A Phase I Study of Concurrent Chemotherapy (Paclitaxel and Carboplatin) and Thoracic Radiotherapy with Swallowed Manganese Superoxide Dismutase Plasmid Liposome Protection in Patients with Locally Advanced Stage III Non-Small-Cell Lung Cancer

Ahmad A. Tarhini,^{1*} Chandra P. Belani,^{2*} James D. Luketich,¹ Athanassios Argiris,¹ Suresh S. Ramalingam,³ William Gooding,¹ Arjun Pennathur,¹ Daniel Petro,¹ Kevin Kane,¹ Denny Liggitt,⁴ Tony ChampionSmith,⁵ Xichen Zhang,¹ Michael W. Epperly,¹ and Joel S. Greenberger¹

Abstract

Manganese superoxide dismutase (MnSOD) is a genetically engineered therapeutic DNA/liposome containing the human MnSOD transgene. Preclinical studies in mouse models have demonstrated that the expression of the human MnSOD transgene confers protection of normal tissues from ionizing irradiation damage. This is a phase I study of MnSOD plasmid liposome (PL) in combination with standard chemoradiation in surgically unresectable stage III non-small-cell lung cancer. Chemotherapy (carboplatin and paclitaxel) was given weekly (for 7 weeks), concurrently with radiation. MnSOD PL was swallowed twice a week (total 14 doses), at three dose levels: 0.3, 3, and 30 mg. Dose escalation followed a standard phase I design. Esophagoscopy was done at baseline, day 4, and 6 weeks after radiation with biopsies of the squamous lining cells. DNA was extracted and analyzed by PCR for the detection of the MnSOD transgene DNA. Ten patients with AJCC stage IIIA (three) and IIIB (seven) completed the course of therapy. Five had squamous histology, two adenocarcinoma, one large cell, and two not specified. Patients were treated in three cohorts at three dose levels of MnSOD PL: 0.3 (three patients), 3 (three patients), and 30 mg (four patients). The median dose of radiation was 77.7 Gy (range 63–79.10 Gy). Overall response rate for the standard chemoradiation regimen was 70% ($n = 10$). There were no dose-limiting toxicities reported in all three dosing tiers. It is concluded that the oral administration of MnSOD PL is feasible and safe. The phase II recommended dose is 30 mg.

Introduction

FOR PATIENTS with unresectable stage III non-small-cell lung cancer (NSCLC), clinical trials conducted in the 1970s established the efficacy of definitive radiation therapy (RT) (Perez *et al.*, 1980). Subsequently, sequential therapy (chemotherapy followed by RT) was tested to avoid overlapping toxicities. The CALGB 8433 trial (Dillman *et al.*, 1990), the Radiation Therapy Oncology Group (RTOG) 88-08 trial (Sause *et al.*, 1995), and the French trial (Le Chevalier *et al.*,

1991) have primarily established that sequential chemotherapy and radiation are superior to radiation alone. In an effort to improve outcomes, subsequent studies compared sequential versus concurrent chemoradiotherapy, taking advantage of the synergistic effect of chemotherapy and RT. In the 1990s, two large randomized trials, RTOG 9410 and the West Japan Lung Cancer Group trial, have demonstrated that chemotherapy with concurrent radiation is superior to sequential administration of chemotherapy and radiation (Furuse *et al.*, 1999; Curran *et al.*, 2002, 2003).

¹University of Pittsburgh Cancer Institute, Pittsburgh, PA 15232.

²Penn State Hershey Cancer Institute, Hershey, PA 17033.

³Emory University, Atlanta, GA 30322.

⁴University of Washington School of Medicine, Seattle, WA 98195.

⁵PharmaReg Consultants, San Leandro, CA 94577.

*A.A.T. and C.P.B. contributed equally to this work as first author.

