low doses (105,106).

TSPs have the potential to decrease the toxicity of gene therapy for cancer and represent a powerful tool for the specific targeting of transgene expression to neoplastic cells. However, they do not increase the efficacy of Ad infection since viruses are dependent on CAR for entry. By combining transcriptional targeting with infectivity enhancement, improved vectors can be developed.

**7. Transductional targeting**

Transductional targeting strategies have the potential to increase gene transfer to target tissues and prevent sequestration to non-target tissues. There are two primary means of transductional targeting: a) genetic; and b) physical. These are sometimes referred to as one-component and two-component targeting, respectively. The former involves the genetic modification of adenoviruses to incorporate ligands, which recognize specific cellular receptors, and/or block native receptor binding.
Genetic transductional targeting. Several areas exist in adenoviruses that are amenable for genetic insertion of ligands. One of these is the HI loop of the fiber, which was used as an insertion site for an integrin binding RGD-4C motif (40,107). RGDTKSSTR is an RGD-4C modified adenoviral vector containing HSVtk for molecular chemotherapy and the human somatostatin receptor subtype-2 (SSTR2) gene for non-invasive imaging (108). The RGD-4C modification allowed enhanced infectivity of ovarian cancer cell lines and primary ovarian tumor cells. This enhancement was also observed in the presence of malignant ascites. Further, clinical treatment was mimicked by administering RGDTKSSTR intraperitoneally, in the presence of malignant ascites from ovarian cancer patients, to mice with disseminated ovarian cancer. A significant survival advantage was seen in comparison to an isogenic non-RGD-4C virus and other controls. Importantly, the virus could be non-invasively imaged in vivo for more than 2 weeks (109). This approach is now undergoing clinical evaluation with ovarian cancer patients with peritoneally disseminated disease (110).

In another study, an adenovirus containing luciferase and the RGD-4C modification was analyzed in comparison to a virus without the modification (111). In stringent preclinical substrates, including primary ovarian tumor cells (in the absence and presence of neutralizing antibodies) and in a murine model of ovarian cancer, increased gene expression was observed with the RGD-modified virus.

Because adenovirus has a propensity to localize to the liver, with potential for hepatotoxicity, untargeting the liver is an important goal (49). In this regard, viruses were created, which lack binding to CAR and to cellular integrins since both play a significant role in liver uptake. These viruses could be shown to be effectively reduced in their liver transduction capability (112).

Fiber chimerism. Not all adenoviral serotypes bind CAR and therefore have a different tropism. Fiber chimerism involves replacing the fiber or knob domain with a knob of a different serotype (113,114). For example, the Ad3 serotype has a yet unidentified receptor and a chimeric Ad5/3 vector increased gene transfer by up to 291-fold in ovarian cancer cell lines and primary tumor cells (115). Importantly, the biodistribution and murine toxicity of the chimeric and RGD modified viruses was not significantly different from the Ad5 based vectors, which have proven safe in clinical trials.

Physical transductional targeting. Physical targeting involves complexing adenovirus with a bispecific molecule, which both blocks binding to CAR and redirects the virus to new specific receptors. A major advantage to using this form of targeting is the abundance of antibodies and ligands that be utilized. For example, basic fibroblast growth factor (FGF2) has been conjugated to the Fab fragment of an anti-knob antibody. This Fab-FGF2 conjugate was able to increase
transgene expression by more than 9-fold in ovarian cancer cell lines, and upon intraperitoneal injection of HSVtk viruses into tumor-bearing mice, survival was prolonged from 36 to 44 days (116). In addition decreased hepatic toxicity was demonstrated (117). These findings led to a clinical trial 44 days (116). In addition decreased hepatic toxicity was demonstrated (117). These findings led to a clinical trial protocol where an Fab-FGF targeted Ad coding for HSVtk will be administered intraperitoneally to ovarian cancer patients with peritoneally disseminated disease (Hemmiink, unpublished data). This strategy resulted in increased survival also in a melanoma xenograft mouse model (117). Other Fab-ligand conjugates have been employed in a similar manner with promising results (22,41,49,118-122).

