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GENE THERAPY FOR LUNG NEOPLASMS

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SYNOPSIS

Both advanced stage lung cancer and malignant pleural mesothelioma are associated with a poor prognosis. Although there have been advances in treatment regimens for both diseases, these have had only a modest effect on their progressive course. Gene therapy for thoracic malignancies represents a novel therapeutic approach and has been evaluated in a number of clinical trials over the last two decades. Strategies have included induction of apoptosis, tumor suppressor gene replacement, suicide gene expression, cytokine based therapy, various vaccination approaches, and adoptive transfer of modified immune cells. This review will consider the clinical results, limitations, and future directions of gene therapy trials for thoracic malignancies.

Keywords

Gene Therapy; Immunotherapy; Lung cancer; Mesothelioma

INTRODUCTION

Approximately twenty years ago, advances in molecular genetics and gene transfer technology made the development of “gene therapy” (the modification of the genetic makeup of cells for therapeutic purposes) a clinical possibility. The disorders originally proposed as targets for gene therapy were the inherited, recessive disorders in which transfer of a normal copy of a single defective gene could potentially prevent or alter the course of a disease. Clear, but slow progress has been made in the areas of genetic diseases such as blindness¹ and inherited immune deficiencies.² It soon became apparent that the range of

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target diseases could be extended to acquired diseases, such as cancer, and many early phase clinical trials have now been conducted in various malignancies. The good news is that most trials have shown good safety. The bad news is that there has been relatively limited efficacy to date.

Despite advances in chemotherapy, radiation therapy, and surgery for lung cancer, the 5-year survival rate remains poor at 15% and has improved only minimally over the last two decades. This coupled with the advances that have been made in the understanding of molecular events leading to cancer development, has led to great interest in gene therapy approaches for thoracic malignancies. Unfortunately, lung cancer is usually metastatic at the time of diagnosis and thus requires systemic, rather than local therapy. Systemic therapy using gene therapy has remained largely impossible to date.

Malignant pleural mesothelioma (MPM) accounts for 80% of mesothelioma cases and usually presents in the fifth to seventh decade of life with dyspnea, pleural effusion, and non-pleuritic chest pain in the context of an asbestos exposure history. With a disease course affected only minimally by current treatments, MPM has a poor prognosis (median survival of 6–18 months) except in the rare cases where it can be completely resected. MPM is a potentially good disease target for gene therapy because the thin layer of mesothelial and malignant cells offers a large surface area for efficient, rapid, and diffuse gene transfer.

The purpose of this article is to review the clinical experience in gene therapy for thoracic malignancies and to reflect on the future directions of this approach.

VECTORS USED IN GENE THERAPY

Gene delivery efficiency is an important requirement for successful gene therapy. To this end, various viral and non-viral vectors have been engineered, including replicating and non-replicating viruses, bacteria, and liposomes.³ Each varies in regards to the targeted cell type, DNA carrying capacity, in-vivo gene transfer efficiency, and inflammatory response induced. Though no one vector is suitable for all diseases, one can tailor the vector to the specific disease of interest.

Adenovirus

The most widely used vector is the recombinant, replication incompetent adenovirus. Several characteristics of the adenoviral vector make it attractive for gene therapy for cancer (but not genetic diseases). It is able to transfect various target cell types, even when non-dividing, with high efficiency rates and is able to accomplish high level, but transient gene transfection.^{4, 5} Adenoviral-based delivery is accompanied by significant local and systemic inflammation - an early innate component involving a cytokine surge and a later acquired immune response involving neutralizing antibodies and cytotoxic lymphocytes. Importantly, the safety record of adenoviral vectors in humans has been excellent.

Retrovirus

The principal advantages of this vector derive from its ability to accomplish efficient gene transfer *in vitro* in a broad range of targeted cells, with the capacity to achieve integration into the host genome and long term expression. However, retroviral vectors can achieve gene transfer only to dividing cells and are labile *in vivo* since complement and other components inactivate the virion.

Lentiviruses

To circumvent the inability of retroviruses to infect non-dividing cells, vector systems based on the lentivirus genus of retroviruses, which includes human immunodeficiency virus (HIV), have been developed.^{6, 7} Because these viruses are more complex than other retroviruses, and because of obvious safety concerns, development has been slow and cautious. We are not aware of the use of lentivirus yet for thoracic malignancies.

Adeno-associated virus

Another viral vector that has generated interest is the adeno-associated virus (AAV)^{8, 9}, a defective parvovirus with a single strand DNA genome and a naked protein coat. AAV has not been associated with any known human disease state, suggesting a significant safety margin for this vector.

Vaccinia/Fowl Pox vectors

Vaccinia is a double-stranded DNA virus whose entire life cycle takes place within the cytoplasm of infected cells. Due to its role in the eradication of smallpox, it has been used extensively in humans and is very safe. Vaccinia is being explored as a vector for delivery of cancer therapeutic genes, as a carrier for tumor antigens and/or immunostimulatory molecules to develop cancer vaccines, and as a replication-selective, tumor-specific oncolytic virus.^{10, 11} The related Fowl Pox vectors have been used primarily as cancer vaccines.

Non-viral vectors

As an alternative to the viral vectors, a variety of non-viral vectors have also been developed for *in vivo* and *in vitro* gene delivery. Several general strategies have been developed to achieve this end, including liposomes, polymers, and molecular conjugates.^{12, 13} For the most part, these strategies appear to be less efficient than the various viral vectors described above and they do not result in prolonged transgene expression.

Antisense therapy

Antisense therapy relies on inhibition of gene expression, accomplished with a targeted oligonucleotide delivered either intravenously or intratumorally leading to diminished transcription of the complementary mRNA. The oligonucleotide is usually modified to enhance stability. More recently, siRNA has been used in preclinical models, but has not yet moved to clinical trials.

CLINICAL TRIALS IN LUNG CANCER

The number of potential cancer gene therapy strategies is limited only by the imagination of investigators and a large number have been proposed and tested in preclinical models. However, many fewer have been tested in clinical trials. These are discussed below and summarized in Tables 1–3.

Replacement of Tumor Suppressor Genes

Tumor suppressor genes may undergo homozygous loss of function by a variety of mechanisms including mutation, deletion, methylation, or a combination of these. The rationale for this approach is to use a gene therapy vector to encode a tumor-suppressor gene that is mutated or absent in the majority of lung cancers. Theoretically, replacement of a non-functional copy of a tumor suppressor gene could lead to suppression of tumor growth or tumor cell death *in vivo*.

