Gene therapy in interventional pulmonology: Interferon gene delivery with focus on thoracic malignancies

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Abstract Gene therapy is a promising treatment for a variety of human diseases—particularly for malignancies—but has not been implemented into routine clinical use because sufficient delivering of therapeutic genes to effectively kill large numbers of cancer cells has proved daunting. Recently, researchers have been focusing on a method called immunogene therapy, which transfers genetic material—such as genes encoding for interferons—to trigger the patient’s own immune system to fight the cancer. Mesothelioma is a particularly good target for gene therapy, because no individual treatment (surgery, chemotherapy, and/or radiation) has proven efficacious, and because mesothelioma tends to remain localized until the late stages of the disease. Even immuno-gene therapy is limited in its ability to destroy large tumors, which is why researchers are investigating combination approaches that combine traditional therapies such as surgery and chemotherapy with immunotherapy.

Keywords Interferon · Gene transfer · Adenovirus · Intrapleural · Mesothelioma · Immunotherapy · Interventional pulmonology

Introduction

Interferons (IFNs) are a class of cytokines that have immunomodulatory effects on macrophage function, antibody production, delayed hypersensitivity reaction, and have anti-viral effects [1•, 2]. IFNs have also been shown to have anti-proliferative effects [1•, 2]. The use of interferons for the treatment of tumors and infections has been studied with varied success. A major limitation in the clinical use of IFNs has been toxic systemic effects and the lack of sustained levels in tissues. The rationale for immunogene therapies such as interferon gene transfer is that transduction of specific genes in tumors and infections will result in high local expression of interferons with minimal elevations of interferon in the peripheral blood [3]. The advantage of such an approach would be to eliminate high systemic toxicity and achieve sustained and high local concentrations of therapeutic cytokines.

In this review we aim to provide the readers with a comprehensive update on the applications of gene therapy using interferon coding sequences, with particular emphasis on clinical trials of interferon genes in thoracic malignancies (Table 1).

Preclinical models of interferon gene therapy for cancer

A number of preclinical studies have shown the potential and promise of the use of interferon gene therapy in cancer. These experimental studies have demonstrated the feasibility and efficacy of this novel anti-neoplastic strategy. Early studies focused on identification of the most effective delivery systems—initially retroviral vectors [4], followed by adenoviral vectors [5]; and more recently there has been

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considerable interest in liposomal delivery systems [6, 7]. This section will discuss the potential of interferon gene therapy in preclinical models for different malignancies.

Brain tumors

Early studies on brain tumor cell lines successfully transferred genes encoding for interferon-gamma (IFN- γ) using a retroviral vector, and demonstrated augmentation of cytotoxic T-lymphocytes (CTL) [4]. Intratumoral interferon-beta (IFN- β) expression has been studied using vectors such as herpes simplex virus [8, 9], liposomes [10], and bone marrow-derived cells [11]. Combination therapies in preclinical models have included IFN- β gene transfer and tumor vaccination with dendritic cells, demonstrating promising results [12]. Other combination therapies for glioma have included IFN- β gene transfer with trichostatin A for neuroblastoma [13] and gamma radiation with IFN- β gene transfer [14].

Melanoma

Initial studies of IFN- γ gene transfer in melanoma models demonstrated enhanced expression of major histocompatibility complex (MHC) classes I and II, resulting in increased cytotoxicity [15]. A similar approach also demonstrated responses in murine models of metastatic melanoma [16]. Pretreatment of melanoma tumor cell cultures with retroviral vector-mediated interferon gene transfer followed by cisplatin therapy resulted in increased apoptosis [17]. Cationic liposome-mediated IFN- β gene transfer has also been studied with and without radiation therapy with promising results in murine models of melanoma [18–20].

Prostate cancer

Several preclinical studies have explored the potential of interferon gene therapy for prostate cancer. Early approaches have included pretreatment of cancer cells with retroviral transfection with the IFN- β gene and subsequent injection of these cells—as well as untreated prostate cancer cells—into mouse models, with evidence of rapid progression of non-transfected tumor compared to IFN- β transfected cells [21]. Direct intratumoral injection of an adenoviral vector carrying the IFN- β gene resulted in the ability to prevent tumor growth and inhibit the growth of metastatic sites, as well as to prolong the longevity of prostate tumor-bearing mice [22]. Studies with IFN- β gene therapy have also demonstrated inhibitory effects on angiogenesis of prostate tumor cells [23] and have suggested biochemical mechanisms for tumor suppression [24]. One group of investigators utilized adenoviral vectors for IFN- γ gene transfer with demonstration of in vitro antitumor activity [5].