Esophageal toxicity has been accepted reluctantly, as a necessary side effect of the benefits of chemoradiotherapy for lung cancer (Choy and Browne, 1995; Belani *et al.*, 1997; Langer *et al.*, 1997a; Curran *et al.*, 2003; Gandara *et al.*, 2003). During treatment of large tumor volumes in the chest, the tumor dose-modifying effects of chemotherapy at the molecular level also appear to be esophageal toxicity-enhancing effects.

Manganese superoxide dismutase (MnSOD) is a genetically engineered investigational biological drug, prepared from an *E. coli* seed stock. The final product (VLTS-582) is a therapeutic DNA/liposome formulation consisting of a double-stranded DNA bacterial plasmid, containing the human MnSOD cDNA, combined with two lipids {cholesterol and DOTIM (1-[2-[9-(Z)-octadecenoyloxy]]-2-[8-(Z)-heptadecenyl]-3-[hydroxyethyl]imidazolinium chloride)}, and formulated in a Tris buffer containing sucrose. Initial *in vitro* pharmacology studies using the murine hematopoietic progenitor cell line 32D cl 3 have demonstrated that the expression of the human MnSOD transgene could protect against irradiation damage (Epperly *et al.*, 1998a). Protection of normal tissues from ionizing irradiation damage by gene therapy has also been demonstrated in the mouse lung (Epperly *et al.*, 1998b; Wheldon *et al.*, 1998). Success has also been achieved in a murine model of radiation-induced esophagitis (Epperly *et al.*, 1999, 2001).

The primary toxicology study with MnSOD plasmid liposome (PL) used rabbits to mimic the route and method of delivery, which entailed swallowing of measured doses of the MnSOD PL. As in the clinical trial, this study also incorporated 14 doses, but on an accelerated schedule of alternate day dosing. The safety margin at the highest dose was approximately ninefold. Two additional toxicology studies were also performed to account for potential systemic exposure via esophageal ulcerations, and an intravenous toxicology study with biodistribution was performed in mice. There was also concern of potential inadvertent inhalation of incompletely swallowed material in patients receiving MnSOD PL; hence, a single-dose lung instillation study was also performed in rabbits. Upon analysis of appropriate histologic, hematologic, and clinical chemistry data, these studies demonstrated no significant toxicologic findings in any dose group.

Therefore, we conducted this phase I study of MnSOD PL gene therapy protection of the esophagus from damage induced by chemoradiotherapy. For concurrent chemotherapy, we used a combination of weekly paclitaxel and carboplatin (Choy and Browne, 1995; Belani *et al.*, 1997; Langer *et al.*, 1997a).

Patients and Methods

Patients

Patients were eligible if they had pathologically documented NSCLC. Patients were required to have American Joint Committee on Cancer (AJCC) stage IIIA (T1/T2 N2 or T3 N1–2) and were considered medically inoperable, or stage IIIB (T4 with any N or any T with N3). Eligible patients were also required to have met the following criteria: age $\geq$ 18 years; ECOG PS 0–1; no prior systemic chemotherapy or radiation to the thorax; adequate hematologic (granulocytes $\geq$ 2,000/ml, platelets $\geq$ 100,000/ml, hemoglobin $>$ 8 gm/dl), hepatic function (bilirubin $<$ 1.5 $\times$ normal), and renal values

(creatinine clearance $>$ 50 ml/min); FEV₁ $>$ 800 cc; and written informed consent.

Study design and treatment

This is a phase I study to assess the feasibility and safety of MnSOD PL in combination with paclitaxel, carboplatin, and thoracic radiotherapy. MnSOD PL was delivered by swallowing on days 1 and 3 of each week of combined chemotherapy and radiation. Radiation (all received three-dimensional conformal radiation) commenced 4–6 hr after the first MnSOD PL dose, at 1.9–2.1 Gy daily 5 $\times$ per week. The total dose was planned at 77.0 Gy with a range of 69–84 Gy in 34–38 fractions over 7–8 weeks. Chemotherapy was given concurrently with radiation and consisted of weekly carboplatin [area under the curve (AUC) 2] and paclitaxel (45 mg/m²) intravenously. Additional cycles of consolidation chemotherapy with carboplatin AUC 6 and paclitaxel 200 mg/m² every 21 days were allowed 1 month after completion of the chemoradiation regimen, off the study for patients who were deemed medically fit, at the discretion of the treating physician.