Tumor-based p53 Therapy: Cellular and animals studies have shown that replacement of the normal p53 tumor suppressor gene in tumor cells induced rapid cell death. The strategy of restoring wild-type p53 expression in lung tumor cells has been evaluated in several early phase clinical trials (Table 1). In the earliest Phase I study, a retrovirus vector carrying wild-type p53 was administered to nine lung cancer patients by direct intratumoral injection.¹⁴ In six cases, there was evidence of increased apoptosis, and tumor regression at the site of injection was observed in three of the patients, but all three had progression of their disease at mediastinal or distant sites. This was the first study to demonstrate the feasibility of tumor suppressor gene replacement mediating local tumor regression.

Phase I studies of p53 replacement with adenoviral vectors (Ad) have also suggested clinical benefit with a few partial responses and several patients with stabilization of disease.¹⁵⁻¹⁷ A large phase I study of Ad.p53 gene transfer delivered intra-tumorally combined with chemotherapy demonstrated safety and evidence of increased apoptosis in transduced tumors when examined histologically.¹⁸ A single-arm phase II study of intratumoral Ad.p53 in combination with radiation demonstrated evidence of tumor regression in 63% (12 of 19) and was well tolerated.¹⁹ However, in a phase II study in subjects with at least two measurable lesions, there was no difference in response rates for lesions treated with Ad.p53/chemotherapy compared with chemotherapy alone, implying Ad.p53 provided little local benefit over chemotherapy.²⁰

Keedy and colleagues used repeated delivery of Ad.p53 by bronchoalveolar lavage (BAL) for patients with bronchioloalveolar carcinoma (BAC).²¹ BAL delivery resulted in transient expression of p53 in 19% (3 of 16), two of whom achieved stable disease. These results suggested that BAL could potentially be used for adenoviral delivery, however, there was considerable toxicity to this approach.

Guan and colleagues performed a non-randomized study with delivery of Ad.p53 alone or in combination with bronchial artery instillation (BAI) of chemotherapy (combination of fluorouracil, navelbine, and/or cisplatin).²² Ad.p53 delivery was performed via direct percutaneous delivery or via BAI. Results were encouraging for the indicator lesion with a 47% objective response rate in the combination group, and an improvement in time to progression when compared to BAI alone.

Although Ad.p53 has been approved for use in China, primarily based on studies of potential utility in head and neck cancers²³, there are no current trials ongoing in the U.S. using this approach in lung cancer. In the opinion of the authors, the lack of a strong bystander effects coupled with the relatively low transfection efficiency of adenoviral vectors, will limit potential application in lung cancer to treating local (i.e. endobronchial) lesions unless more efficient vectors are developed.

FUS1 Replacement: FUS1 is a novel tumor suppressor gene identified in the human chromosome 3p21.3 region where allele losses and genetic alterations occur early and frequently for many human cancers.²⁴ Expression of FUS1 protein is absent or reduced in the majority of lung cancers and premalignant lung lesions. Restoration of wild-type FUS1 function in 3p21.3-deficient non-small cell lung carcinoma (NSCLC) cells significantly inhibits tumor cell growth by induction of apoptosis and alteration of cell cycle kinetics.²⁴ A Phase I clinical trial is underway at MD Anderson Cancer Center (ClinicalTrials.gov Identifier NCT0059605) to evaluate delivery of the FUS1 gene using repeated intravenous injection of liposomal particles. It will be interesting to determine the toxicity of this approach (injected DNA can activate toll-like receptors and induce inflammatory responses) and the efficiency of gene transfer to the lung.

Immuno-gene Therapy Approaches

Immunotherapy is based on the premise that there are intrinsic differences in the protein composition of tumor cells that allow the immune system to recognize tumor cells as “foreign” and kill them. However, established tumors have evolved many ways to evade or overwhelm the immune system and thus some sort of exogenous stimulus is needed to enable the immune system to effectively eliminate tumor cells.²⁵

Although immunotherapy is in its early stages of clinical development, there is now increasing evidence that a variety of cancer immunotherapy approaches can be highly effective under certain circumstances. Examples of successful approaches include: (i) vaccines: based on phase II and phase III data, the FDA has recently approved “Provenge”, a prostate cancer vaccine²⁶, (ii) delivery of activating cytokines or chemokines into tumors, (iii) adoptive T-cell transfer: dramatic anti-tumor responses using adoptive T-cell transfer have been shown in melanoma patients²⁷ and Epstein-Barr virus lymphomas^{28–30}, and (iv) blockade of tumor immunosuppression: phase III data showing improved survival in melanoma patients treated with a blocking antibody (ipilimumab) against the cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4), an immune checkpoint molecule that down-regulates pathways of T-cell activation.³¹

Gene therapy approaches are becoming increasingly important in implementing immunotherapy. Specifically, gene therapy has been used to introduce tumor antigens directly, to introduce tumor antigens into dendritic cells, to modify tumor cells used as vaccines, to introduce cytokines or chemokines into tumor cells *in situ*, and to introduce tumor-specificity to adoptively transferred T-cells. A number of these approaches have been tested in thoracic malignancies (Table 2).

Gene-Modified Tumor Cell-Based Vaccination—The use of “killed” (usually irradiated) tumor cells that are then injected into patients as vaccines against recurrent cancer has been employed for many years with only occasional success. Gene therapy has allowed investigators to modify these tumor cells (either autologous or allogeneic) to enhance immunogenicity.

Transforming Growth Factor-β2 (TGF-β2) antisense vector modified cells: Elevated levels of TGF-β2 are associated with greater immunosuppression and with poorer prognosis in patients with non-small cell lung cancer (NSCLC). In pre-clinical studies, delivery of an antisense gene targeting TGF-β2 to *ex vivo* tumor cells led to inhibition of cellular TGF-β2 expression and increased immunogenicity when these gene-modified tumor cells were used as a vaccine. This strategy of vaccination with irradiated tumor cells modified with a TGF-β2 antisense vector (Belagenpumatucel-L) was evaluated in a phase II trial.³² Each patient received one of three doses per month until disease progression. A dose-related survival advantage was observed with minimal toxicities. Differences in immunological endpoints were also noted with increased cytokine (i.e. IFN-γ, IL-6, IL-4) production and the development of HLA-antibody responses to the vaccine.

In a subsequent trial, 21 patients received Belagenpumatucel-L at a single dose of 2.5×10^7 cells per month.³³ Stable disease was noted in 70%, but no complete or partial responses were observed. This compound is currently being evaluated in a Phase III trial of patients with NSCLC.

Tumor Cells genetically modified to secrete granulocyte-monocyte colony stimulating factor (GM-CSF) (GVAX®): GM-CSF is a cytokine involved in the maturation and proliferation of myeloid progenitor cells and has been shown to stimulate proliferation, maturation, and migration of dendritic cells, which play a major role in induction of T-cell

immune responses against cancer. Preclinical studies have shown that transfection of tumor cells with the GM-CSF gene markedly augmented the ability of these cells to induce anti-tumor immune responses.

