Clinical Cancer Research

Pilot and Feasibility Trial Evaluating Immuno-Gene Therapy of Malignant Mesothelioma Using Intrapleural Delivery of Adenovirus-IFNα Combined with Chemotherapy

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Abstract

Purpose: "*In situ* vaccination" using immunogene therapy has the ability to induce polyclonal antitumor responses directed by the patient's immune system.

Experimental Design: Patients with unresectable malignant pleural mesothelioma (MPM) received two intrapleural doses of a replication-defective adenoviral vector containing the human IFNα2b gene (Ad.IFN) concomitant with a 14-day course of celecoxib followed by chemotherapy. Primary outcomes were safety, toxicity, and objective response rate; secondary outcomes included progression-free and overall survival. Biocorrelates on blood and tumor were measured.

Results: Forty subjects were treated: 18 received first-line pemetrexed-based chemotherapy, 22 received second-line chemotherapy with pemetrexed ($n = 7$) or gemcitabine ($n = 15$). Treatment was generally well tolerated. The overall response rate was 25%, and the disease control rate was 88%. Median overall survival (MOS) for all patients with epithelial histology was 21 months versus 7 months for patients with nonepithelial histology. MOS in the first-line cohort was 12.5 months, whereas MOS for the second-line cohort was 21.5 months, with 32% of patients alive at 2 years. No biologic parameters were found to correlate with response, including numbers of activated blood T cells or NK cells, regulatory T cells in blood, peak levels of IFNα in blood or pleural fluid, induction of antitumor antibodies, nor an immune-gene signature in pretreatment biopsies.

Conclusions: The combination of intrapleural Ad.IFN, celecoxib, and chemotherapy proved safe in patients with MPM. OS rate was significantly higher than historical controls in the second-line group. Results of this study support proceeding with a multicenter randomized clinical trial of chemo-immunogene therapy versus standard chemotherapy alone. *Clin Cancer Res; 22(15); 3791–800. ©2016 AACR.*

Introduction

Malignant pleural mesothelioma (MPM) is a rapidly progressive thoracic neoplasm with high mortality that typically responds poorly to standard medical regimens (1). The current front-line standard-of-care chemotherapy regimen is pemetrexed and cisplatin (or carboplatin), resulting in a median overall survival (MOS) of 12 to 13 months (Supplementary Table S1). Patients with progressive disease may be offered additional agents, including drugs such as gemcitabine or vinorelbine, but second-line treatments for MPM have not demonstrated significant response

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Translational Relevance

Malignant pleural mesothelioma (MPM) is a rapidly progressive thoracic neoplasm with high mortality that typically responds poorly to standard medical regimens. The recent success of cancer immunotherapy makes it important to explore this novel strategy in mesothelioma. This trial describes an approach called "*in situ* immunogene therapy" in which a nonreplicating adenoviral vector (Ad) expressing the immune-activating cytokine IFNα was injected intrapleurally into mesothelioma patients, followed by first- or second-line chemotherapy. Ad.IFN transfects both benign mesothelial and malignant mesothelioma cells, resulting in viral "danger signals" and the production of large concentrations of IFN within the pleural space, providing a powerful stimulus to the immune system. This study demonstrates that this approach was safe, feasible, and well tolerated in patients with MPM. Overall survival rate was significantly higher than historical controls in the second-line group. These results support validation with a larger, multicenter randomized clinical trial.

rates or improvements in survival and have not been approved by the FDA for this indication (1, 2). For patients with MPM receiving second-line chemotherapy, the MOS is approximately 9 months (Supplementary Table S1).

Given these suboptimal results, our group has explored the use of *in situ* immunogene therapy to treat MPM using first-generation, replication-deficient adenoviruses (Ad) administered intrapleurally (3). Our recent work focused on Ad vectors encoding type I *IFN* genes (initially IFNβ, then subsequently IFNα; refs. 4–6). Although type I IFNs have been used with some success in certain tumors (7), and intrapleural IFNγ showed some efficacy in early-stage mesothelioma (8), the high doses required and associated systemic side effects have limited the utility of this approach, a problem potentially overcome by localized delivery of cytokine genes.

After intrapleural injection, Ad.IFN efficiently transfects both benign mesothelial and malignant mesothelioma cells, resulting in the production of large concentrations of IFN within the pleural space and tumor (4–6). Mesothelioma cell transduction with Ad.IFN results in tumor cell death and a powerful stimulus to the immune system, as type I IFNs augment tumor neoantigen presentation/processing in dendritic cells (DC), induce TH1 polarization, and augment cytotoxic $CD8^+$ T-cell function, as well as that of natural killer (NK) cells, and M1 phenotype macrophages (7, 9). The inflammatory response to the Ad viral vector itself also elicits additional "danger signals," further potentiating antitumor immune responses (10). This multipronged approach alters the tumor microenvironment, kills tumor cells, and stimulates the innate and adaptive immune systems.

We previously showed safety, feasibility, and induction of antitumor humoral and cellular immune responses in phase I intrapleural Ad.IFN trials (4–6). We also identified an MTD and demonstrated that two doses of Ad.IFNα2b administered with a dose interval of 3 days resulted in augmented gene transfer without enhanced toxicity. In some patients, this approach appeared to "break tolerance," engendering a long-lasting response (presumably immunologic) characterized by tumor regression at distant sites over months without further therapy. A trial using the same Ad.IFNα2b vector via intravesical instillation in bladder cancer patients has also demonstrated promising results (11).

Although encouraging, the percentage and degree of tumor responses in our phase 1 studies were limited. We attempted to enhance the efficacy of adenoviral immunogene therapy in preclinical models by adding COX-2 inhibition (mitigating the immunosuppressive tumor microenvironment by decreasing PGE2 and IL10 production; ref. 12) and by concomitant/adjuvant administration of chemotherapy (13). This latter approach fits well with the emerging consensus that immune stimulation by certain forms of chemotherapy, by exposure of tumor neoantigens to DCs and depletion of regulatory T cells (Treg), among other mechanisms, is crucial to therapeutic efficacy (14–17). Accordingly, we designed a pilot and feasibility study in patients with MPM who were not candidates for surgical resection to assess the safety and activity of two doses of intrapleural Ad.hIFNα2b (given in combination with high-dose celecoxib), followed by standard first-line or second-line chemotherapy.

Materials and Methods

Study design and patients

In this single-center, open-label, nonrandomized pilot and feasibility trial, there were two primary outcome measures: (i) safety and toxicity and (ii) tumor response (by modified RECIST). Secondary outcomes included progression-free survival (PFS), OS, and biocorrelates of clinical response and multiple immunologic parameters.