The dose of MnSOD PL is based on preclinical data in a mouse mode where 10 μ g of MnSOD PL per 25-g mouse was able to protect the esophagus from irradiation damage. This corresponds to a dose of 28 mg per 70-kg man; therefore, we have chosen a dose of 30 mg as the upper limit for this phase I study. Three dose levels for MnSOD PL were defined based on the dose of MnSOD plasmid DNA: 0.3 mg, 3 mg, and 30 mg per dose. Dose escalation proceeded according to a standard phase I design with three patients initially treated on each tier. If, on any dose tier of MnSOD PL, two of three patients or two of six patients experienced a grade III or IV toxicity due to MnSOD, dose escalation of MnSOD PL would cease. The maximally tolerated dose was defined as the highest dose with fewer than one-third of patients experiencing a dose-limiting toxicity (DLT) due to MnSOD PL. All patients on a tier were required to be observed for 8 weeks after starting treatment before the dose of MnSOD PL was escalated.

To detect the presence of the MnSOD transgene in the esophagus, biopsies were obtained before the first treatment, 3 days after treatment began, and 6 weeks after the final treatment. Biopsies were obtained at the distal esophagus at the gastroesophageal junction, mid-esophagus, and proximal esophagus and immediately frozen in dry ice. DNA was extracted using the DNeasy Blood and Tissue Kit (catalogue no. 69504; Qiagen, Germantown, MD). Polymerase chain reaction (PCR) was performed using primers specific for MnSOD or actin as a PCR control. PCR amplification was performed with GoTaq DNA polymerase (catalogue no. M3001; Promega, Madison, WI) using the following conditions: 2 min at 94°C for DNA denaturation, 35 cycles of 40 sec at 94°C, 40 sec at 56°C, and 60 sec at 72°C, followed by a 10-min extension at 72°C. Ten microliters of the MnSOD and actin PCR products were mixed and electrophoresed in a 1.5% agarose gel with EZ-Vision Loading Buffer (catalogue no. N313; Amresco, Inc., Solon, OH) and visualized under ultraviolet illumination. The MnSOD primers were 5'-CAT CAG CGC TAA GCC AGC for the forward primer and 5'-GTG GTT TAC TTT TTG C for the reverse primer. The actin primers were 5'-ACC AAC TGG GAC GAT ATG GAG AAG A for the forward primer and 5'-TAC GAC CAG AGG CAT ACA GGG ACA A for the reverse primer.

Toxicity and response assessments

The National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0 was used for grading toxicities. RECIST (Response Evaluation Criteria in Solid Tumors) version 1.0 was used to determine the levels of response.

Dose modifications

The drug was to be either held or discontinued due to the occurrence of grade 3–4 toxicities attributable to the study drug. If RT was held due to toxicities, MnSOD was also held and restarted with RT.

Statistical methods

The primary objective was to evaluate safety and to determine the phase II recommended dose of MnSOD PL. A standard phase I design was implemented. The study planned to treat three patients each at three tiers of 0.3, 3, and 30 mg of MnSOD/plasmid DNA. If no DLTs (grade III/IV toxicity due to MnSOD PL) were observed, the dose of MnSOD PL would be escalated to the next tier. If one DLT was observed, the cohort would be expanded to six patients. If two of six patients experienced a DLT, dose escalation would cease and the next lowest dose would be declared to be the maximum tolerated dose (MTD). If none of three or one of six patients experienced DLT at the 30-mg tier, that dose would be defined as the starting dose for the efficacy phase and the MTD would be undefined.

