UPMC CancerCenter

Partner with University of Pittsburgh Cancer Institute

UPCI #01-054: Concurrent Chemotherapy (Paclitaxel and Carboplatin) and Thoracic Radiotherapy with Swallowed Manganese Superoxide Dismutase (MnSOD) Plasmid Liposome (PL) Protection in Patients with Locally Advanced Stage III Non-Small Cell Lung Cancer a Phase I-II Study

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UPCI #01-054: Concurrent Chemotherapy (Paclitaxel and Carboplatin) and Thoracic Radiotherapy with Swallowed Manganese Superoxide Dismutase (MnSOD) Plasmid Liposome (PL) Protection in Patients with Locally Advanced Stage III Non-Small Cell Lung Cancer A Phase I-II Study

SCHEMA

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Paclitaxel 45mg/m^2 and
Carboplatin AUC = 2
Weekly for 7 weeks

Thoracic Radiotherapy 77.0 Gy
For 7-8 weeks/34-38 daily fractions
Beginning on day 1
(1.9-2.1 Gy for a total dose of 77.0 Gy with a range of 69-84 Gy in 34-38 fractions over 7-8 weeks)

MnSOD (PL) twice per week
(MnSOD continues until RT ends)

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1.0 INTRODUCTION

1.1 Background

Locally Advanced unresectable NSCLC

Clinical trials conducted in the 1970's established the efficacy of radiation therapy in patients with locally advanced NSCLC. The optimal dose of radiation was determined to be 60 Gy administered in 2Gy/fraction over 6 weeks (1). Despite undergoing definitive radiation, patients experienced a high incidence of local and distant relapse. This led to the initiation of trials that studied the effect of adding chemotherapy in addition to radiation. Chemotherapy was thought to act in multiple ways that included radiation-sensitization of cancer cells in the field of radiation, and cytotoxic effect on micrometastatic disease. The Cancer and Leukemia Group B (CALGB) 8433 trial evaluated the utility of addition of induction chemotherapy to patients undergoing radiation therapy for locally advanced NSCLC (2). The median survival for patients treated with induction chemotherapy with cisplatin and vinblastine followed by radiation was 13.7 months, compared to 9.6 months for patients treated with radiation therapy alone. Subsequently the RTOG 88-08 trial (3) and the French trial (4) also established that chemotherapy and radiation is superior to radiation alone. In the 1990's, two large randomized trials have demonstrated that chemotherapy with concurrent radiation is superior to sequential administration of chemotherapy and radiation (Table 1) (5,6). In the West Japan Lung Cancer Group trial (5), there was no difference in the rate of local relapse in both the groups and distant relapse occurred in a majority of the patients on both arms.

Table 1: Clinical trials in Locally advanced NSCLC

Trial	*Treatment*	*Median Survival (Months)*	*1-Yr Survival*
RTOG 9410 (19) (N=611)	Sequential	14.6	57%
	Concurrent-QD	17.0	63%
	Concurrent-BID	15.6	61%
Furuse (20) (N=322)	Sequential	13.3	55%
	Concurrent	16.5	64%

Table 2: Long-term benefits from RTOG 9410

	Sequential	*Con-QD RT*	*Con-BID RT*
#Patients	201	201	193
Esophagitis Gr.3/4	4%	25%	47%
Median Survival	14.6 months	17.0 months	15.2 months
4-year Survival	12%	21% (p=0.046)	17%

The results of RTOG 9410 trial which was a 3-arm, randomized phase III trial that evaluated sequential vs concurrent chemoradiation were updated this year (7) (Table 2). Sequential therapy (Arm 1) consisted of two cycles of cisplatin and vinblastine, followed by 60 Gy external beam radiation, administered in once-daily fractions, beginning on Day 50. In arm 2, the same chemotherapy was administered, while radiation was administered concurrently starting on Day 1 of chemotherapy cycle. Arm 3 used hyperfractionated radiation therapy with 69.2 Gy administered as twice-daily fractions. Patients in arm 3 received concurrent chemotherapy that consisted of cisplatin and oral etoposide. A total of 611 patients with unresected stage II/III NSCLC were enrolled between 1994 and 1998. The median survival was superior (17.0 months vs 14.6 months, p=0.038) for patients with concurrent chemoradiation (Arm 2) compared to the sequential arm. Hyperfractionated radiation did not result in improved survival compared to the sequential arm (15.6 months vs 14.6 months). While the incidence of acute toxicity was higher in the concurrent arms, late non-hematological toxicity was identical for patients in all three arms. The 4-year survival on the concurrent chemotherapy and daily standard radiation arm was 21% versus 12% with sequential chemotherapy followed by radiation (p=0.046). Thus, concurrent chemoradiation is the standard of care for patients with locally advanced NSCLC, especially those with a good performance status. Concurrent therapy, while associated with a higher incidence of acute grade 3 esophagitis (up to 20%), results in better overall outcome. While the reduction of acute toxicity remains a major goal for further research efforts, better control of micrometastatic disease will have to be achieved to build upon the survival benefit achieved with concurrent chemoradiation.

The South West Oncology Group investigators evaluated the role of consolidation chemotherapy following concurrent chemoradiation in a phase II trial (SWOG 9504)(8). Initial therapy consisted of cisplatin and etoposide with concurrent radiation (61 Gy) followed by three cycles of consolidation chemotherapy with docetaxel. The non-p53 dependent apoptotic effect of docetaxel, when administered in sequence with cisplatin therapy (which induces apoptosis in a p53 dependent manner) was postulated to improve disease control. This study enrolled 83 eligible patients and demonstrated a median survival of 26 months and median PFS was 16 months. The 1-, 2- and 3-year survival rates were 76%, 54% and 37% respectively. The treatment was well tolerated with a low incidence of grade 3/4 esophageal toxicity during concurrent chemoradiation (17%).

Brain metastasis was the most common site of failure, occurring in 51% of the patients and occurred in almost all patients with distant failure. Based on the impressive results noted in this study, the SWOG investigators have adopted the 9504 regimen as 'the standard' in the Intergroup 0023 trial in which the patients are then randomized to Gefitinib versus observation. The Hoosier Oncology Group is currently performing a randomized phase III trial comparing consolidation chemotherapy with docetaxel following concurrent chemoradiation with concurrent chemoradiation alone.

1.2 Paclitaxel and Carboplatin as Chemotherapeutics

Taxol® (paclitaxel) is obtained via a semi-synthetic process from *Taxus baccata.* Enthusiasm for the use of paclitaxel has been generated over the past few years by its unique mechanism of action and promising anti-tumor activity in patients with advanced solid tumors. Paclitaxel acts as a mitotic inhibitor, blocking cells in the G_2 and M phases of the cell cycle. The inhibition is unique in that the drug enhances the rate and yields of microtubular assembly and prevents microtubular depolymerization (9,10). It has been known for many years that cells in the G_2 and M phase of the cell cycle are particularly sensitive to radiation (11). Tishler et al. showed that 24-hour treatment with 10 nM paclitaxel rendered a radio-resistant astrocytoma cell line susceptible to radiation (12). The enhanced level of cell kill in this study was consistent with the greater radiosensitivity of G_2/M cells. The radiation sensitizing effect of paclitaxel was also observed with only l-hour treatment using 300 nM Taxol, in human leukemia cell line (HL-60*)* and human lung cancer cell line (Calu-3*)* (13).

Carboplatin is active against non-small cell lung cancer and can be used as a radiation sensitizer. The mechanism of radiation sensitization with carboplatin is different from that of paclitaxel. Carboplatin potentially interferes with repair of sublethal radiation injury, whereas paclitaxel recruits cells in the highly radiosensitive G_2/M phase (11). Recent laboratory data suggest a possible synergistic relationship of paclitaxel and carboplatin (14).

1.3 Phase II Study of Concurrent Paclitaxel, Carboplatin and RT Followed by Adjuvant Chemotherapy

In order to further improve local control and reduce distant metastasis, a phase II study of concurrent weekly paclitaxel (50mg/m^2/wkly/7wks*),* carboplatin (AUC 2 wkly/7wks*)* and standard RT, followed by two additional cycles of adjuvant paclitaxel (200mg/m^{2}q3wksx2) and carboplatin (AUC 6 q3wksx2) was designed by the Clinical Oncology Group of Rhode Island (COGRI) (15). The goal was to determine the response rate and toxicity of this regimen. The median survival of 38 patients treated was 20.5 months. The two-year survival was 38%. Esophagitis was a major toxicity in this trial and was seen in 46% of patients (NCI Toxicity Criteria). Pneumonitis occurred in 22.0% of patients. Thus, concurrent radiation therapy (66Gy) with weekly paclitaxel (50 mg/m^2/wkly*)* and carboplatin (AUC 2) followed by two additional cycles of paclitaxel (200 mg/m^2) and carboplatin (AUC 6) could be safely delivered to patients.

1.4 Phase II Study of Weekly Low Dose Paclitaxel, Carboplatin and RT

Dr. Argiris et al. (16) recently completed a phase II study of weekly low dose paclitaxel at 45 mg/m^2 (3-hour infusion) with carboplatin, 100 mg/m^2 and simultaneous standard-dose thoracic radiotherapy (total of 60-65 Gy*)* for patients with locally advanced NSCLC. Thirty-eight patients were enrolled, of which 16 were stage IIIA and 22 were stage IIIB. The salient toxicities included: nine grade 3 leucopenia, three grade 3 mucositis and esophagitis, two grade 3 fatigue and two grade 3 nausea/vomiting. There were no grade 4 toxicities reported. Overall the regimen was well tolerated. There were 12 instances of dose reduction and there was a delay in treatment duration of $\geq$ 1 week in 5 patients. Three patients died as a result of rapidly progressive disease without any evidence of dose-limiting toxicities. The median survival has not yet been reached. The 1-year, 2-year and 3-year actuarial survival rates for this group of patients with locally advanced NSCLC are 63% (95 CI: 44-77%), 54% (95 CI: 35-70%), and 54% (95 CI: 35-70%) respectively.

1.5 Phase I Dose Escalation Study

Another approach to combine carboplatin and paclitaxel with thoracic radiation has been reported in a preliminary manner by Langer et al. from the Fox Chase Cancer Center (17). A total of 32 patients have been entered into a phase I dose escalation regimen in which patients receive two cycles of induction paclitaxel and carboplatin followed by concurrent carboplatin, paclitaxel and thoracic RT. To date the induction dose has been escalated to 225 mg/m^2 for paclitaxel and an AUC of 7.5 for carboplatin. The paclitaxel/carboplatin during radiotherapy has been given every three weeks and has been successfully escalated to 175 mg/m^2 paclitaxel and a carboplatin AUC dose of 5.0. Dose-limiting toxicity has been esophagitis (17).

In summary, there have been several approaches in pilot studies to combine the regimen of carboplatin and paclitaxel with thoracic RT; however, the optimal approach has not yet been identified. When identified, optimal dosing could be tested in a phase III design against the most favorable arm of RTOG 94-10. As with other cisplatin-based regimens, however, it is anticipated that utilizing concurrent chemotherapy and radiation will heighten both hematologic and esophageal toxicity.