Cutaneous T-cell lymphoma

Several studies have determined an imbalance in local cytokine environment in cutaneous T-cell lymphoma [25–28]. This promotes the survival of the tumor cells by viability factors such as interleukin 7 and interleukin 15 [28–30]. It has also been shown that CTCL cells also secrete T-helper 2 (Th2) cytokines such as IL-10 [26, 27, 31]. It is therefore thought that there are immunosuppressive effects such as delayed-hypersensitivity reactions, hypereosinophilia, and elevated immunoglobulin E (IgE) serum levels in patients with CTCL [32] and diminished natural killer (NK) cell activity [33]. Therefore, primary cutaneous lymphomas have been successfully treated clinically with recombinant IFNs, counterbalancing the Th2-skewing state [34]. The short half-lives and toxic effects of these recombinant proteins have prompted preclinical studies involving interferon gene transfer methods to elicit similar effects on tumor growth [32, 35, 36].

Malignant pleural mesothelioma and malignant pleural effusions

It has been nearly 20 years since Smythe and colleagues at the University of Pennsylvania Medical Center first introduced replication-deficient recombinant adenovirus carrying the *Escherichia coli* lacZ gene into human mesothelioma cell lines. These investigators also accomplished effective gene transfer of human mesothelioma xenographs implanted within the peritoneal cavities of immunodeficient mice [37].

Nearly 10 years later, Okada and colleagues also at the University of Pennsylvania Medical Center (2001) first conducted preclinical studies investigating intracavitary delivery of a replication-deficient adenoviral vector encoding for the murine IFN- β gene (Ad.muIFN-beta) in syngenic murine models of malignant mesothelioma [38]. This novel approach to mesothelioma provided evidence of strong CD8(+) T-cell-mediated antitumor effects. The same group also demonstrated that tumor-specific CD4+ T cells were also critical for in vivo tumor eradication by intracavitary interferon- β gene transfer [39]. Kellerman et al. [40] demonstrated similar immunogenic responses in a murine mesothelioma model (AK 7) using IFN- γ gene expression.

Krukltis et al. [41] studied the effects of neoadjuvant immuno-gene therapy prior to surgical debulking of malignant mesothelioma in a syngeneic, immunocompetent murine model. Tumors transduced with replication-incompetent adenovirus encoding for the murine interferon- β gene prior to surgical debulking demonstrated increased long-term tumor-free survival compared to mice who underwent tumor resection without upfront immune-gene delivery.

Suzuki et al. [42] studied the outcome of using chemotherapy (gemcitabine) in conjunction with immuno-gene therapy using an adenovirus expressing IFN- β in syngeneic murine models of mesothelioma and lung cancer. Significant antitumor effects were achieved with treatment of tumor-bearing mice with gemcitabine followed by administration of Ad.mu IFN- β ; however, this effect was diminished in mice bearing larger tumors.

Willmon et al. [43] demonstrated an interesting approach of combining expression of IFN- β gene and the oncolytic vesicular stomatitis virus (VSV) vector in the treatment of mesothelioma. This animal study using a mouse model concluded that the efficacy and safety of the oncolytic VSV vector is enhanced with expression of IFN- β in tumor cell lines.

A number of studies have also evaluated the efficacy of IFN gene therapy in sarcoma [44–48], renal cell carcinoma [49–51], superficial bladder cancer [52–56], and gastrointestinal malignancies such as hepatocellular [57–61] and pancreatic carcinomas [62].

Preclinical models of interferon gene therapy for infectious and inflammatory conditions

HIV

Studies have shown inhibition of HIV replication in cell lines by incorporating IFN- α , IFN- β , and IFN- γ genes under the control of HIV long terminal repeat [63, 64]. Another strategy has been IFN- β gene transduction of CD4(+) T cells to produce a continuous low-dose expression of IFN- β in controlling HIV infection and allowing immune restoration in mouse models [65, 66]. A comparison of interferon gene therapy and gene therapy with other modalities concluded that gene therapy using CD4 immunoadhesin was more efficient in a SCID mouse model, although expression of IFN genes did result in a marked reduction in HIV viral load [67]. More recently, lentiviral vectors containing IFN- β sequences under the influence of the murine moderate H2-kb promoter have been used with success [68].