The vector used in this trial, originally called SCH 721015 (Ad.hIFNα2b), is a clinical grade, serotype 5, E1/partial E3-deleted replication-incompetent adenovirus with insertion of the human IFNα2b gene in the E1 region of the adenoviral genome (6). It was provided by the Schering-Plough Research Institute (Kenilworth, NJ).

Eligibility stipulated: (i) pathologically confirmed MPM; (ii) ECOG performance status of 0 or 1; and (iii) accessible pleural space for vector instillation. Exclusion criteria included pericardial effusion, inadequate pulmonary function [FEV1 < 1 L or <40% of predicted value (postpleural drainage)], significant cardiac, hepatic, or renal disease, or high neutralizing anti-Ad antibody (Nabs) titers (>1:2,000).

The stopping criteria and detailed description of adverse events that served as dose-limiting toxicities (DLT) are described in the Supplementary Methods. In brief, DLTs were defined (using NIC criteria) by any grade 4 toxicity, grade 3 hypotension or allergic reaction, grade 3 nonhematologic toxicity persisting for more than 7 days, persistent cytokine release syndrome, or grade 3 hematologic toxicity persisting for >7 days.

The protocol was approved by the Penn Institutional Review Board (IRB; UPCC 02510), the FDA (BB-IND 13854), and the NIH Recombinant DNA Advisory Committee. Written informed consent was obtained from patients at the time of screening, and the study was registered at clinicaltrials.gov (NCT01119664).

Study design

Eligible patients with MPM underwent tunneled intrapleural catheter insertion under local anesthesia or via thoracoscopy (6). On study days 1 and 4, a dose of 3×10^{11} viral particles Ad.hIFNα2b, diluted in 25 to 50 cc of sterile normal saline, was instilled into the pleural space. Patients were observed in the Clinical and Translational Research Center (CTRC) of the

Table 1. Basic demographics and patient characteristics

	Patients ($n = 40$)
Age in years, ave. (median)	68 (67)
Gender	
Men	29 (72%)
Women	11 (28%)
Stage	
I	3 (8%)
II	3 (8%)
III	16 (40%)
IV	18 (45%)
Histologic type	
Epithelial	30 (75%)
Biphasic	5 (12.5%)
Sarcomatoid	4 (10%)
Lymphohistiocytic	1 (2.5%)
Type of chemotherapy	
First-line pemetrexed/platin	18 (45%)
Second-line repeat pemetrexed/platin	7 (17.5%)
Second-line gemcitabine ± platin	15 (37.5%)

University of Pennsylvania Medical Center (Philadelphia, PA) for at least 24 hours after vector instillation. The vector was administered concomitant with a 14-day course of oral celecoxib (400 mg twice daily starting 3 days prior to vector instillation).

Fourteen days after the first dose of vector, patients initiated outpatient chemotherapy in one of two treatment groups: treatment-naïve patients received standard-dose front-line chemotherapy with pemetrexed and a platinum agent (either cisplatin or carboplatin). Those undergoing second-line chemotherapy primarily received gemcitabine ± carboplatin (Table 1). In addition, the second-line cohort included patients who had undergone pemetrexed-based chemotherapy at least 6 months previously with disease stability or response. These subjects were retreated with pemetrexed, as has been reported in the medical literature (Supplementary Table S1).

Patients were monitored as outpatients through day 190 and thereafter by telephone or electronic medical record. Patients were assessed for antitumor responses every 6 weeks after initial treatment using chest CT scans up until 6 months. If progression was documented at the initial follow-up CT scan (approximately 2 months postvector dosing), then subjects proceeded with other therapeutic options but continued to be followed (Supplementary Table S2). After 6 months, patients were tracked in return visits, by communications with local physicians, and by phone conversations. Times of death and progression were recorded; subsequent treatments and the causes of death were determined where possible.

Radiographic analysis was performed by a board-certified thoracic radiologist (S.I. Katz) blinded to the patients' medical history and other clinical trial results. Modified RECIST measurements were recorded at each exam (18).

Biocorrelates

ELISAs were used to measure IFNα2b levels (PBL Biomedical Laboratories), as well as serum mesothelin–related protein (SMRP) levels (Fujirebio, Inc). Nabs were assessed as described previously (5). To detect induced humoral responses against tumor antigens, we performed immunoblotting against purified mesothelin and extracts from allogeneic mesothelioma cell lines using pre- and posttreatment serum as described previously (4–6). See Supplementary Methods and Supplementary Fig. S1 for details.

Cryopreserved peripheral blood mononuclear cells (PBMC) were collected prior to treatment, 2 days after Ad.IFN instillation (before the second dose) and 15 days after the first dose (just prior to chemotherapy administration). PBMCs were studied from a set of six patients who responded to therapy and six patients who progressed with treatment (Supplementary Table S3). PBMCs were thawed, and the activation of NK cell and T cells was assessed using flow cytometry as detailed in the Supplementary Methods (see also ref. 19).

Formalin-fixed paraffin-embedded sections from original surgical biopsies or previous surgery were available from 18 patients and stained with anti-CD8, anti-CD68, or anti-PDL1 antibodies. Tissue sections were also assessed for RNA levels using NanoString analysis (see Supplementary Methods for details).

Immunogene score. To evaluate the basal "immune activation" state of the tumors, we adapted the recently described "immunoscore" derived from studies used to predict immune responses of melanoma and lung cancer patients to an anticancer MAGE vaccine (20). This study identified 84 genes (mostly related to CD8 T cells and IFN responses) that correlated with response. We had information on 27 of the 61 PCR-validated genes in our NanoString data (see Supplementary Table S4). The sum of the intensity of each of these 27 genes was determined and each tumor ranked from highest expression to lowest expression.

Statistical analysis

Our original Penn IRB approval was for enrollment of 10 to 15 patients in each of the two cohorts: first- and second-line chemotherapy. With a minimum of 11 patients in a treatment stratum, we had 90% power to identify any unanticipated toxicity with prevalence of $\geq$19%; we were ultimately provided with enough vector to treat 40 patients, so we subsequently received IRB approval for a study amendment allowing for a total number of 40 patients, allowing us to treat 18 first-line and 22 second-line patients. This provided us with 90% power to identify any unanticipated toxicity with prevalence of approximately 12%.

Efficacy was determined by estimating objective response rates and distributions of times to progression and death. We summarized the distributions of PFS and OS by Kaplan–Meier curves, comparing curves across strata by the log-rank test.

Statistics used for the flow cytometry data are described in the Supplementary Methods.