1.6 Chemoradiation-Induced Esophageal Toxicity

Esophageal toxicity has been accepted reluctantly, as a necessary side effect of the beneficial radiosensitization of chemotherapy drugs for lung cancer (7, 8, 15-17). With respect to the esophagus 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. A technique by which to selectively protect the esophagus during radiotherapy treatment of NSCLC volume would be of great potential benefit to lung cancer patients by reducing morbidity associated with the treatment and potentially allowing escalated doses of irradiation and/or effective chemotherapeutic agents. New protocols with higher doses would seek to gain a higher percent of complete responders and cured patients.

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 fractionation, and 3) delivery of radiation protective agents to the esophageal tissues. There is little question that improved treatment planning decreases esophageal toxicity (18). 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 (19-21). 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 (22-25). Higher dose fractions neutralize some of the radioprotective benefit of low fraction size (24). Thus, while 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 (24). Radioprotective agents, including sulfahydryl radical scavenging drugs (26), atropine (27) and amifostine (28), 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 issues for these therapies (29).

1.7 Human Manganese Superoxide Dismutase (MnSOD) Transgene

Manganese Super Oxide Dismutase (MnSOD) is a genetically engineered investigational dBiological rug, 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 hMnSOD 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 pharmacology studies demonstrating that expression of the human MnSOD transgene could protect against irradiation damage, were performed in vitro. Murine hematopoietic progenitor cell line 32D cl 3 was transfected with a plasmid containing the human MnSOD transgene (30). Stable clones (1F2 and 2C6) expressing the human MnSOD transgene, as demonstrated by nested reverse transcriptase-polymerase chain reaction (RT-PCR) using primers specific for the human transgene and increased MnSOD biochemical activity, were selected. Irradiation survival curves showed that MnSOD clones 1F2 and 2C6 were more resistant to irradiation as seen by an increased shoulder (n = nbar) (34). Clones 1F2 and 2C6 were also more resistant to irradiation-induced apoptosis. Cells from 32D cl 3, 1F2 or 2C6 were irradiated to 1000 cGy and examined for apoptosis at 0, 6, 24 or 48 hours later. Cell line 32D cl 3 had 29.37% of its cells apoptotic at 24 hours compared to 5.21 and 5.27 for 1F2 and 2C6, respectively (34). Examination of cell cycle analysis following irradiation demonstrated a G2/M phase block at 6 hours followed by a G1/S phase block at 24 hours in all three cell lines . Even though overexpression of MnSOD made the cells more radioresistant, there was no change in cell cycle distribution following irradiation (30).

Protection of normal tissues from ionizing irradiation damage by gene therapy has recently been demonstrated in the mouse lung (31, 32). Delivery of plasmid/liposomes (PL) or adenovirus (32) containing the human Manganese Superoxide Dismutase

(MnSOD) transgene by intratracheal injection prior to irradiation has demonstrated detectable expression of messenger RNA for human MnSOD in both alveolar type II cells and tracheobronchial tree cells in treated mice (31). Increased MnSOD biochemical activity in treated lung tissues was associated with a decrease in radiation-induced messenger RNA levels for acute inflammatory response genes such as IL-1, TNF-α and TGF-β (31). Plasmid/liposome MnSOD gene therapy also decreased the pathologic sequelae of lung irradiation, including organizing alveolitis/fibrosis typically seen at approximately 150 days following irradiation (35). Success has also been achieved in a murine model of radiation-induced esophagitis (33). Intraesophageal administration of MnSOD PL complexes was shown to reduce radiation-induced damage to esophageal epithelial cells and increase MST dramatically compared to untreated or mock-treated control animals (33).

Dosing: Preclinical data in our mouse model has demonstrated that doses as low as 10 ug/ 25 gm mouse was able to protect the esophagus from irradiation damage. This corresponds to a dose of 28 mg/ 70 kg man which is why we are proposing 30 mg as the upper limit for our clinical trial.

1.8 MnSOD Activity in the Pig Esophagus

To demonstrate that MnSOD-PL could transfect the esophagus in a bigger model, pigs were anesthetized so that the esophagus was temporally paralyzed and MnSOD-PL (10 mg of plasmid DNA) was administered through an endoscope 10 cm from the gastroesophageal junction (GE) or at the top of the esophagus were the pig was allowed to swallow the MnSOD-PL (10 mg of plasmid DNA) (4). The pigs were sacrificed 24 hr later with the esophagus removed and cut into 4 sections. In the pig receiving MnSOD-PL only at the site of the PDT treatment, there was increased MnSOD biochemical activity or increased human MnSOD mRNA production as detected by nested RT-PCR using primers specific for the human MnSOD transgene only at the site of administration. Another pig which had been lightly anesthetized was given MnSOD-PL at the top of the esophagus and allowed to swallow the MnSOD-PL was sacrificed and the esophagus removed and divided into 3 sections with increased MnSOD biochemical activity and increased mRNA for the human MnSOD transgene found at all levels of the esophagus. This demonstrates that there is increased MnSOD activity in the pig esophagus following administration of MnSOD-PL.

Significant progress in developing a system of esophagus protection from irradiation damage has been made through designing a method for intraesophageal administration of MnSOD plasmid/liposomes. MnSOD plasmid/liposome gene therapy protection of the esophagus from damage induced by chemoradiotherapy should be a valuable addition to the care of lung cancer patients by reducing treatment-related esophagus toxicity, decreasing the need for hospitalization and hyperalimentation, and potentially allowing dose escalation that locally controls and perhaps cures more patients. Once the MnSOD plasmid/liposome comes into contact with the gastric acid the plasmid/liposome complex would be disassembled with the plasmid DNA being destroyed. Thus, reflux would not result in an increased dosing of the patient.

This trial will help determine whether the increased incidence of esophagitis can be

reduced with the use of Manganese Superoxide Dismutase (MnSOD) transgene therapy. The dose of paclitaxel is based on previous phase I experience and the dose of carboplatin will be dose adjusted for renal function to provide more uniform pharmacokinetics.

2.0 RATIONALE

Esophagitis has been the primary non-hematological toxicity reported with concurrent chemoradiation therapy utilizing paclitaxel and carboplatin (34-43). Supportive care agents and selective approaches are needed to prevent or decrease esophageal toxicity with the promising combined modality regimens. This could lead to overall improved outlook in these patients. Manganese Superoxide Dismutase transgene therapy in liposomes is one way of decreasing the esophageal toxicity of this regimen.

Esophageal damage in response to ionizing radiation results from the local production of toxic free radical species. Enzymatic oxidation of these species protects cells from excessive free radical-induced damage. MnSOD is a principal mediator of this protective effect. Gene therapy-mediated overexpression of MnSOD decreases the expression of inflammatory cytokines in response to radiation and reduces cellular apoptosis, micro-ulceration and esophagitis.

Significant progress in developing a system of esophagus protection from irradiation damage has been made through designing a method for intraesophageal administration of MnSOD plasmid/liposomes. MnSOD plasmid/liposome gene therapy protection of the esophagus from damage induced by chemoradiotherapy should be a valuable addition to the care of lung cancer patients by reducing treatment-related esophagus toxicity, decreasing the need for hospitalization and hyperalimentation, and potentially allowing dose escalation that locally controls and perhaps cures more patients.

This trial will help determine whether the increased incidence of esophagitis can be reduced with the use of Manganese Superoxide Dismutase (MnSOD) transgene therapy. This gene therapy method of effectively protecting the esophagus would reduce treatment-related morbidity and potentially increase the deliverable radiation dose and lead to better disease control.

3.0 OBJECTIVES

3.1 Primary Objectives

3.1.1 To evaluate the feasibility and safety of MnSOD plasmid/liposome transgene given twice per week during concurrent chemotherapy using carboplatin and paclitaxel with thoracic radiation for protection of the esophagus in patients with locally advanced NSCLC.

3.1.2 To evaluate the ability of MnSOD PL transgene given twice per week to reduce the incidence of radiation-induced esophageal toxicity.

3.2 Secondary Objectives

3.2.1 To evaluate the clinical efficacy of the combined treatment modality in locally advanced Stage III NSCLC.

3.2.2 To assess whether chemotherapy/dose intensity and planned radiation therapy intensity can be maintained with the use of the MnSOD plasmid/liposome.

4.0 PATIENT ELIGIBILITY

4.1 Histologically or cytologically documented NSCLC, including squamous cell carcinoma, adenocarcinoma (including bronchoalveolar cell*),* and large cell anaplastic carcinoma (including giant and clear cell carcinomas*)* and poorly differentiated non-small cell lung cancer. Totally resected tumors are excluded.

4.2 Patients must be without evidence of M0.

4.3 Patients with T1 or T2 disease with N2 or T3N1-2 disease (Stage IIIA*)*

4.2 are eligible if they are medically inoperable. Patients with T4 with any N or any T with N3 disease are eligible. Radiographic evidence of mediastinal lymph nodes > 2.0 cm in the largest diameter is sufficient to stage N2 or N3 disease. If the largest mediastinal node is < 2.0 cm in diameter and this is the basis for stage III disease, then at least one of the nodes must be proven positive cytologically or histologically.

4.4 Patients with tumors adjacent to a vertebral body are eligible as long as all gross disease can be encompassed in the radiation boost field. The boost volume must be limited to < 50% of the ipsilateral lung volume.

4.5 Patients with a pleural effusion that is a transudate, cytologically negative and non-bloody are eligible if the radiation oncologists feel the tumor can still be encompassed within a reasonable field of radiotherapy. Patients with exudative, bloody, or cytologically malignant effusions are ineligible. If a pleural effusion can be seen on the chest CT but not on CXR and is too small to tap, the patient will be eligible.

4.6 Radiation Oncology and Medical Oncology Consults must deem patient suitable for protocol treatment.

4.7 Performance Status $\geq$ 70 (Karnofsky Performance Scale; Appendix I).

4.8 Weight loss $\leq$ 10% in 3 months prior to diagnosis.

4.9 Age $\geq$ 18 years.

4.10 No prior systemic chemotherapy, radiation therapy to the thorax, or total surgical resection.

4.11 At least 3 weeks since formal exploratory thoracotomy and patient has recovered from surgery, or 1 week from diagnostic thoracoscopy.

4.12 Required Initial Laboratory Values (see Section 6.1.for required timing):

Granulocytes	≥ 2,000/ml
Platelets	≥ 100,000/ml
Hemoglobin*	> 8 mg/dl
Bilirubin	< 1.5 x normal
Creatinine clearance (24 hour or calculated)	> 50 ml/min
FEV_1	> 800 cc

* Physician can maintain a patient's hemoglobin with the use of Erythropoeitin or transfusions. (Prophylactic use of G-CSF is not permitted.)

4.13 Patients must have a MRI or CT brain within 4 weeks prior to study entry to rule out asymptomatic brain metastases.

4.14 Informed Consent: Each patient must be aware of the neoplastic nature of his/her disease process and must willingly sign a study-specific consent prior to randomization after being informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. (Human protection committee approval of this protocol and consent form is required.)

4.15 No active concurrent malignancy is allowed, except inactive non-melanoma skin cancer or in situ carcinoma of the cervix. Prior cancer is eligible only if the patient has been disease-free for ≥ 5 years.