Herpes simplex virus (HSV)

Early studies showed that innate production of IFN- α protected mice from herpes simplex virus type 1 (HSV-1) corneal disease [69]. A series of studies by Noiskran et al. suggested the efficacy of naked plasmid DNA encoding IFN- α offered protection against ocular HSV-1 infection in murine models [70–72]. These promising results led to comparison of topical application of recombinant IFN- α to plasmid DNA encoding IFN- α in ocular HSV-1 infection in mice [73]. There was no significant difference in efficacy for

prophylaxis; however, topical application of plasmid DNA conferred advantage over recombinant IFN- α when applied post-infection.

Hepatitis B virus (HBV), hepatitis C virus (HCV), and cirrhosis

The success of interferon use in controlling HBV and HCV replication has led to attempts at eliciting tightly regulated tissue-specific gene expression of interferons [74, 75]. The tetracycline-regulated (Tet) gene expression system [76, 77] has been used with success to demonstrate liver-specific expression of IFN- γ following adenoviral gene transfer in mouse models of chronic HBV infection. This bidirectional Tet promoter system resulted in a tightly regulated 1000-fold induction of gene expression that was liver specific and effectively controlled HBV replication in mice, but did not completely eradicate the infection.

A similar approach has been adopted for HCV infection with injection of adenoviral vectors encoding human IFN- α DNA (huIFN- α DNA) into HCV-infected hepatocyte cell lines, resulting in complete disappearance of HCV [78]. Another study by the same group studied adenoviral vector mediated expression of IFN- α in a mouse model of cirrhosis, resulting in substantial improvement in fibrotic changes [79].

Western equine encephalitis virus (WEEV)

In a murine model of WEEV, adenoviral-mediated IFN- α expression demonstrated the capacity for eliciting full protection against various WEEV strains when administered prophylactically, and a delayed progression of infection when given post-infection, suggesting that adenovirus-mediated expression of IFN- α can be an alternative approach for the prevention and treatment of WEEV infection [80].

Cytomegalovirus (CMV)

The combined effect of viral DNA vaccines and IFN- α and IFN- β gene transfer in murine CMV infection has been shown to be better than the partial immune response elicited by the former alone [81]. Expression of several subtypes of interferons administered by DNA inoculation has been shown to be effective in different phases of myocarditis secondary to CMV infection in mice [82].

Pulmonary tuberculosis (PTB)

Use of recombinant adenoviruses encoding murine IFN- γ (AdIFN- γ) for PTB has shown to reduce the bacillary load

in murine models for both drug-sensitive as well as multidrug-resistant (MDR) tubercular strains [83]. This offers a novel and promising approach for a disease that has significant burden on health care especially in developing countries, particularly for MDR-TB, for which standard medical therapy regimens are limited.

Hand foot and mouth disease

Studies have demonstrated complete immunization in swine by use of adenoviral vectors delivering porcine IFN- α genes [84, 85].

Atherosclerosis

While most studies have demonstrated the benefits of interferon expression for their antiviral and anti-proliferative properties, the proinflammatory effects of IFN- γ can be counterproductive in augmenting atherosclerotic plaque formation [86]. IFN- γ neutralization, by repeated injections of a plasmid encoding a soluble IFN- γ construct (sIFN γ), has been shown to reduce lesion progression in ApoE knockout mice [87].

Human clinical trials of interferon gene transfer

Melanoma

The first clinical trial of IFN gene therapy for melanoma was conducted by Abdel-Wahab et al. [88], published in 1997. This phase 1 trial aimed to study the feasibility of active immunization of melanoma patients with IFN- γ gene-modified autologous melanoma cells. Autologous melanoma cells were retrovirally transduced *ex vivo* with the human IFN- γ gene to facilitate autocrine and paracrine secretion of biologically active IFN- γ . These genetically modified cells were first inactivated by irradiation and administered as immunizations to 20 melanoma patients once every 2 weeks for 3 months in a dose-escalating manner. Thirteen of the 20 patients completed the intended protocol. Humoral immunoglobulin G (IgG) response, as measured by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, and radioimmunoprecipitation, was seen in eight of the 13 patients. The humoral response increased progressively with each immunization. Four patients showed clinical responses; in two of the four responding patients clinical efficacy was associated with significant increases in serum IgG response. This study suggested that autologous cellular vaccination using IFN- γ gene transfer for patients with melanoma was safe and potentially efficacious, and induced significant and relevant humoral responses.

