CONFERENCE PAPER

Adenovirus as vehicle for anticancer genetic immunotherapy

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Adenoviruses (Ads) are in the forefront of genetic immunization methods being developed against cancer. Their ability to elicit an effective immune response against tumor-associated antigens has been demonstrated in many model systems. Several clinical trials, which use Ad as vehicle for immunization, are already in progress. Preclinical

studies have also demonstrated the efficacy of combining Ad-mediated immunization with adjuvants such as chemotherapeutic agents and cytokines. Issues related to sero-prevalence and safety of Ads, however, continue to pose a challenge and need to be addressed.

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Introduction

In the past decade, a significant amount of knowledge about the mechanisms regulating the immune response has been accumulated. Several tumor-associated antigens (TAAs) have been cloned and peptides derived from these antigens, which elicit major histocompatibility complex (MHC)/human leukocyte antigen (HLA)-restricted immune responses have been identified. In addition, technical advances have increased our ability to detect and characterize several immunological interactions involved in the immune response.

In the last few years, these achievements have fostered intense research activity to develop strategies to deliver TAAs as potent anticancer vaccines in humans. Studies in animal models and clinical trials have proven the ability of genetic immunization to raise an effective immune response against the TAA-bearing tumor cells. Among the vehicles adopted for gene transduction, Adenovirus (Ad)-based vectors have rapidly gained importance in cancer therapy, and a number of these vectors carrying TAAs are already being tested in the clinical setting.¹ This review will briefly describe the significance of genetic immunization in eliciting an immune response against cancer (self) antigens and focus on the promising use of Ads as vehicles for cancer immunotherapy.

Immune response against cancer

Vaccines against microbial or viral pathogens elicit a protective and/or therapeutic immune response against foreign macromolecules. By contrast, the development of a vaccine against tumors has to account for cancer cells being derived from the host, and that most of their macromolecules are self-antigens present in normal cells. Therefore, in principle, it was considered debatable if the immune system could be induced to precisely target cancer cells without harming normal cells. However, it

was always empirically recognized that a proportion of immunocompetent cancer patients showed spontaneous regression whereas immunocompromised hosts showed a higher cancer incidence. This implied that tumor cells were recognized by the immune system and could be eliminated by the immune response.

Similarly, mice with defined immunological defects exhibited greater susceptibility to spontaneous and induced tumors, with many of these tumors rejected if transplanted into normal hosts.^{2,3} Adoptive transfer of cytotoxic T lymphocytes (CTLs) demonstrated the critical role of T-cell-mediated immunity in the eradication of transplanted tumors in mice.⁴ Lymphocytes and interferon- γ (IFN- γ) were shown to collaborate in protecting mice against development of carcinogen-induced sarcomas and spontaneous epithelial carcinomas.⁵

Discovering the crucial role played by T cells in antitumor immunity prompted the identification of T-cell antigens. Many investigators then developed reverse immunology approaches to identify tumor antigens that are bound by MHC molecules.⁶ These antigens have been characterized for their reactivity with autologous tumor-specific T cells isolated from cancer patients.⁷ In 1997, Sahin *et al* discovered that sera from patients contain antibodies recognizing the autologous tumor, thus revealing the existence of a natural humoral response directed against the tumor.⁸ This finding was exploited to effectively identify B-cell tumor antigens by screening tumor cDNA expression libraries with the autologous sera. A large part of the clones singled out by this approach (SEREX, Serological Identification of Recombinantly Expressed Tumor Antigen) turned out to be T-cell antigens as well. Concomitantly, innovative technologies dramatically improved the set of tools to detect with high sensitivity molecular interactions involved in immunological responses. This improved our ability to characterize and decipher complex events, as T-cell activation or antigen recognition. T-cell assays (tetramers, intracellular cytokine flow cytometry and ELISPOT assay) have emerged as first-line methods for monitoring T-cell induction during vaccination.⁹ They provide the means

to identify the type and quantitatively measure the cell-mediated and humoral immunity (CMI and HI) induced by different immunization protocols.

