ID: UMCC 2015.035

NCT02492867

A Pilot Study of Response-Driven Adaptive Radiation Therapy for Patients With Locally Advanced Non-Small Cell Lung Cancer

A Pilot Study of Response-Driven Adaptive Radiation Therapy for Patients with Locally Advanced Non-Small Cell Lung Cancer

Principal Investigator: Shruti Jolly, M.D.
Department of Radiation Oncology
University of Michigan

Co-Investigators: James A. Hayman, M.D.
Department of Radiation Oncology
University of Michigan

James Balter, Ph.D.
Department of Radiation Oncology
University of Michigan

Gregory Kalemkerian, M.D.
Department of Internal Medicine, Hematology/Oncology
University of Michigan

Theodore S. Lawrence, M.D., Ph.D.
Department of Radiation Oncology
University of Michigan

Martha M. Matuszak, Ph.D.
Department of Radiation Oncology
University of Michigan

Randall K. Ten Haken, Ph.D.
Department of Radiation Oncology
University of Michigan

Muneesh Tewari, M.D., Ph.D.
Department of Internal Medicine, Hematology/Oncology
University of Michigan

Aamer Chughtai, M.D.
Department of Radiology
University of Michigan

Jason Hearn, M.D.
Department of Radiation Oncology
University of Michigan

Caitlin Schonewolf, M.D.
Department of Radiation Oncology
University of Michigan

Primary Statistician: Matthew Schipper, Ph.D.
Department of Radiation Oncology
University of Michigan

Study Drug: None
Version: 05/22/2022

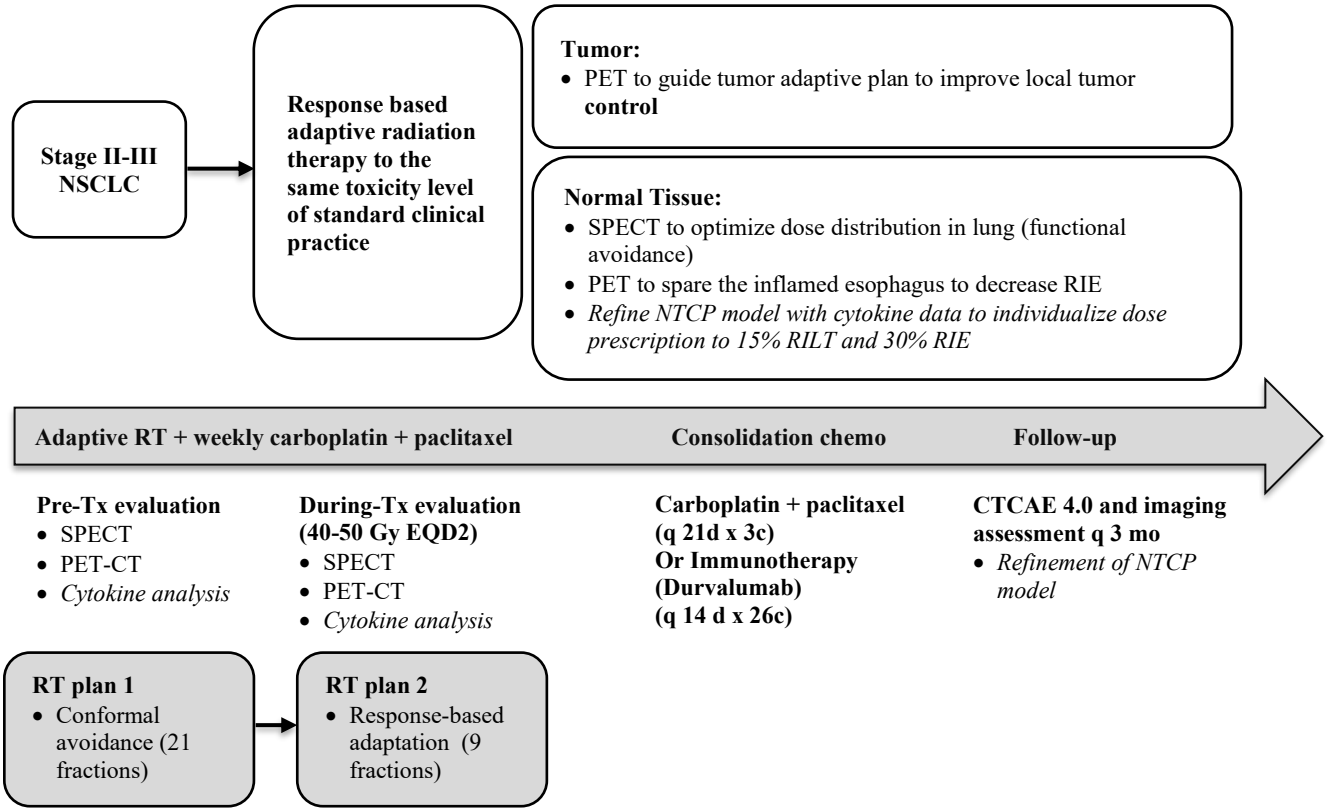
TABLE OF CONTENTS

ABBREVIATIONS

ABC	Active Breathing Control
AE	Adverse Event
ALK	Anaplastic Lymphoma Kinase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CBC	Complete Blood Count
COPD	Chronic obstructive pulmonary disease
CR	Complete response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTO	Clinical Trials Office
CTV	Clinical Target Volume
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
DSMR	Data and Safety Monitoring Report
EQD2	Biologically isoeffective dose at 2 Gy fractions
FDG	18-Fluorodeoxyglucose
FISH	Fluorescence in situ Hybridization
FUNBIPM	Lung Functional Imaging and a Functional BioPhysical Model
GTV	Gross Tumor Volume
H&P	History & Physical Exam
IGRT	Image Guided Radiation Therapy
IMRT	Intensity Modulated Radiation Therapy
IRB	Institutional Review Board
IV (or iv)	Intravenously
LPFS	Local Progression-free Survival
LRPFS	Local Regional Progression-free Survival
MTV	Metabolic tumor volume
NSCLC	Non-small Cell Lung Cancer

NTCP	Normal Tissue Complication Probability
OS	Overall Survival
p.o.	per os/by mouth/orally
PART	Personalized Adaptive Radiotherapy
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression-free Survival
PI	Principal Investigator
PR	Partial Response
PTV	Planning Target Volume
RIE	Radiation induced esophagitis
RILT	Radiation Induced Lung Toxicity
RITT	Radiation Induced Thoracic Toxicity
RT	Radiotherapy
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPECT	Single-photon Emission Computed Tomography
SPGT	Serum Glutamic Pyruvic Transaminase
TKI	Tyrosine-kinase Inhibitor
3D-CRT	Three dimensional conformal radiation therapy
TS	Thymidylate Synthase
UMCCC	University of Michigan Comprehensive Cancer Center
WBC	White Blood Cell

STUDY SCHEMA



STUDY SYNOPSIS

Title	Response-driven Adaptive Treatment in Patients with Non-Small Cell Lung Cancer
Phase	Pilot
Methodology	A pilot study
Study Duration	7 years
Study Center	University of Michigan, Ann Arbor Veterans Affairs Health System
Objectives	To improve local tumor control while maintaining the same rate of treatment toxicity by adapting therapy to the uninvolved lung and esophagus while continuing to adapt therapy to the tumor for patients with Stage II/III NSCLC cancer.
Number of Subjects	55 evaluable patients (up to 70 patients may need to be enrolled in order to obtain 55 evaluable patients)
Inclusion Criteria	NSCLC, unresectable/inoperable stage II-III
Exclusion Criteria	Any component of small cell lung carcinoma, prior radiotherapy to the thorax or not candidate for systemic chemotherapy
Study Regimen	FDG-PET-CT and SPECT guided radiation at the start of treatment. After administration of approximately 60% of the treatment, dose will be intensified to the residual FDG avid tumor identified on the during-RT scans, and V/Q SPECT-CT scanning during-treatment will be used to avoid functionally recovered lung, and FDG-PET to minimize radiation sensitive esophagus.
Duration of Administration	6 weeks of radiation with concurrent chemotherapy
Reference Therapy	Patient enrolled in UMCC 2006.040 standard radiotherapy (60Gy in 30 fractions in 6 weeks)+concurrent chemotherapy
Statistical Methodology	This is a pilot study with a primary aim to obtain preliminary estimates of toxicity and doses achievable with the proposed adaptive treatment strategy. The proportion of patients experiencing G2+ lung toxicity or G2+ esophagitis will be reported with 90% confidence intervals. The doses given to the tumor will be compared to what would have been given to these patients if they had been treated on UMCC 2007-123. In addition, local control will be summarized with Kaplan-Meier methods. An additional secondary aim was regarding imaging uncertainty.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background: Overview of Lung Cancer

Lung cancer is the leading cause of cancer death in the United States and worldwide. In 2012, there were 226,160 new cases and 160,340 deaths related to lung cancer in the United States (1). Approximately, 80-85% of lung cancers are NSCLC, and 40% of these are locally advanced (stage II/III) at diagnosis (2). The current standard of care for these patients is “one size fits all” RT with concurrent chemotherapy and recently data shows benefit of adjuvant immunotherapy when appropriate in uniform regimens (94). Even after concurrent chemoradiation, however, the five year overall survival was still about 15%; almost one half of the patients failed locally (3). The addition of consolidative immunotherapy extends overall survival from 14 to 23 months, suggesting further intensification of therapy is necessary. At the same time, intensification of both radiotherapy and concurrent chemotherapy may result in excessive toxicity or incomplete treatment. Therefore, it is critical to tailor the treatment to each individual’s sensitivity in combination with functional imaging guided response-driven treatment and biomarker guided individualized dose prescription, thus taking into consideration both the tumor and toxicity profile.

1.2 PET Guided Adaptive Radiotherapy to Improve Long-term Tumor Control

1.2.1 Effective Local Radiation Improves Long-term Tumor Control

Local tumor failure remains a major problem after radiation-based non-surgical treatment. Approximately one half of patients with locally advanced NSCLC failed locally (4). The earliest positive dose-response relationship was demonstrated by RTOG 73-01, in which 376 patients were randomized to 40 Gy split-course radiations, a continuous-fractionation 40 Gy, 50 Gy or 60 Gy. The intrathoracic failure rates at 3 years were 44%, 52%, 42% and 33% respectively (5, 6). Although that study did not show improved overall survival with higher dose therapy, secondary analysis from RTOG trials led to the conclusion that local tumor control is significantly correlated with improved survival (6). In an extensive literature review, Vijayakumar, et al. estimated that a dose of 80 Gy was required for 90% local control in lung cancer (7). Using 3D CT-based conformal techniques, several prospective studies have escalated radiation doses much higher than 60–70 Gy (8-20). Higher doses appear to be associated with better local tumor control and survival in medically inoperable or unresectable NSCLC (11, 16, 21). For patients with stage I-III disease, multivariate analysis of our UMCC 9402 demonstrated the radiation dose to be the only significant factor for local tumor control and overall survival in the dose range of 63-103 Gy (16). An increase of 1 Gy was associated with a > 1% improvement in the 5-year tumor control, and a 3% decrease in risk of death. An RTOG secondary analysis of 11 RTOG trials (9/11 treated with concurrent chemoradiation) in 1,356 patients receiving standard 60-69.6 Gy therapy reported that a 1 Gy increase in biologically equivalent dose (BED) was associated with a 4% risk reduction in local failure and a 3% risk reduction of death in patients with stage III NSCLC treated with combined chemoradiation (22) (23). This study took treatment duration into consideration, reporting 2-year and 5-year survival rates of 38% and 15%, with 2-year and 5-year local-regional failure rates of 46% and 52%, respectively. For higher dose ranges, we have demonstrated that radiation dose is a significant factor in patients with larger tumors of early stage NSCLC (24). In patients receiving >70 Gy with concurrent chemoradiation, we recently reported positive dose-survival relationship in the range of 60-100 Gy with concurrent and adjuvant carboplatin and paclitaxel (25). The median rate of local-regional and progression-free survival (95%CI) was 10.7 (Range: 8.4-13.0) months and non-reached (14.1 to date), (p=0.001) for physical doses <70 and >70 Gy, respectively. The median survival was 15.5 (Range: 6.5-24.4) and 41.9 (Range: 18.3-65.5) months (p=0.003), for physical doses < and >=70 Gy, respectively. One Gy of dose escalation was associated with a 3% reduction in the risk of death. All this evidence suggests that **high-dose radiation has the potential to improve local-regional control and overall survival in patients treated with fractionated therapy with concurrent chemotherapy.**

However, it is challenging to deliver high dose RT in the majority of patients with locally advanced NSCLC without exceeding doses to OARS and causing significant side effects. After our clinical trials described above for patients treated with radiation alone or sequential chemoradiation, we designed a study for Stage III patients (UMCC2003073) treated with concurrent chemoradiation followed by adjuvant chemotherapy. We found that, using our standard complication limits, 60% of our patients could not be safely treated beyond 66 Gy. More importantly, RTOG 617, a phase III randomized trial was conducted to compare the survival outcome between 60 and 74 Gy arms without consideration of treatment tolerance in each patient. The first interim results reported significantly worse survival at 1 year in the high-dose arm. **Thus, our pilot study and RTOG 0617 suggest that high dose uniform dose prescription to the entire tumor is limited by the radiation toxicities (4, 8).** Therefore, new strategies are required*.*

We hypothesized that we could develop safer and more effective therapy by adapting treatment to the individual patient's response. With respect to the tumor, we hypothesized, that we could improve outcome by redistributing dose to the more aggressive regions of the tumor, assessed using mid-treatment FDG-PET scanning. With respect to uninvolved organs, we need methods of estimating tolerable radiation doses for the individual patient rather than the population average. Such a strategy requires assessing both global and regional normal lung function and the technology to deliver dose in a manner that minimizes damage to functional lung and esophagus. The background supporting this approach is laid out in the following sections.