Nemunaitis et al. [89] conducted a phase 1 trial of direct *intratumoral* injection of retroviral vector encoding for human IFN- γ (huIFN- γ) in patients with advanced melanoma. The aim of the trial was to determine the feasibility, safety, and efficacy of such an approach. A single injection of a retroviral vector expressing huIFN- γ was administered to 13 patients on a daily basis for 5 consecutive days. Within 1 week of dosing, 12 of the 13 patients underwent resection of the injected lesion to confirm DNA transduction *in situ*; 10 of these samples had viable tissue for analysis. Three of the 10 harvested tumor samples were confirmed to have IFN- γ expression via enzyme-linked immunospot assay (ELISPOT). Interestingly, two patients (one with stage III melanoma and one with breast cancer) had no evidence of local tumor following resection of the injected lesion. No adverse events were attributed directly to the injections.

This study demonstrated the feasibility and safety of retroviral-mediated huIFN- γ injection, with evidence of IFN- γ expression in the tumor cells and anecdotal evidence of local tumor responses. The investigators speculated that multiple vaccinations may result in induction of superior antitumor responses.

The same investigators conducted an open-label, phase 1 inter-patient dose-escalation study to evaluate the safety and efficacy of multiple courses of retroviral-mediated huIFN- γ vaccination [90]. Seventeen patients with metastatic melanoma were divided into six cohorts, with the first three cohorts receiving retroviral huIFN- γ vector by *intratumoral* injection for 5 consecutive days. Patients in the second three cohorts were treated with the same dose levels, but were treated for six cycles—five daily injections every 2 weeks. No adverse events that could be attributed directly to the immunogene therapy were noted. Replication-competent retrovirus was not detected by polymerase chain reaction (PCR) studies. One of nine patients in the study cohort that received a single cycle of treatment achieved a >50% response at the injected site, and the other eight had progressive disease. Three of eight patients in the treatment arm that received 6 cycles of vector injections achieved a >50% response at the injected site, with the remainder maintaining a period of stable disease at the injected site. Median survival of single-injected patients was 150 days, while the eight patients who received multiple cycles of injections had a median survival of 369 days [91]. Overall, the study showed a benefit in clinical and immunogenic response in patients receiving multiple courses of retroviral huIFN- γ vector injections; and the investigators recommended further studies to evaluate the optimal number of injections and duration of treatment; and also to study the effect of IFN- γ gene transfer in conjunction with chemotherapy or other immune therapy in patients with metastatic melanoma [92].

A more recently published pilot study evaluated the role of IFN- β gene transfer for patients with advanced

Table 1 Interferon-based gene therapy trials

Study	Phase	Histology	Total <i>n</i> (<i>n</i> evaluable)	Agent	Delivery	Best clinical response	Additional outcome measures
<i>Immuno-gene therapy</i>							
Abdel-Wahab et al. [88•], 1997	I	Melanoma	20 (13)	Retrovirus IFN- γ transduced autologous melanoma cells	Subcutaneous—multiple doses	4 of 13 showed “clinical responses”	Humoral immune responses seen in 8 of 13 patients
Nemunaitis et al. [90], 1999	I	Melanoma, breast cancer	13 (12)	Retrovirus IFN- γ	Direct intratumoral injection (single)	2 of 12 showed complete local response at time of tumor resection	10 of 12 demonstrated IFN- γ production by ELISPOT on resected tumor
Nemunaitis et al. [91], 2000	I	Melanoma	17 (17)	Retrovirus IFN- γ	Direct intratumoral injection (multiple)	3 of 8 patients receiving 6 cycles had a > 50% local response rate	8 of 17 patients receiving multiple doses had a median survival of 369 days
Mastsumoto et al. [93], 2008	I	Melanoma	5 (5)	Cationic liposomes (IAB-1)	Direct intratumoral injection (single)	1 MR, 1 SD, 3 PD	Intratumoral CD4+ T-cell infiltration
Dummer et al. [34], 2004	I	CTCL/CBCL	7 CTCL/2 CBCL	TG1042 (Ad.IFN- γ)	Repeated intratumoral injection	3 local CR; 2 local PR at injection sites	7 of 9 patients had detectable TG1042 derived IFN- γ mRNA after 1st cycle
Chiocca et al. [103], 2008	I	Glioma	11 (11)	Ad.hIFN- β	Stereotactic intratumoral injection	Tumor recurrence within 4 months of resection	Ad.hIFN- β DNA and hIFN- β expression in tumor tissue at excision
Sterman et al. [112•], 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 et al. [113], 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 1st dose but not 2nd, induction of humoral immune response
Sterman et al. [114], 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