Another transductional targeting modality is the sCAR-ligand conjugate. This conjugate contains the secretory ecto-domain of CAR fused with a targeting ligand. For example, epidermal growth factor (EGF) has been conjugated to sCAR and used to target adenoviruses to cancer cells that overexpress the EGF receptor (123,124). A dose-dependent increase of luciferase expression was reported in cell lines with both a replication-defective and an oncolytic adenovirus (125). When infected cells were infected subcutaneously, only 1% of targeted adenoviral-infected cells were needed to inhibit tumor growth and only 5% were needed to heal tumors. sCAR has also been fused to a single-chain antibody specific for the c-erbB-2 oncoprotein. Again, significant increases in gene transfer were observed (Kashentseva et al, Cancer Res 62: 609-616, 2002).

Although two-component targeting has shown promising results for retargeting adenovirus to new receptors, it may present some disadvantages. Two-component gene delivery systems have more complex pharmacodynamics and -kinetics, and their stability in humans has not yet been studied. Therefore, one-component systems may be more easily applicable to human cancer gene therapy trials.

8. Conditionally replicating adenoviruses (CRAWs)

Although non-replicating first generation adenoviruses have provided high in vitro and in vivo transduction rates and good safety data, clinical trials have suggested that the single agent anti-tumor effect may not be sufficient for all treatment approaches. Although tumor targeting and infectivity enhancement have improved preclinical results dramatically, it is possible that non-replicating agents may require multiple rounds of re-administration. Viruses that replicate and spread specifically inside the tumor have been suggested as a way to improve tumor penetration with an additional benefit of local amplification of effect. To this end, CRAWs have been explored. These viruses are genetically modified to take advantage of tumor specific changes that allow preferential replication of the virus in target cells (126-129). The viral replication cycle causes oncolysis of the cell, resulting in the release of the newly generated virions and subsequent infection of neighboring cells. Thus, the anti-tumor effect is not delivered with a transgene but by the actual replication of the virus. In theory, the oncolytic process continues as long as target cells for the virus persist. There are two main ways to control viral replication. One method is the control of replication regulators, such as the viral early gene E1, with TSPs. The other method involves introduction of deletions in the viral genome that require specific cellular factors to compensate the effects of these deletions. Further, both approaches can be combined with the potential for increased specificity.

Various promoters have been used to control viral replication (130-134). Typically, the TSP is placed to control expression of E1A, the crucial regulator of Ad replication. PSA and kallikrein-2 have been used in the context of prostate cancer and AFP has been used for hepatoma (130,132,135). When the DF3/MUC1 promoter was used to drive expression of E1A in breast cancer cells, replication at levels comparable to wild-type Ad was seen, while in negative cell lines, replication was decreased. A single intratumoral injection of this TSP-controlled CRAW resulted in significant reduction of tumor burden (133). For the treatment of pediatric solid tumors, a CRAW featuring the midkine (MK) promoter was utilized. This CRAW achieved specific and high levels of replication in MK-positive cell lines and was able to induce tumor cell killing in vitro (136). To further increase the oncolytic effect, transgenes for cytokines or prodruk-activating enzymes have been included (133,137,138). The latter approach could also allow abrogation of virus replication in case of toxicity.

Heretofore, two approaches have been utilized for creation of deletion type CRAWs. The first one was ONXY-015, which has two mutations in the gene coding for the E1B 55-kDa protein. The purpose of this protein is binding and inactivation of p53 in infected cells, for induction of S-phase, required for virus replication (139-142). Thus, this virus should only replicate in cells with mutated p53, a common feature in human tumors (139). Initial studies suggest that this agent replicates more effectively in tumor than in normal cells (126,141,143-145). Unfortunately, the function of EIB 55-kDa is not limited to p53 binding (145), which causes inefficient replication of the virus compared with the wild-type adenovirus (139,144,146). In addition, recent studies have suggested replication of ONXY-015 in nontarget normal tissue (Wadler et al, Proc Am Assoc Cancer Res 43: abs. 1097, 2002).