Role of funding source

This study was sponsored by the University of Pennsylvania (Philadelphia, PA), with funding provided by an NCI Program Project grant (NCI P01 CA66726). The academic authors were fully responsible for the design, conduct, and analysis of the trial.

Results

Forty patients with MPM were enrolled on the trial between March 2011 and October 2013. Patient demographics are summarized in Table 1.

Thirty-two patients received two intrapleural doses of Ad. hIFNα2b. Eight patients received only one dose of vector because of: (i) low serum albumin ($n = 1$); (ii) shortness of breath ($n = 2$); (iii) increased serum transaminases ($n = 1$); (iv) supraventricular tachycardia ($n = 1$); or (v) decreased absolute neutrophil count ($n = 3$). In several of the eight cases, wherein patients received a

Table 2. Adverse events related to study treatment

	Grade (number of events)				
Adverse events	**1**	**2**	**3**	**4**	**Total**
Syndrome					
Cytokine release	14	25			39
IFN syndrome[a]	9	2			11
Blood					
Hemoglobin—low	5	3	2		10
Leukocytes—low	7	4			11
Lymphopenia	10	11	13	4	38
Neutrophils—low	5	2	2		9
Platelets—low	10				10
Cardiac					
Supraventricular tachycardia			1		1
Hypertension	1				1
Coagulation					
PTT—high	4	1			5
Constitutional					
Chills—intermittent	2				2
Fatigue	2				2
Anxiety	2				2
GI					
Nausea	2				2
Anorexia	2	1			3
Metabolic					
Albumin—low	19	23			42
ALT—high	4				4
AST—high	7				7
Calcium—low	22	4			26
Creatinine—high	2	1			3
Total bilirubin—high	1	1			2
Potassium	2				2
Neurology					
Insomnia	1				1
Dizziness	1				1
Pain					
Pleural—postvector instillation	1				1
Headache	1				1
Tumor site worsen	1		1		2
Pulmonary					
Cough	1				1
Atelectasis					
Dyspnea on exertion	2	1			3
Hypoxia	1		1		2

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GI, gastrointestinal; PTT, partial thromboplastin time.

[a]IFN syndrome refers to toxicity presumed secondary to IFN production post-vector administration similar to the side effects of systemic IFN administered for hepatitis C. Typically, the syndrome is malaise, loss of appetite, mild nausea, and persistent low-grade fevers.

single dose and were ineligible for repeat dosing, the adverse effects that precluded repeat dosing were at least in part attributable to expected adverse events secondary to the initial vector dose.

All 40 patients were able to begin chemotherapy treatment 14 days after initial vector instillation. Eighteen of 40 patients (45%) received first-line chemotherapy. Twenty-two patients (55%) received second-line chemotherapy with either pemetrexed ($n = 7$) alone or gemcitabine ± carboplatin ($n = 15$). At least four cycles of chemotherapy were delivered to all but 10 of the 40 patients. Chemotherapy was stopped in nine of these 10 patients due to disease progression after one cycle [$n = 1$ (first-line)], two cycles [$n = 6$ (one first line, five second-line)], or three cycles [$n = 2$ (both second-line)]. In the 10th patient, chemotherapy was stopped after one cycle due to development of an acute respiratory decompensation subsequently determined to be unrelated to the protocol.

The study protocol was generally well tolerated. Most patients experienced only expected mild toxicities from the vector and transgene expression, including cytokine release syndrome, nausea, fatigue, anemia, lymphopenia (grade 3–4), and hypoalbuminemia (Table 2). These toxicities typically resolved within 24 to 48 hours of completion of vector dosing and predominantly occurred after the initial vector infusion. We identified 11 patients who had mild symptoms, including temporary malaise, loss of appetite, nausea, and persistent low-grade fevers, for a few days after vector instillation, presumably due to systemic IFN effects. Serious adverse events included pleural catheter infection ($n = 2$); hypoxia ($n = 2$); supraventricular tachycardia ($n = 1$); and esophagitis ($n = 1$); none was directly attributable to the instillation of the vector (Table 2). Local infection related to catheter placement was certainly associated with the study protocol, in which the majority of patients underwent catheter insertion specifically for enrollment in this clinical trial, but adverse effects from the catheter were not directly related to the administration of rAdIFN into the pleural space via the catheter or to the rAdIFN vector itself. The one patient with transitory hypoxia experienced a presumed congestive heart failure exacerbation on the day of repeat vector dosing related to planned withholding of diuretics in anticipation of possible hypotension related to vector instillation. The hypoxia rapidly resolved after diuresis. The episode of supraventricular tachycardia was seen a single patient with massive tumor burden in the right hemithorax and mediastinum compressing both his left and right atria. The esophagitis was noted in a patient who required stereotactic radiotherapy for palliation of a focal region of her left-sided malignant pleural mesothelioma that was compressing her distal esophagus.

There were no treatment-related deaths. Adverse events during the chemotherapy portion of the study were expected and comparable with historical controls (Table 3).

Response rates using modified RECIST1.1 are shown in Fig. 1A and Table 4. For both cohorts combined, we noted stable disease in 62.5% of patients and partial responses in 25% of patients; no complete responses were observed. Only 12.5% had progressive disease following cycle 2. The overall disease control rate (DCR) was 87.5%. Partial responses were seen in 9 of 25 (36%) evaluable patients with pemetrexed-based chemotherapy and 1 of 15 (7%) with gemcitabine-based treatment.

Figure 1B and C show the changes in modified RECIST measurements and SMRP levels, respectively, compared with baseline. For SMRP, 12 of the 27 patients showed more than a 20% increase in SMRP level (Fig 1C, top), whereas 15 of the 27 patients showed a greater than 20% decrease at some time point (Fig. 1C, bottom). Both modified RECIST and SMRP responses were durable.

At the time of submission of this manuscript, 6 of 40 patients remained alive with a minimum follow-up of 24 months. All but two of the deceased patients died from progressive disease, with one patient dying from esophageal perforation status post proton-beam radiotherapy (5 months) and another from a BAP-1 deficiency–related metastatic uveal melanoma (40 months). Figure 2A shows the Kaplan–Meier curve of the entire group. The MOS was 13 months [95% confidence interval (CI), 9–12]; however, we noted a significant "tail" to the curve, revealing a subset of patients with prolonged survival. The survival of the entire cohort at 12 months was 55% (95% CI, 0.38–0.69), at 18 months 40% (95% CI, 0.55–0.25), and at 24 months 25% (95% CI, 0.39–0.13). The PFS was 5.3 months.