Ad adenovirus; *CBCL* cutaneous B-cell lymphoma; *CR* complete response; *CTCL* cutaneous T-cell lymphoma; *MPE* malignant pleural effusion; *MPM* malignant pleural mesothelioma; *MR* mixed response; *PD* progressive disease; *PR* partial response; *SD* stable disease

melanoma using cationic liposomes (IAB-1) to deliver the transgene [93]. Five patients were enrolled and underwent intratumoral injection of cationic liposomes containing a plasmid encoding for human interferon- β . Clinical response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST criteria): one patient had a mixed response; one had stable disease; and three patients showed disease progression. Although clinical efficacy of IFN- β gene transfer using cationic liposomes could not be properly assessed in this small pilot study, there were demonstrable immunological responses as evidenced by the infiltration of CD4+ T cells around tumor cells expressing HLA class II antigen associated with epidermal hyperplasia and melanoma cell apoptosis. After demonstration of safety and feasibility of this strategy in this small pilot study, the authors proposed additional studies with an increase in the number of intratumoral injections of IAB-1 and/or the use of IFN- β gene transfer in conjunction with administration of dendritic cells or other cytokines to enhance the anti-tumor immune response [93].

Cutaneous T-cell lymphoma

Cutaneous T-cell lymphoma (CTCL) is the most common type of primary cutaneous lymphoma, accounting for approximately 65% of cases [94]. Although there are no curative options available for CTCL, many newer treatment strategies have emerged in the past few years [32, 94, 95]. One of the newer approaches tested in human clinical trials involved intratumoral adenovirus-mediated transfer of the human IFN- γ gene [32, 34, 35, 94].

The first human trial for the treatment of CTCL by interferon gene therapy was conducted in 2004 by Dummer et al. [34] using TG1042, a third-generation, non-replicating, adenoviral vector containing a human IFN- γ cDNA insert. This was a phase 1 open-label, dose-escalating trial, investigating the effects of repeated intratumoral administration of TG1042 in seven patients with advanced primary CTCL and two patients with multi-lesional cutaneous B-cell lymphomas. TG1042 was injected into the target lesion once weekly for 3 weeks with no injection in the fourth week (defined as 1 treatment cycle) and thereafter up to 4 cycles, if there was no evidence of progressive disease. Most frequently reported adverse effects were injection site reaction, fatigue, fever, and headache. One patient developed severe colitis requiring hospitalization. No deaths were reported. Seven of the nine patients had detectable TG1042-derived IFN- γ mRNA in the injected lesions after the first treatment cycle. Biopsies of the lesions showed up-regulation of CD8, TIA-1, and CAR following the TG1042 treatment. Systemic immune response such as increased serum levels of interleukin 6, IFN- γ , and neopterin (a marker for IFN- γ -induced macrophage activity) were documented in all patients. Seven patients exhibited a significant increase in anti-

adenovirus neutralizing antibodies (Nabs) after TG1042 treatment. Local tumor responses in target lesions were observed in five of the nine patients (three complete responses and two partial responses), with responses lasting from 1 to 6 months, with a median of 3 months. Based on the promising results of the above trial, Transgene initiated a phase 2 clinical trial in CBCL in 2006 [96].

Malignant glioma

Malignant glioma is an attractive target for gene therapy because it is a highly fatal, locally progressing neoplasm in a background of non-dividing cells (neurons) with rare metastasis outside of the central nervous system, thus allowing delivery of vectors into the desired site [97, 98]. Although several preclinical studies on animal models have shown promise [6, 11, 13], few clinical trials have been published using IFN gene therapy. Most clinical studies have used other gene therapy strategies such as using suicide genes [97–99] and oncolytic viruses [13, 99–101].