1.2.2 FDG-PET to Adapt Radiotherapy

FDG-PET, a Medicare approved tumor functional PET, is widely available in daily oncology practice for diagnosis, staging, radiation treatment planning, and monitoring treatment response. The literature on FDG-PET/CT has been focused on scans performed at approximately 3 months after completion of RT. Limited studies have demonstrated that an early (1-2 months) post-treatment FDG-PET/CT scan is a prognostic factor for survival and is more predictive than CT response, stage, or pretreatment performance status (26). Furthermore, evaluation of post-treatment images is significantly complicated by the presence of variably hypermetabolic inflammatory post treatment changes. Images done during the course of chemoradiation have shown markedly less inflammatory changes, suggesting that during-treatment FDG-PET/CT may allow a less confounded evaluation of response to therapy (27). We and others have demonstrated that *FDG-PET can be performed earlier during the course of therapy to predict post-treatment outcome*. The change in FDG uptake early during the course of chemotherapy was found to be predictive of progression-free and overall survival (28-32). Researchers from the Netherlands reported a large intra-individual heterogeneity in the evolution of FDG uptake during the early course of RT (33). They reported a non-significant increase in the first week ($p=0.05$) and a small but statistically significant decrease in the second week ($p=0.02$) during RT. We have demonstrated a greater and more significant reduction of peak FDG activity at 40-50 Gy (4-5 weeks during the course of fractionated RT) (27). The regions of peak tumor FDG activity during-RT correlated with those seen 3 months post-RT ($R2 = 0.7$; $p < .001$). Investigatorsfrom Stanford University (RSNA 2008) and Princess Margaret Hospital (ASTRO 2008) also studied the role of FDG-PET/CT during RT and reported a heterogeneous reduction of FDG uptake at about 4 weeks during RT. The Stanford group also reported a correlation of FDG uptake during RT with progression-free survival. Indeed, the role of PET/CT in therapeutic monitoring and prediction of outcome is expanding rapidly because of its ability to provide earlier and more robust identification of non or poor responders than is provided by conventional CT. Therefore, ***during-RT FDG-PET/CT potentially can provide important benefits to individual patients by intensifing dose to more resistent tumor, allowing early changes to alternative, more efficacious treatment or by avoiding the unnecessary toxicity related to ineffective therapy.***

We have conducted a series of prospective studies to examine this issue at the University of Michigan. The key findings include the following: 1) FDG uptake and tumor volume were significantly reduced after 40-50 Gy of fractionated RT (27); 2) Adapting the

planned target volume to this decreased tumor size with a fixed composite NTCP of 15% allows escalation of the total dose by 30-102 Gy (mean: 58 Gy) or a reduction in NTCP if the dose remained unchanged (34); 3) PET MTV can be defined reproducibly (35); 4) Reduction in the MTV was greater than the reduction of the CT-GTV during RT (35); 5) Using the MTV during RT, tumor dose can be escalated above 74 Gy while keeping lung NTCP unchanged in a majority of patients with stage III NSCLC (36). Based on these findings, we have conducted a prospective clinical trial of PART using the during-RT FDG-PET in 42 patients. Preliminary results demonstrated that during-RT PET adaptive RT produced **significantly better local control and overall survival at 1 and 2 years than standard RT** in patients with stage III NSCLC treated with concurrent and adjuvant carboplatin and paclitaxel. **This methodody has been adopted in RTOG 1106, which is currently recruiting patients. The proposed study will use this promising methodology to intensify dose to the more resistent active tumor to improve local control.**

1.3 V/Q SPECT, PET, and Biomarkers to Decrease Lung and Esophageal Toxicity

1.3.1 V/Q SPECT to Guide Adaptive Radiotherapy to Decrease Lung Toxicity

V/Q SPECT allows simultaneous imaging of regional blood flow and ventilation for the whole lung. Lung blood flow is typically imaged using macroaggregates of albumin. Both radioactive gases and particulate aerosols labeled with radioactivity have been used for imaging of regional ventilation. V/Q SPECT has a potential to guide RT planning to minimize radiation dose to functional lung. Largely due to limited technology, most earlier studies focused on Q-SPECT at baseline to guide radiation planning for functional region avoidance (37-44). However, ventilation is another important component of lung function. V/Q SPECT provides more information than Q-SPECT alone in the assessment of local pulmonary function at baseline for patients with NSCLC and tumor shrinkage during the course of RT (27) (45). V/Q SPECT can be used to assess the spatial distribution of lung blood flow and ventilation at baseline and during the course of RT. By co-registering with conventional CT images, SPECT images can provide functional information which can be used to refine RT planning. A number of dosimetric planning studies suggested a potential to reduce pulmonary toxicity by use of SPECT with IMRT for functional lung avoidance (38-40, 43). IMRT may produce better PTV90/fV20 ratio and reduce fMLD values compared to 3-DCRT in patients with stage III disease due to a reduction in fV20 for fixed PTV coverage. By comparing IMRT treatment plans generated with and without SPECT guidance for 5 patients, investigators from Duke University found that the Q-SPECT-guided plan produced a more favorable DFH compared with the non-SPECT guided plan. Additionally, the fV20 and fV30 values were reduced for all patients by an average of 13.6% ± 5.2% and 10.5% ± 5.8%, respectively (40).

Modern radiotherapy technology may allow for improved target volume coverage and better selective avoidance of normal tissues. IMRT for NSCLC may lead to a 6–15% absolute decrease of V20 compared to 3-DCRT. The current standard for evaluating the normal tissue sparing effect of modern RT techniques considers the whole lung as a uniform organ. However, patients with NSCLC have frequent respiratory comorbidity particularly in the form of COPD resulting in heterogeneous function within different lung regions, and the presence of the tumor itself may affect local vascular supply and ventilation. Indeed, the majority of patients with lung cancer have some functional defect at baseline. From imaging analysis of over 50 consecutive patients, the V/Q defects were matched, reverse mismatched (V- defect greater than Q-defect), and mismatched (Q-defect greater than V-defect) in 61%, 31% and 8% patients, respectively. The tumor is the leading cause of the defects in ipsilateral lung in 73% patients. The defect scores of ipsilateral lung were greater in patients with central primary tumors than those with peripheral primaries for both V- (2.3±1.1 vs. 1.5±0.8, p=0.017) and Q-SPECT (2.2±0.8 vs. 1.4±0.6, p=0.000). The patients with COPD had greater defect scores in contralateral lung for both V- (1.5±0.7 vs. 1.0±0.8, p=0.006) and Q- SPECT (1.4±0.6 vs. 1.0±0.4, p=0.010). On assessing the potential value of SPECT on RT planning, 39% of patients could have their RT application type changed from SPECT of Q-scan alone to V/Q-scan. Combining using co-registered V/Q SPECT and CT images, we can also classify the lung functional

defects to: Type A defect as a defect from tumor occupation; Type B1 defect as a complete function defect due to COPD or other unrecoverable diseases; Type B2 as reduced lung function due to COPD or other unrecoverable diseases; Type B3 as temporarily dysfunctional lung due to tumor and other potentially reversible conditions; Type C as normal functional lung. ***Specific avoidance of functioning lung (i.e. type C, B3 and B2) defined by a V/Q SPECT scan may decrease dose to functioning regions and thus decrease functional damage of the lung.***

During the course of radiation therapy, lung function changes globally and locally. Our previous study demonstrated a significant improvement in breathing assessed by dyspnea grade during-RT, and remarkable improvements in regional V/Q function by the time a mean dose 45 Gy was delivered. Regional V/Q improvements were mostly in the ipsilateral lung in patients with central tumors, which may be due to tumor volume reduction. Additionally, regional V or Q functional mapping changed most remarkably in B3 regions. An adaptive plan based on V/Q SPECT during-RT may better spare the functioning lung. Significant V/Q SPECT changes during-RT suggest the value of obtaining a V/Q SPECT to re-optimize the treatment plan, especially in patients with central tumors. V/Q functional mapping based on our recently proposed classification on pre-RT V/Q SPECT may guide the potential applications of SPECT on RT planning based on the regional function level, etiology and potential for recovery (45). SPECT may provide an opportunity for RT plan optimization as follows: Type A tumor occupying lung treated to the maximum safe dose; Type B1 regions, with unrecoverable non-functioning “bad” lung, can be given high dose RT without causing a change in the global lung function; B2 regions, with unrecoverable low functioning lung, may be given a high dose without causing remarkable change in the global lung function; Type B3 should be spared whenever possible and may be given high dose RT if it remains nonfunctioning on the during-RT SPECT; The RT dose to type C regions should be minimized to decrease functionally or clinically significant complications. By further studying the functional mapping on the V/Q scan during-RT, the current study to some degree validated our previous functional classification. Type B3 regions, potentially recoverable, were observed in 71.4% (43/56) of patients on pre-RT SPECT. The V/ Q function recovered in 51.2% (22/43) of these regions, accounting for 39.3% (22/56) patients. This group of patients may benefit from V/Q SPECT acquired during-RT for RT plan re-optimization for sparing of functional lung. ***An adaptive plan based on during-treatment SPECT may further optimize PART to avoid high dose radiation to the well-functioning regions, and would thus decrease RILT.***

In summary, we proposed the combination of pre- and during V/Q-SPECT to improve the sparing of healthy lung. V/Q SPECT adds lung ventilation mapping on top of the Q-SPECT, providing more information (including the mechanism for lung function defects and their potential for recovery). During-RT V/Q SPECT allows adaptive-RT because lung function changes globally and locally during-RT, largely due to RT induced tumor volume reduction improving the vascular supply and ventilation(46). **The combination of pre- and during V/Q SPECT can classify the lung into different functional regions, and a strategy to give differential priority to the regions has been developed to minimize lung damage**.

1.3.2 PET-CT to Guide Adaptive Radiotherapy to Decrease Esophageal Toxicity

In addition to RILT, esophagitis is a common side effect of thoracic radiation therapy and a source of considerable morbidity (47). Patients often complain of dysphagia and/or odynophagia in the late course of fractioned radiotherapy that can cause significant complications from dehydration and weight loss leading to interruption of radiation therapy and in rare instances, can result in esophageal perforation or obstruction and death (48, 49). Clinical and dosimetric studies have shown that the dose and volume of esophagus irradiated, as well as the concurrent use of chemotherapy correlate with the severity of esophagitis (50-52), but the predictive value of these correlations is only modest (51-54). Our preliminary results (see below) show that the increase in FDG uptake in esophagus at 40-45 Gy identifies the sensitive esophagus, improves the ability to estimate esophagitis over the predictions made by using maximum esophageal radiation dose alone. **Thus, we propose to adapt treatment in patients with greater esophageal FDG avidity**

midtreatment by decreasing the esophageal dose to keep esophagitis levels at no greater level than that produced by 60 Gy of standard therapy.

1.3.3 Serum Biomarkers to Predict Lung Toxicity and Potentially Guide Adaptive Therapy

While it seems essential to deliver an adequate dose of radiation with chemotherapy to shrink the tumor for disease control, it is important to note that patients often respond to treatment differently in terms of both tumor control and treatment-related toxicity. Strictly limiting lung NTCP to 15% does not predict which 15 of 100 patients will develop complications. Given that DVH parameters are associated with RILT in populations of patients but lack optimal predictive ability for individuals, we hypothesized that the addition of biologic parameters to the model will improve the prediction of post-treatment RILT for individual patients.

Recent insights into the pathogenesis of radiation-induced normal tissue injury have revealed the involvement of a number of pro-inflammatory and pro-fibrogenic cytokines, including TGF-ß1, IL-1ß, IL-6, IL-8, and TNF-α (55). TGF-ß1, a fibrogenic and radiation-inducible cytokine, has been known to play a key role in this process. Data from animal models demonstrate significantly elevated TGF-ß1 mRNA and protein expression within type II pneumocytes and fibroblasts in radiation-sensitive mice after thorax radiation (56-59), which subsequently contributed to an increased TGF-ß1 level in the circulation. TGF-ß1 modulation normally precedes histologically discernible fibrosis. In rats, the expression of TGF-ß1 mRNA and protein in lung peaks within 3 to 6 weeks and coincided with an initial influx of inflammatory cells in bronchoalveolar lavage, while pulmonary fibrosis was not apparent until 8-10 weeks after thoracic radiation (59). In humans, the Duke group reported that plasma TGF-ß1 levels at the end of radiation are correlated with the later onset of symptomatic lung toxicity in patients treated with definitive radiation therapy (57, 60, 61). Unfortunately, end-RT TGF-ß1 correlations have not been consistently reproduced by others (62). We have demonstrated that TGF-ß1 elevation in the middle of treatment (2-4 weeks during-treatment) relative to pre-treatment is highly correlated with late-onset grade $\geq$2 RILT in NSCLC patients (63). This correlation is more important than end-treatment markers, as it provides us an opportunity to adjust treatment accordingly. This finding was recently validated by an independent prospective study (64). IL-8 overexpression was associated with impaired migration of neutrophils which may inhibit the inflammation effect. Hart et al from Duke reported that a lower pretreatment plasma level of IL-8 was an independent risk factor for the development of RILT (65). We have also recently demonstrated that a high level of baseline IL-8 is associated with decreased risk of clinically significant RILT. However, the sensitivity to change and predictive power from any single cytokine alone are limited. We have recently reported that combining TGF-ß1 and IL8 with MLD can provide more accurate prediction of RILT than MLD alone (65)**. Thus, we plan to continue to collect data on serum biomarkers to further refine our biophysical model with the ultimate goal of individualizing radiation dose prescription to isotoxicity of lung estimated by not only MLD, but also a cytokine signature.**

1.3.3.1 Circulating Extracellular RNA, DNA, and Urinary RNA/DNA as Additional Biomarker Approaches for Prediction of Lung Toxicity and Potentially Guiding Adaptive Therapy

Clinically useful blood-based biomarkers based upon circulating cell-free nucleic acids are also under development. For example, circulating tumor DNA is emerging as an exciting new area and has been shown as a promising noninvasive biomarker for early detection, prognosis, and treatment follow-up for various cancers. Likewise, circulating cell-free RNA, including microRNA, is being actively developed as a biomarker approach for multiple oncology applications. New technologies are being developed to increase the sensitivity and specificity of cfNA detection in the blood. Hence, we plan to analyze cell-free nucleic acids in plasma and serum specimens that will be collected to identify biomarkers for prediction or early detection of toxicity, which could be used to adapt therapy.