Table 3. Adverse events related to chemotherapy

	Grade (number of events)				
Adverse events: Chemo related	**1**	**2**	**3**	**4**	**Total**
Blood					
Hemoglobin—low	20	37	10	1	68
Neutrophils—low	2	1	1		4
Lymphopenia	5	1	5	7	18
Neutrophils—low	2		1	2	5
Platelets—low	5	1	2	1	9
Leukocytes—low	6	2	1		9
Constitutional					
Fatigue	5	5			10
Fever in absence of neutropenia	2				2
Weight—loss	2				2
Weight—increase	1				1
Rigor	1				1
Dermatology					
Alopecia	1				1
Hyperpigmentation—nevi	1				1
Rash—pruritic trunk/UE		1			1
Endocrine					
Cushingoid appearance (swelling to face)		1			1
Gastrointestinal					
Anorexia	3	3			6
Nausea	10	1			11
Esophagitis	3		1		4
Diarrhea	2				
Vomiting	1	1			2
Hiccups	1				1
Metabolic					
Albumin—low	19	23			42
ALT—high	1	1			2
AST—high	1	1			2
Calcium—low	5	2	1		8
Sodium—low	6				6
Creatinine	2	1			3
Potassium	1				1
Neurologic					
Dizziness	2				2
Neuropathy	1	1			2
Tinnitus		1			1
Rhinorrhea/rhinitis	2				2
Vertigo	1				1
Other: buzzing in ears	1				1
Other: numbness hand/feet	1				1
Pain					
Arthralgia	1				1
Tumor site		1			1
Headache		1			1
Pulmonary					
Cough	1				1

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; UE, upper extremity.

A number of subgroups were analyzed. Figure 2B shows a significant (log rank, $P = 0.004$) difference in MOS for the 30 patients with epithelial histology (19 months) versus the 10 patients with nonepithelial histology (6.5 months). The 18 treatment-naïve patients treated with front-line chemotherapy had a MOS of 12 months (95% CI, 6–15; Fig. 2C) with a median PFS of 6.5 months (95% CI, 5.5–11.5). Figure 2D shows survival in the 22 patients treated with second-line therapy. The MOS for the second-line cohort was 17 months (95% CI, 6.5–26). Figure 2E is a subgroup analysis of the second-line cohort. In the second-line pemetrexed group ($n = 7$), the MOS was 26 months (the 24-month survival rate was 62% with 3/7 patients still alive) with a median PFS of 8 months (95% CI, 3–∞). In the second-line gemcitabine group ($n = 15$), the MOS was 10 months (95% CI, 4–21) and the median PFS 3.5 months (95% CI, 1.5–5.5). MOS was not significantly associated with gender or age (data not shown).

All potential patients were screened for baseline adenoviral Nab titers. Sixteen percent of the screened patients had titers above our predetermined cut-off value of 1:2,000 and were thus deemed ineligible. Of the 40 patients who participated in the trial, the median titer was 1:100; the distribution of titers is shown in Supplementary Fig. S2.

Biocorrelates

Serum levels of IFNα were measured pre-vector infusion (day 1). Serum IFN was undetectable or very low at baseline in 39 patients; one subject had high circulating levels before therapy (2,100 pg/mL). Roughly, half of the patients ($n = 21$) had detectable levels of serum IFN (15–1,608 pg/mL) on day 2 after vector infusion (Supplementary Fig S3A). Of these patients, the median value was 470 pg/mL. Levels of IFNα in the pleural fluid or the pleural lavage were measured at baseline in 38 patients. No patients had detectable baseline intrapleural IFNα. Pleural levels were much higher than seen in the serum after initial dosing (Supplementary Fig. S3B). We saw no correlation of survival times with the serum or pleural IFN levels (Supplementary Fig. S3C).

Expression of antitumor antibodies in the serum of posttreatment patients was available for analysis in 39 of the 40 patients. In 11 patients, we observed no changes in the number or intensity of antitumor immunoblot bands, in 14 there were minimal changes in tumor bands, and in the remaining 14, there were clear increases in antitumor bands. However, there were no significant differences in survival or in radiographic response rates among these groups (Supplementary Table S5).

We conducted flow cytometry from PBMC in six patients who had good responses (average survival = 23.5 months) and compared results with six patients with poor responses (average survival = 7.2 months; Supplementary Table S3). In previous studies, we had observed increases in the expression of the activation marker CD69 in NK cells after Ad.IFN administration in some patients, suggesting this could be a marker of systemic release of IFNα resulting in activation of the NK cells. Although we observed increases in the percent of NK cells and T cells expressing CD69 3 days after Ad.IFNα instillation in the majority of patients, we detected no significant correlation with clinical responses (Supplementary Table S6). We observed no increases in the NK activation receptors NKp46, NKG2D, NKG2A, and NKp30 [which had predicted response in a DC vaccine trial (21)], nor changes in a CD8 T-cell activation signature ($CD38^{hi}$/HLA-DR^{hi} and $ki67^{hi}$/Bcl-2^{low}; ref. 22). We also noted no differences in baseline levels of CD4 Tregs ($CD4^+/CD25^+/FOXP3^+$cells) or changes in the induction of these cells. Increases in the expression of ICOS on CD4 cells have been associated with responses in patients with melanoma treated with anti-CTLA4 antibody (23); however, we saw no significant changes in these markers (data not shown).

Finally, we investigated whether the "immunogenicity of the tumor microenvironment" could predict responses to immunotherapy (refs. 20, 24, 25; using pathologic material from pretreatment biopsies available in 18 patients). Using IHC, we noted no significant correlations with either the degree of lymphocyte (CD8 staining), or macrophage (CD68 staining) infiltration, nor expression of PD-L1 with survival (Supplementary Figs. S4A, S4B, and S5). Slides were also used to produce