The first reported clinical trial of IFN gene transfer for glioma was by Eck et al. [102•], who reported the results of an open-label, inter-patient dose escalation toxicity study of adenoviral IFN- β gene transfer in patients with recurrent or progressive malignant glioma who were undergoing surgical tumor excision. Chiocca et al. [103] subsequently evaluated the safety and biological activity of a human IFN- β (hIFN- β)-expressing adenovirus vector (Ad.hIFN- β) in 11 patients with malignant glioma in a phase 1 clinical trial. Stereotactic injections of Ad.hIFN- β were performed in a dose-escalating manner followed by surgical removal of tumor 4–8 days post vector instillation. Most of the adverse events seen were not attributed to the Ad.hIFN- β vector except for a grade 4 confusional state in one patient. All patients had tumor recurrence within 4 months, and 10 patients died between 6 and 46 weeks after enrollment in the trial with a median survival of 17.9 weeks. Post-treatment histopathology showed evidence of dose-related induction of apoptosis of malignant glioma cells and a substantial increase in intratumoral inflammatory infiltrates. Ad.hIFN- β DNA and hIFN- β expression were demonstrated within tumor tissue. The study supported the safety and feasibility of such an approach with the caveat that the patient who experienced a confusional state had direct contact between Ad.hIFN- β and CSF.

Malignant pleural mesothelioma and metastatic pleural effusions

Several clinical trials in the early 1990s demonstrated evidence of the safety and efficacy of systemic and intracavitary administration of recombinant IFN- α , IFN- β , and IFN- γ in patients with early-stage malignant pleural mesothelioma (MPM) [104–106]. Progress with this approach, however,

was hampered due to problems such as drug availability, short in vivo half-life and lack of sustained tissue levels of the recombinant proteins, toxic effects of systemic administration, and limitation of demonstrable clinical effects to early-stage patients [104, 105].

Intrapleural IFN- β administration for metastatic pleural effusions showed promise and reasonable safety profile, but with minimal clinical benefit, likely due to several of the same issues that limited application of recombinant type I and type II interferons in mesothelioma [106, 107]. To overcome these hurdles, a new area of interest in the past two decades has been expression of interferon(s) in tumor cells using gene transfer techniques.

The promise shown by the preclinical studies as well as human trials with other forms of immunogene therapy [108–111] led to the first human trial of interferon- β gene therapy for MPM and MPE published in 2007 [112]. The study, conducted at the University of Pennsylvania Medical Center, evaluated the safety and feasibility of a single-dose intrapleural IFN- β gene transfer using an adenoviral vector (Ad.IFN- β) in patients with malignant pleural mesothelioma and metastatic pleural effusions. Ad.IFN- β was administered through an indwelling pleural catheter in varying doses in two cohorts of patients—MPM (seven patients) and MPE (three patients). Subjects were evaluated for toxicity, gene transfer, immune responses, and anti-tumor responses via 18-fluorodeoxyglucose (FDG)-positron emission tomography (PET) scans and chest CT scans. Intrapleural Ad.IFN- β was generally well tolerated with transient lymphopenia as the most common side effect. Other side effects included hypoxia and liver function abnormalities. Gene transfer was documented in 7 of the 10 patients by demonstration of IFN- β mRNA or protein expression in pleural fluid. Cytokine levels in the pleural space were highly variable at baseline and after response to gene transfer. Antitumor immune responses were demonstrated in seven of the 10 patients and included the detection of cytotoxic T cells (one patient), activation of circulating natural killer cells (two patients), and humoral responses to known (simian virus 40 large T antigen; mesothelin) and unknown tumor antigens (allogenic mesothelioma homogenates). Four of 10 patients showed meaningful clinical responses defined as disease stability and/or regression on 18-FDG PET and CT scans at day 60 after vector infusion.

This study demonstrated several important aspects of IFN gene transfer in human subjects. Firstly, administration of intrapleural Ad.IFN- β was feasible and largely well-tolerated. The maximal tolerated dose of viral particles was also determined in these 10 patients (9×10^{11} viral particles). Secondly, intrapleural Ad.IFN- β administration resulted in successful gene transfer as evidenced by the production of IFN- β measured on ELISA of pleural fluid. Lastly, the study also demonstrated that a single intrapleural

dose of IFN- β vector was able to induce measurable anti-tumor immune response in most patients and anecdotal clinical responses in some patients as quantified by modified Response Evaluation Criteria in Solid Tumors (RECIST).