Genetic vaccination and gene delivery vectors

Several vaccination strategies employ nongenetic methods (ie cells, proteins, or peptides) or genetic methods (ie naked DNA, virus-mediated delivery) to elicit an immune response against a single or multiple TAAs. Whole-cell vaccines using allogeneic, autologous, or gene-modified tumor cells have been the most popular nongenetic approach. These vaccines have been tested in combination with adjuvants for several years with modest results.¹⁰ The immune response was limited and most of the cancer trials showed a clear lack of antitumor activity.¹¹ The most likely reason for their low success rate is that tumor cells are poor antigen presenters *per se*.

A second group of nongenetic vaccines include those using whole proteins or peptides.¹² Administration of antigenic peptides derived from different cancer antigens used either alone or in combination with cytokines (IL-2, IL-12, granulocyte macrophage colony stimulating factor (GM-CSF)) and/or adjuvants (ie incomplete Freund's adjuvant) have led to the induction of strong specific CD8⁺ T-cell responses.^{13,14} Enhanced immunologic effects were observed in clinical studies using dendritic cells (DCs) loaded with peptides derived from melanoma antigens (ie MAGE-3, Melan A/MART-1) that were related with disease stabilization and regression of metastatic melanoma lesions in vaccine trials in single patients.^{15,16} Preclinical and clinical studies have shown that tumor-derived peptides have an enhanced ability to bind to MHC molecules and lead to enhanced immune responses.¹⁷ The limitation of this methodology is that it requires knowledge of the HLA haplotype and antigen for the vaccine to be effective on each individual. In addition, the number of antigens that can be administered by this method is also limited as there may be antigen-variant escape mutants of the tumor.

Recognition of the critical role played by the CMI response to effectively control tumor growth and spread of metastases supported the development of genetic vaccine strategies. Here, the gene coding for the target antigen is expressed intracellularly, thus having the potential of inducing a specific T-cell response. As the cell machinery ensures correct folding and post-translational modifications, these approaches can induce antibodies against native antigen structures. Several different gene delivery vehicles have been designed and tested through diverse procedures with the goal of identifying the most effective routes for delivering the selected TAA to the immune system and inducing CD8⁺ and/or CD4⁺ T-cell responses against defined cancer antigens. These delivery vehicles largely overlap with those employed for gene therapy studies and essentially fall into two categories –nonviral and viral-mediated delivery.

In the early 1990s, it was reported for the first time that injecting plasmid DNA expressing a gene in the mouse muscle results in the synthesis of the gene-encoded protein and generates an immune response. Mice immunized with DNA encoding an internal conserved protein of influenza A, nucleoprotein (NP), developed both NP-specific antibodies and MHC class I-restricted CTL.¹⁸ Similarly, intramuscular immunization of mice

with a DNA plasmid encoding hemagglutinin (HA), a surface glycoprotein of influenza A, was shown to generate antibody responses.^{19,20} The technology proved successful as a mean to elicit an immune response against the protein encoded by the gene which could be a TAA.²¹ DNA-based vaccination is highly attractive as, in most cases, it does not raise an immune response against the vehicle and lacks toxicity. The vaccine can be produced on a large-scale through reproducible and simple procedures. Naked DNA plasmid vaccines elicited measurable response against the target TAA and proved broadly effective in mouse models.^{22–24} However, preclinical studies in larger animals revealed an unexpected drop of immunogenicity and early clinical results confirmed the need for increased potency.^{25,26} For instance, in different clinical trials against follicular lymphoma and metastatic colorectal cancer, plasmid DNA evoked little or no humoral or cell-mediated response in a majority of the patients.^{27,28} A clear improvement was achieved by following DNA injection into the muscle with an electrical stimulation of defined characteristics under anesthesia.²⁹ This protocol enhances gene expression and increases both T- and B-cell response up to 50-fold, compared to control injection. The tolerability of this treatment, which still awaits a thorough evaluation, deserves a note of caution.