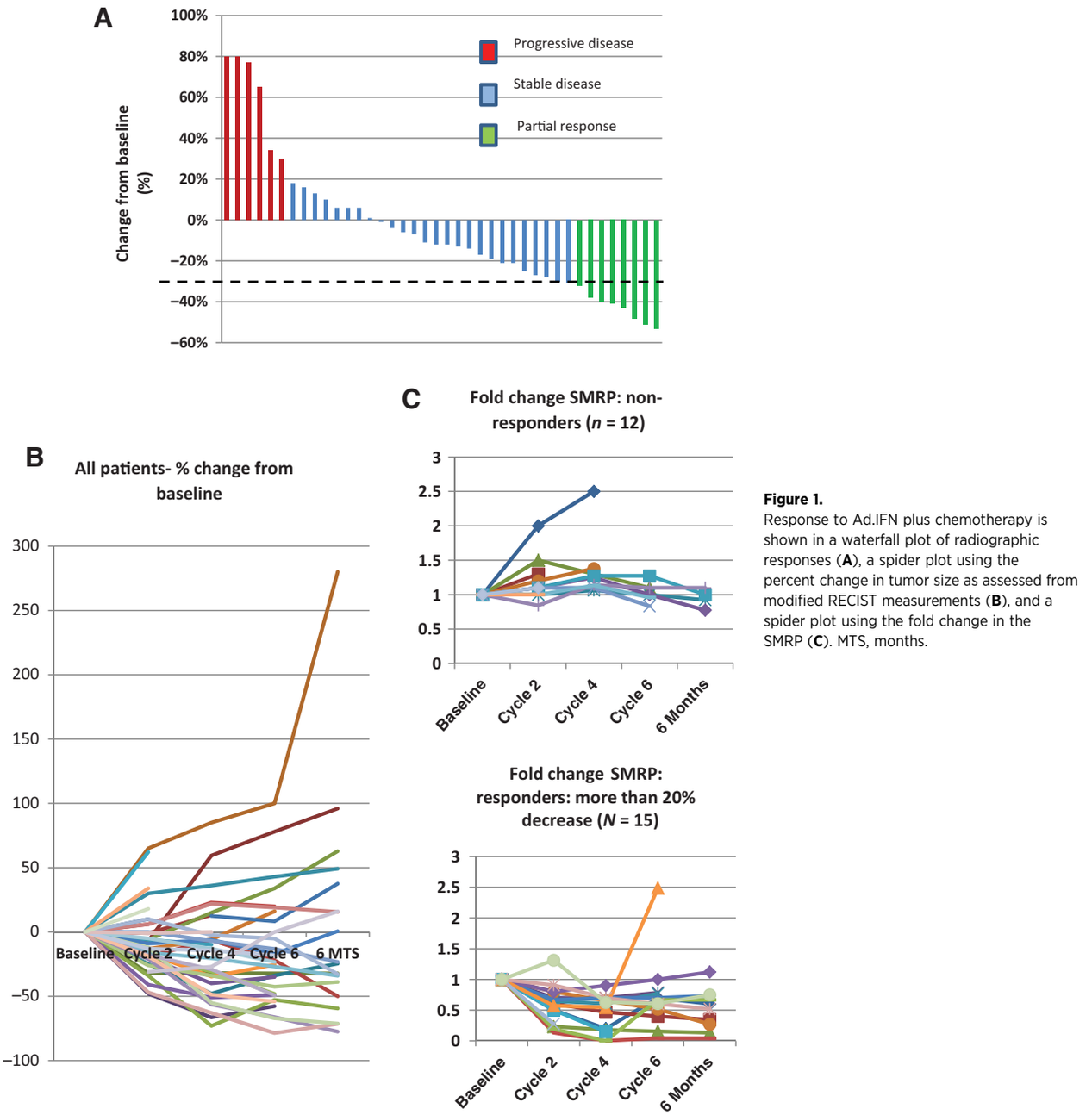

Figure 1.
Response to Ad.IFN plus chemotherapy is shown in a waterfall plot of radiographic responses (**A**), a spider plot using the percent change in tumor size as assessed from modified RECIST measurements (**B**), and a spider plot using the fold change in the SMRP (**C**). MTS, months.

RNA that was interrogated for 600 immune response–related genes using NanoString technology. We had information on 27 of the 61 PCR-validated genes from a recently published immune response gene signature (20). These markers are primarily T-cell and IFN-induced genes (see Supplementary Table S4). When the MPM specimens were ranked for intensity of expression of these genes, there was no significant correlation with survival (Supplementary Fig. S6).

Discussion

The rationale for this trial was to induce antitumor immune responses using an approach called "*in situ* vaccination," a strategy where the tumor site itself is used as a target and becomes the source of antigen. We used the strong immune-potentiating activity of an adenoviral vector expressing an activating transgene (IFNα) to both induce immunogenic cell death and change the tumor microenvironment towards an immunostimulatory state. In addition, we attempted to further alter the tumor microenvironment by inhibiting the potent immunosuppressive molecule PGE2 (26) by administering a COX-2 inhibitor, celecoxib. Most cancer vaccines, however, require multiple administrations of antigen ("boosts") for optimal efficacy (27, 28). As the induction of neutralizing Ad antibodies prevented us from giving more than two, closely spaced doses of vector, we provided our "boost" by taking advantage of the observations that certain types of

Table 4. Responses

Patient group	Chemotherapy	# Pts	Response rate (%)	Stable disease %	DCR %	Median PFS (mo)	Median OS (mo)	OS 1 yr (%)	OS 18 mo %	OS 24 mo %
All patients		40	25	62.5	87.5	5.3	13	55	40	25
Naïve	Pem/cis	18	28	55	83	6.5	12	55	28	17
Pretreated	All second line	22	14	77	91	4.0	17	59	50	32
	Prior Pem- repeat Pem	7	28	72	100	8.0	26	86	86	57
	Prior Pem repeat GEM	15	7	80	87	3.5	10	47	33	20

Abbreviations: cis, cisplatin; GEM, gemcitabine; Pem, pemetrexed.

chemotherapy can cause cell death in an immunogenic context, thus stimulating a primed antitumor response (14–17). This is, therefore, one of the first clinical trials to formally employ a combination of *in situ* genetic immunotherapy and chemotherapy.

Our multipronged combination approach proved to be both feasible and safe in the majority of patients enrolled. In our study, 32 of 40 patients tolerated the combination therapy without evidence of serious adverse events; the majority of adverse events related to vector dosing were attributable to the initial dose; and seven of the eight patients who had serious adverse events after initial dosing were able to safely complete the course of celecoxib and chemotherapy. Only a single patient did not proceed with further chemotherapy dosing, and this was because of the esophagitis related to radiotherapy, as described previously.

On the basis of our prior clinical trials involving repeated intrapleural dosing of recombinant Ad vectors expressing type I IFN genes (AdIFNβ and AdIFNα; refs. 4–6), the majority of the observed toxicities were related to cytokine release syndrome secondary to the initial vector dose. In this study, one of the primary outcome measures was the safety of sequential therapy with rAdIFN/celecoxib and chemotherapy. We did not believe that there would be substantial differences between the combination of one dose of rAdIFN and chemotherapy and that of two doses. As we had seen radiographic responses with single doses of AdIFN in prior phase I clinical trials (4–6), it was reasonable from both a safety and efficacy perspective to allow patients to proceed in the study after only the initial rAdIFN dose.