Encouraged by the results of a single-dose intrapleural IFN- β gene transfer, the same group conducted a phase 1 trial at the University of Pennsylvania Medical Center to determine whether using two doses of Ad.IFN- β vector would be superior to a single dose [113]. Ten patients with MPM and seven with MPE (underlying primary malignancies: 3 lung cancer, 2 ovarian cancer, and 2 breast cancer) received two doses of Ad.IFN- β through an indwelling pleural catheter. Because of potential safety concerns, the trial used a dose interval of 14 days initially for 13 patients. After demonstrating no additional toxicity, the remaining four patients were treated with 7-day dosing interval. Repeated doses were generally well tolerated. The most common adverse events were lymphopenia, hypoalbuminemia, hypotension, anemia, hypocalcemia, and a mild cytokine release syndrome characterized by fever, rigors/chills, nausea, and tachycardia. Two unusual adverse effects were also detected. One of the patients developed an asymptomatic elevated partial thromboplastin time (PTT) after the first dose of vector and therefore did not receive the second dose. Subsequent investigations revealed an elevated anticardiolipin antibody level. Another patient developed pericardial tamponade and was successfully treated by therapeutic pericardiocentesis. Pericardial fluid analysis did not reveal malignant cells or IFN- β and was negative for adenovirus testing via PCR or viral cultures [113].

In this repeat dose gene transfer study, high levels of IFN- β were detected in pleural fluid after the first dose; however, only minimal levels were seen after the second dose. Lack of intrapleural IFN- β expression after the second dose correlated with the rapid induction of neutralizing Ad antibodies (Nabs) within 8–14 days of initial vector instillation. Antibody responses against tumor antigens were induced in most patients. At 2 months, modified RECIST responses for the enrolled subjects were as follows: 1 partial response, 2 stable disease, 9 progressive disease, and 2 non-measurable disease. One patient died after 1 month. By PET scanning, two patients had mixed responses and 11 had stable disease. There were seven patients with survival times longer than 18 months. Overall, repeated intrapleural instillation of Ad.IFN- β vector was safe, induced immune responses, and some evidence of clinical responses. However, rapid development of Nabs prevented effective gene transfer after the second dose, even with a dose interval as short as 7 days [113].

Based on these results, investigators at the University of Pennsylvania Medical Center planned to evaluate a shortened dosing interval by administering a second dose of Ad-IFN- β vector 3 days after the first dose, prior to the expected peak of Nabs production. Due to withdrawal of Ad.IFN- β vector

supply by the industry collaborator, however, the investigators applied the above principles using a recombinant, replication incompetent adenovirus vector expressing the human interferon- α 2b gene (Ad.IFN- α 2b) obtained from Schering-Plough/Merck (SCH721015) [114]. The reasoning behind this move was that the vector backbones were virtually identical and the biologic activities of both type I interferons—IFN- α and IFN- β —were very similar.

Adenovirus vector expressing the human interferon- α 2b gene (Ad.IFN- α 2b) was instilled on study days 1 and 4 via a tunneled pleural catheter. The first vector dose was 1×10^{12} viral particles of Ad.hIFN- α 2b (since the investigators had no experience with this new vector, for safety reasons, they chose an initial dose that was lower than the maximal tolerated dose from prior clinical trials of Ad.IFN- β). This dose was further reduced to 3×10^{11} after the first three patients developed severe flu-like symptoms with the first dose of vector instillation. Subjects were assessed for anti-tumor responses approximately 60 days after initial treatment using CT and PET scans. Pleural fluid and serum IFN- α 2b levels, mesothelin-related protein (SMRP) levels, and Nabs were measured using standard techniques. If progressive disease was documented any time after 2 months, patients received other anti-tumor therapies [114].

In general, Ad.IFN- α 2b vector instillation was well tolerated. Most patients developed some degree of fever and chills. High and sustained serum IFN- α levels were sometimes associated with protracted “flu-like symptoms” lasting 7–10 days. Pleural catheter-related infections occurred in two patients and both were treated successfully with antibiotics. No further infections were observed after the trial was amended so that subsequent patients had their pleural

catheters removed the day following the second vector instillation [114].