4.16 No serious medical or psychiatric illnesses that would prevent informed consent. Patients with post-obstructive pneumonia are eligible. Patients with an active serious infection or other serious underlying medical condition that would otherwise impair their ability to receive protocol treatment are ineligible.

4.17 Prior significant allergic reactions to drugs containing cremophor, such as cyclosporine, or vitamin K are not eligible. A significant reaction may be defined as, but is not limited to, the description of grade ≥ 3 allergic reactions using the CTC.

4.18 No history of serious cardiac disease that is not adequately controlled. Patients with documented myocardial infarction within 6 months prior to study entry, congestive heart failure, unstable angina, clinically significant pericardial effusion or arrhythmia are ineligible. An ECG must be done within 4 weeks prior to study entry on all patients.

4.19 Nonpregnant, nonlactating female patients. Patients of childbearing potential must implement an effective method of contraception during the study. All women of childbearing potential must have a pre-study negative serum or urine pregnancy test within 7 days prior to study entry.

5.0 EVALUATIONS

5.1 History (including baseline symptoms*),* weight, performance status, WBC/granulocytes, Hgb, platelet count, SGOT or SGPT, alkaline phosphatase, total bilirubin, albumin,

glucose and creatinine (24 hour or calculated clearance*).* These must be obtained within 2 weeks prior to study entry.

5.2 A serum or urine pregnancy test must be obtained within 7 days prior to study entry for females of childbearing potential.

5.3 EKG will be performed within 4 weeks prior to study entry.

5.4 Pre-study radiographic assessments such as CT or MRI and bone scans will be used to document study tumor size and absence of metastases. Pre-study radiographic assessments must be obtained within 4 weeks of study entry.

5.5 The method used to document the tumor (CT or MRI*)* should be used consistently for all evaluations. If tumor was subtotally resected during thoracotomy, the CT or MRI must be done after surgery.

5.7 Weekly: Weight, toxicity evaluation, Hgb, WBC, differential.

5.8 Every 3 Weeks: Physical exam, weight, performance status, toxicity evaluation, Hgb, WBC, differential, BUN, creatinine, creatinine clearance, alk. Phos., bilirubin, SGOT, CA++, PO4, blood sugar.

5.9 1 month follow-up: Physical exam, tumor measurement, weight, performance status, Hgb, WBC, differential, BUN, creatinine, creatinine clearance, alk. phos., bilirubin, SGOT, blood sugar.

5.10 Post-treatment as clinically indicated: Electrolytes, EKG, CXR.

5.11 Four weeks post-treatment, then every 3 months thereafter for 2 years, and then as needed or at recurrence to document indicator lesions with quantitative measurements (please ask for quantitative measurements on radiology requests): Chest CT scan.

5.12 If indicated and feasible: Histological or unequivocal cytological diagnosis.

5.13 Four weeks post-treatment: Pulmonary function tests (PFT). Additional PFTs will be performed as needed for assessment of radiation pneumonitis.

5.14 Every 3 months for 2 years: Physical exam, tumor measurement, weight, performance status, Hgb, WBC, differential, BUN, creatinine, creatinine clearance, alk. Phos., bilirubin, SGOT, blood sugar.

5.15 Every 6 months for 2 more years: Physical exam, tumor measurement, weight, performance status, Hgb, WBC, differential, BUN, creatinine, creatinine clearance, alk. Phos., bilirubin, SGOT, blood sugar.

6.0 STUDY PARAMETERS AND SERIAL OBSERVATIONS

If a cycle is missed or a subject's treatment and/or testing days need to be rescheduled due to the subject's inability to comply with the study calendar (i.e., hospitalizations, business and vacation travel plans, illness, transportation issues, holidays, family emergencies, etc.), a window of ± one (1) week is available for rescheduling of treatment and procedures per the discretion of the treating physician investigator, and as discussed with the principal investigator. In addition, at the discretion of the Principal Investigator, certain tests/procedures may be performed up to 7 days in advance of the scheduled date to allow ready access to the result(s), reduce financial burden on the subject (i.e. non-UPMC insurance coverage) or reduce travel inconvenience.

6.1 Observations and tests to be performed and recorded before, during and after treatment as indicated below.

Parameters	Pre-Treatment and Treatment Regimen				Post -Treatment Regimen*****		
	Pre-Treatment or Day 1	Day 4	Weekly	Every 3 weeks	1 month follow-up	Every 3 months for 2 years	Every 6 months for 2 years
Pregnancy test	X						
History and Physical	X			X	X	X	X
Tumor measurement	X				X	X	X
Weight	X		X	X	X	X	X
Performance status	X			X	X	X	X
Toxicity evaluation	X		X	X			
Hgb, WBC	X		X	X	X	X	X
Differential	X		X	X	X	X	X
Urinalysis	X						
BUN, creatinine	X			X	X	X	X
Creatinine clearance	X			X	X	X	X
Alk. Phos, Bilirubin, SGOT	X			X	X	X	X
CA^{++}, PO_4	X			X			
Blood sugar	X			X	X	X	X
Electrolytes	X				X*	X*	X*
Brain MRI	X						
EKG	X				X*	X*	X*
CXR	X				X*	X*	X*
Chest CT Scan	X				X**	X**	X**
Histological or unequivocal Cytological diagnosis	X				X***		
PFT	X				X****		

NOTE: On days that the MnSOD-PL is given with chemotherapy, the order in which they should be given is MnSOD-PL administration, chemotherapy then radiation treatment. **HOWEVER**, on Day 1, there is a 4-6 hour wait before the radiation treatment (sec. 9.1) (chemotherapy still should be given after the MnSOD-PL).

* Post-treatment and as clinically indicated.

** Four weeks post-treatment completion, then every 3 months thereafter for 2 years, and then as needed or at recurrence to document indicator lesions with quantitative measurements (please ask for quantitative measurements on radiology requests).

*** If indicated and feasible, to document histological complete response.

**** Four weeks post treatment, and thereafter as indicated (i.e., additional PFTs for assessment of radiation pneumonitis)

***** For those patients that go on to receive additional chemo after their protocol treatment, they will be followed as "standard of care" and for research purposes will only need to be followed for radiologic response and survival, once they are past the point of 8 weeks post radiation therapy treatment.

7.0 CHEMOTHERAPY

7.1 Paclitaxel

7.1.1 Availability:

Paclitaxel is commercially available. Paclitaxel is a natural product obtained via a semi-synthetic process from *Taxus baccata*. Improved solubility requires a mixed solvent system with further dilutions of either 0.9% sodium chloride or 5% dextrose in water. Paclitaxel is supplied in a sterile concentrated solution, 6 mg/ml, and is available in 5 ml (30 mg) and 16.7 ml (100 mg) multidose vials. Each milliliter contains 6 mg paclitaxel, 527 mg of Cremophor EL (polyoxyethylated caster oil) and 50% dehydrated alcohol, USP, 50%. The contents of the vial must be diluted just prior to clinical use. Commercial supplies of Paclitaxel will be used for this study.

7.1.2 Solution Preparation:

Paclitaxel must be diluted prior to infusion. Paclitaxel should be diluted in 0.9% sodium chloride or 5% dextrose injection to a final concentration of 0.3 to 1.2 mg/mL. Infusions should be mixed as closely as possible to the start of each infusion since paclitaxel stability after 27 hours at room temperature in solution is unknown. Paclitaxel must be prepared in glass, polypropylene, or polyolefin containers due to leaching of diethylhexylphthalate (DEHP) plasticizer from polyvinyl chloride (PVC) bags. Paclitaxel will be administered using non-PVC tubing and connectors such as the IV administration sets that are polyethylene lined. In-line filtration must be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 microns (e.g., IVEX-HP and IVEX-II, Abbot). Nothing else is to be infused through the lines where paclitaxel is being administered. Solutions exhibiting excessive particulate formation should be discarded.

The Chemo Dispensing Pin™ device or similar devices with spikes should not be used with vials of paclitaxel since they can cause the stopper to collapse resulting in the loss of sterile integrity of the paclitaxel solution.

7.1.3 Storage and Stability:

Intact vials should be stored at room temperature (2-25°C or 36-77°F). Shelf life of the vials stored under appropriate conditions corresponds to the manufacturer's expiration date on each vial. All solutions of paclitaxel exhibit a slight haziness directly proportional to the concentration of drug and time elapsed since preparation. When prepared as above, solutions of paclitaxel (0.3 – 1.2 mg/ml) are stable for 27 hours.

7.1.4 Calculating Dosage of Paclitaxel:

The dosage will be calculated using the patient's actual weight at each treatment visit. The dosage will be rounded to the nearest 5 mg. In calculating surface areas, actual heights and weights should be used. That is, there will be no adjustment to "ideal" weight. This principle applies for individuals whose calculated surface area is 2.2 m^2 or less. In those rare cases where a patient's surface area is greater than 2.2 m^2, then 2.2 m^2 should be used for calculating the dosage for this trial.

7.1.5 Premedication Prior to Paclitaxel:

All patients will receive the following premedications 30 to 60 minutes prior to the paclitaxel infusion:

- Dexamethasone 10 mg IV * or 20mg po 12 and 6 hours prior to Paclitaxel in the event the parental form of dexamathasone is not available.
- Diphenhydramine 50 mg IV push
- An H_2 blocker IV (e.g. Ranitidine 50 mg; Cimetidine 300 mg)

* If patients do not have any reaction with the first dose, then the dose of dexamethasone can be increased to 20 mg prior to the weekly dose of paclitaxel.

Premedication can be adjusted per institutional guidelines or physician discretion.

7.1.6 Administration of Paclitaxel

Paclitaxel, at the appropriate dose and dilution, will be given as a 1-hour (45 mg/m^2) continuous IV infusion. Paclitaxel will be administered using non-PVC tubing and connectors, such as the IV administration sets (polyethylene or polyolefin) that are used to infuse parenteral Nitroglycerin and/or fat emulsion. A 0.22 micron filter must be placed on the distal end of the infusion line. Nothing else is to be infused through the line where paclitaxel is being administered.

7.1.7 Adverse Effects:

The following adverse events are expected with the administration of paclitaxel. For complete information, see package insert.

- *Hematologic:* Myelosuppression
- *Gastrointestinal:* Nausea and vomiting, diarrhea, stomatitis, mucositis
- *Cardiac:* Arrhythmia, heart block, ventricular tachycardia, hypotension, myocardial infarction (MI)
- *Neurologic:* Peripheral neuropathy, seizures
- *Allergy:* Anaphylactoid and urticarial reactions (acute), flushing, rash, pruritus
- *Other:* Alopecia, malaise, arthralgia, myalgia, elevated

SGOT, alkaline phosphatase and bilirubin

- *Note:* Cardiac toxicities are rare and continuous cardiac monitoring is not required except for patients with serious conduction abnormalities or other underlying, serious cardiac risk factors

7.1.8 Recommended Management of Hypersensitivity Reactions

Mild Symptoms: (e.g., mild flushing, pruritus)

Complete paclitaxel infusion. No treatment required.