In terms of clinical efficacy in first-line patients, our response rate, median PFS, MOS, and 1-year survival were similar to those previously reported in the literature with combination chemotherapy alone (See Supplementary Table S1). However, our disease control rate was higher than reported with chemotherapy, and there was a "tail" on the Kaplan–Meier curve, representing a subset of patients with prolonged survival. This was observed

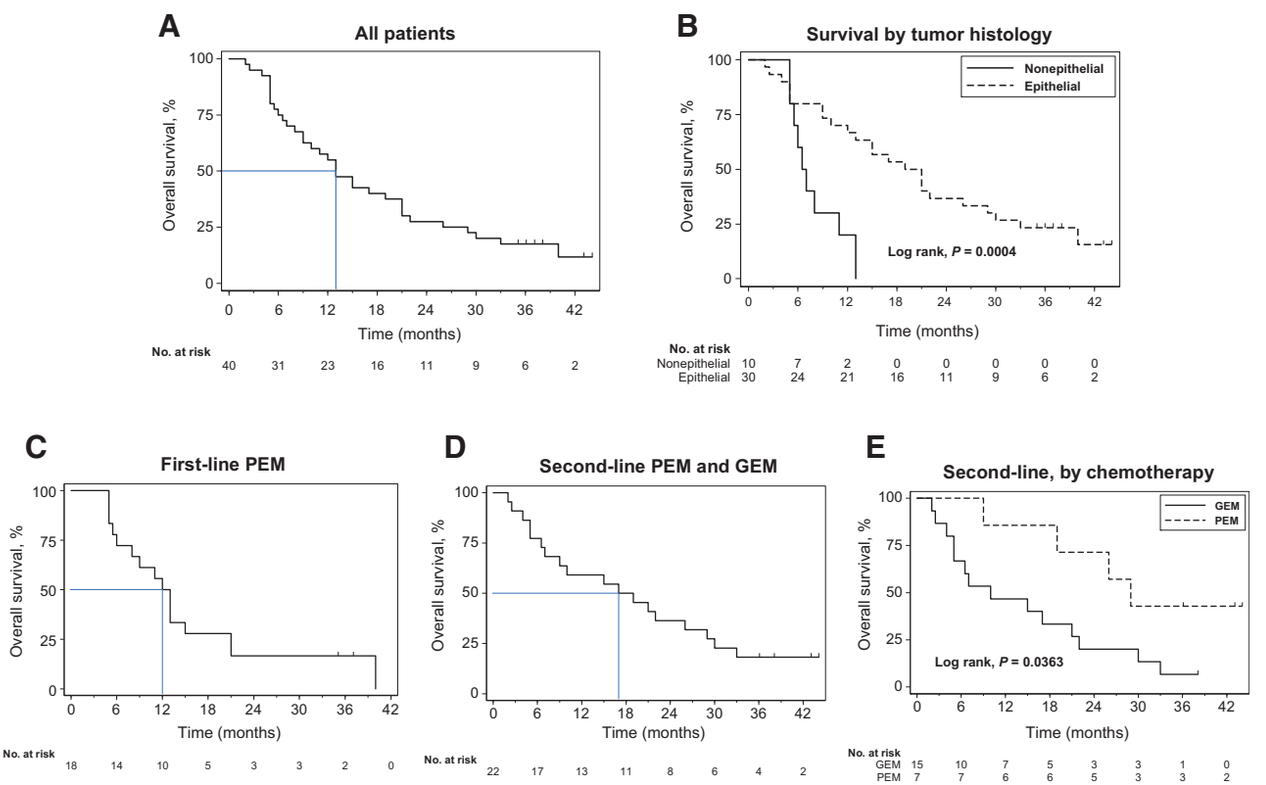

Figure 2.
Kaplan–Meier plots for survival for all subjects ($n = 40$; **A**) or subjects segregated by tumor histology [nonepithelial ($n = 10$) vs. epithelial ($n = 30$; **B**)], subjects receiving first-line therapy with pemetrexed (PEM; $n = 18$; **C**), subjects receiving second-line therapy ($n = 22$; **D**), and second-line subjects segregated by type of chemotherapy [gemcitabine (GEM) based ($n = 15$) vs. pemetrexed based ($n = 7$; **E**)].

despite the fact that only 11 of the 18 patients (61%) in our first-line cohort had the more favorable epithelial histology [a proportion lower than any of the reported trials (Supplementary Table S1)]. Although the numbers are small, the MOS in the epithelial, front-line group was 15 months versus only 8 months in the nonepithelial patients ($P < 0.05$).

We believe that the lack of improvement in MOS seen in the front-line pem/plat/rAdIFN group compared with historical controls was due to several factors, including higher percentage of nonepithelioid tumors, pretreatment with surgery and/or palliative radiotherapy, and selection of late-stage patients as early-stage patients with mesothelioma were shunted into concurrent trials of radical pleurectomy and photodynamic therapy at our institution. Surgery for mesothelioma was not nearly as well established in 2003 at the time of publication of the study by Vogelzang and colleagues, and therefore, many of the patients receiving chemotherapy in that trial would have been considered for surgical intervention at the present time.

Although the response rate and median PFS in second-line patients were similar to those from previously reported trials, the DCR and MOS were almost double those reported in similar second-line chemotherapy trials (Supplementary Table S1). Similar to the front-line patients, we found a "survival tail" on the Kaplan–Meier plots. Approximately 20% of second-line patients receiving gemcitabine-based chemotherapy were alive at 24 months, suggesting a prolonged immunologic phenomenon. Of special interest, however, was the finding that the seven second-line patients undergoing retreatment with pemetrexed had an especially impressive DCR of 100%, response rate of 28%, a PFS of 8 months, and an MOS of greater than 25 months. For a comparison with this specific patient population, we were able to find data from three clinical trials (which included a total of 103 patients) that administered pemetrexed as second-line therapy in patients who had previously responded to pemetrexed (Supplementary Table S1). Although this group clearly has especially good response characteristics (with average reported response rates of 18%, PFS of 5.1 months, and MOS of 11.7 months), the patients in this trial responded to a much more impressive degree (see above).

The presence of patients with durable stable or slowly progressive disease resulting in prolonged survival has been observed in other immunotherapy trials (29). For example, recent studies using anti-CTLA antibodies have shown this pattern in melanoma and mesothelioma (30, 31). This pattern is consistent with observations that the effects of immunotherapy are frequently delayed, can show mixed patterns of response, and may not result in increased PFS or MOS while still engendering improved long-term survival rates (29, 32). Our long-term response data using radiographic measurements and SMRP levels, and the prolonged "stable disease" seen in many of our patients, are similar to other immunotherapy trials.