Gene-based vaccination using viral vectors is emerging as one of the most promising approaches.³⁰ This success stems from the intrinsic ability of the virus to initiate immune responses, with inflammatory reactions occurring as a result of the infection creating the danger signal necessary for immune activation. In addition, viruses efficiently transduce a wide range of cells. Several viruses have been modified for use as cancer vaccine vehicles. DNA viruses such as fowlpox, canarypox, modified vaccinia Ankara, Ad, Adeno-associated virus, herpes simplex, and lentivirus have been more popular than RNA viruses such as retrovirus.³¹

Human clinical trials have been initiated (National Cancer Institute Physician's Data Query website) and recent results suggest that viral vectors can stimulate antigen-specific T cells in human subjects as it has been demonstrated with poxvirus (ALVAC) against colorectal cancers.³² The same trials have demonstrated that recombinant viruses are safe in humans, and can break immune tolerance against self and/or weakly immunogenic tumor antigens. In many cases, viral vectors can be grown at high titers and manufactured on a large scale. The efficiency of viral-mediated therapy can be enhanced by adding many different adjuvant therapies, routes of inoculation, and boosting procedures. Safety issues (toxicity, potential replication of competent viruses, etc.) remain a concern.

Ad as a vector for gene delivery

Adenoviral vectors have the potential to become the dominant gene delivery vector.³³ Ads are nonenveloped double-stranded DNA viruses whose capsid is mainly composed of pentons (penton base and fiber monomers) and hexons. The viral cycle begins with the attachment of the fiber to a cellular receptor, which in the case of the widely used Adenovirus serotype 5 (Ad5) is the Coxsackievirus and Adenovirus receptor (CAR) followed by the interaction of the penton base with proteins belonging to the integrin family.³⁴ Ad5 can transduce a

wide range of cells under both resting and proliferating conditions.³⁵ First-generation Adenoviral vectors (FG-Ad) are perhaps the most widely studied vectors. Deletion of the E1 and E3 viral genes generates replication-deficient virus with limited pathogenicity. FG-Ads are stable vehicles which can be easily propagated at high titers (exceeding 10^{11} infectious units/ml) in cells complementing the deleted functions. The 36 kb viral genome can be readily manipulated by homologous recombination to accept cDNA sequences up to 7.5 kb. The viral genome remains extrachromosomal, a state that minimizes the risk of insertional mutagenesis. They have a wide host-range and are currently divided into three genera with further subdivision into species (also termed subgenera or subgroups) A to F. Division of human serotypes, based mainly on immunological criteria, has historically been the basis of classification.³⁶ The *in vivo* distribution of CAR makes it impossible to restrict infection solely to the targeted cells. While widespread distribution of CAR favors virus dissemination, some important therapeutic targets (eg certain tumor cells and smooth muscle cells) are refractory to infection because they express only limited amounts of CAR. Adenoviral vectors with broadened tropism are already in development.³⁷ Since they deliver their genome to the nucleus and can replicate with high efficiency, they are prime candidates for the expression and delivery of therapeutic genes.

FG-Ad are an attractive choice in cancer vaccine therapy. Following transduction of the cell, intracellular expression of the TAA allows processing into both MHC class I and II pathways. This results in a balanced activation of CD4⁺ and CD8⁺ T cells, as well as the induction of antibody responses.³⁸ Therefore, adenoviral vectors can be used in human vaccines to deliver high antigen concentrations, promote effective processing and MHC expression, and stimulate potent cell-mediated immunity. Adenoviral vectors have shown good results in animal models and clinical trials are already in progress. In this review, some data will be reported.

Ad mediated immunization in spontaneous mouse tumor models

Genetic immunization against cancer using sophisticated vaccines has been investigated and in mice some of these treatments have been very effective. Unfortunately, results observed in mice are not always confirmed in clinical applications.³⁹ One of the reasons for this discrepancy is that the transplantable tumor model in mice is too artificial for predicting the outcome in a clinical trial. Transgenic mice that develop spontaneous tumors instead provide a defined model for assessing the effectiveness of vaccination and the significance of the immune response they elicit.⁴⁰ Here, we will briefly describe two examples of Ad treatment in mice spontaneous tumor models.