Recent studies have demonstrated that nucleic acid and other analytes in urine may serve as markers for systemic (i.e. non-urinary tract) disease status. This includes microRNA, cell-free (cf) trans-renal DNA fragments, potentially other diverse species of RNA, and other biomarker types. Hence, we also plan to study urine cfDNA and extracellular RNA as a source of potential biomarkers.

1.3.3.2 Circulating and Infiltrating Immune Cell Infiltrates as Additional Biomarker Approaches for Prediction of Lung Toxicity and Potentially Guiding Adaptive Therapy

The number and function of circulating hematopoietic populations can provide insight into the functional state of the immune system. The ratio of T cell reinvigoration to tumor burden has been hypothesized to serve as a biomarker for response to immune checkpoint blockade in metastatic melanoma patients (94). Tumor infiltration of T cells has been hypothesized to alter radiotherapy toxicity and efficacy (95). Peripheral blood functional immunophenotyping will be performed by flow cytometry. Multiparametric flow cytometry analysis will include enumeration and functional evaluation of antigen presenting cell subsets, innate lymphoid cell subsets, myeloid derived suppressor cells, and T cell subsets (including NKT cells, CD8+ effector cells, and CD4+ helper cells). Cytokine secretion, proliferation, immunoinhibitory ligand expression, and transcription factor profile will be quantified by flow cytometry in T cell subsets in hopes of developing new clinically useful biomarkers..

Certain features of anti-tumoral immunity may be best assessed through examination of tumor tissue. It has been suggested that the Immunoscore (multiparametric immunohistochemistry and automated imaging for of CD3+ T cells density, CD8+ cytotoxic T cells density, and CD45RO+ memory T cells presence) in tumor tissue may have improved prognostic potential as compared to TMN systems in colorectal cancer (96). Further, preliminary studies have begun examining the hypothesis that the pre-treatment immune infiltrate or mid-treatment alteration in immune infiltrate induced by chemoradiation impact the tumor microenvironment and PDL1 expression (97). We plan to perform quantitative multiparametric immune immunohistochemical tumor evaluation on patients at UM with diagnostic core needle biopsies in hopes of developing clinically useful biomarkers to predict treatment or toxicity (98).

1.3.4 Summary of Normal Tissue Adapation Strategy

In summary, patients receiving the same doses of RT often have very different levels of toxicity or toxicity patterns, largely due to their biologically different intrinsic sensitivity to radiation damage (66). We propose to use functional imaging (V/Q SPECT) and dosimetric parameters (mean lung dose) to guide RT planning at baseline for RILT estimation. We will continue to collect data on serum biomarkers to refine our biophysical model such that we can ultimately adapt treatment in patients with a cytokine signature predictive of RILT by decreasing lung dose to keep RILT levels at no greater level than that produced by 60 Gy of standard therapy. Additionally, we will use during RT PET scan to identify sensitive esophagus (for sparing) for radiation plan optimization. **By identifying high risk patients and adjusting OAR dose limits to the threshold of tolerance, we anticipate a significant reduction in the incidence of toxicity from UMCC2007123 without compromised tumor control by applying the model to optimize radiation planning.**

1.4 Combination of Chemotherapy and/or Immunotherapy in NSCLC

The standard of care for patients with stage III unresectable NSCLC is combined chemoradiotherapy. The results with RT alone for stage III tumors that are deemed unresectable or marginally resectable are poor, with five-year survival rates of 5%–7% (6, 67, 68). Radiation therapy alone is only used to treat patients who cannot tolerate chemotherapy. The addition of neoadjuvant chemotherapy resulted in a 2–4 month extension in median survival and 8%–20% improvement in 2–3-year overall survival (69,

70) in at least three randomized trials: CALGB 9433 (67), RTOG8808/ECOG4588 (68), and a French study (71). The French study also reported a significant reduction in distant recurrence rate.

1.4.1 Sequential vs. Concurrent Chemoradiotherapy Several prospective randomized trials examining the treatment of patients with stage III unresectable NSCLC have demonstrated superior results with concurrent chemoradiotherapy compared to sequential chemoradiotherapy, with a 2–3 month extension in median survival and 7%–10% improvement in 3–5-year survival, albeit with increased toxicity (72-74). In patients with locally advanced NSCLC who are medically fit, **concurrent chemoradiotherapy is considered as a standard therapy**.

1.4.2 Adjuvant or Induction Chemotherapy and Immunotherapy with Concurrent Chemoradiotherapy Although there is general agreement on the principle of using combined modality therapy with a concurrent regimen for stage III unresectable NSCLC, there is controversy over the optimal approach and sequence in this population. A number of phase II and III trials have evaluated the use of either induction or consolidation chemotherapy (75-80). Researchers from the University of North Carolina reported a median survival of 24 months in patients treated with induction paclitaxel/carboplatin (CP) followed by concurrent chemoradiotherapy. However, CALGB 39801, a phase III study comparing concurrent chemoradiotherapy alone to induction CP followed by concurrent chemoradiotherapy failed to show a significant survival difference between the two arms, with a median survival of 14.0 months in the induction arm versus 11.4 months in the concurrent alone arm (P = .154) (81). A median survival of 26 months, the best survival result reported thus far for unresectable stage III NSCLC, was reported from a phase II trial (SWOG 9504) using docetaxel as consolidation chemotherapy (79). The median overall survival from the locally advanced multiple modality protocol (LAMP) was 13.0, 12.7, and 16.3 months with sequential chemoradiotherapy, concurrent chemoradiotherapy after induction CP, and concurrent chemoradiotherapy followed by consolidation CP, respectively (78). Although the LAMP was not powered to definitively address differences between arms, it suggested that concurrent chemoradiotherapy followed by consolidation chemotherapy resulted in the best median survival. Large phase III trials are still warranted to determine the optimal combination of chemotherapy and radiotherapy and a recent HOG study failed to show a benefit of adjuvant docetaxol (82). The PACIFIC trial recently showed that adjuvant administation of durvalumab (anti-PDL1 antibody) in locally advanced NSCLC cancer patients who had not progressed during chemoradiation resulted in a 28% response rate, a improvement from 5.6 to 16.8 months in progression free survival, and an improvement in median overall survival from 14.6 months to 23.2 months (94). Thus the current practice in patients with good performance status is concurrent chemotherapy followed by consolidation immunotherapy and or chemotherapy in the United States (80). Therefore, this trial will use concurrent chemoradiation followed by adjuavant immunotherapy and or chemotherapy.

1.5 Rationale

The standard therapy for patients with unresectable NSCLC is concurrent chemoradiation [(83)] with a fixed “uniform” of radiation in the dose range of 60-66 Gy. It was estimated that a dose of 100-180 Gy BED was associated with 90% of long-term tumor control (84). However, adequate target coverage also requires increased treatment of normal structures and thus increases radiation toxicities. During-RT FDG-PET can potentially be used for prediction of treatment outcome, evaluation of response during treatment, and adaptation or alteration of the remaining treatment (27). Our preliminary results from the UMCC2007-123 trial, which has reached its planned enrollment of 42 patients, demonstrated that adapting the planning target volume to the decreased tumor volume with a fixed composite NTCP of 17.2% allows for a substantial escalation of the total BED to the active tumor and with significantly improved overall survival (P=0.03) and local tumor control at 2 years (P<0.001) relative to standard RT in patients with stage III NSCLC treated with concurrent

and adjuvant carboplatin and paclitaxel. However the toxicity of radiation administered by UMCC2007-123 appears to be greater than that produced by standard therapy. RILT, a key dose-limiting toxicity of treatment (55, 85-87), was about 5% higher than standard dose RT. RIE was about 10% higher than that of standard dose RT. To decrease lung damage, V/Q-SPECT images will be used to guide RT planning so that radiation is directed to the non-functional lung regions (38-40, 43) which may produce more favorable dose functional volume histograms compared to non-SPECT guided plans (40). Our preliminary results strongly suggest that avoiding V/Q SPECT-CT functional regions in pre- and during- RT can minimize dose to functional lung. For radiation esophagitis which is a source of considerable morbidity (47), we have demonstrated that the increase in FDG uptake in esophagus at 40-45 Gy identifies the sensitive esophagus, improves the ability to estimate esophagitis over the predictions made by using maximum esophageal radiation dose alone. We will thus adapt the treatment to avoid radiating the sensitive esophagus. Finally, we will use baseline IL-8 and TGF-ß1 in the middle of treatment (2-4 weeks during-treatment) to adjust treatment, as only these two cytokines have been independently validated by 2 centers for their predictive values. In summary, in this pilot study, we minimize treatment of functioning lung from the beginning of treatment using V/Q SPECT-CT scanning. After administration of approximately 60% of the treatment, we will not only intensify dose to the residual FDG avid tumor but also use V/Q SPECT-CT scanning during-treatment to avoid functionally recovered lung, and FDG-PET to minimize radiation sensitive esophagus. In addition, patients who are unusually sensitive to radiation will be identified at baseline (by measuing IL-8) and during treatment (by measuring TGF-ß1) so that the remainder of their treatment course can be modified and toxicity kept to acceptable levels. **We hypothesize that by adapting radiation therapy to the uninvolved lung and senstive esophagus, we can maintain the dose to the FDG-avid tumor that we achieved in UMCC2007-123 while decreasing grade 2 and above RILT and RIE from the UMCC 2007-123 so that causing no greater overall toxicity than expected from standard therapy (60 Gy uniformly delivered to the tumor).**

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 Establish the feasibility of the proposed adaptive treatment strategy

2.1.2 To obtain preliminary estimates of the toxicity of and doses achievable by the proposed response-driven adaptive treatment strategy in patients with Stage II/III NSCLC.

2.2 Secondary Objectives

2.2.1 To obtain preliminary estimates of local regional tumor control from an adaptive approach, which will aid in the design of a subsequent randomized phase II trial.

2.2.2 To determine the residual uncertainties in V/Q SPECT scans related to the accuracy with which persistent tumor subvolumes and the spatial distribution of local function of uninvolved lung could be mapped to guide plan modification.

2.2.3. To determine the time to local regional progression and death in patients treated with this response-driven adaptive regimen.

2.2.4. To refine our biophysical NTCP model to individualize and adapt radiation dose prescription to minimize lung injury.

2.3 Endpoints

2.4.1 Primary Endpoints

2.4.1.1 Grade 2 and above lung toxicities, as defined via NCI's CTCAE version 4.0 in appendix);

24.1.2. Grade 2 and above esophageal toxicities, as defined via NCI's CTCAE version 4.0 in appendix);

2.4.1.3 Comparison of delivered dose to dose that would have been administered using the criteria described in UMCC2007-123.

2.4.2 Secondary Endpoints

2.4.2.1 Time to local progression, which will be defined as the time from start of treatment to time of local regional progression on PET.

2.4.2.2 Overall survival time, which will be defined as the time from start of treatment to death. Patients alive at their last follow-up will be censored at the date of last follow-up.

2.4.2.3 Other tumor control measures: tumor response (based RECIST criteria).

3.0 PATIENT ELIGIBILITY

Subjects must meet all of the inclusion and exclusion criteria to be enrolled to the study. Study treatment may not begin until a subject is enrolled.

3.1 Inclusion Criteria

3.1.1 Patients must have FDG-avid and pathologically proven Stage IIA-IIIB non-small cell lung cancer (NSCLC) (according to AJCC staging, 8th edition).

3.1.2 Patients must be considered unresectable or inoperable

3.1.3 Patients must be 18 years of age or older.

3.1.4 Patients must have Karnofsky performance score ≥ 70.

3.1.5 Patients must have adequate organ and marrow function as defined below:

WBC	≥3,000/mm3
absolute neutrophil count	≥1,500/mm3
platelets	≥100,000/mm3
total bilirubin	≤3.0 mg/dl
AST (SGOT) and ALT (SGPT)	≤4 X institutional upper limit of normal
Creatinine	≤2.0 mg/dl

3.1.6 Patient must be willing to use effective contraception if female with reproductive capability for 3 months post last dose of study treatment.

3.1.7 Patients must be informed of the investigational nature of this study and given written informed consent in accordance with institutional and federal guidelines

3.2 Exclusion Criteria

3.2.1 Patients with any component of small cell lung carcinoma;

3.2.2 Patients with evidence of a malignant pleural or pericardial effusion;

3.2.3 Prior radiotherapy to the thorax such that composite radiation would significantly overdose critical structures, either per estimation of the treating radiation oncologist or defined by failure to meet normal tissue tolerance constraints;

3.2.4 Patients cannot tolerate concurrent chemotherapy

3.2.5 Pregnant women are excluded from this study because radiation has the potential for teratogenic or abortifacient effects;

3.2.6 Prisoners are excluded for this study.

4.0 SUBJECT SCREENING AND REGISTRATION PROCEDURES

Patient registration for this trial will be centrally managed by the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log.

It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the Clinical Trials Office. After patient eligibility has been determined, a copy of the **completed** Eligibility Worksheet together with all the pertinent de-identified source documents will be submitted by the requesting site to the Clinical Trials Office, either by fax or by email to CTSU-Oncology-Multisite@med.umich.edu. **Note:** Expired studies need to be repeated if out of specified time frame.

A Multi-Site Coordinator of the Clinical Trials Office, who acts as the registrar, will review the submitted documents and process the registration. Sites should inform the Multi-Site Coordinator of a potential registration by 5 p.m. on the day prior to registration. Same day registrations cannot be guaranteed.

An email will be sent by the registrar to the requesting site registrar to confirm patient registration and to provide the study identification number that has been assigned to the patient. In addition, a copy of the completed Eligibility Worksheet signed and dated by the registrar, will be sent back to the requesting site registrar.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not have study identification number assigned to them, and will not receive study treatment.