Despite our extensive investigations, we were unable to identify potential biomarkers that might provide prognostic and/or mechanistic information. This may be due to the fact that circulating cells or factors may poorly reflect processes within tumors, the implication being that the most useful biomarkers will need to be found from tumor biopsy specimens. This may be especially true for types of immunotherapy (such as ours) that generate polyclonal responses against unknown antigens, compared with vaccines where responses against a known specific antigen can be measured in the blood.

It is of interest to speculate on how Ad.IFN therapy might interface with checkpoint inhibitory blockade, an approach showing promise in mesothelioma (31). In contrast to checkpoint blockade therapy with anti-PD-1 or anti-PD-L1 antibodies, the expression of PD-L1 and the preexisting immune signature of the tumor did not predict response to Ad.IFN. Given that *in situ* vaccination presumably works by inducing immune responses rather than simply amplifying existing endogenous immunity, Ad.IFN may be especially useful in those patients with minimal endogenous immune responses or low expression of PD-L1 and might be even more efficacious when combined with anti-CTLA4 or anti-PD1 antibodies. Preclinical studies to test these hypotheses are underway.

As our study was relatively small, nonrandomized, and conducted at a single center, it is important to recognize several potential limitations to the interpretation of the results. There is substantial heterogeneity in the clinical course of mesothelioma. A recently published registry study detailing the survival of patients with MPM posited that the MPM population can be divided into two groups: one with a short survival time (9–12 months) and another small group that survives considerably longer (33). Any early-stage clinical trial, such as ours, is subject to possible selection bias, including bias towards a good ECOG performance status and a clinical status sufficient to tolerate access to the pleural cavity for intrapleural delivery of the Ad.IFN vector. Importantly, many of our patients received subsequent therapies with uncertain impact on ultimate survival (see Supplementary Table S2). In addition, with the limitations of our study design, we cannot differentiate the individual effects of PGE2 inhibition with celecoxib and induction of antitumor immune responses by intrapleural delivery of rAdIFN. Although there are independent effects of each intervention in preclinical models, we demonstrated synergy in antitumor effects with the combination of celecoxib and adenoviral gene therapy (ref. 16 and unpublished data). Given the rarity of malignant pleural mesothelioma, the urgent need to rapidly test new approaches, and the challenges of accruing patients into large, randomized clinical trials, particularly into a four-armed clinical trial (chemo alone, chemo + rAdIFN, chemo + celecoxib, and chemo + rAdIFN/celecoxib), we chose to study the rAdIFN/celecoxib combination as a "single" experimental intervention. We have also observed in pilot studies that celecoxib administration can mitigate some of the symptoms of cytokine release syndrome that can be seen in some patients receiving rAdIFN; thus, there is an additional rationale for a brief period of celecoxib administration in future trials even in the absence of effects upon the tumor microenvironment.

As our trial was nonrandomized, our results can only be interpreted in the context of previously published studies with the presumption that the smaller second-line trials had the same sort of patient populations and similar biases as our trial. Using this admittedly imperfect comparator, a particularly interesting finding in our study was that patients with mesothelioma who received second-line chemotherapy (especially second-line pemetrexed) did extremely well when the chemotherapy was given subsequently to a priming protocol of immunogene therapy via Ad.IFN *in situ* vaccination plus targeted blockade of immunosuppression by concomitant administration of celecoxib. As for the second-line pemetrexed patients, it is clear that this group fared better in terms of MOS than the second-line gemcitabine cohort (and, ironically, even better than first-line pemetrexed recipients). We were likely selecting patients with more favorable tumor

biology, given that they had a durable (at least 6-month) initial response to pemetrexed prior to disease progression. In addition, those patients who failed to respond to pemetrexed/platin and then received gemcitabine likely had a worse overall tumor biology than the treatment-naïve patients in the first-line cohort. Therefore, there were selection biases in both directions in the second-line arm of the trial. Perhaps most importantly, these same biases are present in every second-line chemotherapy trial in mesothelioma, and our reported OS rates in second line are superior to prior reports of retreatment with pemetrexed as well as with gemcitabine (see Supplementary Table S1).

These results raise several interesting, but as yet unanswered questions: (i) why did second-line patients respond so much better than first-line recipients? (ii) why do patients receiving a repeat course of pemetrexed perform better than those on the second line gemcitabine? (iii) is it possible that the patients who initially responded to pemetrexed and were then retreated have been preselected as long-term stable disease? and (iv), if the immune response is to be credited with the difference in survival, then why are there no markers of immune responsiveness that correlate with this outcome? A biopsy subsequent to therapy would have been helpful in determining intratumoral markers of immune responsiveness but was not included in this clinical protocol. Hopefully, some of these questions can be answered in future studies.

We do not yet know the optimal chemotherapy regimen for "immunologic priming" in mesothelioma. The potential role of chemotherapy in combination with immunotherapy is multifold and includes tumor cell death resulting in presentation of tumor neoantigens to DCs; decreased numbers of myeloid-derived suppressor cells and Tregs; overall T-cell depletion allowing increased space in the existing T-cell repertoire for tumor-specific cytotoxic T cells; and increased T-cell trafficking into the tumor microenvironment (14–17). Our laboratory has spent considerable effort in evaluating these characteristics of both pemetrexed and gemcitabine in syngeneic, immunocompetent murine models of malignant mesothelioma and has demonstrated significant synergy for both chemotherapy agents with murine versions of rAdIFN. We selected pemetrexed for first-line therapy in this clinical trial in large part because of its accepted role as the standard-of-care chemotherapy agent for front-line therapy in mesothelioma; gemcitabine is a well-accepted second-line agent for mesothelioma. It is possible, however, that gemcitabine may be a more effective agent to use in front-line therapy with rAdIFN than pemetrexed, and we hope to answer this question in future human clinical trials.

In conclusion, our study shows that the combination of intrapleural Ad.IFNα2b vector, celecoxib, and systemic chemotherapy proved to be safe, feasible, and well tolerated in patients with MPM. Disease control and survival rates observed in this study, especially in the second-line therapy, compared favorably with historical data. Obviously, the value of our approach needs to be validated with a larger, multicenter randomized clinical trial. Such a study is being planned in the second-line setting, where no therapy has yet been shown to enhance survival in patients with mesothelioma.

Disclosure of Potential Conflicts of Interest

J.P. Stevenson reports receiving commercial research grants from Verastem. No potential conflicts of interest were disclosed by the other authors.