MnSOD PL

The investigational agent comprises a plasmid encoding human MnSOD complex with a cationic lipid. The plasmid/lipid is resuspended at 3 mg of plasmid DNA per milliliter.

A pNGVL3 (plasmid obtained from the National Gene Vector Laboratory)-MnSOD plasmid was constructed by excising the human MnSOD transgene from the pRK5-MnSOD plasmid (Wong *et al.*, 1989), cutting the 3' end of the transgene with *PvuI* endonuclease, and blunt-ending the 3' end by incubating with T4 DNA polymerase for 5 min at 37°C. The 5' end of the MnSOD transgene was cut with *EcoRI*, and the MnSOD transgene (750-bp fragment) was isolated by electrophoresis on a 1% agarose gel. The plasmid was cut with *Bam*HI restriction endonuclease and blunt-ended with T4 DNA polymerase as described above. The pNGVL3 plasmid was cut with *EcoRI* and isolated by electrophoresis on a 1% agarose gel. The MnSOD transgene was ligated into the pNGVL3 plasmid using the Boehringer-Mannheim (Mannheim, Germany) Rapid DNA Ligation Kit. DH5a *Escherichia coli* cells were transformed by mixing 5 µl of ligation mixture with 50 µl of DH5a *E. coli*-competent cells on ice for 5 min, heat-shocking the cells for 30 sec at 37°C, incubating on ice for 2 min, and plating on L-agar plates containing 10 mg/ml kanamycin. Clones were isolated and expanded, and plasmids were isolated. The MnSOD transgene was isolated by cutting the plasmids with *EcoRI* and *Bgl*II, electrophoresing on a 1% agarose gel, isolating the 750-bp DNA fragment, and sequencing the isolated fragment. Comparisons of the DNA sequence from the isolated fragments and the published sequence (Heckl, 1988) identified one of the clones as containing MnSOD in the correct orientation (Valentis, Burlingame, CA).

TABLE 1. PATIENT DEMOGRAPHICS AND BASELINE DISEASE CHARACTERISTICS (*n* = 12)^a

Variable	No. of patients
Age (years)	
Median	66
Range	50–78
Sex	
Female	6
Male	6
Performance status (ECOG)	
0	5
1	7
Prior therapies	
Chemotherapy	0
Radiation	0
AJCC stage	
IIIA	3
IIIB	9
Histology (all NSCLC)	
Squamous	5
Adenocarcinoma	4
Large cell, poorly differentiated	1
Not otherwise specified	2

^aTwo patients (both IIIB and adenocarcinoma) withdrew consents after the first esophagoscopy, one of whom had received one dose of MnSOD PL and one cycle of chemotherapy before consent withdrawal. Therefore, a total of 10 patients completed the course of therapy per study protocol.

Results

Patient characteristics

Twelve patients with AJCC stage IIIA (three) and IIIB (nine) NSCLC were enrolled. Baseline patient and disease characteristics are shown in Table 1.

Treatment details

Two patients (both IIIB and adenocarcinoma) withdrew consents after the first esophagoscopy; one of these had received one dose of MnSOD PL and one cycle of chemotherapy before withdrawal. Therefore, 10 patients completed the course of therapy per study protocol and were treated in three cohorts at three dose levels of MnSOD PL: 0.3 mg (three patients), 3 mg (three patients), and 30 mg (four patients). The fourth patient treated with the 30-mg dose is part of the phase II expansion phase of the study, but has been included in this phase I analysis to further support the safety of the phase II recommended dose.

Eight of 10 patients received all 14 doses of MnSOD PL. One patient (at 0.3 mg) received a total of 13 doses of MnSOD PL, missing dose number 3 after being hospitalized with grade 4 hypercalcemia. Another patient (30 mg) missed the second dose of MnSOD PL because of a complicated second esophagoscopy (bronchospasm and difficult intubation) requiring hospitalization and treatment interruption. Three patients required one reduction of the chemotherapy doses by 50% secondary to grade 2 neutropenia. The total dose of radiation ranged between 63.00 Gy and 79.80 Gy (median 77.7 Gy; Table 2).