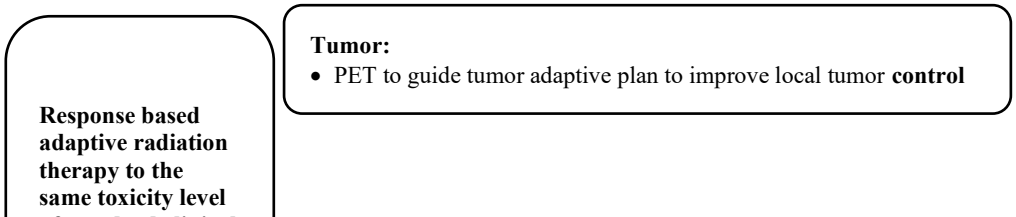
The completed, signed, and dated Eligibility Checklist will be retained in the patient's study file.

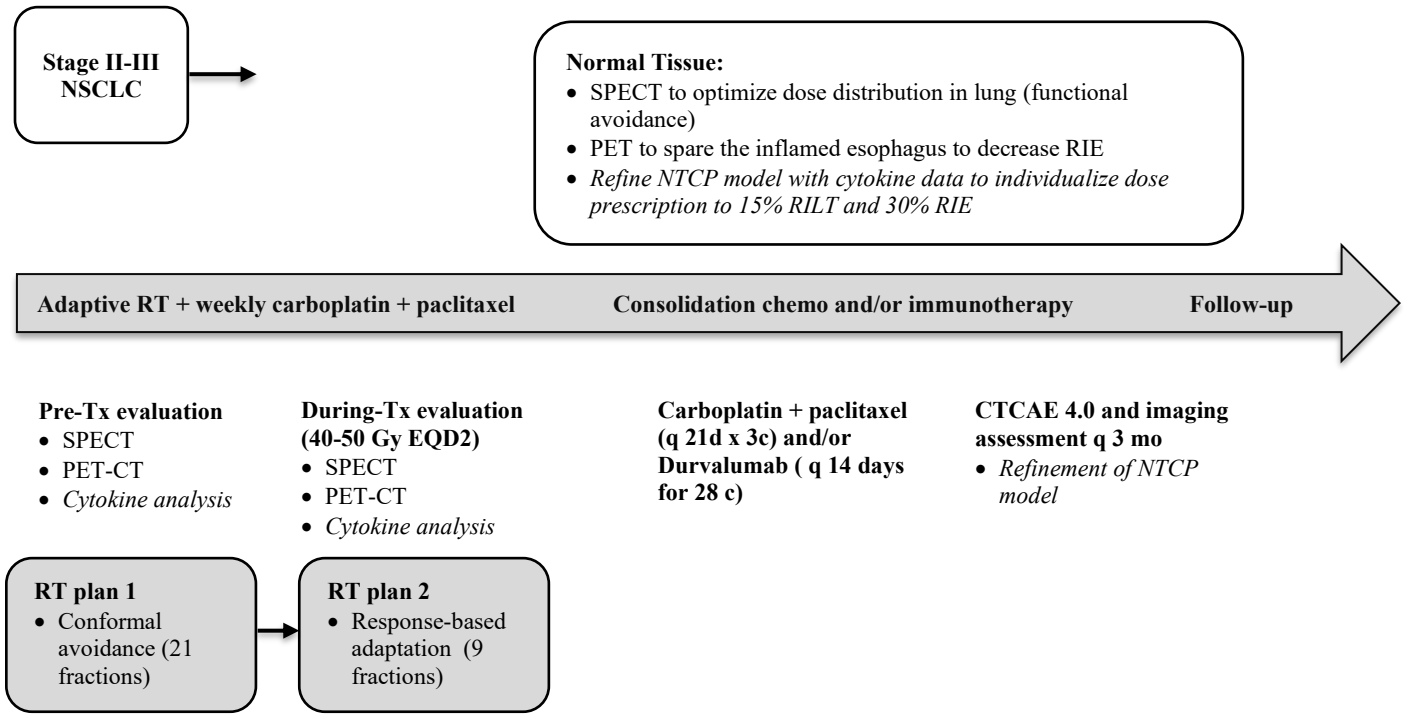
5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

Protocol treatment must start within 14 business days of enrollment to the study.

This project is a pilot therapeutic clinical trial that assesses the patient after approximately 60% of the treatment has been delivered, and adapts the latter 40% of the treatment using the knowledge of (1) the mid treatment FDG-PET scan to deliver a high dose of radiation to the active regions of the tumor (as was done per UMCC2007123); (2) V/Q SPECT-CT scanning both at the beginning of therapy as well as mid treatment to adapt local dose to the lung; (3) the mid treatment FDG-PET scan to assess esophageal toxicity. **This first trial will establish the feasibility of incorporating V/Q SPECT-CT (to predict local lung function) and FDG-PET (to predict esophageal toxicity)** while redistributing a high dose of radiation to the active regions of the tumor (determined by mid treatment FDG-PET, as was done per UMCC2007123). In addition, we will continue to collect data on plasma biomarkers (pre- and during-treatment) to further refine our biophysical NTCP model for lung injury. The goal is to incorporate this model into the adaptive treatment schema by identifying patients **who are unusually sensitive to radiation by changes in biofluid biomarkers (such as cytokine levels, circulating RNA/DNA, and/or urinary biomarkers) so that the remainder of the treatment course can be modified and toxicity can be kept to acceptable levels**. Our overall goal is to deliver similar radiation doses to the FDG-PET avid tumor that we gave in UMCC2007-123 while causing no greater toxicity of lung and esophagus than standard therapy (60 Gy uniformly delivered to the tumor), i.e. ~15% grade 2 and above RILT (4, 78, 88), ~30% grade 2 and above RIE (4, 89).





5.1.1 Radiation Therapy

5.1.1a **Radiation Therapy Schema**:

Patients will receive treatment 5 days per week, in once daily fractions, for 30 treatments with dose per fraction individually adapted over the final 9 treatments to intensify dose to active tumor while limiting normal tissue toxicity to an estimated grade ≥2 pneumonitis rate of 15% and grade ≥2 esophagitis rate of 30%. **The radiation dose will be delivered in daily fractions of ≥2.2 Gy, with the treatment duration limited to 30 fractions, and total radiation dose limited to 66-80.4 Gy physical dose.**

The first part of treatment (21 fractions: 46.2 Gy at 2.2 Gy per fraction) will be delivered to a target defined using pre-treatment CT and PET with the pretreatment V/Q SPECT-CT scan used to optimize the radiation dose distribution to minimize dose to functional lung (conformal avoidance RT). This will be followed by an adaptive course of radiation (9 fractions: 2.2 – 3.8 Gy per fraction) based on individual FDG-PET response (tumor and esophagus) as well as individual V/Q SPECT response (lung). Patients will receive a total tumor dose up to 80.4 Gy, which will be constrained by dose limits of the thoracic organs-at-risk (OARs). Specifically, using population based NTCP models we will limit predicted grade 2 and above RILT to ≤15% and grade 2 and above esophagitis to ≤30%, the rates of the standard practice (4, 90).

5.1.1a.1 **Use of FDG-PET scans to intensify dose to active tumor**:

Patients will undergo a FDG-PET-CT at baseline and after delivery of 40-50 Gy EQD2 (approximately 18 fractions). The first 21 fractions of radiation will be given based on a target defined at the start of radiation, and the remaining dose will be delivered to the target defined by FDG-PET acquired during the course of radiation. All patients receive a tumor dose up to a ≤15% NTCP for estimated RILT and the dose limits of other OARs per standard practice. All points in the dose distributions are corrected to their EQD2 using the linear quadratic model on a tissue by tissue basis. The radiation dose is delivered in daily

fractions of ≥2.2 Gy, with the treatment duration limited to 30 fractions, and the total radiation dose limited to 66-80.4 Gy physical dose; 64-102 EQD2 for lung toxicity assessment (alpha/beta = 2.5 Gy); 66-92 EQD2 for tumor control (alpha/beta = 10 Gy).

5.1.1a.2 **Use of V/Q SPECT-CT scans to spare functional lung:**

The pre-RT SPECT will be used during the optimization of the initial plan to minimize dose to functional lung. A priority based, lexicographic ordering strategy (38, 91) will be used to prioritize sparing functional lung relative to other targets and OAR objectives. The lung will be divided into five regions as summarized in Table 5.1.1a.2. The actual classification of each lung region will be determined by our nuclear medicine radiologists and radiation oncologists according to the functional intensity of V/Q SPECT-CT and its geometric location relative to the tumor in CT are also shown, as well as other clinical information. For treatment planning optimization, doses associated with regional based intensities (surrogate for function) within each region classification will be used to preferentially distribute dose in the lung tissue according the desire for avoidance given in **Table 5.1.1a.2.**

Table 5.1.1a.2. SPECT-CT functional classification of lung functional regions

Region	Lung function	Intensity in V/Q SPECT	Usually location relative to the tumor in CT	RT Plan dose avoidance
A	Truly bad	Very low	On tumor	No avoidance
B1	Truly bad	Very low	Proximal to tumor	No avoidance
B2	Not so bad	Low	Proximal to tumor	Low priority avoidance
B3	Recoverable	Low or very low	Distal to tumor	Medium priority avoidance
C	Good	High	Not on tumor	High priority avoidance

The V/Q SPECT-CT scan during treatment will be used to update the classified lung regions as shown above table from the scan obtained pretreatment. The scan during treatment can improve the estimate of the functional lung regions by determining whether the B1 region defined by the pretreatment scan remains poor, or if the B3 region has recovered after 40- 50Gy of RT. If the B3 region in pretreatment scan does recover, it would be redefined as the C region in the adaptive plan. Our experience in 75 cases showed that the above classification based on pre-treatment V/Q SPECT-CT is about 80% reproducible on the V/Q SPECT-CT during radiation. The new regions will be used in adaptive radiation optimization to minimize functional lung dose through the same strategy as the initial plan.

5.1.1a.3 **Use of FDG-PET to spare esophagus scans:**

The FDG-PET-CT scan during treatment will be used to optimize treatment planning to minimize radiation to FDG avid esophagus. Instead of using post-treatment PET to retrospectively correlate with radiation esophagitis (92), we will identify those patients with severe radiation-induced esophagitis on the during-RT PET scan to guide adaptive planning (93).

5.1.1b. **Radiation Volume Definitions**

Treatment volume for the first plan (initial 46.2 Gy given in 2.2 Gy/Fx in 21 fractions): The initial gross tumor volume (GTV) is **a composite volume** based on both the GTV from the pretreatment CT (CT) and the GTV from the pretreatment PET (PET1).

- **The CT-based GTV (GTV_CT1)** includes the primary tumor mass and any hilar or mediastinal lymph nodes ≥ 1 cm on the simulation CT scan (breath hold technique

or 4D-CT [ITV]), plus any abnormal findings detected on bronchoscopy and/or mediastinoscopy, if applicable.

- **The PET-based GTV (GTV_PET1)** of both primary tumor and nodal disease will be contoured using a threshold technique. The mean PET intensity of a 1.5 cc volume in the aortic arch will be calculated and then multiplied by 1.5 to determine the minimum threshold for auto-contouring of the PET-based GTV. Care will be taken to review the autocontoured GTV_PET volumes, as editing may be required to remove regions outside known gross tumor, such as the esophagus, which may demonstrate uptake, especially on the during treatment PET, secondary to esophagitis.
- **The final pretreatment GTV (GTV_CT1+PET1)** will consist of a composite of the GTV_CT1 and the GTV_PET1.
- **The pretreatment clinical target volume (CTV_CT1+PET1)** will consist of the composite GTV (GTV_CT1+PET1) with an approximate 0.5 cm margin for microscopic extension. Radiographically uninvolved supraclavicular, paratracheal and subcarinal lymph nodes will NOT be intentionally included in the CTV.
- **The pretreatment planning target volume (PTV_CT1+PET1)** will consist of the CTV_CT1+PET1 plus a minimum of a 0.5 cm margin for set-up error.

Treatment volume for the second plan (during treatment): During treatment target volumes will be defined based on a repeat PET-CT scan acquired after delivery of 40-50 Gy EQD2 (approximately 18 fractions).

- The during treatment GTV (GTV_PET2) will be defined using the same auto-contouring using methodology as was used to define the pretreatment PET GTV (GTV_PET1).
- GTV_PET2 will be directly expanded to a PTV (PTV_PET2) with a uniform expansion of at least 0.5 cm.

Treatment volume structures and doses:

Structure Name	Description	Preferred Dose covering 95% volume	Acceptable Variation‡
Plan 1			
GTV_CT1+PET1	GTV*_CT1 + GTV_PET1	n/a	
CTV_CT1+PET1	GTV_CT1+PET1 + 0.5 cm	n/a	
PTV_CT1+PET1	CTV_CT1+PET1 + 0.5 cm (minimum)	46.2 Gy or above	41.6 - 46.2 Gy
Final composite plan (plans 1 + 2)			
GTV_CT1+PET1	GTV*_CT1 + GTV_PET1	n/a	
CTV_CT1+PET1	GTV_CT1+PET1 + 0.5 cm	n/a	
PTV_CT1+PET1	CTV_CT1+PET1 + 0.5 cm (minimum)	56.0 Gy or above	50.4 - 56.0 Gy
PTV_PET2	GTV_PET2 + 0.5 cm (minimum)	Up to 80.4 Gy†	5-10% less of desired dose

* ITV (internal target volume for 4D-CT scans)

† Constrained by dose limits of the thoracic organs-at-risk (OARs).

‡ The minimum dose within the PTV can fall below the 90% of the prescription dose but underdosing must be confined to areas of overlap with critical OARs. In those regions, the minimum dose to the PTV should be equal to the maximum allowed dose to the OAR.

5.1.1c. **Radiation Technique:**

Position/Immobilization/Simulation: Patients will be positioned and immobilized using standard techniques. Simulation CT scans of the chest will be performed using either 4D-CT or breath-hold techniques (for tumors with respiratory motion > 1 cm and patient tolerance permits).