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References

1. Ai J, Stevenson JP. Current issues in malignant pleural mesothelioma evaluation and management. Oncologist 2014;19:975–84.
2. Zucali PA, Simonelli M, Michetti G, Tiseo M, Ceresoli GL, Collovà E, et al. Second-line chemotherapy in malignant pleural mesothemioma: Results of a retrospective multicenter survey. Lung Cancer 2012;75:360–67.
3. Vachani A, Moon E, Wakeam E, Albelda SM. Gene therapy for mesothelioma and lung cancer. Am J Respir Cell Mol Biol 2010;42:385–93.
4. Sterman DH, Recio A, Carroll RG, Gillespie CT, Haas A, Vachani A, et al. A phase I clinical trial of single-dose Intrapleural INF-beta gene transfer for malignant pleural mesothelioma and metastatic pleural effusions: high rate of antitumor immune responses. Clin Cancer Res 2007;13:4456–66.
5. Sterman DH, Recio A, Haas AR, Vachani A, Katz SI, Gillespie CT, et al. A phase I trial of repeated Intrapleural adenoviral-mediated interferon-beta gene transfer for mesothelioma and metastatic pleural effusion. Mol Ther 2010;18:852–60.
6. Sterman DH, Haas A, Moon E, Recio A, Schwed D, Vachani A, et al. A trial of intrapleural adenoviral-mediated Interferon-α2b gene transfer for malignant pleural mesothelioma. Am J Respir Crit Care Med 2011;184:1395–9.
7. Ferrantini M, Capone I, Belardelli F. Interferon-alpha and cancer: mechanisms of action and new perspectives of clinical use. Biochimie 2007;89:884–93.

8. Boutin C, Nussbaum E, Monnet I, Bignon J, Vanderschueren R, Guerin JC, et al. Pleural treatment with recombinant gamma-interferon in early stage malignant pleural mesothelioma. Cancer 1994;74:2460–7.
9. Arico E, Belardelli F. Interferon-alpha as antiviral and antitumor vaccine adjuvants: Mechanisms of action and response signature. J Interferon Cytokine Res 2012;32:235–47.
10. Majhen D, Calderon H, Chandra N, Fajardo CA, Rajan A, Alemany R, et al. Adenovirus-based vaccines for fighting infectious diseases and cancer: progress in the field. Hum Gene Ther 2014;25:301–17.
11. Dinney CP, Fisher MB, Navai N, O'Donnell MA, Cutler D, Abraham A, et al. Phase I trial of intravesical recombinant adenovirus mediated interferon-α2b formulated in Syn3 for bacillus Calmette-Guerin failures in nonmuscle invasive bladder cancer. J Urol 2013;190:850–6.
12. Haas AR, Sun J, Vachani A, Wallace AF, Silverberg M, Kapoor V, et al. Cyclooxygenase-2 inhibition augments efficacy of a cancer vaccine. Clin Cancer Res 2006;12:214–22.
13. Fridlender ZG, Sun J, Singhal S, Kapoor V, Cheng G, Suzuki E, et al. Chemotherapy delivered after viral immune-gene therapy augments antitumor efficacy via multiple immune-mediated mechanisms. Mol Ther 2010;18:1947–59.
14. Lake RA, Robinson BWS. Immunotherapy and chemotherapy – a practical partnership. Nat Rev Cancer 2005;5:397–405.
15. McCoy MJ, Nowak AK, Lake RA. Chemoimmunotherapy: an emerging strategy for the treatment of malignant mesothelioma. Tissue Antigens 2009;74:1–10.
16. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008;8:59–73.
17. de Biasi AR, Vliiena-Vargas J, Adusumilli PS. Cisplatin-induced antitumor immunomodulation: a review of preclinical and clinical evidence. Clin Cancer Res 2014;20:5384–91.
18. Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Ann Oncol 2004;15:257–60.
19. Stevenson JP, Kindler HL, Papasavvas E, Sun J, Jacobs-Small M, Hull J, et al. Immunological effects of anti-transforming growth factor-beta (TGF-beta) antibody GC1008 in cancer patients with malignant pleural mesothelioma (MPM). Oncoimmunology 2013;8:e26218.
20 Ulloa-Montoya F, Louahed J, Dizier B, Gruselle O, Spiessens B, Lehmann FF, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. J Clin Oncol 2013;31:2388–95.
21. Osada T, Clay T, Hobeika A, Lyerly HK, Morse MA. NK cell activation by dendritic cell vaccine: a mechanism of action for clinical activity. Cancer Immunol Immunother 2006;55:1122–31.
22. Miller JD, van der Most RG, Akondy RS, Glidewell JT, Albott S, Masopust D, et al. Human effector and memory CD8+ T cell responses to smallpox and yellow fever vaccines. Immunity 2008;28:710–22.
23. Ng Tang D, Shen Y, Sun J, Wen S, Wolchok JD, Yuan J, et al. Increased frequency of ICOS+ CD4 T cells as a pharmacodynamic biomarker for anti-CTLA-4 therapy. Cancer Immunol Res 2013:1:229–34.
24. Ji RR, Chasalow SD, Wang L, Hamid O, Schmidt H, Cogswell J, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2012;61:1019–31.
25. Wang E, Bedognetti D, Marincola FM. Prediction of response to anticancer immunotherapy using gene signatures. J Clin Oncol 2013;31:2369–71.
26. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. Semin Immunopathol 2013;35:123–37.
27. Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, et al. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol 2014;11:509–24.
28. Palena C, Schlom J. Vaccines against human carcinoma: strategies to improve antitumor immune responses. J Biomed Biotechnol 2010;2010: 380697.
29. Madan RA, Gulley JL, Fojo T, Dahut WL. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. Oncologist 2010;15:969–75.
30. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from Phase II and Phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol 2015;33:1889–94.
31. Calabrò L, Morra A, Fonsatti E, Cutaia O, Amato G, Giannarelli D, et al. Tremelimumab for patients with chemotherapy-resistant advanced malignant mesothelioma: an open-label, single-arm, phase 2 trial. Lancet Oncol 2013;14:1104–11.
32. Hoos A, Eggermont AM, Janetzki S, Hodi FS, Ibrahim R, Anderson A, et al. Improved endpoints for cancer immunotherapy trials. J Natl Cancer Inst 2010;102:1388–97.
33. Linton A, Pavlakis N, O'Connell R, Soeberg M, Kao S, Clarke S, et al. Factors associated with survival in a large series of patients with malignant pleural mesothelioma in New South Wales. Br J Cancer 2014;111:1860–69.