Initial clinical trials in lung cancer used a patient-specific vaccine platform with intradermal vaccination of irradiated autologous tumor cells that were virally engineered to secrete GM-CSF.^{34, 35} In the first trial of metastatic NSCLC, GM-CSF was transduced into autologous tumor cells with the use of adenoviral vector before irradiation and patient vaccination.³⁴ A few clinical responses were observed and several lines of evidence suggested a strong immune response. In a majority of patients, immunization elicited the development of a delayed hypersensitivity reaction to irradiated, autologous, non-transfected tumor cells. In a study that included both early and late stage patients, a similar strategy resulted in similarly promising results – several clinical responses were observed with a similar demonstration of immunological outcomes.³⁵

In an attempt to produce a vaccine with a more consistent rate of GM-CSF production, the next trial employed the use of a vaccine composed of unmodified, but irradiated autologous tumor cells mixed with a GM-CSF-secreting bystander cell line.³⁶ This approach eliminated the need for viral transduction and potentially allowed for more precise and higher rates of GM-CSF secretion. Although vaccine GM-CSF secretion with this approach was considerably higher than with the autologous vaccine, the frequency of vaccine site reactions, tumor responses, and survival were all less favorable with the bystander vaccine.

Based on lack of compelling evidence for efficacy in lung cancer and in other tumor types (such as prostate cancer) being studied, the GVAX approach has been abandoned at this time in lung cancer, although studies in pancreatic cancer are ongoing.

B7.1/HLA Vaccination.**B7:** 1(CD80) is responsible for co-stimulation of T-cells during priming by an antigen presenting cell (APC). Tumor cells transfected with B7.1 and foreign HLA molecules have been shown to stimulate an immune responses leading to T-cell activation. The strategy of treatment with an allogeneic lung cancer cell line vaccine transfected with B7.1 and HLA-A1 or -A2 was evaluated in a phase I trial of 19 patients with advanced NSCLC.³⁷ Overall, one patient had a partial response, and five had stable disease; however, the median overall survival in this group with a very poor prognosis was an impressive 18 months. In the six responders, the CD8 T cell titers to tumor cell stimulations remained elevated up to 150 weeks after cessation of therapy. Based on the encouraging results of these trials in heavily pretreated patients, a trial is currently ongoing in patients with stage IIIB/IV disease who fail first line chemotherapy (ClinicalTrials.gov Identifier: NCT00534209).

α (1,3)Galactosyltransferase: The gene encoding α (1,3)-galactosyltransferase (α GT), which catalyzes the synthesis of α Gal epitopes on glycoproteins and glycolipids, is inactive in humans but is functional in other mammalian cells. The human immune system produces anti- α Gal antibodies, which is the major mechanism responsible for hyperacute rejection of xenotransplants. A phase I trial evaluated the use of allogeneic NSCLC tumor cells that were retrovirally modified to express α GT.³⁸ A total of 17 patients with advanced disease received up to 7 intradermal treatments, which were well tolerated and resulted in a 10–14 fold increase in serum anti- α Gal titers. A total of 6 patients had prolonged stable disease. A Phase II trial is currently underway at the NCI (Clinical Trials.gov Identifier NCT-00075790).

Gene-Modified Dendritic Cell-Based Vaccination—Dendritic cells (DC) are the most potent antigen presenting cells in the immune system and have been used as vaccine

vehicles. DC can be generated *ex vivo* from blood monocytes. One immunotherapy approach has been to “load” immature, phagocytic DC with antigen using purified protein, cell extracts, mRNA, or gene therapy vectors, “mature the cells”, and then inject these DC subcutaneously. A second approach has been to modify DC *ex vivo* with chemokines or cytokines and inject them directly into tumors where they can take up antigen, migrate to lymph nodes and induce immune responses.

Ad.p53: Given that wild-type p53 protein has a brief half-life and is therefore present in very low levels in normal cells, whereas mutant p53 has a significantly prolonged half-life and is present in much greater quantities in tumor cells, p53 protein has been proposed as a good tumor antigen for a vaccine. Encouraging results with p53-based gene therapy have been attained with the combination of p53-transduced DC with standard chemotherapy.³⁹ In a phase I study, 29 small cell lung cancer (SCLC) patients were vaccinated with DC’s transduced with Ad.p53 resulting in one partial response and seven patients with stable disease. However, of the 21 patients receiving subsequent second-line chemotherapy, a 62% response rate was observed, considerably higher than the historical response rate seen with second line therapy in SCLC. A slight survival advantage (12.1 months vs. 9.6 months) was observed for patients exhibiting an immune response to vaccination. These results are promising, especially given the low survival rates and poor prognosis associated with advanced SCLC. A phase II trial is ongoing at H. Lee Moffitt Cancer Center (ClinicalTrials.gov Identifier NCT00617409).

CCL21: CCL21 is a CC chemokine that is expressed at high levels in high endothelial venules and T-cell zones of spleen and lymph node where it exerts potent attraction of naive T cells and mature DC promoting T cell activation.⁴⁰ Preclinical studies showed that DC transduced with CCL21 and injected into tumors had potent activity against lung cancers. A Phase I clinical trial is now ongoing at UCLA in which DC derived from a leukopheresis sample are being transduced with an adenoviral vector encoding human CCL21 and then injected intratumorally under CT-guidance or bronchoscopy (ClinicalTrials.gov Identifier NCT00601094). Results have not been published yet.

Vaccination Using Viral Vectors to Encode Tumor Antigens—A number of trials have taken advantage of the strong innate and acquired immune responses to viruses by using viral vectors to encode tumor antigens to attempt to generate anti-tumor immune responses.

MUC-1 Vaccination: MUC-1 is a tumor-associated mucin-type surface antigen normally found on epithelial cells in many tissues. Targeting of MUC-1 in lung cancer has been attempted in various ways including both gene and non-gene therapy approaches. A vaccinia virus construct containing the coding sequences for MUC-1 and IL-2 (TG4010) was evaluated in a two-arm phase II trial of 65 patients with stage IIIB/IV NSCLC.⁴¹ All patients were required to have MUC-1 antigen expression on the primary tumor or a metastases. Arm 1 consisted of upfront combination therapy with TG4010 and cisplatin/vinorelbine, whereas arm 2 used TG4010 monotherapy followed by combination therapy at progression. In Arm 1 (44 patients), partial response was observed in 29.5% with a one year survival rate of 53%. Arm 2 had two patients (of 21 total) with stable disease for more than 6 months with TG4010 monotherapy, but given the lack of efficacy this arm was closed early. MUC1-specific responses were measured with the enzyme-linked immunosorbent spot (ELISpot) assay (a method for measuring secretion of antigen-specific antibodies) and were observed in 57% of patients (12 of the 21) with either partial response or stable disease. Additionally, 4 out of 5 patients who developed an ELISpot response during the study achieved disease control. Detectable MUC-1 specific responses were associated with significantly longer time

to progression and overall survival. Based on the encouraging results of combination therapy, additional studies of TG4010 and chemotherapy are being performed in the first-line setting (Clinical Trials.gov identifier NCT00415818).