Response

Overall response rate for the standard chemoradiation regimen was 67% (*n* = 10). Seven patients (70%) had a partial

TABLE 2. TREATMENT DETAILS

Patient	MnSOD PL (mg)	CT dose reduction	Radiation dose (total; GY)
1	0.3	No	77.70
2	0.3	No	69.30
3	0.3 ^a	Yes ^b	69.30
4	3	Yes ^b	79.80
5	3	No	79.80
6	3	No	63.00
7	30	No	79.80
8	30	Yes ^b	73.50
9	30	No	79.10
10	30 ^a	No	79.80

CT, chemotherapy.

^aThese patients missed one dose of MnSOD PL. All other patients received 14 doses.

^bReason for dose reduction: grade 2 neutropenia.

response; of these, three have already progressed (after 6, 9.5, and 11 months). One patient had stable disease (lasting 7.5 months), and two had progression.

Safety

Table 3 summarizes adverse events by severity. Grade 2 dyspepsia and a grade 1 rash in one patient and grade 1 constipation and hyponatremia in another patient were considered to have a possible relationship to MnSOD PL. None of the other reported toxicities were considered possibly, probably, or definitely related to MnSOD PL. There were no DLTs reported in all three dosing tiers. The 30-mg dose was defined as the starting dose for the efficacy phase.

PCR for the detection of MnSOD transgene

PCR results for the detection of the MnSOD transgene in the esophagus of the first nine patients are summarized in Fig. 1. For two of these patients (3 and 4), biopsies were not obtained following treatment due to patient refusal. For patient 10, the second esophagoscopy was complicated by bronchospasm and difficult intubation, and thus the procedure was terminated without a biopsy. This patient also declined the third planned biopsy.

MnSOD PCR product was not detected in the samples. These data were supplemented with very precise serial dilution of positive control plasmid clearly defining the lower limited detectability of the transgene, which is the minimum level that would have been detected at the expected time point of the second biopsy during the first week of MnSOD PL gene therapy (after the second swallow).

Discussion

Esophagitis has been the primary nonhematological toxicity reported with concurrent chemoradiation therapy using paclitaxel and carboplatin (Choy *et al.*, 1996, 1997, 1998a, 1999; Langer *et al.*, 1997b; Lau *et al.*, 1997, 1999; Socinski *et al.*, 1997, 1998; Mattson, 1998). Attempts to prevent irradiation-induced esophagitis during lung cancer chemoradiotherapy have usually focused on three approaches: (1) avoidance of esophageal irradiation by optimized treatment planning and dose distribution, (2) improved techniques of irradiation

TABLE 3. SUMMARY OF ADVERSE EVENTS BY SEVERITY (*n* = 12 PATIENTS)

Hematologic type	All grades (no. of patients)	Grade 3/4 (no. of patients)
Anemia	4	
Lymphopenia	1	1
Neutropenia	4	
Thrombocytopenia	2	
Nonhematologic type	All grades (no. of patients)	Grade 3/4 (no. of patients)
Cardiovascular		
Atrial fibrillation	1	1
Hypotension	1	
Supraventricular tachycardia	1	
Thrombosis/embolism	2	2
Constitutional		
Fatigue	8	2
Weight loss	2	
Insomnia	1	
Gastrointestinal		
Anorexia	3	1
Constipation	3 ^a	
Dehydration	1	1
Diarrhea	2	
Dyspepsia	1 ^a	
Dysphagia	7	
Esophagitis	6	
Esophageal stricture (post Rx)	1	1 ^b
Hoarseness	2	
Nausea	3	
Vomiting	1	
Infection		
Thrush	1	
Metabolic/laboratory		
Hypercalcemia	1	1
Hyperglycemia	4	1
Hyperkalemia	1	
Hypoalbuminemia	1	
Hypokalemia	1	
Hypocalcemia	1	
Hyponatremia	3 ^a	
Neurologic		
Neuropathy-sensory	2	
Somnolence	1	1
Involuntary movement/restlessness	1	
Pulmonary		
Cough	5	
Dyspnea	8	2
Hemoptysis	1	
Pneumonitis	1	1 ^c
Renal		
Skin		
Rash (dermatitis)	3 ^a	
Other		
Allergy (paclitaxel)	2	
Hemorrhoidal bleed	1	1
Pain (headache, hip, extremity)	4	