Treatment Planning: All patients will undergo CT- and PET-based treatment planning for conformal radiation therapy. GTV definition, CTV margin, and PTV margin are as described above. The treatment technique and number of fields will be optimized individually. Dose volume histograms will provide objective criteria for prescription dose selection based on the potential for normal tissue damage. Suitable treatment plans will minimize thoracic normal tissue doses while also providing the highest prescription dose with acceptable target volume coverage.

Target Volume Coverage: The expectation is conformal treatments, which minimize lung dose and meet all normal tissue constraints. As a guideline, a conformity index (ratio of the volume of the prescription isodose surface to the PTV) of < 1.5 is desirable. For treatment plans limited by the dose to normal lung (the standard case), the prescription isodose surface should encompass at least 95% of each PTV or the lowest dose limit of OARs, if any of them is lower than the prescription dose. It is desirable for the minimum PTV dose not to fall below 90% of the prescription dose and for the maximum PTV dose not to exceed 110% of the prescription dose. For PTVs which overlap or come near other critical OARs, greater PTV dose heterogeneity is acceptable. The table above describes the target dose objectives.

Organs at Risk Tolerances: All critical organs (listed below) will be contoured in the treatment planning system when they are in the radiation field. Lung, spinal cord, esophagus, heart, pericardium and brachial plexus contours should be based on the published atlas on organs at risk available on the RTOG web site: http://www.rtog.org/CoreLab/ContouringAtlases.aspx. If constraints cannot be met, the prescription dose may be decreased heterogeneously accordingly. Tradeoffs in target coverage and OAR dose limits may be made at the discretion of the treating physician on a case-by-case basis.

Structure Description	Metric	Goal
Lungs minus (GTV_CT1+PET1)	Max Dose (Gy, 0.03 cc)	⩽ 110 % Rx Dose
	Mean Dose (Gy)	⩽ 20 Gy
	Vol > 20 Gy (%)	⩽ 35 %
	Vol > 5 Gy (%)	⩽ 65 %
Heart/ Pericardium	Max Dose (Gy, 0.03 cc)	⩽ 70 Gy
	Mean Dose (Gy)	⩽ 30 Gy
	Vol > 30 Gy (%)	⩽ 50 %

	Vol > 40 Gy (%)	≤ 35 %
Esophagus	Max Dose (Gy, 0.03 cc)	≤ 74 Gy
	Mean Dose (Gy)	≤ 34 Gy
Spinal Cord + 3 mm	Max Dose (Gy, 0.03 cc)	≤ 50 Gy
Brachial Plexus	Max Dose (Gy, 0.03 cc)	≤ 63 Gy

Radiation Dose Calculations: Dose calculations will be performed using modern dose calculation algorithms (listed on the IROC Houston web site at http://irochouston.mdanderson.org) with inhomogeneity corrections that take into account the density differences within the irradiated volume. Non-validated dose calculation algorithms (i.e. simple pencil beam) will not be allowed for this study. For free-breathing treatment, dose calculations will be performed on an untagged or average scan generated from 4DCT data. For breathing controlled treatments, dose calculations will be performed on the CT taken at the breath hold state to be used for treatment.

Treatment Equipment: Megavoltage equipment is required with effective photon energies of 6 MV (preferred) or higher. Inverse-planned, external beam techniques are preferred including intensity modulated radiation therapy and volumetric modulated radiation therapy. Daily image guidance is required, preferably with volumetric imaging.

5.1.2 Chemotherapy Administration

5.1.2.1 **Concurrent chemotherapy:**

For patients with stage II or III disease and good performance status, chemotherapy will be administered weekly concurrent with radiation. Carboplatin (AUC 2, IV) and Paclitaxel (40-50 mg/m2, IV) will be started on week 1 of thoracic radiotherapy and will be continued weekly for six weeks. Patients may receive chemotherapy on any day of the week from Monday to Friday, but the day of administration should remain constant during the course of chemoradiotherapy. A one-day shift in the day of weekly chemotherapy infusion (or more at the discretion of the physician) will be allowed if necessary.

Weekly Concurrent Chemotherapy Regimen

Agent	Dose	Route	Infusion Time
Paclitaxel	40-50 mg/m^2	IV	60 minutes
Carboplatin	AUC 2	IV	30 minutes

Paclitaxel 40-50 mg/m^2 IV will be given by one hour infusion. Paclitaxel is mixed in non-PVC containers per the usual guidelines of the pharmacy.

Carboplatin will be given at AUC 2 over 1/2 hour immediately after paclitaxel using the Calvert formula: calculated dose of carboplatin (mg) = target AUC x (GFR + 25) (GFR as per the Cockroft-Gault or Jelliffe formula). 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.

*Note: Occasionally, the infusions may take longer than the stated time above. If Paclitaxel is given within 90 minutes and Carboplatin is given within 60 minutes, these will not be considered deviations.

Prior to receiving carboplatin and paclitaxel, all patients should receive standard pre-medication. One standard that is recommended is:

- Dexamethasone 20 mg orally 12 and 6 hours before paclitaxel or 20 mg IV just prior to paclitaxel
- Diphenhydramine 50 mg IV (or equivalent) prior to paclitaxel
- Cimetidine 300 mg IV (or equivalent, ranitidine 50 mg or famotidine 20 mg) prior to paclitaxel
- Granisetron 2 mg orally (or equivalent) prior to chemotherapy

5.1.2.2 **Consolidation Chemotherapy Or Immunotherapy:**

Consolidation chemotherapy will start approximately 4-6 weeks after the completion of radiotherapy when esophagitis and chemo-induced neuropathy are grade 1 or less, and ANC > 1500 and platelet count > 100,000. The decision to receive cytotoxic chemotherapy or immunotherapy will be at the discretion of the medical oncologist. If cytotoxic chemotherapy is to be administered, Carboplatin (AUC 6, IV) and Paclitaxel (200 mg/m^2, IV) will be given on day 1. This will be repeated every 21 days for a total up to 3 cycles. Administration of carboplatin and paclitaxel and standard pre-medications have been described in section 5.2.1. However, during consolidation chemotherapy, paclitaxel 200 mg/m^2 will be administered over 3 hours. If immunotherapy is to be given (permitted for stage III patients only, that have not progressed following concurrent chemoradiation), durvalumab (10 mg/kg) will be given on day 1. This will be repeated every 14 days for a total of 26 cycles. Patients will be monitored for infusion reactions and informed of risk for a delayed infusion reaction. While these are current standard dosing regimens for both chemotherapy and immunotherapy, treating physician may use discretion to alter the treatment regimen as needed.

Consolidation Chemotherapy Regimen

Agent	Dose	Route	Infusion Time	Days for administration
Paclitaxel	200mg/m^2	IV	3 hours	q 21 days × 3 cycles
Carboplatin	AUC 6	IV	½ hour	q 21 days × 3 cycles

Consolidative Immunotherapy Regimen

Agent	Dose	Route	Infusion Time	Days for administration
Durvalumab	10 mg/kg	IV	1 hour	q 14 days × 26 cycles

5.1.3 Supportive Care Guidelines

Nutritional support is recommended for all patients. Supportive care with medications will be determined by participating physician, based on each individual situation. Suggested management for acute radiation pneumonitis includes bed rest, bronchodilators, and corticosteroids. Oxygen and even assisted ventilation may be necessary for severe cases. Treatment of esophagitis varies with the severity of the patient's symptoms. Diet adjustment and narcotic management may be sufficient for grade 2 esophagitis. Nutritional

support via gastric tube or jejunostomy tube may be initiated upon development of grade 3-4 esophagitis, per mutual preference of the physician and patient.

5.1.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue through completion of concurrent chemoradiotherapy and consolidation chemotherapy or until one of the following criteria applies:

- Local-regional disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

If treatment is interrupted due to a non-dose-limiting adverse event or any reason other than toxicity, such as a holiday, bad weather, or a transportation problem, the duration of therapy will be extended accordingly. If a patient misses a day of radiation and chemotherapy, then the weekly chemotherapy should be delivered the next day and the missed radiation fraction will be given after the completion of planned treatments.

5.2 Delays/Dose Modifications during consolidation chemotherapy or immunotherapy.

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events Table (Section). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

Dose of radiation and chemotherapy / immunotherapy will be modified independently.

5.2.1 Radiation dose modifications

Radiation treatment will be stopped if a patient develops severe lung toxicity at any point during the course of radiation therapy. It will be the decision of the treating physician if the patient should continue protocol treatment and the timing of restart treatment.

Following the mid-treatment scans, a radiation treatment break of one day will be permitted in the event that additional time is needed for treatment planning and QA.

5.2.2 Chemotherapy / Immunotherapy dose modifications: All toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0. Dose modifications or delays in administration of chemotherapy and/or immunotherapy may be based on results from local laboratories for concurrent chemotherapy. For consolidative chemotherapy or immunotherapy, dose modifications should be made as per standard of care, or as per discretion of the treating medical oncologist. All dose modifications and alterations will be recorded in the patient's medical chart. A maximum of three dose reductions will be allowed per patient.

5.2.2.1 Chemotherapy dosage modifications for toxicity during **concurrent chemoradiotherapy**.

Hematologic Toxicity	Peripheral Neuropathy	Dysphagia	Paclitaxel Dose	Carboplatin Dose

ANC≥1000	AND	Platelet≥80K	AND	Grade 0-1	AND	Grade 0-1	100%	100%
ANC≥1000	AND	Platelet≥80K	AND	Grade 2	AND	Grade 0-1	50%	100%
ANC 500-999	AND	Platelet 50-79K	OR	Grade 2	OR	Grade 2	50%	50%
ANC<500	OR	Platelet<50K	OR	Grade 3-4	OR	Grade 3-4	Hold*	Hold*
Neutropenic fever	OR	Neutropenic fever	OR	Grade 3-4	OR	Grade 3-4	Hold*	Hold*

*Resume chemotherapy when relevant toxicity becomes ≤ grade 1.

5.2.2.3 **Hematological Toxicity:** The absolute neutrophil count must be ≥ 1500/mm3 and the platelet count must be ≥ 100,000/mm3 to receive chemotherapy on Day 1 of each cycle.

Dose modifications must be made according to the criteria specified in the table below:

		Previous Cycle		**Next Cycle**	
ANC nadir (/mm^3)		Platelet nadir (/mm^3)	Fever/Sepsis	Carboplatin Dose	Paclitaxel Dose
≥ 500	AND	≥ 50,000	No	No reduction	No reduction
< 500	OR	< 50,000	No	One dose level	One dose level
< 1000	AND	Any	Yes	One dose level	One dose level

Note: Granulocyte colony-stimulating factor (G-CSF) may be used in case of hematological toxicity. If G-CSF is used, it should be used in accordance with the American Society of Clinical Oncology (ASCO) guidelines.

Treatment with carboplatin and paclitaxel may be delayed for up to 2 weeks until the ANC is ≥ 1500/mm3 and the platelet count is ≥ 100,000/mm3. Patients should begin the next cycle as soon as possible after appropriate hematologic recovery.

No dose reductions will be made for anemia. Patients may be supported with packed red blood cell transfusions and/or erythropoietin.

5.2.2.4 **Hepatic Dysfunction**
The SGOT or SGPT and bilirubin values on Day 1 of each cycle should be used to determine the dose of paclitaxel on the next cycle.

SGOT/SGPT (Day 1 of each cycle)		**Bilirubin (Day 1 of each cycle)**	**Paclitaxel Dose**
≤ 4 × upper limit of normal	AND	≤ 3.0 mg/dl	No change
> 4 × upper limit of normal	OR	> 3.0 mg/dl	Hold dose*

*If paclitaxel is held due to hepatic toxicity, carboplatin should also be withheld and administered when the paclitaxel is resumed. No dose reductions of carboplatin will be made for hepatic toxicity. If recovery of hepatic toxicity exceeds 2 weeks, consolidation chemotherapy with paclitaxel and carboplatin should be discontinued.

5.2.2.5 **Neurologic Toxicity**

Paclitaxel doses should be modified for neurologic toxicity based upon the worst grade experienced during the preceding cycle. Dose modifications made for neurotoxicity are permanent reductions.

Sensory Neuropathy (CTC Grade)	Paclitaxel Dose	Carboplatin Dose
0-1	No change	No change
2	one dose level	No change
≥ 3	Hold*	No change

*May restart with dose reduction of two dose levels when neuropathic toxicity improves to ≤ grade 1.

5.2.2.6 **Hypersensitivity Reactions:** Patients who have had a mild to moderate hypersensitivity reaction to paclitaxel have been successfully re-challenged, but careful attention to prophylaxis and bedside monitoring of vital signs is recommended.

Symptoms should be managed as follows:

Grade 1 - Complete paclitaxel infusion. Supervise at bedside. No treatment required.

Grade 2 - Stop paclitaxel infusion. Give diphenhydramine 25 mg IV and dexamethasone 10 mg IV. Resume paclitaxel infusion after recovery of symptoms at a low rate, 20 ml/hour for 15 minutes, then 40 ml/hour for 15 minutes, then, if there are no further symptoms, resume the paclitaxel infusion at full dose rate until complete. If symptoms recur, stop paclitaxel infusion and after full recovery continue with carboplatin.

Grade 3 or 4 - Stop paclitaxel infusion. Give diphenhydramine IV and dexamethasone IV, as above. Add epinephrine or bronchodilators if indicated. The patient should not be rechallenged with paclitaxel and will discontinue protocol treatment.

5.2.2.7 **Other Toxicity:**

For any drug-related Grade 3 or 4 toxicity not mentioned above except anemia, lymphopenia, or nausea, treatment with paclitaxel and carboplatin should be withheld for a maximum of 14 days until the toxicity improves to ≤ Grade 1. Treatment may then be resumed at a one dose level reduction. For Grade 1 or 2 toxicities, no dose reduction should be made.