L523S Vaccination: L523S is an immunogenic lung cancer antigen expressed in approximately 80% of lung cancer cells. In a phase I study, 13 patients with early stage NSCLC (stage 1B, IIA, and IIB) were treated with two doses of intramuscular recombinant DNA (pVAX/L523S) followed by two doses of Ad.L523S given 4 weeks apart.⁴² This vaccination schedule was used in an attempt to develop an immune response against the recombinant protein and thereby achieve a more substantial immune response to L523S. Although the regimen was tolerated only one patient demonstrated a L523S-specific antibody response.

Adenovirus encoding B-galactosidase (Ad. β -gal): Another strategy attempted early on in lung cancer was to use a replication deficient adenovirus containing the lacZ marker gene, which encodes the enzyme β -galactosidase (Ad. β -gal).⁴³ This initial dose-escalation trial was designed to investigate feasibility and tolerance of adenoviral vectors. Although B-galactosidase is not a true tumor antigen there was the possibility of anti-tumor responses due to “antigen spreading” from immune responses induced by the adenovirus or the transgene. A total of 12 patients with advanced lung cancer were treated with a single dose of Ad. β -gal and concomitant chemotherapy. One complete response was observed which lasted greater than 16 months after the last dose of gene therapy. Some patients at the highest dose level had CD4 and cytotoxic T lymphocyte responses against both β -galactosidase and adenoviral particles.⁴⁴

A separate group conducted two phase I studies of Ad. β -gal or Ad.IL-2 delivery in a total of 21 patients with NSCLC.⁴⁵ β -galactosidase activity was measurable in most post-treatment biopsy samples, however only low levels of IL-2 mRNA were detected. No clinical responses were noted.

Anti-sense Therapy

The rationale behind anti-sense therapy is that this technology offers the possibility of down regulating a wide variety of molecules that have been shown to promote lung cancer tumor growth. Anti-sense trials with three different targets have been published (Table 3). The first used was aprinocarsen, a 20-mer oligonucleotide that binds to the mRNA for protein kinase C- α (PKC- α) and inhibits its expression. Aprinocarsen was shown to be generally safe in patients with lung cancer, and had modest activity in combination with chemotherapy.^{46–48} However, a Phase III trial of chemotherapy with or without aprinocarsen as first line therapy did not demonstrate enhanced survival and did show some toxicity.⁴⁹ Phase I studies in various advanced cancer patients with Raf antisense molecules demonstrated a few patients with prolonged stable disease and one patient with a significant response.^{50–52} Unfortunately, two phase II studies in lung cancer (total of 26 patients) failed to demonstrate any significant anti-tumor activity.⁵³ A third set of studies targeted Bcl-2, an apoptotic inhibitor that is overexpressed by many tumors including 80–90% of SCLCs and is associated with increased resistance to chemotherapy. Although two Phase I trials demonstrated promising results^{54, 55}, a Phase II study of standard chemotherapy with or without a bcl-2 antisense oligonucleotide (oblimersen) demonstrated poorer overall survival in the experimental arm and greater hematologic toxicity.⁵⁶

In general, these anti-sense approaches have not been very successful. This is likely due to the fact that the oligonucleotides even after modification are relatively unstable and thus difficult to deliver in adequate amounts and that they, in general, lack bystander effects.

siRNA, which is more efficient, has promise, but still suffers from the same issues of adequate delivery to tumors and lack of bystander effects.

Clinical Trials including Incidentally Treated Lung Cancer Subjects—Numerous other strategies have been employed in the cancer gene therapy and in some of these trials, patients with lung cancer have been included in either Phase I or II studies. In this section, we examine some of these approaches and the results as they specifically pertain to lung cancer and the results of these strategies more generally.

Onyx-015: One additional approach to the use of p53 replacement has been the use of Onyx-015, an oncolytic adenovirus designed to replicate specifically in p53-mutant cells. When used alone, onyx-015 had limited efficacy, though some responses were noted in patients with head and neck cancer.^{57, 58} Onyx-015 has also been used in combination with Enbrel, a TNF- α antagonist, used to inhibit viral clearance. This study noted suppression of TNF- α secretion with some evidence of reduced viral clearance, but clinical efficacy was limited. Further trials are needed to evaluate higher Enbrel doses with potentially greater suppression of TNF- α .⁵⁹

Carcinoembryonic Antigen (CEA): Vaccine strategies have been devised by several groups that target CEA, a tumor marker known to be upregulated in many malignancies, particularly adenocarcinomas. The first such strategy used canarypox virus engineered to express both the co-stimulatory molecule B7 (to enhance response) and CEA.⁶⁰ In this phase I trial, 18 patients with metastatic tumors were treated including three with primary lung cancer. The vaccine was shown to be safe, but there were minimal clinical responses noted in the patients with lung cancer.

A second phase I trial of a virally-encoded CEA vaccine included 58 advanced cancer patients including 9 with primary lung cancer.⁶¹ Eight cohorts received different combinations of two vectors (recombinant fowlpox and recombinant vaccinia virus) and GM-CSF. The vaccines also encoded 3 co-stimulatory molecules to enhance immune response (B7, ICAM-1, and LFA-3, designated TRICOM). One patient with SCLC achieved a pathologic complete response and significant immune responses were also observed. One-year survival was higher in subjects with CEA-specific T-cell responses (83% vs. 41%).

The CEA molecule has also been used in conjunction with gene-modified dendritic cell (DC) vaccination.⁶² Dendritic cells were transfected with a recombinant fowlpox vector encoding CEA and TRICOM. Although clinical responses were limited, immune responses were documented in most patients receiving DC-based anti-CEA therapy. Several ongoing phase II and III trials using the fowlpox-CEA/TRICOM vaccine are being conducted in advanced NSCLC patients.