The second group of deletion mutants have a 24 bp deletion in the constant region 2 (CR2) of the E1A gene (141,147,148). This domain of the E1A protein is responsible for binding the retinoblastoma tumor suppressor/cell cycle regulator protein (Rb), which allows Ad to induce S-phase entry (149). Therefore, viruses with this type of deletion have reduced ability to overcome the G1-S checkpoint and replicate efficiently only in cells where this interaction is not necessary, e.g. tumor cells defective in the Rb-p16 pathway (147,150). Appropriately, this pathway seems to be inactive in almost all human tumors (151). It has been shown that replication of CR2-deleted viruses is attenuated in non-proliferating normal cells (147,150). Interestingly, abrogation of replication was also demonstrated when Rb was re-introduced into otherwise permissive cells (147).

Adenoviruses with mutations in CR1 and CR2 domains of E1A were found to replicate selectively in tumor cells expressing human papillomavirus E6 and E7 oncoproteins (152). Further, CRAWs featuring an additional mutation in the binding site for p300, cell cycle regulator, have been described (153). These viruses were further modified by replacing the natural promoter of E4 by a TSP (154).
9. Targeted conditionally replicating adenoviruses

Non-targeted CRAds infect cells mostly based on their CAR-level, which may be highly variable in clinical cancers. Nevertheless, even such first generation CRAds have shown evidence of clinical utility (49). These initial successes suggest that if efficiency of infection and specificity of replication of the agents could be enhanced, further improvements in clinical efficacy could be gained. This is corroborated by demonstration of the close association between infectivity and oncolytic potency (48,125,155). Consequently, infectivity enhanced CRAds have been constructed, with impressive preclinical efficacy. Ad5-D24RGD features an RGD-4C modification of the fiber (148,156), and displays similar oncolytic potency to wild-type virus in ovarian cancer cells. Further, this virus is able to replicate in ovarian cancer primary cell spheroids and results in significantly prolonged survival in an aggressive orthotopic ovarian cancer model (156). These developments have led to clinical trial protocols, where glioma and ovarian cancer patients will be treated with the Ad5-D24RGD virus (Hemminki, unpublished data).

A major problem in assessing CRAd efficacy and safety preclinically is the lack of an appropriate animal model. Human serotype Ads or CRAds do not replicate productively in commonly used animal models. Therefore, meaningful safety data is difficult to obtain, and efficacy data may be skewed due to deficient immune responses in xenograft models.

10. Cancer trials with CRAds

The first cancer trials with replicating adenoviruses were done shortly after the virus was detected in the 1950s. Various serotypes of wild-type adenoviruses were applied intra-tumorally, intra-arterially, intra-tumorally and intra-arterially in combination or intravenously into patients with cervical carcinoma. The overall response rate, measured as formation of necrotic areas, was 65% (157). The authors do not describe severe side effects, but relapse was common. The first trial with a CRAd started nearly 50 years later and was predicated on the development of viral agents where replication was more selective for the target tissue (Table I).

The first CRAd used in clinical trials was the ONYX-015 virus. In a limited number of biopsy specimens, replication of the agent in tumor cells was demonstrated and the safety data was excellent (158,159). Since oncolysis can be synergistic...
to the effects of radiation or chemotherapy (140,141), it seems obvious to combine CRAd approaches with these treatments. Recent clinical studies show promising results and impressive safety data (159-161). The most exciting data is from a phase II study utilizing a combination of intratumorally injected ONYX-015 with simultaneous cisplatin and 5-fluorouracil chemotherapy in 30 evaluated patients with advanced stage head and neck cancer (160). Eighty-three percent of the tumors responded; in 63% the response was objective with more than 50% tumor size reduction. Partial response was shown in 36%, and 27% showed a complete response. This is of interest since head and neck cancer is often refractory to available treatments. Though patients had several tumors, only one was chosen for viral injection. In the follow-up, the non-injected tumors relapsed more frequently than the injected tumors. Similar data were obtained in another study with a similar approach (161). A phase III trial with ONYX-015 is currently under way (161).