6 DRUG INFORMATION

6.1 Paclitaxel (Taxol)

Description: Paclitaxel is a poorly soluble plant product from the western yew, Taxus brevifolia. Improved solubility requires a mixed solvent system with further dilutions of either 0.9% sodium chloride or 5% dextrose in water.
Mechanism of Action: Paclitaxel affects microtubule formation during interphase and mitosis with a mechanism distinct from the vinca alkaloids.

Human Toxicology: Hematologic toxicity includes myelosuppression. Gastrointestinal toxicities include nausea and vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis,

ischemic colitis, neutropenic enterocolitis, increased liver function tests (SGOT, SGPT, bilirubin, alkaline phosphatase) hepatic failure and hepatic necrosis. Cardiac complications may include arrhythmias, heart block, ventricular tachycardia, myocardial infarction (MI), bradycardia, atrial arrhythmia, hypotension, hypertension, and lightheadedness. Neurologic morbidity may manifest as sensory changes (taste), peripheral neuropathy, seizures, mood swings, hepatic encephalopathy, encephalopathy, sensation of flashing lights, blurred vision, and scintillating scotoma. Anaphylactoid and urticarial reactions (acute), flushing, rash, and pruritus are also possible. In addition, alopecia, fatigue, arthralgia, myopathy, myalgia, infiltration (erythema, induration, tenderness, rarely ulceration), and radiation recall reactions are described.

Formulation: Paclitaxel is available in sterile solution concentrates in 5, 16.7, 25 and 50mL multi-dose vials in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol, USP, 50%. Paclitaxel will be diluted at the appropriate dose, in D5W, USP, in 5% polyolefin containers due to leaching of diethylhexphthalate (DEHP) plasticizer from polyvinyl chloride (PVC) bags and intravenous tubing by the Cremophor vehicle in which paclitaxel is solubilized. NOTE: Formation of a small number of fibers in solution (NOTE: acceptable limits established by the USP Particular Matter Test for LVP's) have been observed after preparation of paclitaxel. Therefore, in-line filtration is necessary for administration of paclitaxel solutions. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 microns (eg: Millex-GV Millipore Products) into the IV fluid pathway distal to the infusion pump. Although particulate formation does not indicate loss of drug potency, solutions exhibiting excessive particulate matter formation should not be used.

Storage and Stability: Paclitaxel vials should be stored between 2°-25°C (36°-77°F). Vials will be labeled with shelf-life. All solutions of paclitaxel exhibit a slight haziness directly proportional to the concentration of drug and the time elapsed after preparation, although when prepared as described above, solutions of paclitaxel (0.3-1.2 mg/ml) are physically and chemically stable for 27 hours.

Supplier: Commercially available and should be purchased by a third party.

Premedications: Dexamethasone, diphenydramine (or equivalent) and an H2-receptor antagonist are recommended before paclitaxel administration.

6.2 Carboplatin (Paraplatin)

Description: Carboplatin is supplied as a sterile lyophilized powder.

Mechanism of action: Carboplatin inhibits DNA synthesis through intracellular platinum complexes to form intrastrand, interstrand and protein cross-linking through covalent binding of DNA molecules. Carboplatin is considered to be cell cycle phase-nonspecific, but recent studies have shown complex and variable effects on the cell cycle.

Human Toxicology: Adverse effects include myelosuppression, nausea, vomiting, peripheral neuropathy, ototoxicity, hepatic toxicity, electrolyte imbalance, hypomagnesaemia, hypercalcemia and allergic reaction.

Formulation: Carboplatin is supplied as a sterile lyophilized powder available in a single-dose vial containing 50 mg, 150 mg, and 450 mg of carboplatin for administration by intravenous infusion. Each vial contains equal parts by weight of carboplatin and mannitol. 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:

Vial Size	Diluent Volume
50 mg	5 ml

150 mg	15 ml
450 mg	45 ml

These dilutions all produce a carboplatin concentration of 10 mg/ml.

Storage and Stability: Unopened vials of carboplatin are stable for the life indicated on the package when stored at controlled room temperature and protected from light. When prepared as directed, carboplatin solutions are stable for eight hours at room temperature; since no antibacterial preservative is contained in the formulation, it is recommended that carboplatin solutions be discarded eight hours after dilution.

Supplier: Commercially available and should be purchased by a third party

Premedication: Antiemetics and hydration are recommended before carboplatin administration.

6.3 Immunotherapy / Durvalumab

Supply and Storage: Durvalumab Injection is a clear to opalescent, colorless to slightly yellow solution supplied in a carton containing one single-dose vial either as:

- 500 mg/10 mL (NDC 0310-4611-50)
- 120 mg/2.4 mL (NDC 0310-4500-12)

Store in a refrigerator at 2°C to 8°C (36°F to 46°F) in original carton to protect from light. Do not freeze. Do not shake.

Mechanism of action: Durvalumab inhibits PDL1. This alters immune checkpoints to potentiate anti-tumoral immunity. Expression of programmed cell death ligand-1 (PD-L1) can be induced by imflammatory signals (e.g., IFN-gamma) and can be expressed on both tumor cells and tumor-associated immune cells in the tumor microenvironment. PD-L1 blocks T-cell function and activation through interaction with PD-1 and CD80 (B7.1) By binding to its receptors, PD-L1 reduced cytotoxic T-cell activity, proliferation, and cytokine production.
Durvalumab is a human immunoglobulin G1 kapp (IgG1k) monoclonal antibody that binds to PD-L1 and blocks the interaction of PD-L1 with PD-1 and CD80 (B7.1). Blockade of PD-L1/PD-1 and PD-L1/CD80 interactions releases the inhibition of immune responses, without inducing antibody dependent cell-mediated cytotoxicity (ADCC).
PD-L1 blockade with durvalumab led to increased T-cell activation in vitro and descreased tumor size in co-engrafted human tumor and immune cell xenograft mouse models.

Human Toxicology: Adverse effects include autoimmune disease, including endocrine, neurologic, gastrointestinal, pulmonary, and dermatologic.

Formulation:

- Injection: 500 mg/10 mL (50 mg/mL) solution in a single dose vial.
- Injection: 120 mg/2.4 mL (50 mg/mL) solution in a single dose vial.

Storage and Stability: Per package insert.

Supplier: Commerically Available and should be purchased by a third party.

Premedication: No premedication is indicated for the administration of Cycle 1 of durvalumab. However, patients who experience an infusion-related reaction (IRR) with Cycle 1 of durvalumab may receive premedication with antihistamines or antipyretics/analgesics (e.g., acetaminophen) for subsequent infusions. Metamizole (dipyrone) is prohibited in treating durvalumab-associated IRRs because of its potential for causing agranulocytosis.

7 TOXICITIES TO BE MONITORED AND REPORTED

7.1 Radiation Induced Lung Toxicity

Lung toxicity includes radiation pneumonitis and clinical fibrosis will be reported. Grade 2 and above are the study endpoint. .Grade 3 and above are considered to be severe. Diagnosis and grading of radiation pneumonitis and clinical fibrosis are listed on the table below.

Table 7.1. Diagnosis and Grading System for Radiation Pneumonitis and Clinical Fibrosis

	Radiation Pneumonitis	Clinical Fibrosis
Grade 1	Minimal or mild symptoms of dry cough AND/OR dyspnea on exertion; AND without evidence of tumor progression or other etiology; AND with radiographic evidence of acute pneumonitis	Radiographic evidence of radiation fibrosis without or with minimal dyspnea
Grade 2	Persistent dry cough requiring narcotic antitussive agents or steroid; AND/OR dyspnea with minimal effort but not at rest; AND without evidence of tumor progression or other etiology; AND with radiographic evidence of acute pneumonitis, and requiring steroid for treatment	Radiographic evidence of radiation fibrosis; AND dyspnea with minimal effort but not at rest, not interfering with activities of daily living
Grade 3	Severe cough, unresponsive to narcotic antitussive agent; AND/OR dyspnea at rest; AND with radiographic evidence of acute pneumonitis; AND requiring oxygen (intermittent or continuous) for treatment	Radiographic evidence of radiation fibrosis AND dyspnea at rest, interfering with activities of daily living; AND home oxygen indicated
Grade 4	Radiation pneumonitis causes respiratory insufficiency requiring assisted ventilation	Radiation fibrosis causes respiratory insufficiency, requiring assisted ventilation
Grade 5	Radiation pneumonitis directly contributes to the cause of the death	Radiation fibrosis directly contributes to the cause of the death

7.2 Esophageal Toxicity

Esophageal toxicity, including dysphagia and odynophagia, will be graded per CTCAE4.0, Severe acute esophageal toxicity is defined as persistent grade 3 or higher esophageal toxicity occurring within 3 months of the start of radiation therapy. According to the RTOG acute radiation morbidity scoring criteria for esophagitis, grade 3 is defined as severe dysphagia or odynophagia with dehydration or weight loss > 15% from treatment baseline, requiring a feeding tube, IV fluids, or hyperalimentation. Grade 4 is defined as esophagitis causing life-threatening consequences, such as perforation, obstruction, or fistula formation. Grade 5 is severe esophagitis directly contributing to death. Persistent grade 3 esophageal toxicity is defined as esophageal toxicity dependent on a feeding tube, IV fluids, or hyperalimentation longer than 6 weeks after the completion of radiation therapy.

The incidence of severe acute esophageal toxicity is expected to be lower than 5%. Since only pneumonitis is modeled by the NTCP function, doses to the lung will not be adjusted if excess severe esophageal toxicity occurs. Instead, the normalization dose to the esophagus will be adjusted if at least 2 of the first 10 patients, or 4 of the first 20 patients, or 5 of the first 30 patients experience severe acute esophageal toxicity as described.

7.3 Other Toxicities

Lung and esophageal toxicities will be graded using CTCAE v4.0. These will include, but not be limited to: cough, dyspnea, pneumonitis, radiation pneumonitis, radiographic or clinical pulmonary fibrosis and esophagitis. Lung and esophageal toxicities of grade $\geq$ 2

will be collected in the database for analysis. Other toxicities will also be monitored and reported using the NCI CTCAE) v4.0.

8.0 CORRELATIVE TRANSLATIONAL STUDY

We will collect data to investigate the role of biomarkers for treatment outcome prediction.

Blood and urine will be collected on the following schedule:

- 4 weeks prior to radiation therapy (RT)
- Day 1 – 1 hour post radiation
- Day 2 – prior to radiation
- Day 5 – prior to radiation
- Day 5 – 1 hour post radiation
- Thereafter, blood and urine will be collected weekly during chemoradiation.
  Blood and urine will also be collected at ~1 month and at ~ 3 months post-RT, and every ~3 months through one year post treatment, then every ~6 months through four years post treatment, and at five years post treatment.
- Subjects who do not provide some samples, miss time-points or decline any or all of the sample collections will not be reported as protocol deviations.

The absolute concentration of circulating, cfNA tends to be very low which typically requires analyses of larger volumes of blood than for other approaches such as measurement of protein biomarkers or cytokines. In addition, for different analyte types, different collection tubes and preservatives are needed (eg, EDTA tubes for extracellular RNA and Streck tubes for circulating tumor DNA). We therefore propose to collect:

1 x 10 mL of blood will be collected for serum isolation.
2 x 10 mL of blood in EDTA (purple top) tubes will be collected to use for preservation and subsequent recovery of plasma and blood cells. Tubes will be stored and transported at room temperature.
1 x 10 mL of Streck Cell-Free DNA BCT tube (brown top) will be collected for preservation and subsequent recovery of cfDNA from plasma.

Platelet-poor plasma will be obtained for cytokine and proteomic assays; plasma and/or serum samples will be used for metabolomics, microRNA and other extracellular RNA profiles, circulating tumor DNA, cell death assays and other markers as indicated; buffy coat will be used for genomic and/or transcriptomic studies. Urine will be used for analysis of RNA, DNA, protein, and other biomarkers.

RNA and DNA biomarkers, whether cell-free or cell-associated, will be analyzed using methods including quantitative PCR, digital PCR, and next-generation sequencing. Bioinformatic analyses may be used in these profiling studies.

Plasma TGF-β1 will be measured by molecular specific Enzyme Linked Immune Sandwich Assay (ELISA). The levels of plasma cytokines will be measured by ready to use kits, such as LINCOplex (microsphere-based sandwich immunoassay) for the concentrations of 29 proinflammatory cytokines, including G-CSF, IL-1α, IL-1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1, TGF-α, and TNF-α. RILT will be diagnosed and graded based on CTCAE 4. The plasma proteomes will be compared using a multiplexed quantitative proteomics approach involving ExacTag labeling, RP-HPLC and LC-ESI-MS/MS.

For genomic studies, we will focus our efforts on (but not limited to) gene specific SNPs of TGFβ1, tissue plasminogen activator (tPA) and angiotensin-converting enzyme (ACE), which are associated with radiation-induced thoracic toxicity such as RILT. Genetic variations within functional locus of these genes will be assessed for in each patient by using gene specific PCR

technology. Such SNP studies will be performed using polymerase chain reaction (PCR) and allele specific primers. Variance components models will be used to identify the differential protein expression between patients with and without toxicity. Bioinformatic methodology may be applied for data analysis.

For cellular studies (from archival tissue as a block or slides), we will focus our efforts on (but not limited to) CD8 T cell effector number and function, which have been shown to correlate with radiation induced local control and toxicity. Flow cytometric analysis will be performed in patients with and without toxicity. Immunohistochemical evaluation of T cells will be performed in patients with and without toxicity. Bioinformatic methodology may be applied for data analysis.

Since this is a prospective study, we anticipate advancement in experimental technology and preliminary results. Other techniques and tests also will be applied if they are found to be superior to the ones stated above. Blood markers (cytokine, proteomic, cell-free DNA/RNA, immune cell alterations, and genomics) during early course of treatment will be correlated to clinical outcome in tumor control and treatment related thoracic toxicity.

If patients are also participating in UMCC 2015.006, or other protocols with a similar biospecimen collection schedule, only one set of specimens will be collected.