TNFerade: TNF- α is a pro-inflammatory cytokine that has been shown to possess direct cytotoxic effects on tumor endothelium and can increase procoagulant effect on tumor vasculature. In a Phase I trial, 36 patients (including 5 with lung cancer) with accessible tumors were injected with adenoviral encoded TNF under the control of a radiation inducible promoter (TNFerade), followed by local radiation therapy.⁶³ A significant proportion of patients had objective tumor responses (43%), and in patients with synchronous lesions, TNFerade plus radiation was more efficacious than radiation alone. This approach effectively created high local levels of TNF- α for maximal antitumor effect while avoiding without significant systemic toxicity. Other anti-TNF therapy trials that have included NSCLC have not noted significant clinical responses.⁶⁴

Melanoma differentiation-associated gene (*mda-7*): *mda-7*, also known as IL-24, is a tumor suppressor gene whose gene product can induce apoptosis in several cancer types. Cunningham and colleagues carried out a phase I trial in which 22 patients with advanced cancer were treated with intratumoral adenoviral-*mda-7* (Ad.*mda-7*).⁵⁰ Though response was only evaluable in one cohort of patients, all injected lesions exhibited intratumoral *mda-7* DNA, RNA and protein and apoptosis, as analyzed by TUNEL assay, correlated with MDA-7 protein expression. The one lung cancer patient achieved stable disease and survived 180 days after treatment.

Clinical Trials in Mesothelioma and Malignant Pleural Disease—Gene therapy studies in MPM and malignant pleural disease has been facilitated by the fact the pleural space is easily accessible and amenable to biopsy allowing delivery of study vector/gene and fluid sampling to confirm successful gene transfer. Access and assessment of the pleural space have also been enhanced by the availability of indwelling tunneled pleural catheter systems. Accordingly, several groups have used a variety of gene therapy approaches in an attempt to improve treatment of these diseases (Table 4).

Suicide Gene Therapy

Suicide gene therapy involves transduction of tumor cells with a gene encoding for a specific enzyme that induces sensitivity to an otherwise benign agent. In essence, a “prodrug” is transformed into a toxic metabolite by the enzyme introduced into the cells with subsequent accumulation leading to tumor cell death or “suicide”.⁶⁵ An advantage of suicide gene therapy is the induction of a “bystander effect”—the killing of neighboring cells not transduced with the vector. A commonly studied suicide gene is the herpes simplex virus-1 thymidine kinase (*HSVtk*) gene which makes transduced cells sensitive to the nucleoside analog gancyclovir (GCV). GCV is metabolized poorly by mammalian cells and thus it is usually non-toxic. However, after conversion to GCV-monophosphate by *HSVtk*, it is metabolized rapidly by endogenous kinases to GCV-triphosphate which acts as a potent inhibitor of DNA polymerase and competes with normal mammalian nucleosides for DNA replication.^{65, 66}

Sterman and colleagues initiated a series of Phase I clinical trials of adenovirus (Ad.*HSVtk*/GCV) gene therapy in advanced MPM patients to assess toxicity, gene transfer efficiency, and immune response induction.^{67–69} Subsequent to a single intrapleural administration of Ad.*HSVtk* vector, GCV was given intravenously twice daily for two weeks.

Trials using both a “first-generation” Ad vector and a more advanced-generation (E1/E4-deleted) adenoviral vector that allowed increased vector doses due to decreased contamination with high levels of replication-competent adenovirus were performed. Dose-related intratumoral *HSVtk* gene transfer was demonstrated in 23 of 30 patients, with those treated at a dose equal to or greater than 3.2×10^{11} particle forming units (pfu) having evidence of *HSVtk* protein expression at tumor surfaces and up to 30–50 cell layers deep. Overall, the therapy was well-tolerated with minimal side effects and dose-limiting toxicity was not reached. Anti-tumor antibodies and anti-adenoviral immune responses, including high titers of anti-adenoviral neutralizing antibody and proliferative T-cell responses were generated in both serum and pleural fluid. A number of clinical responses (i.e. survival of more than 3 years) were seen at the higher dose levels with two patients showing long periods of survival (one seven years and one still alive after 10 years).⁶⁹ One of the two surviving patients had demonstrable reduction of tumor metabolic activity as assessed by serial 18-fluorodeoxyglucose positron emission tomography (¹⁸FDG PET) scans over several months. This relatively long response period was likely due to induction of a secondary immune bystander effect of the Ad.*HSVtk*/GCV instillation.

Harrison et al. conducted a Phase I trial using irradiated ovarian carcinoma cells retrovirally-transfected with HSVtk (PA1-STK cells) that were instilled intrapleurally followed by GCV for 7 days.⁷⁰ Minimal side effects were seen and ⁹⁹Tc radiolabeled PA1-STK cells demonstrated preferential adhesion to the tumor lining the chest wall. There were also some post-treatment increases in the percentage of CD8⁺ T lymphocytes in the pleural fluid. However, no significant clinical responses were seen.

Cytokine Gene Therapy

The rationale for cytokine gene therapy is that high level expression of immunostimulatory cytokines (such as interleukin-2 [IL-2], IL-12, tumor necrosis factor [TNF], GM-CSF, or interferons [α , β , or γ]) from tumor cells will activate the immune system *in situ* resulting in a more effective anti-tumor immune response without having to target specific antigens. The advantages of gene therapy over systemic administration of these agents includes lower toxicity, higher local concentrations, much longer persistence of the cytokine, and advantages relating to cytokine secretion by the tumor cell itself.

Robinson and colleagues conducted the first clinical trial of intratumoral cytokine gene delivery in MPM patients using a recombinant partially replication-restricted vaccinia virus (VV) that expressed the human IL-2 gene.⁷¹ Serial VV-IL-2 vector injections over a period of 12 weeks into chest wall lesions of six patients with advanced MPM resulted in minimal toxicity with no demonstrable evidence of vector spread to patient contacts. Though no significant regression of tumor was seen, modest intratumoral T-cell infiltration was detected on post-treatment biopsy specimens. As measured by reverse transcriptase polymerase chain reaction (rtPCR), VV-IL-2 mRNA was detected in biopsy specimens for up to six days post-injection (though declined to low levels by day 8) despite the generation of significant levels of anti-VV neutralizing antibodies.⁷¹

Vero cells, which are immortalized monkey fibroblasts capable of expressing human proteins, have also been studied as a cytokine delivery vector in humans. Fourteen MPM patients received four courses of injections of Vero cells expressing IL-2. The treatment was well tolerated with no significant adverse effects. Levels of circulating IL-2 were detected in half of the patients with one patient demonstrating transient tumor regression and one with disease stabilization for four months. To our knowledge, this approach is not being pursued further.⁷²

Tan et al. conducted a Phase I clinical trial of ten patients with malignant pleural effusions and persistent disease despite conventional therapy.⁷³ Tumor-infiltrating lymphocytes (TILs) acquired from the patients' effusions underwent retroviral-mediated IL-2 gene transfer before being infused back into the patients' chest cavities. The treatment was well tolerated with transient mild fever being the most common adverse effect. Six of the 10 patients were effusion-free for at least 4 weeks after treatment. One patient had both resolution of effusion as well as regression of the primary tumor.