Moderate Symptoms: (e.g., moderate rash, flushing, mild dyspnea, chest discomfort)

Stop paclitaxel administration. Give intravenous dexamethasone 10 mg and diphenhydramine HCl 25 mg. Resume paclitaxel infusion after recovery from symptoms at 20/ml/hr for 15 minutes, then 50 ml/hr for 15 minutes, then if no further symptoms, at full dose rate until infusion complete. If symptoms recur, stop paclitaxel infusion. The patient should not receive additional Paclitaxel for that week.

Severe symptoms: (e.g., hypotension requiring pressor therapy, IV fluids, angioedema, respiratory distress requiring broncho-dilation therapy, generalized urticaria)

Stop paclitaxel administration. Give intravenous diphenhydramine HCl 25 mg and dexamethasone as above. Add adrenaline (1:1000) or broncho-dilators as indicated. Contact Study Coordinator and report as a serious adverse event (See Section 11.2). In the case of severe symptoms occurring, the patient should not receive additional paclitaxel and is to be taken off of the study.

7.2 Carboplatin (Paraplatin® - NSC #241240)

7.2.1 Availability:

Carboplatin is commercially available as a sterile lyophilized powder in single-dose vials containing 50 mg, 150 mg, or 450 mg of carboplatin. Each vial contains equal parts by weight of carboplatin and mannitol. Commercial supplies of carboplatin will be used for this study.

7.2.2 Preparation:

Immediately before use, the content of each vial must be reconstituted with either sterile water for injection, USP, 5% dextrose in water, or 0.9% sodium chloride injection, USP, according to the following schedule: (These dilutions all produce a carboplatin concentration of 10/mg/ml)

Vial Strength	Diluent Volume
50 mg	5 ml
150 mg	15 ml
450 mg	45 ml

When prepared as directed, the resultant carboplatin solutions are stable for eight hours at room temperature and protected from light. Because no antibacterial preservative is contained in the formulation, it is recommended that carboplatin solutions be discarded eight hours after dilution.

NOTE: Aluminum reacts with carboplatin, causing precipitate formation and loss of potency. Therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin.

7.2.3 Storage and stability:

Unopened vials of carboplatin are stable for the life indicated on the package when stored at controlled room temperature (59° - 86°F) and protected from light. When prepared, carboplatin solutions are stable for 8 hours at room temperature.

7.2.4 Administration:

Carboplatin will be administered as an IV infusion over 30 minutes after paclitaxel. Carboplatin will be adjusted for renal function to achieve a calculated AUC (area under curve) as defined by the following Calvert Formula. Carboplatin dose will be based on calculated GFR (glomerular filtration rate) based on measurement of creatinine clearance or calculated creatinine clearance. See Appendix II for Carboplatin Dosing Worksheet.

Calvert Formula for Carboplatin Dose:

AUC Dose = 2.0 X (GFR + 25)

Substitute GFR by calculated creatinine clearance (Cockroft-Gault):

$$\text{GFR} = \frac{(140 - \text{Pt. Age})\ (\text{Weight in Kg})}{\text{Serum Creatinine} \times 72} \times 0.85\ (\text{females})\ \underline{\text{or}}\ 1.0\ (\text{males})$$

By the end of 2010, all clinical laboratories in the US will use the new standardized Isotope Dilution Mass Spectrometry (IDMS) method to measure serum creatinine. The IDMS method appears to underestimate serum creatinine values compared to older methods when the serum creatinine values are relatively low (e.g., ~0.7 mg/dL). Measurement of serum creatinine by the IDMS-method could result in an overestimation of the Glomerular Filtration Rate (GFR) in some patients with normal renal function. If the total carboplatin dose is calculated based on IDMS-measured serum creatinine using the Calvert formula, carboplatin dosing could be

higher than desired and could result in increased drug-related toxicity.

The current label for carboplatin provides safe dosing instructions that are based on actual GFR measurements. Provided that actual GFR measurements are made to assess renal function, carboplatin can be safely dosed according to the instructions described in the label.

(http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=13328)

If a patient's GFR is estimated based on serum creatinine measurements by the IDMS method, as recommended by the FDA physicians should consider capping the dose of carboplatin for desired exposure (AUC) to avoid potential toxicity due to overdosing. Based on the Calvert formula described in the carboplatin label, the maximum doses can be calculated as:

Total Carboplatin Dose (mg) = (target AUC) x (GFR +25) [Calvert formula]

Maximum Carboplatin Dose (mg) = target AUC (mg•min/mL) x (150 mL/min)

The maximum dose is based on a GFR estimate that is capped at 125 mL/min for patients with normal renal function. No higher estimated GFR values should be used.

For a target AUC = 6, the maximum dose is 6 x 150 = 900 mg
For a target AUC = 5, the maximum dose is 5 x 150 = 750 mg
For a target AUC = 4, the maximum dose is 4 x 150 = 600 mg
For a target AUC = 2, the maximum dose is 2 x 150 = 300 mg

7.2.5 Toxicities:

Some of the expected adverse events from carboplatin are listed below. For further description of adverse events see Package Insert.

Hematologic:	Myelosuppression
Gastrointestinal:	Nausea, vomiting, diarrhea, weight loss Constipation, gastrointestinal pain
Metabolic:	Electrolyte imbalances, hypomagnesemia, hypocalcemia, hyponatremia, hyperuremia
Hepatic toxicity:	Elevated alkaline phosphatase, SGOT, and total bilirubin, CNS peripheral neuropathies (mild parerethises, clinical ototoxicity and other sensory abnormalities are rare)
Genitourinary:	Renal tubular damage, renal insufficiency, impotence, sterility, amenorrhea, gynecomastia
Allergy:	Anaphylactoid and urticarial reactions

	(acute), flushing, rash, pruritus and rarely hypotension or bronchospasm
Other:	Alopecia, pain, asthenia and mucosal side effects, decreased serum electrolytes values (sodium, magnesium, calcium and potassium)

8.0 MANGANESE SUPEROXIDE DISMUTASE TREATMENT PLAN

8.1 Characteristics of MnSOD Plasmid/Lipid Complex

The investigational agent comprises a plasmid encoding human MnSOD complex with a cationic lipid. The plasmid/lipid is re-suspended at 3 mg plasmid DNA per ml.

8.2 Study Design

This study aims to assess the feasibility and safety of MnSOD plasmid liposome (PL) in combination with Paclitaxel, Carboplatin and thoracic radiotherapy by dose escalation in 3 cohorts of 3 patients each. It further aims to assess the efficacy of MnSOD PL in protecting the esophagus by estimating the incidence of grade 3 or 4 esophagitis in a group of patients treated at the MTD. MnSOD PL will be delivered by swallowing on days 1 and 3 of each week of combined chemotherapy and radiation.

8.3 MnSOD PL Dose Escalation

Three dose levels are defined based on dose of MnSOD plasmid DNA: level 1, 0.3 mg per dose; level 2, 3 mg per dose; and level 3, 30 mg per dose. Dose escalation will proceed according to a standard phase I design with 3 patients initially treated on each tier. Patients will be closely monitored for toxicities due to MnSOD plasmiod/liposome Paclitaxel, Carboplatin or thoracic radiotherapy. If, on any dose tier of MnSOD plasmid liposome, 2 of 3 patients or 2 of 6 patients experience a grade III or IV toxicity due to MnSOD, dose escalation will cease. The maximally tolerated dose is defined as the highest dose with fewer than 1/3 of patients experiencing a dose limiting toxicity due to MnSOD . All patients on a tier must be observed for 8 weeks after starting treatment before escalating the dose of MnSOD. The decision to escalate to the next higher dose will be made by the principal investigator and IND Sponsor.

8.4 Study Drug Discontinuation and Toxicity Management

Doses of study drug will be held or discontinued due to the occurrence of grade 3-4 nausea/vomiting, or grade 3-4 rash/desquamation.

Rash/Desquamation: If a patient experiences grade 3-4 rash/desquamation, the MnSOD will be discontinued and the patient will be treated at the physician's discretion. The patient should continue to receive XRT and chemotherapy if MnSOD is discontinued, and should be followed as per protocol. The CTCAE v 3.0 grading criteria for rash/desquamation is provided below:

CTCAE Grade for Rash/Desquamation				
	1	2	3	4

Rash/ Desquamation	Macular or papular eruption or erythema without associated symptoms	Macular or papular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering < 50% of the body surface area (BSA)	Severe, generalized erythroderma or macular, papular or vesicular eruption; desquamation covering ≥ 50% of BSA	Generalized exfoliative, ulcerative, or bullous dermatitis

Nausea/Vomiting: Grade 1 nausea/vomiting occurring on days when no chemotherapy is planned should be treated symptomatically as necessary. If grade 2-3 nausea vomiting occurs, the patient may be treated with antiemetics as required. If a patient experiences grade 4 nausea/vomiting or recurrent grade 3 nausea/vomiting related to the MnSOD and after adequate antiemetic medication, the MnSOD will be discontinued. The patient should continue to receive XRT and chemotherapy if MnSOD is discontinued, and should be followed as per protocol.

Retreatment: Retreatment with MnSOD can occur if the grade 3-4 nausea/vomiting or grade 3-4 rash/desquamation resolves to grade 1 within 2 weeks.

If RT is held due to toxicities, MnSOD should be held and restarted with RT therapy and continue to be given 2X week with one day in between (ex. Day 1 and 3 or Day 2 and 4).

8.5 MnSOD Efficacy Assessment

Once the starting dose is determined, 27 additional patients as described in section 12.0 will be treated with MnSOD plasmid at that dose. The proportion of patients with grade 3 or 4 esophagitis will be assessed as described in section 12.0.

8.6 Safety Monitoring During the Evaluation of Efficacy

In the interest of patient safety we will also apply a stopping rule to the efficacy phase of the study: If 6 the first 12 patients treated at the highest dose of MnSOD P/L, experience a grade III or grade IV toxicity due to therapy, the study will cease, the FDA will be notified and a comprehensive safety review will be undertaken.

9.0 RADIATION TREATMENT PLAN

9.1 Radiation Dose

Radiation therapy will commence 4 to 6 hours after the first MnSOD PL therapy. This will be administered at 1.9-2.1 Gy daily 5 times a week. The total dose will be 77.0 Gy with a range of 69-84 Gy in 34-38 fractions over 7-8 weeks.

9.2 Treatment Techniques

9.2.1 All doses are to be prescribed and calculated assuming a heterogeneous patient. There will be corrections for heterogeneity used in the definitions of these doses.

9.2.2 The doses shall be prescribed and calculated according to the following ICRU recommendations for external treatments using photons and electrons.

9.2.2.1 At mid-separation on the central rays for two opposed coaxial equally weighted beams.

9.2.2.2 At the center of the target volume on the central rays for two opposed coaxial unequally weighted beams.

9.2.2.3 At the point of intersection of the central rays for two or more intersecting beams that are not coaxial.

9.2.2.4 At the center of the target volume for complex treatment arrangements which are not covered above.

9.2.2.5 At the depth of maximum dose for a single-electron beam with an electron beam energy chosen such that the minimum percent dose at 3.0 cm depth is 90%.

9.3 Target Volumes

9.3.1 Two different target volumes shall be considered: the initial large field target volume consisting of primary and mediastinum, and the boost target volume consisting of the primary, involved nodes and nodes $\geq$ 2.5 cm. in diameter only. In treating the initial fields, various sets of fields may be used. Target volumes should be based on original tumor volume.