9.0 STUDY PROCEDURES AND CALENDAR

9.1 Pre Treatment Clinical Evaluation

The following clinical evaluations should be done within the specified times prior to patient enrollment:

9.1.1 Complete history and physical examination should be performed within 4 weeks;
9.1.2 Weight and Karnofsky performance status should be evaluated within 4 weeks;
9.1.3 CBC with differential, complete chemistry panel including alkaline phosphatase, creatinine, serum albumin, total bilirubin and AST/ALT should be done within 4 weeks;
9.1.4 CT scan (if possible with IV contrast) of chest should be done within 6 weeks;
9.1.5 MRI or CT of the brain with contrast should be done within 6 weeks;
9.1.6 A total body FDG-PET scan is required within 2 weeks from CT simulation. PET scan performed outside hospital is allowed if it can be transferred to our treatment planning system and the image is quality assured. A bone scan is optional, pending the decision of the treating physician;
9.1.7 A V/Q SPECT scan is required within 2 weeks from CT simulation;
9.1.8 Complete pulmonary function tests should be done within 6 weeks;
9.1.9 Blood samples for biomarkers (such as IL-8 and TGF-ß1) should be drawn within 4 weeks.
9.1.10 Quality of life questionnaires will be collected within 4 weeks

All the tests above, except biomarker measurements and questionnaires, are part of routine staging work-ups.

9.2 <u>Pretreatment and during treatment studies</u>

Procedure	Pretreatment	Weekly During Chemotherapy / RT
History and physical	Within 4 wks	X
Weight and KPS	Within 4 wks	X
Tumor measurement	Based on treatment planning CT	

Chest CT*	Within 6 wks	
CBCP, differential	Within 4 wks	X
Electrolytes, creatinine, liver function tests	Within 4 wks	As Clinically Indicated
Complete pulmonary function tests	Within 6 wks	
CT Simulation (re-planning)		X[a]
Head CT or MRI	Within 6 wks	
PET scan	Within 2 wks, before or after, treatment planning CT	X[a]
SPECT scan	Within 2 wks, before or after, treatment planning CT	X[a]
Blood drawing for biomarkers	Within 4 wks	X[b]
Urine collection for biomarkers	Within 4 wks	X[b]
Toxicity evaluation	Within 4 wks	X
Quality of Life Questionnaires	Within 4 wks	X[c]
Tissue Evaluation	At diagnostic biopsy[d]	

* High-resolution CT is preferred. Upper abdomen preferred but not required.

a. PET-CT scan, SPECT-CT scan, and re-planning CT Simulation scan will be repeated after patient has received 40-50 Gy EQD2 of radiotherapy

b. In addition to pre-treatment samples, blood and urine will be collected for biomarkers at the following time points:
   - Day 1 – 1 hour post-radiation
   - Day 2 – prior to radiation
   - Day 5 – prior to radiation
   - Day 5 – 1 hour post-radiation
   - Thereafter, blood and urine will be collected weekly during chemoradiation

c. Quality of life questionnaires will be done pre-treatment, mid-treatment (3rd week of treatment), and also the last week of treatment. Since patients can be seen in a variety of clinics, missed QOLs will not be reported as a protocol deviation.

d. For UM patients only, tissue collected from diagnostic biopsy will be assessed for immunohistochemical analysis as described in section 8.0.

9.3 Follow-up calendar

Procedure	Time since the end of RT	1, 3 months*	6,9,12 months*	18,24,30,36 months*
	Frequency of Follow-up		Q 3 months	Q 6 months
History and physical		X	X	X
Weight and KPS		X	X	X
Tumor measurement		3 mo only	X	X
Chest CT**		X[a]	X[a]	X
CBCP, differential		X	X	X
Electrolytes, creatinine, liver function tests		X	X	X
Pulmonary function tests		X[b]	X[b]	
Toxicity evaluation		X	X	X
Blood and urine collection for biomarkers [c]		X	X	X
Consolidation Chemotherapy or Immunotherapy		X[d]		
Quality of life questionnaires[e]		X	X	X

*Timing of follow-up: From 2 months to 15 months = (+/-) 3 week window. For 18 months to 36 months = (+/-) 1 month window.

** High-resolution and IV contrast enhanced CT.

a: Chest CT will be done approximately 1 month after last chemotherapy consolidation cycle, or as per standard of care during consolidation immunotherapy. Upper abdomen preferred but not required. At 6 months and later, chest CT will be done every 6 months.

b: Pulmonary function tests will be done at 3 and 12 months .

c: If a patient experiences severe toxicity (as defined in 9.1 and 9.2) and returns for clinical evaluation at a time not designated on study calendar, TGF ß1 and other molecules will be drawn.

Blood and urine will be collected at each of the timepoints indicated in the table above.

d: Consolidation Chemotherapy per 5.1.2.2 or Immunotherapy per 5.1.3.

e. Questionnaires will be given to patients at follow-up visits 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, and 36 months. Since patients can be seen in a variety of clinics, missed QOLs will not be reported as a protocol deviation.

Note: PET scans, bronchoscopies, pulmonary function tests, and head CTs or MRIs may be done post-treatment as part of routine clinical care, to assist with disease or toxicity evaluation. If these are done, the results may be used for research purposes.

9.4 Off Study Conditions

9.4.1 Patients may be removed from study at any time by patient request or at the discretion of the investigator.

9.4.2 Patients who exhibit tumor progression, metastatic disease, or move on to other treatment modalities, will discontinue all study calendar procedures. Such patients will be

medically managed. For the purposes of the research, they will continue to be followed for toxicity, further progression, and survival, by reviewing and collecting standard clinical information. These patients may be treated with other agents.

10.0 STATISTICAL CONSIDERATIONS

Overview/Design:
This is a single arm pilot study of adaptive radiation therapy in patients with stage II/III NSCLC. A total of 55 evaluable patients will be enrolled (up to 70 patients may need to be enrolled in order to obtain 55 evaluable patients). A patient will be considered evaluable if they complete treatment and a minimum of 6 months of follow up. This will allow thorough analysis of treatment toxicity, which can be observed as late as six months after radiation treatment. The adaptive elements to their treatment include re-planning to redistribute dose through normal tissue based on SPECT scan and within tumor based on FDG PET.

Objectives/Analysis:
Primary aims:
Establish the feasibility of the proposed adaptive treatment strategy

To obtain preliminary estimates of the toxicity of and doses achievable by the proposed response-driven adaptive treatment strategy in patients with Stage II/III NSCLC.

The first primary aim is feasibility which is defined as the ability to successfully deliver the full treatment including all adaptations. In the simplest analyses, feasibility will simply be summarized as the proportion of patients for whom the intended treatment was feasible. Feasibility is defined as the ability to successfully replan the patient based on the mid-treatment imaging. Whether or not this occurs will be recorded for each enrolled patient. The reasons for lack of feasibility for any patients will be investigated individually.

Two types of toxicity are of primary interest, lung toxicity and esophageal toxicity. The proportion of patients who experience these types of toxicity will be summarized by grade, and the proportion of patients who experience G2+ lung toxicity and the proportion of patients who experience G2+ esophageal toxicity will be calculated and reported with 90% confidence intervals. In terms of achievable doses, the mean dose to the PET avid region will be summarized across patients and the proportion of patients able to receive doses of at least 70 and 75 Gy will be reported. In addition, we will generate the treatment plan (and hence dose to PET avid region) each of these patients would have received had they been treated on UMCC 2007-123, which redistributed dose to the PET avid region but not through normal tissue. These dose values will then be compared to the doses actually given to assess for any mean differences.

Secondary aim 1: To obtain preliminary estimates of local control from an adaptive approach.

Local control is defined as the absence of local/regional progression (as defined in section 2.3) and will be summarized as a time-to-event endpoint. Patients without local progression will be censored at the last date they were assessed for local progression. Local control will be summarized with the Kaplan-Meier method. Our plan is follow this pilot trial with a randomized phase II trial where the primary aim would be to demonstrate improved local control in the adaptive arm (similar treatment to that on this protocol) relative to the control arm at the same overall level of toxicity. Data from this pilot trial will provide an estimate of the local control achievable with the proposed adaptive treatment strategy that will be important to have when designing and powering the phase II study.

Secondary aim 2: To determine the residual uncertainties in V/Q SPECT scans related to the accuracy with which persistent tumor subvolumes and the spatial distribution of local function of uninvolved lung could be mapped to guide plan modification.

The mapping is done by (deformable) image registration using standard in-house software. For any given mapping method (e.g. from SPECT-CT space to the patient's planning CT), there are a series of locations (either "voxels" of a given size or subregions) that contain functional information, and their use in the anatomic space of the patient (for treatment planning/delivery) requires a spatially-variant transform, with varying accuracy at different locations. We will characterize the uncertainly in local mapping using a large number of expert-extracted landmark locations, visibly assessed on the reference (e.g. planning CT) and test (e.g. guiding CT for SPECT) images. By manually measuring these locations, we have a reference library of known transformations (locations for both scans) for characterizing the uncertainty in any mapping method. These locations are not laid out on a uniform spatial grid, but they should be dense (on the order of 10^2 locations per case, with ~15 cases planned for lung and potentially as many for liver if needed as a secondary goal). We will utilize two methods for characterizing the error or uncertainty in the V/Q SPECT scans.

1) As a function of distance from the landmarks (e.g. interpolation).
2) A function of local driving information, which depends on the nature of alignment (e.g. intensity-driven seeks out intensity gradients, FEM uses forces applied to specific surfaces).

Justification of Design:

The primary aims are to demonstrate feasibility and to obtain a preliminary estimate of the toxicity associated with the adaptive treatment strategy. With 35 patients, the half-width of the 90% confidence interval will be at most 0.11 with greater than 80% probability when the true probability of toxicity is 15%. A related goal is to ensure that the adaptive treatment strategy is not overly toxic. If the true rate of Grade 2+ lung toxicity is 15%, then 35 patients gives 80% power to rule out toxicity rates of 30% or higher at a type 1 error rate of 10%.

11.0 REPORTING ADVERSE EVENTS

11.1 Adverse Event definitions

11.1.1 An Adverse Event is any untoward medical event that occurs in a patient who has received an investigational treatment, and does not necessarily have a causal relationship with the investigational treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational treatment, whether or not related to the treatment.

11.1.2 Pre-existing diseases or symptoms or abnormal laboratory values present upon recruitment are not considered an AE even when observed during the further course of the study. However, every worsening of a pre-existing condition is considered as an adverse event.

11.1.3 All grade 3 and above AEs will be collected, in addition to all grade 1 and above lung/thoracic/treatment AEs. The NCI CTCAE v4.0 will be utilized to grade AE's for AE reporting.

11.1.4 During the course of an adverse event, severity and/or causality and/or seriousness may change. For CRF documentation this adverse event represents one entity from onset to resolution and the worst of the observed categories shall be attributed.

11.1.5 When event reoccurs after it disappeared, it should be handled as a new AE. However, AEs that occur intermittently can be recorded as one AE.

11.1.6 A serious adverse event (SAE) shall be defined as an adverse advent which fulfills one or more of the following criteria:

- Results in death

- Is immediately life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent 1 of the outcomes listed above.

Any events or hospitalizations that are unequivocally due to progression of disease should not be reported as a SAE. The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigators and will be labeled Definitely related to treatment, Probably related to treatment, Possibly related to treatment, Unlikely related to treatment or Not related to treatment.

11.1.7 Only adverse events deemed serious and related will be reported to the IRB and the PI within 10 days of awareness of the event (see below SAE reporting procedures). All other events will be noted in the patient's medical record.

11.1.8 Adverse events will no longer be reported if the patient has another lung-directed therapy or starts chemotherapy.

11.1.9 The following types of hospitalizations do not constitute SAEs:

- Hospitalization or Emergency room visits secondary to expected cancer morbidity: Admission for palliative care or pain management
- Planned hospitalizations for surgical procedures, either related or unrelated to the patient's cancer.

11.2 Serious Adverse Event Reporting

All serious adverse events (SAEs) and unanticipated problems (UPs) related to the study therapy, will be reported to the Principal Investigator and also to the Coordinating Center. SAEs and UPs must be reported to the Coordinating Center within 10 days of first awareness of the event. Events should be reported using the CTO SAE form as available in the study database (Velos) A copy of the **CTO SAE form** should be sent to the Coordinating Center via fax or email to CTSU-Oncology-Multisite@med.umich.edu within 10 days of the site's knowledge of the event.

Contact information for Principal Investigator SAE Reporting:

Name: Shruti Jolly, MD
Telephone: 734-936-7810
Fax: 734-763-7370
Email: snrutij@med.umich.edu

Follow-up information must also be reported within 10 days of receipt of the information by the investigator.

All SAEs and UPs will be reported to the IRB per current institutional standards.

The Coordinating Center will disseminate information regarding SAEs and UPs to the participating sites within 5 days of review of the information by the Coordinating Center's Principal Investigator (or designee in the event of extended absence).

12.0 DATA AND SAFETY MONITORING

This trial will be monitored in accordance with the NCI approved University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Plan. This committee is responsible for monitoring the safety and data integrity of the trial.

Each participating site is required to have its own Data and Safety Monitoring Committee (DSMC) for the study. The study specific Data and Safety Monitoring Committee (DSMC), consisting of the protocol investigators, data manager or designee and other members of the study team involved with the conduct of the trial, will meet quarterly or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness.

These meetings are to be documented by the site data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the site principal investigator. Each site is required to submit the completed DSMR to the Multi-Site Coordinator at the University of Michigan Clinical Trials Office on a quarterly basis together with other pertinent documents.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of both the sites data manager or study coordinator and the site principal investigator. These reports are to be sent to the University of Michigan Clinical Trials Office within 7 calendar days of awareness of the event and on a quarterly basis with the Protocol Specific Data and Safety Monitoring Report.

For the specimen collections for biomarker analysis, we anticipate that patients may not be able to provide all requested specimens at every planned collection. This may result in missing specimens, however we will not consider this a protocol deviation.