Based on strong preclinical data^{74, 75}, a Phase I Ad.Interferon- β (Ad.IFN- β) dose escalation trial in MPM (seven patients) and metastatic pleural malignancies (three patients) was undertaken.^{76, 77} Gene transfer was detected in 7 of the 10 patients by measurement of pleural fluid IFN- β mRNA or protein. Anti-tumor immune responses, including humoral responses to known tumor antigens (e.g. SV40 Virus Tag, mesothelin) and unknown tumor antigens were elicited in 7 of 10 patients. Four patients demonstrated meaningful clinical responses defined as disease stability and/or partial regression on ¹⁸F-DG-PET and CT imaging two months after vector administration.

In light of the encouraging results, a second study was performed with the aim of augmenting these immunologic and clinical response.⁷⁸ Based on preclinical studies showing enhanced effects after two doses⁷⁹, a second Phase I trial involving two administrations of Ad.IFN- β (levels ranging from 3×10^{11} to 3×10^{12} viral particles) via an indwelling pleural catheter separated by one to two weeks was conducted in seventeen patients (10 with MPM and 7 with malignant pleural effusions). Again, overall treatment was well tolerated and anti-tumor humoral immune responses similar to that seen in the initial trials were induced. Several patients had meaningful clinical responses (mixed and/or partial responses) as determined by pre- and post-vector delivery PET/CT scans. However, high anti-adenoviral neutralizing antibodies titers were detected, even with a dose interval as short as 7 days, inhibiting effective gene transfer of the second dose.

A third Phase I trial of Ad.IFN (α instead of β , solely as a result of changes in corporate sponsorship) has just been completed.⁸⁰ To avoid the effects of rapidly developing neutralizing antibodies to adenovirus, the protocol was modified to deliver the two Ad.IFN- α vector doses three days apart. Preliminary results show prolonged and high IFN- α protein expression in pleural fluid and serum. No clinical responses were seen in the 4 subjects with advanced disease. However, evidence of disease stability or tumor regression was seen in the remaining 5 patients, including one dramatic example of partial tumor regression at sites not in contiguity with vector infusion (Figure 1).

Based on preclinical studies showing synergy between Ad.IFN and systemic chemotherapy, our group at Penn has initiated a trial in which Ad.IFN- α is being administered in combination with first- or second-line chemotherapy for MPM patients. Additionally, in light of animal studies demonstrating a benefit of debulking surgery in combination with immunotherapy⁸¹, a neoadjuvant surgery trial involving vector administration to MPM patients followed by maximal cytoreduction and adjuvant chemo-radiotherapy is also being planned.

Tumor-based p53 therapy

There are two reports from China of using Ad.p53 to treat malignant effusions. Both studies noted more control of the effusion in patients who received the Ad.p53 based gene therapy, but did not report any data on tumor response rates or survival.^{82, 83} Thus, it is possible that this approach may have only been useful in achieving pleurodesis without any significant effect on the primary tumor.

SUMMARY and FUTURE DIRECTIONS

In the past two decades, much experimentation using gene therapy has been done pre-clinically, and some clinical trials for thoracic malignancies have been performed. In general, these trials have shown safety, but only intermittent efficacy. *In vivo* gene transfer has been clearly achievable, but with the vectors currently available, it has been very difficult to transduce more than a small percentage of tumor cells, and this is usually only accomplished by local injection. This limitation has thus doomed the approaches that do not have strong bystander effects (i.e. oncogene inactivation or replacement of tumor suppressor genes).

One potential approach that could avoid these problems is secretion of anti-tumor substances such as anti-angiogenic agents. The development of vectors that can induce long term *in vivo* expression, such as AAVs or lentiviruses, may make this feasible.

However, the primary direction of the field has been a shift toward “immuno-gene therapy” (Figure 2). This strategy requires only enough gene transduction to stimulate an endogenous

immune response and create a strong bystander effect. Promising approaches involve using gene therapy to stimulate anti-tumor responses by a vaccine or by delivering immunostimulatory cytokines. Although these strategies seem to be successful in initiating anti-tumor immune responses, investigators are beginning to recognize that they are limited by large tumor volumes and significant immuno-inhibitory networks created by the tumors involving cytokines such as TGF- β , interleukin-10, prostaglandin E2, and vascular-endothelial cell growth factor (VEGF) and inhibitor cells such as T-regulatory cells and myeloid derived suppressor cells.⁸⁴ Future trials are going to likely require combination approaches that stimulate the immune system, reduce tumor burden (surgery and/or chemotherapy) and “inhibit the inhibitors” (with agents such as COX-2 inhibitors or anti-CTLA4 antibodies).

Another major direction of the field is to use adoptive transfer of gene-modified autologous lymphocytes that have been altered *ex vivo* by using retroviruses or lentiviruses to augment their ability to attack lung cancer or mesothelioma cells. This can be done by transfection of T-cell receptors with altered specificity or by the introduction of totally artificial chimeric T-cell antigen receptors (CARs) that use single chain antibody fragments to define antigen specificity and intracellular fragments of both the T-cell receptor and accessory molecules (such as CD28 or 4-1BB) to enhance activation.⁸⁵ A group at Baylor University has begun a clinical trial (see ClinicalTrials.gov, identifier NCT00889954) that is targeting HER-2-positive lung cancers with T-cells directed to this antigen that have been modified with a chimeric receptor. These cells are also being modified to be resistant to TGF- β . Our group and a group at Memorial Sloan Kettering Cancer Center are designing CARs to target T-cells to the tumor antigen mesothelin for use in the treatment of MPM. The approach has worked well in preclinical models⁸⁶ and a clinical trial has been initiated at the University of Pennsylvania.

Gene therapy for lung cancer and MPM has not yet reached clinical practice. An appropriate analogy may be the development of monoclonal antibodies where it took more than 20 years from discovery to actual clinical applications. Despite what some perceive as a slow start, we feel that progress is clearly being made and this therapeutic tool will find its place in the anti-cancer armamentarium in the next decade.

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Figure 1. Regression of mediastinal tumor after intrapleural Ad.IFN α

Panel A: Pretreatment PET/CT image demonstrating a pleural tumor nodule near the anterior mediastinum (circled), Panel B: Regression of nodule 6 months after treatment with Ad.IFN α .