Initially, recombinant adenoviral vectors became common in the transduction of tumor antigens into DCs because of their high efficacy in inducing both humoral and cell-mediated immune responses.^{41,42} Sharma *et al*⁴³ used a murine model of spontaneous bronchoalveolar cell carcinoma to assess the efficiency of dendritic cells transduced with a reAd5-expressing interleukin 7 (DC-Ad5IL7). These transgenic mice (CC-10Tag), expressing the SV40 large T antigen under the *Clara* cell (nonciliated

epithelial cells of the pulmonary airways) promoter, develop bilateral multifocal pulmonary adenocarcinomas and die at 4 months as a result of progressive pulmonary tumor burden. Injection of DC-Ad5IL7 in the axillary lymph node region weekly for 3 weeks led to a marked reduction in tumor burden with extensive lymphocytic infiltration of the tumors and enhanced survival. These data provide a strong rationale for further evaluation of DC-Ad5IL7 in regulation of tumor immunity and its use in lung cancer genetic immunotherapy.

Another example of an adenoviral vaccine study using transgenic mice that develop autochthonous tumor comes from our recent work.²⁴ We investigated the efficiency of a xenogeneic vaccination in mice using an adenoviral vector expressing p185 (erbB-2/HER2, human homolog of rat *neu* oncogene). Inbred BALB/c mice engineered to overexpress the activated (G664E) *neu* oncogene under the control of the mouse mammary tumor virus promoter (MMTV-LTR; referred to as *neuT* mice) were used as experimental model system.⁴⁴ The transgene is prevalently expressed in mammary glands in these mice. At 3 weeks of age, female *neuT* mice start a process of rapid development of tumors involving all the mammary glands. p185 is a xenogeneic protein in *neuT* mice since it differs in more than 11% of the amino-acid residues when compared to both rat (r-p185) and the endogenous mouse proteins.⁴⁵ We showed that Ad5-mediated immunization induced a much higher response than plasmid DNA followed by electrostimulation. The T-cell response elicited against p185 was only partially crossreactive with the corresponding rat epitopes because of sequence variations. Thus it was not surprising that only a partial impact on tumor development could be detected.

Immunization with Ad5 expressing the rat p185 HER2/*neu* protein induced a strong Th1-skewed humoral and CD8⁺ cell-mediated response. In this case, we observed a significant impact on tumor progression in vaccinated mice. Although strong, this response declined with time indicating that additional immunizations are required to ensure a life-long immunity. However, Ad5 readministration exhibited a weak efficacy because of neutralizing antibodies raised by previous injections.

These observations point toward protocols employing different adenoviral vectors which elicit noncrossreacting antibody response. Alternatively, a response elicited by Ad5 can be effectively sustained by repeated DNA injections, thanks to lack of response against the DNA vehicle. In fact, although DNA injection *per se* is poorly immunogenic, it could sustain response induced by Ad5.⁴⁶ In a symmetric manner, 'priming' by DNA injection elicits a response much greater than that induced by Ad5 alone. This has been demonstrated by DNA in combination with modified vaccinia ankara (MVA).⁴⁷ We predict that a mixed modality immunization, where the response induced by one or multiple Ad vectors is accompanied by a series of plasmid DNA injections with adjuvant cytokines is likely to be an effective vaccination protocol.^{48,49}

Ad mediated immunization in non-human primate models

The search for more spontaneous mouse models specific for each tumor type is a hard but strongly recommended one.

This is important also because there non-human primate models are not available for cancer vaccine at the present time. Most of the current Ad-based treatments are being directly tested in humans after mice immunization studies.

Non-human primates have been mostly used to demonstrate the efficacy of vaccination against non-self-antigens in the context of infectious diseases. Among these studies some have demonstrated the efficacy of adenoviral gene delivery of viral antigens to induce specific immune responses.^{50–52} However, an important study demonstrated the feasibility of using Ad-mediated gene delivery for cancer immunotherapy. Here, the adenoviral vector was used to immunize cottontop tamarins with the Epstein–Barr Virus (EBV) envelope glycoprotein gp340/220 to induce protective immunity against EBV-induced lymphoma. Four tamarins were immunized with three intramuscular injections of Ad5-gp340, one with a nonrecombinant Ad5 alone and one was untreated and used as negative control. Results showed that only the animals that received the Ad5-gp340 developed antigen-specific antibodies, whereas the Ad5 wild-type immunized animals generated only anti-Ad5 capsid antibodies as expected. Following intramuscular and intraperitoneal challenge with a 100% tumorigenic dose of EBV, all the tamarins vaccinated with Ad5-gp340 were protected from developing EBV-induced disease. The animals that received either no vector or Ad5-wt needed to be killed 1 month after challenge due to tumor burden in the lymphnodes. In this model, it appeared that the cell-mediated immune response played a major role in protection from tumor development, as the sera from the immunized animals did not neutralize EBV *in vitro*.⁵³ Shiver *et al* comparatively assessed several vaccine vector delivery systems (plasmid DNA, modified vaccinia Ankara (MVA) virus, and a replication incompetent Ad5 vector expressing the simian immunodeficiency virus gag protein) for their ability to elicit such immune responses in monkeys. They demonstrated that the Ad5 vector elicited the most effective responses when used either alone or as a booster inoculation after priming with a DNA vector.⁵⁴