Another CRAd used in clinical studies is CV-706, where viral replication is under the control of the PSA promoter (162). In a phase I study with 20 patients, very good safety data could be obtained. In addition, preliminary evidence of viral replication and anti-tumor effect could be observed.

11. Model systems - primary cells, spheroids

The preclinical development of novel approaches with replication competent viruses is limited by assay substrates. As a result of adaptation to growth in vitro, established cell lines may have undergone geno- and phenotypic changes, resulting in a disconnect between data obtained from cell lines and clinical specimens. The translational approach from the bench to the clinic requires models that reproduce the patient phenotype as closely as possible. In this regard, the isolation of pure cancer cells from patient samples is an attractive concept. A method for the isolation of primary ovarian tumor cells from the ascitic fluid of patients diagnosed with ovarian adenocarcinoma has been described. Primary neoplastic populations of up to 96% purity have been isolated in this manner (163). Unfortunately, primary tumor cells are difficult to analyze ex vivo for virus replication due to their limited viability in culture (Casado et al., Proc ASCO 20: abs. 253, 2001), which is approximately 7 days and too short for typical assays. In addition, monolayers may not reflect virus dissemination characteristics appropriately as most human solid tumors are three dimensional. Therefore, model systems based on three-dimensional aggregates or spheroids of un-passaged and purified ovarian cancer cells have been developed to overcome these obstacles (164). Spheroids were viable for more than 4 weeks and allowed quantitation of CRAd replication.

12. Future prospects

With increasing understanding of the molecular reasons for cancer, gene therapy has emerged as a logical potential therapeutic option. Following initial optimism and subsequent disappointment, rigorous preclinical and basic research is now beginning to result in clinically feasible approaches. Considering that the immunogenicity of Ad, while useful for mounting an immune response to the tumor, has the potential for severe or even fatal toxicity when large doses are administered (165), it is important to note that safety has been extremely good in cancer trials. Further, there are exciting preliminary results suggesting efficacy (62,79,160). Importantly, the feasibility of gene therapy for correction of disease phenotypes has been demonstrated in other fields of medicine (166-169). What these successes shared in common is the rational approach investigators took for incrementally developing their gene delivery tools. Thus, the clinical breakthroughs were based on advances in vector development. It remains to be seen if consistent improvements in cancer gene therapy reagents can eventually deliver similar clinical success. With regard to treatment of cancer, the key issue remains improving tumor transduction. Fortunately, we have increasingly powerful tools to address this problem, including replication competent systems, infectivity enhancement and targeting strategies.

13. Conclusions

Current treatment options are limited for many types of human carcinomas and especially therapy of advanced disease is often palliative. In the recent decades, we have seen dramatic improvements in the treatment of patients with early disease, due to innovative chemotherapy regimens, refined radiotherapy and advanced operative techniques. However, there has been no real benefit for advanced stage cancer patients. Thus, there is a need for new and innovative therapeutic approaches, which may be able to overcome these limitations. Although gene therapy has proven to be a potential candidate, due to preliminary evidence of clinical utility, there are still obstacles to overcome. Considering the synergistic or additive effect many gene therapy approaches have with existing treatments such as radiation or chemotherapy, it is likely that the first routine clinical applications will be combination treatments with existing modalities. Further, it is noteworthy that the side effect profile of adenoviral treatments have little or no overlap with radiation or chemotherapy.

Acknowledgements

This study was supported by Deutsche Forschungsgemeinschaft Grant BA2076/1-2, the Damon Runyon-Walter Winchell Cancer Research Fund, the Sigrid Juselius Foundation, the Emil Aaltonen Foundation, the Maud Kuistila Foundation, the Finnish Medical Foundation, the US DOD PC991018, The Lustgarten Foundation LF043, the NIH (P50 CA83591, P50 CA89019, R01 CA83821).

References


1170


1999.

1997.

1996.

1995.

1994.

1993.


1990.

1989.


1987.

1986.