Figure 2. Immuno-Gene Therapy Paradigm

This approach relies on only enough gene transfer to induce immunity and generate a strong bystander effect. The anti-tumor immune responses are augmented by complementary approaches to target large tumor volumes or the immunosuppressive networks present in most tumors.

p53 Gene Therapy Trials

Table 1

Study	Phase	Histology	Total No. (# evaluable)	Vector	Delivery	Best Clinical Response (%) [*]	Additional Outcome Measures
Roth ¹⁴ 1996	I	NSCLC	9 (7)	Retroviral p53	Intratumoral (single injection)	3 (33%) with PR	All pts. progressed at distant lesions. 8 pts. with evidence of gene transfer
Schuler ¹⁵ 1998	I	NSCLC	15 (15)	Ad.p53	Intratumoral (single injection)	0 with PR 7 (47%) with SD	6 pts. with evidence of gene transfer
Swisher ¹⁶ 1999	I	NSCLC	28 (25)	Ad.p53	Intratumoral (repeated injection)	2 (7%) with PR 16 (57%) with SD	18 pts. with evidence of gene transfer
Nemunaitis ¹⁸ 2000	I	NSCLC	24 (23)	Ad.p53	Intratumoral + cisplatin (up to 6 cycles)	2 (8%) with PR 17 (71%) with SD	6 pts. with evidence of gene transfer
Schuler ²⁰ 2001	II	NSCLC	25 (25) [‡]	Ad.p53	Intratumoral + chemo. (up to 3 cycles)	IT injected lesion: 13 (52%) with PR Comparator lesion: 12 (48%) with PR	No statistical difference between two groups. 17 pts. with evidence of gene transfer
Swisher ¹⁹ 2003	II	NSCLC	19 (17)	Ad.p53	Intratumoral (3 injections) + radiation	1 (5%) with CR 11 (58%) with PR 3 (16%) with SD	Biopsies of treated lesion three months after injection showed no viable tumor in 12 (63%) of 19 pts.
Fujiwara ¹⁷ 2006	I	NSCLC	15 (13)	Ad.p53	Intratumoral only, every 28 days (9 pts.) Intratumoral + cisplatin, every cycle (6 pts.)	1 (7%) with PR 10 (66%) with SD	The one PR was in AD.p53 only group
Keedy ²¹ 2008	I	BAC	29 (23)	Ad.p53	BAL, multiple instillations	16 (70%) with SD	3 pts. with evidence of gene transfer. 8 pts. with subjective improvement in pulmonary symptoms.
Guan ^{22,‡} 2009	II	NSCLC	58 (58)	Ad.p53	Vector only: Intratumoral injection or BAI Combo: Vector + Chemotherapy	Vector only: 15 (38%) with PR, 8 (42%) with SD Combo: 2 (11%) with CR; 7 (37%) with PR, 18 (46%) with SD.	No statistical difference in objective response between groups.

Abbreviations used: NSCLC – non-small cell lung cancer; Ad – adenovirus; BAL – Bronchoalveolar lavage; CR – complete response; PR – partial response; SD – stable disease

^{*} Best clinical response is noted for injected lesion only, unless otherwise noted.

[‡] This study included patients that had two similar lesions, allowing for a comparison of the injected lesion to a comparator lesion

[‡] This study involved vector delivery by either by intratumoral injection or bronchial artery instillation (BAI); chemotherapy delivered via BAI

Table 2

Immuno-gene Therapy Trials

Study	Phase	Histology	Total No. (# evaluable)	Vaccine	Delivery	Best Clinical Response (%)	Additional Outcome Measures
TGF-β2a							
Nemunaitis ³² 2006	II [†]	NSCLC	75 (40)	antisense gene-modified allogeneic tumor cell vaccine (belagenpumatucel-L)	Intradermal	Dose Level 1: 1 of 11 (6%) with PR Dose Level 2: 3 of 11 (27%) with PR Dose Level 3: 2 of 13 (15%) with PR	Dose related overall survival differences were found between cohorts
Nemunaitis ³³ 2009	II	NSCLC	21 (20)	belagenpumatucel-L	Intradermal	70% with SD	
GM-CSF							
Salgia ³⁴ 2003	I	NSCLC	37 (34)	adenoviral modified autologous tumor cells engineered to secrete GM-CSF (GVAX)	Intradermal	1 (3%) with MR 5 (15%) with SD	Presence of CD4 ⁺ /CD8 ⁺ /CD20 ⁺ cell infiltration associated with tumor regression or disease stabilization
Nemunaitis ³⁵ 2004	I/II	NSCLC	83 (43)*	adenoviral modified autologous tumor cells engineered to secrete GM-CSF (GVAX)	Intradermal	3 (9%) with CR, 1 (3%) with PR, 2 (6%) with MR, 7 (21%) with SD	Vaccine associated GM-CSF secretion was associated with improvement in overall survival
Nemunaitis ³⁶ 2006	I/II	NSCLC	86 (49)	autologous tumor cells with bystander cell line genetically modified to secrete GM-CSF (bystander GVAX)	Intradermal	No CR/PR, 4 (8%) with MR 7 (14%) with SD	Progression-free survival and median survival was better for subjects with injection site reactions
B7.1/HLA-A							
Raez ³⁷ 2004	I	NSCLC	19 (19)	AD100 adenocarcinoma cell line modified to express HLA-A1/A2 and B7	Subcutaneous	1 (7%) with PR, 5 (26%) with SD	Overall survival times ranging from 23 – 40+ months. 16 of 17 pts. had a measurable CD8 response
Alpha(1,3)galactosyltransferase							
Morris ³⁸ 2005	I	NSCLC	7 (7)	3 irradiated lung cancer cell lines modified by retroviral transfer of murine α (1,3)galactosyltransferase	Intradermal	No CR/PR, 4 (57%) with SD	Data presented in abstract form only
p53							
Antonia ³⁹ 2006	II	SCLC	29 (29)	DC-Ad-p53	Intradermal	1 (3%) with PR, 7 (24%) with SD	Association between p53 specific T-cell response and response to 2 nd line chemotherapy (post-study).
MUC-1							
Ramlau ⁴¹ 2008	II [†]	NSCLC	65 (53)	TG4010: vaccinia virus containing coding sequences for MUC-1 & IL-2 Arm 1: TG4010 + IV chemo. Arm 2: TG4010 alone (phase I), then TG4010 + IV chemo (phase II)	Subcutaneous	Arm 1: 13 (35%) with PR, 12 (32%) with SD Arm 2: Phase I: 0 with PR, 2 (9.5%) with SD	MUC-1 specific immune response was associated with longer TTP and improved overall survival

Study	Phase	Histology	Total No. (# evaluable)	Vaccine	Delivery	Best Clinical Response (%)	Additional Outcome Measures
L523S							
Nemunatis ⁴² 2006	I	NSCLC	13 (12)	pVAX/L523S (expressive plasmid) followed by Ad.L523S	Intramuscular	Phase II: 1 (7%) with CR, 1 (7%) with PR 12 early stage subjects (Stage IB-IIIB): median follow-up of 290 days: 2 recurrences; 1 second primary.	Limited evidence of L523S-directed immune activation

Abbreviations used: NSCLC – non-small cell lung cancer; SCLC – small cell lung cancer; CR – complete response; PR – partial response; SD – stable disease

* Response data in table is for 33 patients of the 43 evaluable.