Clinical studies

Immunomodulatory factors delivered by Ad5 are in advanced phase of several clinical trials. One of the strategies adopted to augment antitumor responses tries to increase immune activation by promoting recruitment and activation of professional antigen-presenting cells (APCs) at the tumor site. GM-CSF is a cytokine capable of enhancing immune response through different routes. On one hand it induces differentiation of bone marrow-derived APCs (macrophages), essential for TAA processing and peptide presentation to T cells.⁵⁵ This cytokine is also able to activate quiescent DCs via GM-CSF receptors. *In vitro*, GM-CSF-differentiated DCs upregulate MHC class I and II molecules, as well costimulatory molecules such as B-7 and ICAM.⁵⁶ For these reasons, GM-CSF gene transduction of autologous tumor cells has been tested in multiple human cancers such as melanoma, prostate, renal cell carcinoma, pancreatic cancer and metastatic non-small-cell lung carcinoma. It is worth highlighting that in all these studies a significant immunological activity has been reported.^{57–62} Other group of clinical trials explore the efficacy of GVAX, a GM-CSF gene-modified tumor

vaccine. To make this vaccine, autologous tumor biopsies were taken from each patient, processed to a single cell suspension by enzymatic digestion followed by overnight transduction with an E1/E3-deleted adenoviral vector encoding GM-CSF. The cells were then irradiated, frozen and injected as needed. Recently a phase I/II trial was performed in which 33 patients with NSCLC, which were refractory to standard treatment, have received GVAX vaccination consisting of $5\text{--}100 \times 10^6$ irradiated tumor cells per dose every 2 weeks for 3–6 doses. The results demonstrated not only that GVAX was safe, but also that three of the 33 patients had shown radiological complete responses that lasted for more than 6 months. All three patients are still alive. This was the first report of such a remarkable achievement in refractory metastatic NSCLC patients with an immunomodulatory therapy and strongly supports additional studies using this strategy.^{63,64}

A novel cancer selective apoptosis inducing cytokine gene, initially called mda-7 (melanoma differentiation antigen), and now known as IL-24 has recently showed promises in a phase I/II trial.⁶⁵ This is an interesting cytokine that appears to be expressed in normal cells and to be lost in melanoma cells, suggesting that it may have minimal risk of toxicity for normal cells.⁶⁶ Its physiological role in the immune system needs to be elucidated. However, IL-24 belongs to the IL-10 family but several studies suggest that it may likely have an antagonistic effect to the anti-inflammatory activity of IL-10. In fact, PBMCs treated with IL-24 secrete high levels of TNF- α as well as IL-6 and IFN- γ and these cytokines are down-regulated when IL-10 is added with IL-24. Results from this phase I/II trial have demonstrated that it is safe and immunohistochemical analyses of the resected tumor after injection revealed protein expression, which correlated with apoptosis.⁶⁷ A phase II clinical trial in melanoma in-transit lesions is planned to assess further the anticancer activity of this gene.