^aFor one patient with grade 2 dyspepsia and a grade 1 rash, and for another patient with grade 1 hyponatremia and grade 1 constipation, these were considered possibly related to MnSOD PL or the chemoradiation regimen.

^bPatient in cohort 1, receiving MnSOD at 0.3 mg.

^cPatient in cohort 2, receiving MnSOD at 3 mg.

FIG. 1. PCR detection of MnSOD transgene in the first nine patients. DNA was extracted from biopsies obtained from three levels of the esophagus before treatment began, 3 days after the first treatment, and 6 weeks after the last treatment. PCR was performed using primers specific for MnSOD transgene or actin with the PCR products mixed and electrophoresed. (A) Patients 1 and 2. (B) Patients 3 and 4. (C) Patients 5 and 6. (D) Patients 7 and 8. (E) Patient 9 and the MnSOD standard curve. For each patient, 1 represents the biopsy taken before treatment, 2 is the biopsy taken 3 days after the beginning of the treatment, and 3 is the biopsy taken 6 weeks after the treatment was completed. The letter P is the biopsy taken at the proximal portion of the esophagus, M is from the mid-esophagus, and D is from the distal portion from the gastroesophageal junction. NC is a no-DNA PCR control. For two of the patients (3 and 4), biopsies were not obtained following treatment. MnSOD PCR product was not detected in the samples. An MnSOD standard curve of PCR products is shown in panel E, where A represents the product from 10 μ g of MnSOD plasmid DNA, B is product from 1 μ g, C is product from 0.1 μ g, D is product from 0.01 μ g, E is product from 0.001 μ g, F is product from 0.0001 μ g, and G is product from 0.00001 μ g.

fractionation, and (3) delivery of radiation protective agents to the esophageal tissues. There is little question that improved treatment planning decreases esophageal toxicity (Choy *et al.*, 1998b). Minimizing the volume irradiated while still allowing enough margin to include variations in lung cancer localization during respiration and use of multifield conformal techniques, including use of the multileaf collimator, have provided benefits in decreasing treatment-related toxicity (Byhardt *et al.*, 1998; Choi *et al.*, 1998; Maguire *et al.*, 1998). A comparison of hypofractionation or hyperfractionation regimens with conventional fractionation has revealed that multiple small fractions may decrease esophageal toxicity, but there is a requirement for a higher total dose of radiation to obtain the same likelihood of tumor control (Jeremic *et al.*, 1993; Oetzel *et al.*, 1995; Greenberger *et al.*, 1996; Bahri *et al.*, 1999). Higher-dose fractions neutralize some of the radioprotective benefit of low fraction size (Jeremic *et al.*, 1993). Thus, although a decrease in total irradiation dose in the setting of chemoradiotherapy may minimize esophageal toxicity, the duration and extent of local control of NSCLC are usually compromised (Jeremic *et al.*, 1993). Radioprotective agents, including sulfhydryl radical-scavenging drugs (Grdina *et al.*, 1991), atropine (Byhardt *et al.*, 1993), and amifostine (Nagler and Laufer, 1998), have been tried intraorally or intravenously with some success. However, the depth of penetration of orally delivered drugs, duration of protection, and the inability to translate an *in vitro* radioprotective effect to a comparable effect *in vivo* remain challenging issues for these therapies (Newton *et al.*, 1996).