9.3.2 No part of the primary lesion and ipsilateral hilar and mediastinal lymph nodes (within a 2.0 cm margin*)* will receive a dose less than 45.0 Gy from the initial fields. In cases where the central rays of the initial fields do not intercept the center of the boost target volume it should not exceed the prescribed dose by more than 15%.

9.3.3 Deviations of the daily dose of up to 5% are allowed. In patients for whom the difference in dose to the initial target volume and to the boost target volume is greater than 5%, a change in the boost dose is allowed. As an example of the above, assume that a patient receives 43.0 Gy to the center of the boost target volume when 45.0 Gy has been delivered at the intersection of the central rays of the initial fields. The center of the target volume has received 2.0 Gy less than prescribed. This patient could then receive one extra fraction of 2.0 Gy to the boost field.

9.4 Irradiation Portals

The irradiation target volume must be defined by the individual shaped ports with

secondary lead blocking or tailor-made blocks.

9.4.1 Target Volume of Primary Tumor:

Includes complete extent of visible primary tumor as defined radiographically with a minimum of 2.0 cm and the maximum of 2.5 cm margin around the mass.

9.4.2 Target Volume of Lymph Nodes - The following nodes must be included:

9.4.2.1 Supraclavicular lymph nodes -- if primary, is upper lobes and mainstream bronchus lesions. It is acceptable to treat the ipsilateral supraclavicular nodes only;

9.4.2.2 Ipsilateral hilar lymph nodes -- always (2 cm margin);

9.4.2.3 Superior mediastinal lymph nodes (above carina) -- always (ipsilateral 2 cm margin);

9.4.2.4 Subcarinal lymph nodes (include the contralateral mainstream bronchus and extend field at least to 5 cm below carina) – always;

9.4.2.5 Inferior mediastinal nodes to the diaphragm (to bottom of
9.4.2.6 T-l0 vertebral body) for patients with lower lobe lesions or
9.4.2.7 inferior mediastinal involvement;

9.4.2.6 Contralateral hilar lymph nodes--for patients with contralateral mediastinal, subcarinal, or contralateral hilar involvement (1.0 cm margin).

9.5 Technical Factors

9.5.1 Beam Energy

Megavoltage equipment is required with minimum peak photon energies of 6 MeV. Electrons with at least 90% dose at 3 cm depth mav be used to boost supraclavicular lymph nodes. The dose should be specified dmax.

9.5.2 Treatment Distance

Minimal treatment distance to skin should be greater than 100 cm for SSD technique and minimum isocenter distance should be 100 cm for SAD techniques.

9.5.3 Blocking

In the case of x-ray beams, the primary collimation may be used, and blocking will be required only for shaping of the ports to exclude volume of tissues that are not to be irradiated.

9.5.4 Compensating Filter or Wedges

In the case of a large sloping contour, such as usually encountered when treating

upper lobe tumors in large patients, compensating filters are recommended. A wedge may also be used as a 2 dimensional tissue compensator. If necessary, appropriate reduction in field size must be done to avoid excessive irradiation to critical structures.

9.5.5 Therapy Interruptions

If interruptions of therapy up to one week (5 consecutive days*)* become necessary, irradiation should be completed to the prescribed doses. Total dose, number of fractions, and elapsed days should be carefully reported. Every effort should be made to minimize the length of treatment interruptions. See Section 9.6.4.

9.5.6 Radiotherapy interruptions or delays will be permitted only for febrile neutropenia or grade 4 esophagitis/mucositis. Interruptions longer than 3 days should be discussed with Dr. Joel S. Greenberger.

9.5.7 Treatment Planning

9.5.7.1 Treatment planning should be performed in accordance with the prescribing doses to each target volume, together with restrictions in dose to normal tissues as given in Section 9.6.1. Treatment planning simulation is required. It is recommended that CT-based treatment planning be utilized whenever possible.

9.5.7.2 One set of composite isodose distributions in the transverse plane passing through the midlevel of the boost target volume should be submitted. Sagittal dose distributions are encouraged.

9.5.7.3 In addition to the isodose distribution, the following specific points of dose calculations should be included:

1) The Spinal Cord Dose: If compensating filters are not used, the point at which the spinal cord dose to be calculated is 2 cm below the superior margin of the posterior field. If compensating filters or wedges are used then the point of maximum dose to the spinal cord must be determined. Maximal spinal cord dose should not exceed 48.0 Gy at any level.

2) Subcarinal Nodes: Are assumed to be at mid-plane.

3) Ipsilateral Normal Lung Dose: This is to be calculated at the level of the central rays of the boost fields at the point of maximum dose in the lung which lies at least 2 cm outside the projected border of the initial treatment fields in the ipsilateral lung.

4) Contralateral Normal Lung Dose: This is to be calculated at the level of the central rays of the boost fields at the point of maximum dose in the lung which lies at least 2 cm outside the

projected border of the initial treatment fields in the contralateral lung.

5) Maximum Normal Tissue Dose: This is to be calculated at level of the central rays of boost fields as the maximum total dose at least 2 cm outside of the target volume.

9.5.8 Localization Films:

All fields treated require filming on simulator (conventional or CT sim) units. Portal verification shall be done for all treatment fields. Copies of both simulator and portal fields will be reviewed by Dr. Greenberger.

9.6 Anticipated Side Effects or Toxicities

9.6.1 Suggested Maximum Doses to Critically Sensitive Normal Structures

Organ	Maximum Dose
Spinal Cord *(Maximum Dose)*	48.0 Gy
Heart	
Entire Organ	45.0 Gy
< 50%	50.0 Gy
Esophagus	60.0 Gy

9.6.2 The dose to the spinal cord must be limited to 48.0 Gy. A posterior spinal cord shield will not be an acceptable technique. Oblique or lateral field arrangements with custom shielding are recommended to limit spinal cord dose.

9.6.3 Reversible alopecia, bone marrow toxicity, skin pigmentation and esophagitis are expected side effects of radiation therapy, while radiation-induced myocarditis or transverse myelitis rarely will occur at doses lower than 50.0 Gy. Radiation pneumonitis will occur in 100% of patients within the first six months after initiation of treatment so it is essential to spare as much normal lung as possible.

9.6.4 Treatment interruptions are strongly discouraged; however, radiation therapy may be interrupted for up to two weeks (10 consecutive treatment days*)* for significant (> grade 3) esophagus toxicity: i.e., inability to tolerate liquids and requiring parenteral feedings. Sucralfate slurries may provide symptomatic relief of mucositis and esophagitis.

9.6.5 Post-treatment pneumonitis thought due to radiation should be treated with prednisone after excluding microbial causes.

10.0 TOXICITIES AND MANAGEMENT

10.1 Hematologic Toxicity

10.1.1 Concurrent Chemoradiotherapy

Give the following percents of dose:

Granulocytes ml	Or	Platelets/ml	Carboplatin	Paclitaxel
>1.5		>75	100%	100%
1.0-1.49		50- 74.9	50%	50%
<1.0		<50	0 *	0 *

*omit this dose, repeat counts weekly, and resume chemotherapy based on the above table.

When a dose reduction is required no dose re-escalation will be performed subsequently. Any patient who does not tolerate the 50% dose reduction of paclitaxel and carboplatin, will be taken off study treatment.

10.1.2 Renal Toxicity

A >25% change in the serum creatinine will warrant a recalculation of the carboplatin dose.

10.1.3 Neurotoxicitv/CNS Toxicity- (use CTC Criteria):

Grade	Carboplatin	Paclitaxel
0-2	100%	100%
3	0*	0*

*discontinue until symptoms improve to Grade ≤ 1, then treat with 75% of both agents.

10.1.4 Ototoxicity: For clinically evident hearing loss, discontinue carboplatin.

10.1.5 Hypersensitivity Reactions (Institutional Guidelines may be used.) (e.g.,flushing, rash, pruritus, dyspnea, bronchospasm, rigor, hypotension, fever)

- Mild symptoms
  Complete paclitaxel infusion. No treatment required.

- Moderate symptoms
  Stop paclitaxel infusion. Give intravenous dexamethasone 10 mg and chlorpheniramine 10 mg. Resume paclitaxel infusion after recovery of symptoms at 20 mls/hr for 15 minutes, then 50 mls/hr for 15 minutes, then if no further symptoms, at full dose rate until infusion complete. If symptoms recur, stop paclitaxel infusion. The patient should receive no additional paclitaxel for that week, but may be retreated after discussion with the Study Chair.

- Severe symptoms e.g.,
  - hypotension requiring therapy
  - angieodema
  - respiratory distress requiring therapy
  - generalized urticaria

Stop paclitaxel infusion. Give intravenous chlorpheniramine and dexamethasone as above. Add adrenaline (1:1000*)* or broncho-dilators as indicated. Report as a serious adverse event (see Section 11.0). In the case of severe symptoms, the patient should not receive additional paclitaxel.

10.1.6 Cardiotoxicity:

If a patient develops chest pain or arrhythmia other than asymptomatic sinus brachycardia, the paclitaxel infusion should be stopped and patients should not receive further paclitaxel. For asymptomatic sinus brachycardia, the infusion need not be stopped but the patient should be followed carefully.

10.1.7 Gastrointestinal Toxicity (use RTOG Acute Morbidity Criteria)

For infield acute toxicities (Use RTOG Acute Criteria [see web site links in Adverse Event section]).

Infield	Severity/toxicity Scale	XRT	Modifications	
			Taxol	Carboplatin
Esophagus / pharynx (on day of XRT)	4/RTOG Acute	Hold	Hold	Hold
Esophagus / pharynx (on day of chemo admin)	3/RTOG Acute	no mod	Hold	Hold
Esophagus/pharynx (on day of chemo admin)	2/RTOG Acute	no mod	no mod	No mod
Skin	4/RTOG Acute	Hold	Hold	Hold
Skin	3/RTOG Acute	no mod	no mod	No mod

For out of field acute toxicities (use scoring criteria specified)

Infield	Severity/toxicity Scale	XRT	Modifications	
			Taxol	Carboplatin
Stomatitis	$\geq$ 3/CTC	No mod	Hold	Hold
Stomatitis	< 3/CTC	No mod	No mod	No mod
Weight loss or dehydration with severity as described	> 15% or requirement for IV tube feeding (grade3/RTOG)	No mod	Hold	Hold

For Grade 4 infield esophagitis:

Radiotherapy and chemotherapy should be interrupted. Reevaluate patient weekly.

For Grade $\geq$ 3 esophagitis/pharyngitis, dermatitis or other in-field radiotherapy related toxicity, on day of chemotherapy administration during any treatment week:

Omit paclitaxel until toxicity resolves to grade $\leq$ 2. Then restart the rest of the treatment plan with paclitaxel at 50% of prior dose. Do not reduce carboplatin.

Radiotherapy should be interrupted only for grade 4 infield toxicity and resumed when that toxicity has decreased to grade ≤ 2. If treatment is interrupted for > 2 weeks, the patient should be removed from study treatment. If the patient experiences esophagitis so that IV fluid support is needed, insertion of a feeding tube should be considered. Radiotherapy and chemotherapy are to be held as outlined above.