Cytokines as adjuvants

Many studies have looked for cytokines able to improve the effectiveness of cancer vaccination. Unfortunately, the clinical use of recombinant cytokines with immunomodulatory effects in human patients has been disappointing, possibly because of the short *in vivo* half-life of the protein. To overcome this obstacle, cytokines have been delivered as genes using different vectors. Thus, including a cytokine gene in a DNA prime/Ad5 boost protocols yields a potent immune response in mice and partially compensate for antivector immunity.⁶⁸ Several studies have used an adenoviral vector to deliver cytokines.⁶⁹ In many cases, the goal was to optimize an immunoresponse by combining tumor antigen presentation with immunostimulatory cytokine. Chen *et al*⁷⁰ showed that genetic immunization using DCs transduced *ex vivo* with an Ad5 expressing ErbB-2/neu gene induces protective and therapeutic immunity against a breast tumor cell line overexpressing ErbB-2/neu. This protection was further enhanced by cotransducing DCs with Ad5 expressing IL-12. Along the same line, Ad5-mediated IL12 delivery was shown to enhance antitumor effect in a mouse tumor challenge model when used as an adjuvant for human papillomavirus E7 subunit vaccine.⁷¹

In other studies cytokines delivered using an adenoviral vector are successfully associated with other treatments,

as radiotherapy or antiangiogenic treatments.^{72,73} The latter case is described by Gyorffy *et al*⁷⁴ who used intratumor delivery of adenoviral vectors to induce a selective antitumor response by combining murine angiostatin, a potent angiogenesis inhibitor, with murine IL-12, a powerful immunostimulant and angiostatic cytokine. Coinjection of these components strongly increased the tumor regression. These mice were resistant to tumor challenge and developed a strong CTL response.

Alternative and retargeted adenoviral vectors for cancer immunotherapy

Modification of natural Ad5 tropism (retargeting) to increase immunogenicity by targeting DC or other specific cell types is a promising strategy to improve vaccine effectiveness and avoid side effects. To achieve this, many approaches have been adopted. Worgall *et al*⁷⁵ demonstrated that addition of an RGD motif to the Ad5 fiber knob increases the efficiency of infection of DCs and leads to enhanced cellular immune responses against the Ad5-transferred transgene, suggesting that the RGD capsid modification may be useful in developing Ad-based vaccines.

We validated a phage display system for the selection of cell-binding peptides in the context of the Ad5 knob domain and their subsequent incorporation on Ad5 surface for extending viral tropism to infection refractory cells. This phage display system was used to create a library in which a large collection of ligands was surveyed in the knob context. Panning this library on CAR-negative NIH3T3 cells identified clones that bind a novel cell surface receptor on NIH3T3.³⁷ This work shows that some selected fiber mutations dramatically increase the Ad5 transduction efficiency for mouse and human primary immature DCs.

A couple of approaches have been reported that approached the loading of DC *in vivo*. Zhang *et al*⁷⁶ targeted TAA to CD40 receptor. This was achieved by generating an adenoviral vector encoding a fusion protein between an amino-terminal TAA sequence fused to the CD40 ligand (CD40L). Another strategy uses fiber protein from different viral serotypes or viruses called pseudotyping.^{77–79} Mercier *et al*⁸⁰ developed a gene-delivery vehicle in which the potent Ad5 vector was genetically engineered to display the mucosal-targeting $\sigma 1$ protein of reovirus type 3 Dearing (T3D). Ad-T3D $\sigma 1$ transduces primary cultures of human DCs substantially more efficiently than does Ad5.

Issues related to adenoviral vaccine development

The major limitation of Ads in cancer immunotherapy is the fact that they are ubiquitous and constantly infect human beings. Consequently, the majority of normal individuals have neutralizing serum antibodies against the most common Ad serotypes. The presence of high anti-Ad antibody titres against prevalent serotypes in humans makes this kind of cancer vaccine therapy ineffective in that it becomes harder to break tolerance and to generate a long lasting immune response against the TAA.⁸¹ Diverse strategies are being tested to overcome pre-existing immunity.¹ A number of studies used

increased vaccine dose and/or a prime-boost approach in order to overcome the pre-existing immunity associated with Ad5 vaccination.⁸² Coating with polymers has been proposed to increase the half-life of the virus and, most importantly to avoid the host generation of neutralizing Abs.⁸³

A potential solution to the problem of pre-existing immunity is to use vaccine vectors derived from Ad serotypes that are rare in humans. Alternative Ad serotypes with low prevalence in humans have been investigated.^{84–86} Recently, Boroush *et al* demonstrated the potential utility of Ad serotype 35 as a candidate vaccine vector that is minimally suppressed by anti-Ad5 immunity. These studies suggest that using Ad vectors derived from immunologically distinct serotypes may be an effective and general strategy to overcome the suppressive effects of pre-existing anti-Ad5 immunity.^{87,88} Alternatively serotypes from other non-human species (eg canine, ovine, and chimpanzee Ads) have been considered.^{89–93}

In addition to the pre-existing immunity against Ads present in humans and the generation of neutralizing antibodies that occurs postvaccination, another phenomenon that may compromise the efficacy of adenoviral immunization for gene transfer purposes is the host immune response to the virus.⁸¹ In fact, this may induce the production of proinflammatory cytokines such as IFN- γ and TNF- α that can inhibit the expression of the transgene, when this is under the human cytomegalovirus promoter.