Successful gene transfer and high levels of pleural fluid and serum IFN- α levels were induced, even in patients who received a lower dose of vector. Furthermore, there was evidence that the second Ad.IFN- α 2b dose resulted in successful gene transfer, unlike prior trials with Ad.IFN- β . All patients had markedly increased anti-Ad Nab titers (Nabs) 1 week after Ad vector instillation; however, low levels of Nabs were measured at the time of the second vector dose (day 4). There were no increases in humoral responses to mesothelin or SV40 virus large T antigen (two defined mesothelioma-associated antigens). There were, however, other encouraging immunologic responses such as new or increased intensity bands on immunoblots containing extracts of mesothelioma cell lines in seven of eight patients, systemic NK cell activation, and up-regulation of the activation marker CD69 on circulating NK cells [114].

At the time of first radiographic assessment (60 days), three subjects had progressive disease (two had died), four had stable disease, and two had partial responses using modified RECIST criteria. Two patients had sufficient improvement that they were subsequently able to undergo successful radical pleurectomy (RP), with no signs of recurrence as of 7 and 19 months postoperatively, respectively. One patient, who has been previously treated with radical pleurectomy and chemotherapy, had a very impressive radiographic response in that many of the pleural-based malignant foci had regressed on PET/CT by 2 months after vector instillations. On 6-month follow-up PET/CT, post Ad.IFN- α 2b, many lesions had completely resolved, all at sites distant from vector instillation [114] (Fig. 1).

Fig. 1 PET/CT scan images pre and post intrapleural interferon- α gene delivery in patient with recurrent mesothelioma status post prior right radical pleurectomy and intrapleural photodynamic therapy and adjuvant chemotherapy with new sites of disease in mediastinal, hilar, and peri-celiac nodes, right lateral hemithorax, and anterior mediastinum. Scans 6 months post localized intrapleural instillation of SCH721015 adenoviral interferon- α vector demonstrate marked regression at disease at all sites with complete resolution of 2 centimeter diameter hypermetabolic mass in the anterior mediastinum. This mass was at a site quite distant from the local area of intrapleural vector delivery

Overall this study provided indirect comparison between Ad.IFN- α 2b and Ad.IFN- β vector and highlighted similarities (generally well tolerated and similar anti-tumor humoral immune responses) and differences (pleural and serum IFN- α concentrations were much higher at equivalent, or even lower, vector doses). It is not yet clear if the perceived higher potency of Ad.IFN- α 2b translates into better anti-tumor activity. The most important outcome of the study was the demonstration of lower levels of Nabs at the shortened dosing interval with prolonged intrapleural expression of the interferon transgene. The combination of a better dosing strategy as above as well as the higher potency and sustained levels of IFN- α may result in better anti-tumor response in future clinical trials [114].

Conclusions and future directions

Interferon gene therapy is an attractive and exciting strategy in treatment of many viral infections and tumors. Better vectors, both viral and non-viral, have been developed and a fair number of clinical trials using interferon gene therapy have been attempted in a variety of malignancies. These trials have mostly documented safety, successful gene transfer, and induction of anti-tumor humoral and cellular immune responses, but showed only limited efficacy. It must be noted though that the clinical trials have used this approach after completely exhausting all available standards therapies, and therefore the patients enrolled had by definition advanced malignancies and immune systems that had been buffeted by multiple prior cycles of systemic chemotherapy. Given the complex nature of pathogenesis of many malignancies, the future may lie in combination gene therapy approaches. Use of oncolytic viruses and interferon gene transfer, for example, could improve outcome by facilitating greater expression of cytokine genes within target tumors [43]. Use of other immunostimulatory molecules such as other cytokines or even tumor vaccines could offer promising outcomes and perhaps improve efficacy of each other [115].

Multimodality cancer treatment—ie, surgical debulking, chemotherapy, and/or radiotherapy—in conjunction with interferon gene transfer may provide improved long-term outcomes. Based on preclinical trials, the group at Penn has initiated a phase 2a clinical trial of repeated dose intrapleural Ad.IFN- α in combination with standard chemotherapy for patients with MPM. In addition, a neoadjuvant surgery trial involving vector administration to patients with MPM followed by maximal cytoreduction and adjuvant chemotherapy is also being planned [116].

Although it has been more than 20 years since the advent of immunogene therapy, there has been slow progress with its foray into clinical practice. However, experts in this field

are increasingly optimistic about the future of immunogene therapy as a therapeutic modality, particularly in neoplastic diseases [116, 117].

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