If there is a decline of performance status score to a level of Karnofsky Performance Scale (Appendix I) ≤ 40 for greater than 2 weeks while under treatment, radiotherapy should be held with no further chemotherapy administered. Reevaluate patient after one week for resumption of radiotherapy.

10.2 Acute Radiation Morbidity Scoring Schema

RTOG	Acute Radiation Morbidity Scoring Criteria				
	0	1	2	3	4
Pharynx and Esophagus		Mild dysphagia or odynophagia may require topical anesthetic or non-narcotic analgesics may require soft diet	Moderate dysphagia or odynophagia may require narcotic analgesics, puree or liquid diet	Severe dysphagia or odynophagia With dehydration or weight loss (>15% from pre-treatment baseline) requiring NG tube feeding, IV fluids or hyperalimentation	Complete obstruction, ulceration, perforation, fistula

10.3 Late Radiation Morbidity Scoring Schema

RTOG	Late Radiation Morbidity Scoring Schema				
	0	1	2	3	4
Esophagus	NONE	Mild Fibrosis Slight difficulty in swallowing solids no pain swallowing liquids	Unable to take solid food normally Swallowing semi-solid food Dilation may be indicated	Severe fibrosis Able to swallow only liquids May have pain on swallowing Dilation required	Necrosis/perforation/fistula

10.4 Other Toxicity:

Reevaluate all other toxicities that are grade ≥ 3 (except alopecia, nausea, vomiting, fatigue, and anorexia), reduce carboplatin and paclitaxel by 25% in the subsequent cycle if considered possibly, probably, or definitely related to carbo and taxol. Use CTC scale. If Chemotherapy drugs are held, schedule may be changed to give carbo and taxol on day 1 or day 3 of each week.

10.5 Evaluation of Radiation Reactions

Esophageal Toxicity: Esophagitis will be assessed by subjective and objective criteria. Patients will be evaluated immediately on the day of plasmid/liposome administration for subjective side effects, including tingling sensation in the esophagus, difficulty swallowing pure liquids (Nursing Service Questionnaire based on RTOG schedule for evaluation of esophagitis), and will be asked to report in great detail their food consumption for the 72 hours after plasmid/liposome administration.

10.5.1 Acute Radiation Esophagitis: There is a potential for radiation effects resulting from inflammation of the esophagus that include ulceration, secondary, infection, stricture formation and perforation. The toxic effects may be acute or late (44, 45, 46). Typically, acute radiation esophagitis occurs within 2 to 3 weeks after initiation of XRT. It may last for several weeks after completion of treatment. Initial symptoms include a feeling of fullness in the throat or a sore throat, which promotes dysphagia. The patient may then develop sharp pain along the esophagus, and there may be symptoms of gastric reflux. Secondary infections of the mucosal lining may occur due to tissue inflammation, ulcerations, and the immunocompromised status of the patient (47). Secondary infection in the mucosal lining often occurs in patients with mucositis and esophagitis (48). Because acute radiation esophagitis produces difficulty in swallowing, nutritional intake may be reduced, and patients may lose weight. For milder cases, there can be dietary modifications such as soft foods or liquid diets. More sever cases require feeding tubes or total parenteral nutrition.

Acute radiation esophagitis will be evaluated by the investigator or other health care professional according to the toxicity evaluations described above (pre-treatment or day 1, weekly during the pre-treatment and treatment regimen), or until the radiation esophagitis resolves. We also plan to monitor acute radiation esophagitis daily with the utilization of a daily diary for the patient. The diaries will be collected prior to each planned chemotherapy dose, at the end of therapy, at each follow-up visit, or until the radiation esophagitis resolved, whichever is later.

Acute radiation esophagitis is defined as the development of esophagitis in a patient actively receiving XRT that cannot definitively be ascribed to another process. If there is another cause of the esophagitis (e.g., infection), there must still be some degree of esophagitis present after the treatment for the other cause. If the signs and symptoms of esophagitis resolve after treatment for the other

cause, then the patient did not have radiation esophagitis. We will assess acute radiation esophagitis as follows:

1. At each evaluation, ask whether the patient has pain or difficulty swallowing.

    - If yes, determine location of the pain.
    - The pain must involve the esophagus to be esophagitis, whether or not there is oral or pharyngeal involvement.
    - If the patient does not have pain or if the pain does not involve the esophagus, then the patient does not have esophagitis.

2. Is there an alternate etiology for the acute esophagitis?

    - If no, then the patient has acute radiation esophagitis.
    - If yes, then proof (e.g., culture) must be documented in the primary source documents, and the alternate etiology must be treated.
    - If the esophagitis does not resolve completely after treatment for the alternate etiology, the patient does have acute radiation esophagitis.

3. The grade of the acute esophagitis is as follows:

    - Grade 1: Normal diet.
    - Grade 2: Patient can eat and swallow modified diet (e.g., soft, pureed or liquid)
    - Grade 3: Patient unable to eat or hydrate orally (e.g., may require a feeding tube, IV hydration, peripheral protein sparing alimentation or central hyperalimentation)
    - Grade 4: Life-threatening.

4. Once the patient develops acute radiation esophagitis, they must be assessed daily during XRT and then weekly until the process resolves.

    - Determine whether the patient has acute esophagitis that continues since last assessment.
    - If yes, determine grade
    - If no, determine when esophagitis resolved.

5. Record the date and grade of the initial onset of acute radiation esophagitis; the date and grade whenever a change in severity of the acute radiation esophagitis occurred (worse or better), and the date when the radiation esophagitis completely resolved.

The incidence and severity of acute radiation esophagitis is a primary efficacy endpoint of the study.

10.5.2 Chronic Radiation Esophagitis: Chronic radiation esophagitis is esophagitis lasting at least 4-6 weeks, with continued difficulty swallowing and a need for dilation. Chronic radiation esophagitis will be assessed by the same monitoring procedures

enumerated above, with emphasis on the duration of radiation esophagitis.

10.5.3 Radiation Pneumonitis – Radiation pneumonitis is an interstitial pulmonary inflammation that can develop after thoracic XRT. The severity and extent of damage to normal lung tissues depends on the volume of normal lung included in the XRT field; the total dose and fraction of XRT, and previous or concomitant therapy (i.e., chemotherapy) that may influence the time and severity of the radiation changes (49, 50) Radiation pneumonitis can be either acute or chronic. Acute radiation pneumonitis occurs within 1 to 6 months of XRT. Symptoms can include low-grade fever, cough and fullness in the chest. These symptoms usually resolve without long-term effects. Chronic radiation pneumonitis is permanent changes of radiation fibrosis and it typically takes 6 months to evolve. This is clinically a more significant syndrome for patients, and it may result in progressive dyspnea and mortality.

The radiographic hallmark of radiation pneumonitis is a localized infiltrate corresponding to a previous XRT treatment field (51, 52, 53, 54). The severity of radiation pneumonitis is graded based on radiologic changes, pulmonary function tests (i.e., DL_{CO} or FEV_1), and symptomatology and its affect on activities of daily living. Radiation pneumonitis will be evaluated by the investigator or other health care professional during the follow-up visits described above (every 3 months for 2 years, and then every 6 months for 2 more years) or until the radiation pneumonitis resolves.

We define radiation pneumonitis as a new interstitial infiltrate within the radiation field that cannot be completely and definitively attributed to another process. If another potential cause for the infiltrate is present (e.g., and infection), there must still be at least a residual interstitial infiltrate that is new from baseline within the radiation field after a treatment for the other potential cause. If the infiltrate resolves completely after the treatment, then the patient did not have radiation pneumonitis. If the new infiltrate exists both inside and outside of the radiation port and, after treatment, is still present inside and outside the radiation port, then the patient has radiation pneumonitis.

The following information will be used to assess radiation pneumonitis in this study:

1. Is there an interstitial infiltrate on CT scan that was not present at baseline?
   - If yes, determine the location of the infiltrate (i.e., only within the XRT port, only outside the XRT port, or both within the XRT port and outside the port)

2. Is there an alternate etiology for the interstitial infiltrate?
   - If yes, proof (e.g., culture) must be documented in the primary source documents, and the alternate etiology must be treated.

3. Determine whether the patient meets the criteria for radiation pneumonitis (following treatment of any documented alternate etiology,

if present):

Interstitial Infiltrate		
Within XRT Port	Outside XRT Port	Diagnosis
+	-	Radiation pneumonitis
-	-	No radiation pneumonitis
+	+	Radiation pneumonitis

4. Radiation pneumonitis will be graded based on the radiographic changes within the XRT field on the high-resolution chest CT scan.
   - Grade 1: Minimal radiographic findings (or patchy changes) and estimated radiographic proportion of irradiated lung volume that is fibrotic of < 25%
   - Grade 2: Patchy changes and estimated radiographic proportion of irradiated lung volume that is fibrotic of 25%-<50%
   - Grade 3: Dense or widespread infiltrates/consolidation and estimated radiographic proportion of irradiated lung volume that is fibrotic of 50%-<75%
   - Grade 4: Estimated radiographic proportion of irradiated lung volume that is fibrotic is ≥75%; honeycombing

5. Radiation pneumonitis will be graded based on results of pulmonary function tests (DLCO or FEV_1) as follows:
   - Grade 1: DL_{CO} or FEV_1: ≥75% to <90% of baseline
   - Grade 2: DL_{CO} or FEV_1: ≥50% to <75% of baseline
   - Grade 3: DL_{CO} or FEV_1: ≥25% to <50% of baseline
   - Grade 4: DL_{CO} or FEV_1: <25% of baseline

6. Radiation pneumonitis will be graded based on symptomatology and its affect on activities of daily living:
   - Grade 1: Asymptomatic
   - Grade 2: Symptomatic (coughing, dyspnea), not interfering with activities of daily living
   - Grade 3: Symptomatic (coughing, dyspnea), interfering with activities of daily living; oxygen indicated
   - Grade 4: Life-threatening; ventilatory support indicated

7. The final grade of radiation pneumonitis is the highest grade of one of the following:
   - Radiographic changes on high-resolution chest CT scan
   - The pulmonary function tests
   - Symptomatology and its affect on activities of daily living

Acute radiation pneumonitis: Occurs within 1-6 months following XRT.
Chronic radiation pneumonitis: Occurs >6 months following XRT.

The incidence and severity of acute and chronic radiation pneumonitis is a primary efficacy endpoint of the study.

10.6 Long-Term Follow-up

Patients will be seen for follow-up at the end of treatment, 1 month after completion of treatment, and then every 3 months for 2 years, and then every 6 months for 2 years. Thereafter, they should be followed for recurrence and survival at the discretion of the investigator. Recurrence and survival will be documented.

10.6.1 Autopsy

To obtain vital information about the safety and effectiveness of the gene transfer treatment, at the time of patients death, no matter what the cause, permission for an autopsy will be requested of the patients family.

11.0 ADVERSE EVENTS

11.1 Definitions of Adverse Events

An adverse event is defined as any noxious, pathologic, or unintended change in anatomic, physiologic, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of the clinical trial, regardless of their relationship to study agent. Adverse events include (1) an exacerbation of a pre-existing condition, (2) an intercurrent illness, (3) any drug interaction, (4) any event related to a concomitant medication, and (5) pregnancy.