Safety is a major concern, especially for clinical use of Ad as vaccine vehicle.⁹⁴ Their extensive use for gene therapy protocols in most cases showed no significant side effects following vaccination or clinical application.⁹⁵ However, the dramatic case of the death of a young patient at Pennsylvania's Institute for Human Gene Therapy has renewed the attention on potential risks. The patient, suffering from an inherited enzyme deficiency was injected with a massive dose of the recombinant Ad5 into his liver. This generated an inflammatory reaction, which eventually killed the patient in a few days. Although 'deviation' from the protocols were reported, the reaction of the patient was considered unusual.⁹⁶ In addition, it must be stressed that much lower amounts of virus is used for vaccination protocols.

Concluding remarks

Ad is now a vector of choice for anticancer vaccination. Its biology has been extensively investigated and epidemiological data indicate that it has a very low pathogenicity. The viral genome can be easily manipulated to accept cDNAs up to 7.5 kb. Conditionally replicating and stable Ads have an elevated safety profile, can be grown to high for large scale production and elicit a potent immune Th1-skewed response.⁹⁷ They can be designed to deliver as TAAs of choice as well as helper molecules such as cytokines.

Further improvement should be achieved by choosing the best combination of transgenes as well as the modality of immunization and the immunization schedule. This association could lead to the generation of a more effective protocol, which (a) elicits a potent and persistent immune response against the TAA in order to break tolerance; (b) generates a cell-mediated immune-response that is required for the vaccine to have a

Table 1 Recent reports on Ad as vehicle for anti-cancer genetic immunotherapy

Ad expressing	Ex vivo	In vivo
TAAs	Cho et al ⁴¹ , Dietz and Vuk-Pavlovic ⁴² , Chen et al ⁷⁰ , Broder et al ⁹⁹ , Okada et al ¹⁰⁰ , Chan et al ¹⁰¹ , Okada et al ¹⁰²	Gallo et al ²⁴ , Ragot et al ⁵³ , Zhang et al ⁷⁶ , Duraismamy et al ¹⁰³
Immunostimuli	Sharma et al ⁴³ , Salgia et al ⁵⁸ , Simons et al ⁵⁹ , Soiffer et al ⁶⁰ , Nemunaitis ⁶³ , Nemunaitis et al ⁶⁴ , Tatsumi et al ¹⁰⁴ , Satoh et al ¹⁰⁵ , Friesse et al ¹⁰⁶ , Meziane et al ¹⁰⁷	Fisher et al ⁶⁵ , Gopalkrishnan ⁶⁷ , Hendren et al ⁶⁹ , Ahn et al ⁷¹ , Tatsumi et al ¹⁰⁴ , Trudel et al ¹⁰⁸ , Merritt et al ¹⁰⁹ , Andarini et al ¹¹⁰ , Dummer et al ¹¹¹ , Liu et al ¹¹² , Khorana et al ¹¹³
Immunostimuli in association with radio- or chemotherapy	Lumniczky et al ¹¹⁴ , Trudel et al ¹¹⁵	Oh et al ⁷² , Lohr et al ⁷³ , DeLong et al ¹¹⁶ , Merritt et al ¹¹⁷

clinically relevant effect; (c) is able to avoid neutralization by pre-existing Abs, and (d) does not induce the generation of new antivector antibodies.⁹⁸

Data from literature (Table 1) strongly support the idea of Ad as an effective vehicle for anticancer immunotherapy in general. It has been shown to be especially useful in combination with other treatments. A combinatorial approach using molecular targeting strategies or adjuvant therapy could be the best way toward developing feasible genetic therapy against cancer, in the near future.

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