Gene therapy-mediated overexpression of MnSOD decreases the expression of inflammatory cytokines in response to radiation and reduces cellular apoptosis, microulceration, and esophagitis. Liggitt, Epperly, Greenberger, and colleagues have previously demonstrated modulation of radiation-induced cytokine elevation associated with esophagitis and esophageal stricture by MnSOD PL gene therapy (Epperly *et al.*, 2001). Radiation of the esophagus of C3H/HeNsd mice with 35 or 37 Gy of 6 MV x-rays induces significantly increased RNA transcription for interleukin 1, tumor necrosis factor- α , interferon- γ inducing factor, and interferon- γ . These elevations are associated with DNA damage, apoptosis of the esophageal basal lining layer cells *in situ*, and microulceration leading to dehydration and death. Intraesophageal injection of clinical-grade MnSOD PL 24 hr prior to irradiation mediated a significant decrease in induction of cytokine mRNA by radiation and decreased apoptosis of squamous lining cells, microulceration, and esophagitis (Epperly *et al.*, 2001). These data provide support for translation of this strategy of gene therapy to decrease the acute and chronic side effects of radiation-induced damage to the esophagus. To determine whether the human esophagus can be similarly transfected, the same group conducted a study demonstrating that human esophageal sections can be similarly transfected with MnSOD PL complex *in vitro* and thereby protected against ionizing irradiation-induced apoptosis (Epperly *et al.*, 2000).

None of the treated patients has experienced a grade III or IV toxicity that was considered related to MnSOD PL. Therefore, an MTD was not defined, and the highest dose tested (based on preclinical data) (30 mg) was defined as the phase II recommended dose. This corresponds to a protective dose level based on our preclinical data in a mouse model where a dose of MnSOD PL of 10 μ g/25-g mouse

(corresponding to a dose of 28 mg/70-kg man) was able to protect the esophagus from irradiation damage.

The PCR product was not detected in all of the esophageal samples. These data were supplemented with very precise serial dilution of positive control plasmid clearly defining the lower limited detectability of the transgene, which is the minimum level that would have been detected at the expected time point of the second biopsy during the first week of MnSOD PL gene therapy (after the second swallow). It is very likely that the level of transgene was below detectable levels in the biopsy samples obtained because there was patchy distribution after the esophageal swallow of the transgene. In our mouse models, MnSOD transgene expression was demonstrated in less than 50% of the esophageal cells by *in situ* nested PCR, but still resulted in a radioprotective effect (Epperly *et al.*, 2001). As the amount of tissue biopsied represents such a small portion of the esophagus, it is possible that the material biopsied would not be expressing the MnSOD transgene. It is also possible that samples taken had already rid the cells of the transgene and the transgene product, as is known to have happened cyclically in the mouse model (Epperly *et al.*, 2001). Foreign intracellular DNA recognition and clearance have been postulated to be a fundamental arm of the innate immune response (Ishii and Akira, 2006). Recently, Stenglein and colleagues have suggested a model in which foreign DNA restriction is a distinct and important physiological function of the APOBEC3 proteins (Stenglein *et al.*, 2010). A3A is induced by DNA detection and interferon in phagocytes and triggers the degradation of foreign DNA by a cytidine-deamination and uracil-excision mechanism.

Therefore, the esophagoscopy has added no information regarding the ability to detect the clearance of the transgene.

A phase II efficacy study has been initiated at the University of Pittsburgh Cancer Institute. The clinical endpoint will be the proportion of radiation-induced grade III/IV esophageal toxicity.

Author Disclosure Statement

All authors have nothing to disclose.

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Address correspondence to:
Dr. Joel S. Greenberger
UPMC Cancer Pavilion
5150 Centre Ave., 5th Floor
Pittsburgh, PA 15232

E-mail: greenbergerjs@upmc.edu
and

Dr. Ahmad A. Tarhini
UPMC Cancer Pavilion
5150 Centre Ave., 5th Floor
Pittsburgh, PA 15232

E-mail: tarhiniaa@upmc.edu

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