A pre-existing condition is defined as one that is present prior to or at the start of the study and is to be reported as part of the patient's medical history. It should be reported as an adverse event if the frequency, intensity, or the character worsens during study treatment.

A Serious Adverse Event (SAE) is any experience that suggests significant hazard, contraindication, side effect, or precaution. A serious adverse event includes any experience that:

- Is fatal or immediately life threatening.

NOTE: the term "life threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of event.

- Is severely or permanently disabling.
- Requires or prolongs hospitalization.
- Is a congenital anomaly/birth defect
- Clinically serious based on patient outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning

The Investigator is responsible for reporting all adverse events that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance. All events will be recorded on appropriate clinical report forms with notation of duration, severity, and outcome. Event severity is rated mild, moderate, or severe, and will be coded according to the National Cancer Institute's Common Toxicity Criteria Version 3.0. The Common Toxicity Criteria of the NCI, Version 3.0 can be found at the web site for the National Cancer Institute:

http://ctep.info.nih.gov/reporting/ctc.html

The web site for RTOG/EORTC criteria for determining causal relationship is:

www.rtog.org/members/toxicity/late.html

11.1.2 All adverse events, regardless of causal relationship, are to be recorded in the case report forms and source documentation. The Investigator must determine the intensity of any adverse events according to the CTC and RTOG/EORTC criteria and their causal relationship.

11.1.3 Adverse events resulting in the removal of the patients from the study will be followed until resolution.

11.2. Reporting of Adverse Events

11.2.1 Reporting of adverse events to the FDA

Written IND Safety Reports

The investigator-sponsor will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the investigator-sponsor's receipt of the respective adverse event information.

For each written IND Safety Report, the sponsor-investigator will identify all previously submitted IND Safety Reports that addressed a similar adverse event experience and will provide an analysis of the significance of newly reported adverse event in light of the previous, similar report(s).

Follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the relevant information is available. If the results of the sponsor-investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the investigator-sponsor will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

11.2.2 Telephoned IND Safety Reports

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the investigator-sponsor will notify the responsible review division of the FDA by telephone or facsimile transmission of any observed or volunteered adverse event that is 1) *associated with the use of the investigational drug or study treatment(s)*; 2 *fatal or life-threatening*; and 3) *unexpected*.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the investigator-sponsor's initial receipt of the respective human adverse event information.

11.2.3 Reporting adverse events to the responsible IRB

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the investigator-sponsor will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator-sponsor's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the investigator-sponsor's receipt of the respective information.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the sponsor-investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator-sponsor will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

11.2.4 Withdrawal of subjects due to adverse events

Patients may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document patient outcome, if possible. The investigator should inquire about the reason for withdrawal, request the patient to return for a final visit, if applicable, and follow-up with the patient regarding any unresolved adverse events.

If the patient withdraws from the trial and also withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be collected.

A discontinuation occurs when an enrolled patient ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation:

1. Withdrawal due to adverse event. When a discontinuation is due to a serious adverse event (SAE), the serious adverse event must be reported in accordance with the reporting requirements.
2. Patients may decide to withdraw from the study at any time. Patients who withdraw from treatment should be followed for outcome, and their subsequent treatments should be recorded.
3. Patients must be discontinued if the investigator believes it to be in the patient's best interest.

4. Patients who become pregnant must not receive further treatment in this study. Pregnant patients should be followed for the duration of the pregnancy, and the outcome of the pregnancy should be recorded.
5. Patients who begin new investigational therapy, chemotherapy, cytokine therapy or immunotherapy must not receive further treatment in this study.
6. Patients may be discontinued from the study for poor compliance at the discretion of the investigator.

The final evaluation required by the protocol will be performed at the time of study discontinuation. The investigator will record the reason for study discontinuation and provide or arrange for appropriate follow-up (if required) for the patient.

11.3. Additional OBA reporting guidelines for gene transfer studies:

NIH Guidelines for Research Involving Recombinant DNA Molecules

Investigators who have received approval from FDA to initiate a human gene transfer protocol must report any serious adverse event meeting regulatory reporting guidelines immediately to the local Institutional Review Board, Institutional Biosafety Committee, Office for Protection from Research Risks (if applicable), NIH/ORDA, and FDA, followed by the submission of a written report filed with each group. Reports submitted to:

Office of Recombinant DNA Activities,
National Institutes of Health/MSC 7010
6000 Executive Blvd., Suite 302,
Bethesda, Maryland 20892-7010,
Phone: 301-496-9838.

Initiation of the Clinical Investigation

No later than 20 working days after enrollment (see definition of enrollment in Section I-E-7) of the first research participant on a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the FDA Investigational New Drug Application (IND) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

Additional Clinical Trial Sites

No research participant shall be enrolled (see definition of enrollment in Section I-E-7) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Annual Reporting

Investigators shall comply with annual data reporting requirements. Annual data report forms will be forwarded by NIH OBA to investigators. Information submitted in these annual reports will be evaluated by NIH OBA and the RAC, and possibly considered at a future RAC meeting. Information obtained through the annual data reporting process will be included in the NIH Human Gene Transfer Information System to: (1) provide clinical trial information; (2) provide administrative details of protocol registration; (3) provide annual status reports of protocols; (4) facilitate risk assessment of individual applications of human gene transfer; and (5) enhance public awareness of relevant scientific, safety, social, and ethical issues.

11.4. Additional University of Pittsburgh IBC Reporting Guidelines:

1. This IBC continuing approval is granted only for the research procedures specified in the materials submitted for IBC review. Any proposed modifications to the research procedures (including the procedures addressed in Appendix M of the *NIH Guidelines)* must be prospectively reviewed and approved by the IBC.

2. Upon continuing approval from the University of Pittsburgh IRB, you must provide to the rDNA Office one copy of the IRB continuing approval letter and the final IRB approved version of all consent forms associated with the protocol.

3. You must comply with the annual data reporting requirements of the *NIH Guidelines*, and when possible, copies of the report should be forwarded to the rDNA Office.

4. You must report ***any*** adverse events observed during the conduct of this research to the rDNA Office, in accordance with the accepted requirements for such events. **Please note that *all* Adverse Events must be reported to the IBC regarding of the reporting criteria for the IRB.**

Go to http://www4.od.nih.gov/oba/rac/documents1.htm for detailed review reporting requirements and forms.

11.2.1 Adverse Drug Reactions (ADR)

Adverse Drug Reactions (ADR) to be reported:

- Unknown Grade 2, 3, or 4

- Known Grade 4
- Any ADR which is both serious (life-threatening, fatal) and unexpected.
- Any increased incidence of a known ADR that has been reported in the package insert or the literature
- Any death on study regardless of the relationship to any of the study drugs.

** Prompt reporting of all adverse events is mandatory.

12.0 EVALUATION OF TOXICITY AND RESPONSE

12.1 Data Safety Monitoring Plan

A data safety plan for this study is directed by the PI, who will oversee the adverse events and data to evaluate the toxicities seen in each patient. The PI and the named investigators on this study and clinical research coordinator meet at least once a month to review and discuss study data to ensure subject safety. Decisions to continue treating subjects at the current dose level and/or if the trial accrual should continue will also be discussed during these meetings. Any information regarding increased risks to study subjects that is discovered during these meetings will be forwarded immediately to the IRB and all other designated regulatory agencies (i.e.; FDA, IBC, OBA, NIH and etc.) Any required modifications necessary to ensure patient safety will be discussed and will be submitted to the IRB. All serious adverse events, regardless of their relationship will be reported to the IRB according to the established guidelines for gene transfer studies according sections 3.3, 3.4, 3.5 and 3.6 of the University of Pittsburgh IRB reference manual. All serious adverse events will also be reported to the sponsor and /or other regulatory agency as per their requirements. All protocol violations will also be reported to the IRB and all other designated regulatory agencies as required by the study protocol. All study data reviewed and discussed during these meetings will be kept confidential. Any breaches in confidentiality will be reported to the IRB and the other designated regulatory agencies assigned to this protocol.

12.2 General Toxicity Criteria

NCI Common Toxicity Criteria – See web site link in Adverse Events section.

12.4 Tumor Response

12.4.1 Complete Response

Disappearance of all clinical evidence of tumor, determined by two observations not less than 4 weeks apart.

12.4.2 Partial Response

At least a 30% decrease in the sum of the longest diameter of target lesions for at least 4 weeks without increase in size of any area of known malignant disease or appearance of new areas of malignant disease.

12.4.3 Progressive Disease

At least a 20% increase in the sum of the longest diameter of target lesions taking as reference the smallest sum longest diameter since the treatment started or the appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

12.4.4 Stable Disease

Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started or the persistent of one or more nontarget lesions.

13.0 STATISTICAL CONSIDERATIONS

13.1 Objectives

The primary objective of this study is to evaluate the safety, feasibility and efficacy of swallowed MnSOD plasmid/liposome for protecting the esophagus from toxicity associated with concurrent chemotherapy and radiotherapy. The study will be conducted in two phases, Phase I for safety and feasibility and Phase II for efficacy. Secondary objectives will evaluate the clinical response to the combination of chemoradiotherapy with esophagus protection by MnSOD plasmid/liposome.

13.2 Endpoints

For the safety and feasibility phase, the endpoint will be the proportion of toxicities attributed to administration of MnSOD plasmid liposome. This will exclude esophagitis as well as hematologic and other toxicities normally attributed to the chemotherapy (Paclitaxel and Carboplatin) and thoracic radiotherapy. For the efficacy phase, the clinical endpoint will be the proportion of radiation - induced grade III/IV esophageal toxicity. Grade III/IV toxicity definitions will be by the RTOG scale (appendix). The secondary endpoints will include clinical assessments of objective response rate by RECIST criteria, and Kaplan-Meier estimates of time to progression, time to progression conditional on clinical response, and overall survival from the first day of treatment.

13.3 Design

The first phase of this study will treat 3 patients each at 3 sequential tiers of .3, 3, and 30 mg of MnSOD/plasmid DNA. If no toxicities are observed the dose of MnSOD PL will be escalated to the next tier. If one toxicity is observed the cohort will be expanded to 6 patients. If two of 3 patients experience a grade III or IV toxicity due to MnSOD, dose escalation will cease and the next lowest dose will be declared to be the MTD. If none of 3 or 1 or 6 patients experience grade III/IV toxicity due to MnSOD at the 30 mg tier, that dose will be defined as the starting dose for efficacy phase and the MTD will be undefined.

The second phase will treat 27 patients at the highest dose of the first phase (expected dose is 30 mg MnSOD/plasmid). The goal of this phase is to estimate the proportion of patients with grade III/IV esophagitis and not to select a starting dose for a randomized trial. Accordingly, the statistical objectives call only for estimation with confidence intervals based on the observed radiation-induced toxicity rates. The sample size was selected by practical considerations and is not intended to insure precision of the estimated toxicity rates, to guarantee a selected power for a hypothesis test, or to make a decision based on a hypothesis test.