[†] Randomized phase II study

Table 3

Antisense Therapy Trials

Study	Phase	Histology	Total No. (# evaluable)	Agent	Delivery	Best Clinical Response (%)	Additional Outcome Measures
<i>Protein Kinase C (PKC)-α</i>							
Villalona- Calero ⁴⁶ 2004	I/II	NSCLC	Phase II portion -55 (49)	Antisense oligonucleotide to PKC-α (Aprinocarsen)	IV Aprinocarsen +IV gemcitabine/ cisplatin	Phase II: 1 (2%) with CR, 15 (31%) with PR, 25 (49%) with SD	Median overall survival of 8.9 months
Vansteenkiste ⁴⁷ 2005	II*	NSCLC	Experimental arm – 9 (6) Control arm – 9 (9)	Aprinocarsen	IV Aprinocarsen + IV gemcitabine/ cisplatin vs. Gemcitabine/ cisplatin alone	Experimental arm – 1 (17%) with PR, 2 (34%) with SD Control arm – 4 (44%) with PR, 2 (22%) with SD	Study terminated early due to publication of Phase III study by Paz- Ares (2006)
Ritch ⁴⁸ 2006	II	NSCLC	36 (29)	Aprinocarsen	IV Aprinocarsen + IV gemcitabine/ cisplatin	9(31%) with PR, 11 (38%) with SD	Median overall survival of 8.3 months Severe thrombocytopenia in most pts.
Paz-Ares ⁴⁹ 2006	III	NSCLC	Experimental Arm – 342 (280) Control Arm – 328 (289)	Aprinocarsen	IV Aprinocarsen + IV gemcitabine/ cisplatin vs. IV gemcitabine/ cisplatin alone	Experimental – 3 (1%) with CR, 78 (28%) with PR, 101 (36%) with SD Control – 2 (1%) with CR, 99 (34%) with PR, 104 (36%) with SD	Overall HR 1.05 (0.88 –1.25)
<i>C-raf Kinase</i>							
Coudert ⁵³ 2001	II	NSCLC, SCLC	NSCLC – 18 (15) SCLC – 8 (5)	Antisense oligonucleotide to c-raf-kinase (ISIS 5132)	Continuous 21d. IV infusion of ISIS 5132	0 CR/PR	C-raf status not assessed.
<i>Bcl-2</i>							
Rudin ⁵⁴ 2002	I	SCLC	12 (11)	Antisense oligonucleotide to Bcl-2 (G3139)	IV G3139 + IV paclitaxel	0 CR/PR, 2 (18%) with SD	Suppression of Bcl-2 protein in PBMC noted in 1 pt. with prolonged SD.
Rudin ⁵⁵ 2004	I	SCLC	16 (14)	G3139	IV G139 + IV carboplatin/ etoposide	12 (86%) with PR, 2(14%) with SD	No evidence of Bcl-2 suppression in PBMC
Rudin ⁵⁶ 2008	II*	SCLC	56 (48)	G3139	Arm A: IV cisplatin/ etoposide + IV G3139 Arm B: IV cisplatin/	Arm A: 1 (2%) with CR, 24 (59%) with PR, 10	Overall HR 2.1 (1.1 – 4.1)

Study	Phase	Histology	Total No. (# evaluable)	Agent	Delivery	Best Clinical Response (%)	Additional Outcome Measures
					etoposide alone	(24%) with SD Arm B: 2 (13%) with CR, 7 (47%) with PR, 2 (13%) with SD	

Abbreviations used: NSCLC – non-small cell lung cancer; SCLC – small cell lung cancer; PBMC – peripheral blood mononuclear cells; CR – complete response; PR – partial response; SD – stable disease

* Randomized Phase II study

Table 4

Mesothelioma Gene Therapy Trials

Study	Phase	Histology	Total No. (# evaluable)	Agent	Delivery	Best Clinical Response (%)	Additional Outcome Measures
<i>Suicide Gene Therapy</i>							
Sterman ⁶⁷ 1998	I	MPM	21 (21)	Ad.HSVTK+ IV GCV	Intrapleural - single dose	6 (29%) with SD	Successful gene transfer in 11 pts.
Sterman ⁶⁸ 2000	I	MPM	5 (5)	Ad.HSVTK + IV GCV + IV solumedrol	Intrapleural (Ad.HSVTK) - single dose	N/A	Corticosteroids had no effect on cellular and humoral responses to Ad vector
Harrison ⁷⁰ 2000	I	MPM	16 (16)	PA1-S TK (HSVTK gene modified ovarian cancer cell line) + IV GCV	Intrapleural - up to 3 doses	N/A	PA1-S TK cells adhered preferentially to mesothelioma cells
<i>Cytokine Gene Therapy</i>							
Mukherjee ⁷¹ 2000	I	MPM	6 (6)	Vaccinia virus expressing IL-2	Intratumoral - 3-12 doses	No significant tumor regression observed	VV-IL-2 mRNA detected for up to 3 weeks after each injection
Pitako ⁷² 2003	I	MPM	14 (14)	Vero-IL-2 cells	Intratumoral - multiple doses	1 (7%) with PR, 1 (7%) with SD	7 pts. with increased levels of serum IL-2 receptor
Sterman ⁷⁷ 2007	I	MPM MPE	MPM - 7 (7) MPE - 3 (3)	Ad.IFN β	Intrapleural - single dose	1 (10%) with CR, 2 (20%) with PR, 4 (40%) with SD	Successful gene transfer, induction of humoral/innate immune response
Sterman ⁷⁸ 2010	I	MPM MPE	MPM - 10 (10) MPE - 7 (7)	Ad.IFN β	Intrapleural - two doses	3 (18%) with PR/MR 11 (61%) with SD	Successful gene transfer with 1 st dose but not 2 nd , induction of humoral immune response
Sterman ⁸⁰ 2011	I	MPM	9 (9)	Ad.IFN α	Intrapleural- two doses	2 (22%) PR, 4 (44%) with SD	Ad.IFN α induced much higher levels of gene transfer than Ad.IFN β . Induction of humoral/innate immune response

Abbreviations used: Ad- adenovirus; MPM – malignant pleural mesothelioma; MPE – malignant pleural effusion; GCV – ganciclovir; CR – complete response; PR – partial response; MR – mixed response; SD – stable disease