In the efficacy phase of the study the following stopping rule will be enforced: If 6 the first 12 patients treated at the highest dose of MnSOD P/L, experience a grade III or grade IV toxicity due to any component of therapy (Paclitaxel, Carboplatin, radiation or MnSOD, the study will cease, the FDA will be notified and a comprehensive safety review will be undertaken.

A maximum of 45 patients may be treated on both phases of the study – up to 18 on the

first phase and 27 on the second.

13.4 Characteristics of the Design

The phase I portion of the study will treat 3 patients on each of three increasing dose tiers - .3, 3, and 30 mg MnSOD plasmid/liposome using a standard phase I design with 3 patients initially enrolled on each dose tier. All patients will receive a fixed dose of paclitaxel, carboplatin and radiotherapy. While no grade III or IV toxicities are expected due to the MnSOD plasmid or liposome, a standard phase I design will be implemented to stop escalation if necessary and to define an MTD. The table below shows the probability of declaring each of 3 dose tiers as the MTD under 3 different scenarios.

Probability of Declaring a Dose Tier as the MTD with a Standard Three Patient Phase I Design

Dose of MnSOD PL	If the underlying toxicity rate is:	Prob. of Declaring MTD	If the underlying toxicity rate is:	Prob. of Declaring MTD	If the underlying toxicity rate is:	Prob. of Declaring MTD
.3 mg	.02	.004	.05	.026	.20	.291
3 mg	.04	.017	.10	.091	.30	.358
30 mg	.05	.025	.15	.164	.40	.241
MTD not defined	----	.952	----	.717	----	.108

For the phase II portion of the study, inference about the proportion of grade III/IV esophagitis will be made from the observed proportion of grade III/IV esophagitis among 30 total patients treated at the highest dose of MnSOD P/L in the trial (tentatively set as 30 mg). The estimate will combine 3 - 6 patients treated on the first phase and the 24-27 patients treated on the second phase. Inference about the true but unknown proportion of grade III/IV esophagitis will be based on 90% confidence intervals. The following table shows the bounds of the exact 90% confidence intervals for a binomial proportion with a sample of 30 patients.

Confidence Intervals and Operating Characteristics for N = 30

Number of Observed Grade III/IV Toxicities	Point Estimate of Toxicity Rate	90% Confidence Interval	Exact one sided p value for testing rate = .25	Power* to Detect Rate = Point Estimate
0	.000	.000 - .095	.0002	1.000
1	.033	.002 - .149	.002	.984
2	.067	.012 - .195	.021	.862
3	.100	.028 - .239	.037	.647
4	.133	.047 - .280	.098	.421
5	.167	.068 - .319	.203	.238
6	.200	.091 - .358	.348	.123
7	.233	.115 - .394	.514	.057

* power of a one tailed test with type I error = .05

The baseline grade III/IV esophagitis rate is estimated to be .25 in the absence of protective therapy. The table also shows the operating characteristics of a one tailed hypothesis test for testing a rate of .25 vs selected lower rates. The test power shown in the last column applies to a one tailed hypothesis test with alpha = .05. With 30 patients this test would reject the null hypothesis that the underlying esophagitis rate is .25 in favor of one of the lower rates shown in column 2 whenever 3 or fewer grade III/IV esophagitis events occur. This test provides acceptable power (> .80) for detecting an esophagitis rate of .078 or lower.

Although MnSOD PL is expected to reduce the rate of grade III/IV esophagitis, a stopping rule has been introduced to protect against higher-than-expected esophagitis. The stopping rule will permit stopping the trial if 6 of the first 12 patients treated at the phase II dose of MnSOD PL experience grade III or IV esophagitis. We note the probabilities of stopping the study pursuant to this rule are .046, .054, and .613 if the true rate of grade III/IV esophagitis is 10, .25 and .50, respectively. Thus the stopping rule provides some protection for patients if the underlying toxicity rate is increased to .50 but is unlikely to occur if the toxicity rate is successfully reduced to .10.

13.5 Proposed Data Analysis

Primary data analysis will compute the point estimate and confidence intervals of the phase II grade III/IV esophagitis rate on an intent-to-treat per patient basis Information regarding all grades of radiation-induced esophageal toxicity on both phases of the study will be tabulated and summarized. Summary information will include grade, time of onset, duration of onset, treatment cycle and requisite dose reductions. Compliance with radiation therapy and chemotherapy dose completion statistics on an intent-to-treat basis will be compiled for each patient.

Data analyses to address secondary objectives include the objective response rate with confidence intervals as well as survival analysis applied to time to disease progression, time to disease progression conditional on clinical response, and overall survival. The definitions of clinical response are based on the Response Evaluation Criteria in Solid Tumors (RECIST). On an exploratory basis time to progression and overall survival will be analyzed by log-rank tests or time-dependent Cox models. Potential predictors include pre-treatment clinical and pathologic factors, total chemotherapy and radiotherapy dosages and clinical response.

14.0 PROTECTION OF HUMAN SUBJECTS/REGULATORY REQUIREMENTS

Protection of Human Subjects

- Investigator will obtain written, legally effective, informed consent of all trial subjects. The informed consent process will be carried out by the physician investigator and the clinical research coordinator.
    - The prospective subject will be provided ample opportunity to consider participation in the trial.
    - There will be no coercion or undue influence.
    - Information provided to the subject will be in language that is understandable.
    - The subject will be given ample opportunity to ask questions during the initial consent

process and throughout participation in the trial.
    - The consent process continues throughout the course of a subject's participation in the trial. Research subjects will be fully informed of any new information that may affect their willingness to continue study participation.
    - In addition to the consent form, the consent process will be documented in the subject's medical record.
    - Informed consent is obtained prior to initiation of any procedures that are performed solely to determine study eligibility.
- Protocol deviations should not occur, but where necessary due to extenuating circumstances will be properly documented and reported.
- Toxicities and adverse events will be properly monitored, documented and reported in accordance with sponsor and IRB guidelines.

Monitoring/Auditing

UPCI assumes a responsibility for quality assurance throughout the duration of the study. The program manager, Experimental Therapeutics, or other designated staff will perform regular quality assurance reviews of the conduct of clinical trials in the program. Reasonable intervals for monitoring are determined at the outset of each study, and are dependent upon the complexity of the study and the rate of enrollment. Study progress, case report form completion and compliance with GCP standards are monitored. Under the direction of the investigator, the program manager is responsible for facilitating the monitoring process, as well as completion of study report forms for the FDA, IRB and other relevant review requirements.

Monitoring for all UPCI studies is performed at the Hillman Cancer Center (UPCI Clinical Research Services) as the central location for the performance of the trial. For this trial, potential subjects from satellite offices will be referred to the central location.

Subject Screening and Enrollment

As specified in the protocol, screening tests to assess whether a prospective study subject is eligible for inclusion in a clinical trial are performed.

- Check for proper and timely consent form execution.
- Review the eligibility in the protocol (or a study-specific eligibility checklist) by verifying each requirement in a source document (patient medical record).
- Check lab work and scans against eligibility requirements (values and timeframes).
- Protocol exceptions should not exist, but if there are ambiguities in determining eligibility or if extenuating circumstances have allowed an exception: Check for proper documentation, including rationale, investigator signature and reporting to IRB.

Source Documents

Source documents include all information in original patient records and certified copies of original records of clinical findings, observations or other activities in a clinical trial. Examples of source documents include physician notes, progress notes, laboratory reports and tumor measurements. A complete set of source documents will be maintained for each trial subject.

Protocol Treatment

- Check all aspects of research subject treatment against protocol-specific requirements (procedures and timelines).
- Verify baseline disease status and responses; verify that all tests to assess response were performed per protocol.
- Check body surface area calculations.
- Verify doses and dates of treatment (protocol, source documents, CRFs).
- Assess and verify dose modifications against the protocol.
- Check radiation dose calculation and administration.
- For adverse events:
    - Verify toxicities and grades (source documents, CRFs).
    - Verify lab values.
    - Verify performance and documentation of tests to assess toxicity (per protocol requirements).
    - Verify documentation and reporting to appropriate entities.

Adverse Event Reporting

Adverse event reporting will occur per Section 11.3 above.

- Adverse events will be reported and documented on Form FDA 3500A (MedWatch form) to the FDA. This includes serious related and serious unrelated adverse events.
- Information for this form will be abstracted from source documents and assessments made by the investigator (i.e., causality).
- Serious adverse drug experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
- Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure or the risk information described in the general investigational plan of the current protocol and consent form.
- The FDA will be notified by facsimile of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days of the knowledge of the information.
- Local requirements will be followed for IRB reporting of adverse experiences.

Compliance with the Protocol

- The trial will be conducted in compliance with the protocol approved by all regulatory authorities, including the FDA and the University of Pittsburgh IRB.
- No changes will be made to the protocol or protocol conduct without prior approval of the FDA and IRB, except where necessary to avoid immediate danger.
- Requests for modifications will be in accordance with requirements established by the FDA and IRB. Such modifications will be requested under the direction of the investigator, with appropriate preparation and tracking by regulatory and administrative staff.
- Any protocol deviations will be documented and reported to the IRB.

Correction of Errors

- All corrections on a CRF or a source document are made in a way that does not obscure the original entry. Incorrect information will be stricken with a single line. The correct data is inserted, dated and initialed by authorized study personnel.

Investigational Product

Under the direction of the investigator, the investigational drug pharmacist is responsible for carrying out all aspects of verification and compliance with regulatory requirements pertaining to the investigational product. The investigational drug pharmacist:

- Assures that storage and conditions are acceptable, and that supplies are sufficient throughout the trial.
- Assures that investigational products are supplied only to those subjects who are eligible to receive it, and at specified dosages.
- Assures that study-specific tracking is performed; NCI drug accountability forms are utilized.
- Assures that subjects are provided with necessary instruction on proper use, handling, storing and returning the investigational product.
- Assures that the receipt, use and return of the investigational product is controlled and well documented.
- Assures that the disposition of unused investigational product complies with applicable regulatory and internal requirements.

Study Reports

As required, annual reports will be provided to the FDA and IRB. At the completion of the study (after all subjects have completed protocol therapy and follow-up time points, and after a final internal audit occurs) a final report will be generated. This will also serve as a termination notice to the IRB.

Conflict of Interest

Dr. Joel Greenberger, a co-investigator on this clinical trial, has a significant financial interest in the development of this clinical trial's study treatment. Please see Appendix IV for further details.

15.0 APPENDICES

Appendix I Karnofsky Performance Scale
Appendix II Carboplatin Dosing Worksheet
Appendix III Study Team – Organizational Chart
Appendix IV Standard conflict of interest management plan for Human Subject Research

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Appendix I: Karnofsky Performance Scale

The Karnofsky Performance Scale Index allows patients to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the worse the survival for most serious illnesses.

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Appendix II: Study Team – Organizational Chart

Protocol Title: Concurrent Chemotherapy (Paclitaxel and Carboplatin) and Thoracic Radiotherapy with Swallowed Manganese Superoxide Dismutase (MnSOD) Plasmid Liposome (PL) Protection in Patients with Locally Advanced Stage III Non-Small Cell Lung Cancer, A Phase I-II Study (UPCI 01-054)

Organizational Chart

Implementation of Trial in Accordance with International Conference on Harmonisation (ICH); Good Clinical Practice (GCP) Guidelines.