The Clinical Trials Office is responsible for collating all the Data and Safety Monitoring Reports from all the participating sites, and providing the information to the Data Safety Monitoring Board.

13.0 DATA MANAGMENT

All information will be recorded locally and entered into Case Report Forms (CRFs) on the web-based Velos data management system of the University of Michigan. Online access will be provided to each site by the Coordinating Center.

CRFs will be reviewed and source verified by the MSC during annual monitoring visits and prior to and between visits. Discrepant, unusual and incomplete data will be queried by the MSC. The investigator or study coordinator will be responsible for providing resolutions to the data queries, as appropriate. The investigator must ensure that all data queries are dealt with promptly.

The data submission schedule is as follows:

- At the time of registration
  - Subject entry into Velos
    - Subject Status
    - Demographics
- During study participation
  - All data should be entered online within 10 business days of data acquisition. Information on Serious Adverse Events must be entered within the reporting timeframe specified in Section *11.2* of the protocol.

Long term data will be collected periodically either by chart review or by contacting the patients.

All study information should be recorded in an appropriate source document (e.g. clinic chart).

14.0 QUALITY ASSURANCE AND AUDITS

The Data Safety Monitoring Board can request a ‘for cause’ audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues. A “for cause” audit would be

conducted by the Quality Assurance Review Committee (QARC) of the University of Michigan Comprehensive Cancer Center.

A regulatory authority may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Clinical Trials Office that such a request has been made.

15.0 CLINICAL MONITORING PROCEDURES

Clinical studies coordinated by The University of Michigan Comprehensive Cancer Center (UMCCC) must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements.

This study will be monitored by a representative of the Coordinating Center of the UMCCC. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the Coordinating Center. This will be done as an actual site visit; teleconference, videoconference, or web-based meeting after the site has been given access to the study database and assembled a study reference binder. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting. Study–related questions or issues identified during the site initiation meeting will be followed-up by the appropriate UMCCC personnel until they have been answered and resolved.

Monitoring of this study will include both 'Centralized Monitoring', the review of source documents at the Coordinating Center and 'On-site Monitoring', an actual site visit. The first 'Centralized' visit should occur after the first subject enrolled completes chemotherapy/RT. The study site should send the de-identified source documents to the Coordinating Center for monitoring. 'Centralized' monitoring may be requested by the Coordinating Center if an amendment requires changes to the protocol procedures. The site will send in pertinent de-identified source documents, as defined by the Coordinating Center for monitoring.

The first annual 'On-site' monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The annual visit may be conducted as a 'Centralized' visit if less than three subjects have enrolled at the study site. The type of visit is at the discretion of the Coordinating Center. At a minimum, a routine monitoring visit will be done at least once a year, or once during the course of the study if the study duration is less than 12 months. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the Coordinating Center representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The Coordinating Center expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner. For review of study-related documents at the Coordinating Center, the site will be required to ship or fax documents to be reviewed.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit.

16.0 REFERENCES:

1. Siegel R, Desantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012;62(4):220-41. Epub 2012/06/16. doi: 10.3322/caac.21149. PubMed PMID: 22700443.

2. Tyldesley S, Boyd C, Schulze K, Walker H, Mackillop WJ. Estimating the need for radiotherapy for lung cancer: an evidence-based, epidemiologic approach. Int J Radiat Oncol Biol Phys. 2001;49(4):973-85. Epub 2001/03/10. doi: S0360-3016(00)01401-2 [pii]. PubMed PMID: 11240238.

3. Aupérin A, Le Péchoux C, Rolland E, Curran WJ, Furuse K, Fournel P, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(13):2181-90. doi: 10.1200/JCO.2009.26.2543. PubMed PMID: 20351327.
4. Curran WJ, Jr., Paulus R, Langer CJ, Komaki R, Lee JS, Hauser S, et al. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. J Natl Cancer Inst. 2011;103(19):1452-60. Epub 2011/09/10. doi: 10.1093/jnci/djr325
djr325 [pii]. PubMed PMID: 21903745; PubMed Central PMCID: PMC3186782.
5. Perez CA, Stanley K, Rubin P, Kramer S, Brady L, Perez-Tamayo R, et al. A prospective randomized study of various irradiation doses and fractionation schedules in the treatment of inoperable non-oat-cell carcinoma of the lung. Preliminary report by the Radiation Therapy Oncology Group. Cancer. 1980;45(11):2744-53. PubMed PMID: 6991092.
6. Perez CA, Pajak TF, Rubin P, Simpson JR, Mohiuddin M, Brady LW, et al. Long-term observations of the patterns of failure in patients with unresectable non-oat cell carcinoma of the lung treated with definitive radiotherapy. Report by the Radiation Therapy Oncology Group. Cancer. 1987;59(11):1874-81. PubMed PMID: 3032394.
7. Vijayakumar S, Chen GT. Implementation of three dimensional conformal radiation therapy: prospects, opportunities, and challenges. Int J Radiat Oncol Biol Phys. 1995;33(5):979-83. PubMed PMID: 7493859.
8. Hayman JA, Martel MK, Ten Haken RK, Normolle DP, Todd RF, 3rd, Littles JF, et al. Dose escalation in non-small-cell lung cancer using three-dimensional conformal radiation therapy: update of a phase I trial. J Clin Oncol. 2001;19(1):127-36. PubMed PMID: 11134205.
9. Maguire PD, Marks LB, Sibley GS, Herndon JE, 2nd, Clough RW, Light KL, et al. 73.6 Gy and beyond: hyperfractionated, accelerated radiotherapy for non-small-cell lung cancer. J Clin Oncol. 2001;19(3):705-11. PubMed PMID: 11157021.
10. Rosenzweig KE, Sim SE, Mychalczak B, Braban LE, Schindelheim R, Leibel SA. Elective nodal irradiation in the treatment of non-small-cell lung cancer with three-dimensional conformal radiation therapy. Int J Radiat Oncol Biol Phys. 2001;50(3):681-5. PubMed PMID: 11395236.
11. Bradley JD, Ieumwananonthachai N, Purdy JA, Wasserman TH, Lockett MA, Graham MV, et al. Gross tumor volume, critical prognostic factor in patients treated with three-dimensional conformal radiation therapy for non-small-cell lung carcinoma. Int J Radiat Oncol Biol Phys. 2002;52(1):49-57. PubMed PMID: 11777621.
12. Belderbos JS, De Jaeger K, Heemsbergen WD, Seppenwoolde Y, Baas P, Boersma LJ, et al. First results of a phase I/II dose escalation trial in non-small cell lung cancer using three-dimensional conformal radiotherapy. Radiother Oncol. 2003;66(2):119-26. PubMed PMID: 12648783.
13. Narayan S, Henning GT, Ten Haken RK, Sullivan MA, Martel MK, Hayman JA. Results following treatment to doses of 92.4 or 102.9 Gy on a phase I dose escalation study for non-small cell lung cancer. Lung Cancer. 2004;44(1):79-88. PubMed PMID: 15013586.
14. Bogart JA, Aronowitz JN. Localized non-small cell lung cancer: adjuvant radiotherapy in the era of effective systemic therapy. Clin Cancer Res. 2005;11(13 Pt 2):5004s-10s. PubMed PMID: 16000604.
15. Bradley J, Graham MV, Winter K, Purdy JA, Komaki R, Roa WH, et al. Toxicity and outcome results of RTOG 9311: a phase I-II dose-escalation study using three-dimensional conformal radiotherapy in patients with inoperable non-small-cell lung carcinoma. Int J Radiat Oncol Biol Phys. 2005;61(2):318-28. PubMed PMID: 15667949.
16. Kong FM, Ten Haken RK, Schipper MJ, Sullivan MA, Chen M, Lopez C, et al. High-dose radiation improved local tumor control and overall survival in patients with inoperable/unresectable non-small-cell lung cancer: long-term results of a radiation dose escalation study. Int J Radiat Oncol Biol Phys. 2005;63(2):324-33. PubMed PMID: 16168827.
17. Rosenzweig KE, Fox JL, Yorke E, Amols H, Jackson A, Rusch V, et al. Results of a phase I dose-escalation study using three-dimensional conformal radiotherapy in the treatment of inoperable nonsmall cell lung carcinoma. Cancer. 2005;103(10):2118-27. PubMed PMID: 15830346.
18. Chen M, Hayman JA, Ten Haken RK, Tatro D, Fernando S, Kong FM. Long-term results of high-dose conformal radiotherapy for patients with medically inoperable T1-3N0 non-small-cell lung cancer: is low incidence of regional failure due to incidental nodal irradiation? Int J Radiat Oncol Biol Phys. 2006;64(1):120-6. Epub 2005/10/04. doi: S0360-3016(05)01160-0 [pii]
10.1016/j.ijrobp.2005.06.029. PubMed PMID: 16198503.
19. Kong FM, Pan C, Eisbruch A, Ten Haken RK. Physical models and simpler dosimetric descriptors of radiation late toxicity. Semin Radiat Oncol. 2007;17(2):108-20. Epub 2007/03/31. doi: S1053-4296(06)00108-1 [pii]

10.1016/j.semradonc.2006.11.007. PubMed PMID: 17395041.
20. Bradley JD, Paulus R, Graham MV, Ettinger DS, Johnstone DW, Pilepich MV, et al. Phase II trial of postoperative adjuvant paclitaxel/carboplatin and thoracic radiotherapy in resected stage II and IIIA non-small-cell lung cancer: promising long-term results of the Radiation Therapy Oncology Group--RTOG 9705. J Clin Oncol. 2005;23(15):3480-7. PubMed PMID: 15908657.
21. Rengan R, Rosenzweig KE, Venkatraman E, Koutcher LA, Fox JL, Nayak R, et al. Improved local control with higher doses of radiation in large-volume stage III non-small-cell lung cancer. Int J Radiat Oncol Biol Phys. 2004;60(3):741-7. PubMed PMID: 15465190.
22. Machtay M, Hsu C, Komaki R, Sause WT, Swann RS, Langer CJ, et al. Effect of overall treatment time on outcomes after concurrent chemoradiation for locally advanced non-small-cell lung carcinoma: analysis of the Radiation Therapy Oncology Group (RTOG) experience. Int J Radiat Oncol Biol Phys. 2005;63(3):667-71. PubMed PMID: 15927409.
23. Machtay M, Bae K, Movsas B, Paulus R, Gore EM, Komaki R, et al. Higher biologically effective dose of radiotherapy is associated with improved outcomes for locally advanced non-small cell lung carcinoma treated with chemoradiation: an analysis of the Radiation Therapy Oncology Group. Int J Radiat Oncol Biol Phys. 2012;82(1):425-34. Epub 2010/10/29. doi: 10.1016/j.ijrobp.2010.09.004. PubMed PMID: 20980108.
24. Zhao L, West BT, Hayman JA, Lyons S, Cease K, Kong FM. High radiation dose may reduce the negative effect of large gross tumor volume in patients with medically inoperable early-stage non-small cell lung cancer. Int J Radiat Oncol Biol Phys. 2007;68(1):103-10. Epub 2007/03/17. doi: S0360-3016(06)03660-1 [pii]
10.1016/j.ijrobp.2006.11.051. PubMed PMID: 17363189.
25. Kong FM, Haken RT, Hayman J, Ramnath N, Hassan K, Wang J, et al. Personalized High Dose Radiation (>70 Gy) Is Significantly Associated with Better Local Regional Control and Overall Survival in Non-small Cell Lung Cancer Treated with Concurrent Chemoradiation. Int J Radiat Oncol Biol Phys. 2011;81(2, supplement):S594.
26. Hicks RJ, Mac Manus MP, Matthews JP, Hogg A, Binns D, Rischin D, et al. Early FDG-PET imaging after radical radiotherapy for non-small-cell lung cancer: inflammatory changes in normal tissues correlate with tumor response and do not confound therapeutic response evaluation. Int J Radiat Oncol Biol Phys. 2004;60(2):412-8. Epub 2004/09/24. doi: 10.1016/j.ijrobp.2004.03.036. PubMed PMID: 15380574.
27. Kong FM, Frey KA, Quint LE, Ten Haken RK, Hayman JA, Kessler M, et al. A pilot study of [18F]fluorodeoxyglucose positron emission tomography scans during and after radiation-based therapy in patients with non small-cell lung cancer. J Clin Oncol. 2007;25(21):3116-23. Epub 2007/07/20. doi: 10.1200/JCO.2006.10.3747. PubMed PMID: 17634490.
28. Weber WA, Petersen V, Schmidt B, Tyndale-Hines L, Link T, Peschel C, et al. Positron emission tomography in non-small-cell lung cancer: prediction of response to chemotherapy by quantitative assessment of glucose use. J Clin Oncol. 2003;21(14):2651-7. PubMed PMID: 12860940.
29. Nahmias C, Hanna WT, Wahl LM, Long MJ, Hubner KF, Townsend DW. Time course of early response to chemotherapy in non-small cell lung cancer patients with 18F-FDG PET/CT. J Nucl Med. 2007;48(5):744-51. PubMed PMID: 17475962.
30. Hoekstra CJ, Stroobants SG, Smit EF, Vansteenkiste J, van Tinteren H, Postmus PE, et al. Prognostic relevance of response evaluation using [18F]-2-fluoro-2-deoxy-D-glucose positron emission tomography in patients with locally advanced non-small-cell lung cancer. J Clin Oncol. 2005;23(33):8362-70. PubMed PMID: 16293866.
31. de Geus-Oei LF, van der Heijden HF, Visser EP, Hermsen R, van Hoorn BA, Timmer-Bonte JN, et al. Chemotherapy response evaluation with 18F-FDG PET in patients with non-small cell lung cancer. J Nucl Med. 2007;48(10):1592-8. PubMed PMID: 17873138.
32. Dimitrakopoulou-Strauss A, Hoffmann M, Bergner R, Uppenkamp M, Eisenhut M, Pan L, et al. Prediction of short-term survival in patients with advanced nonsmall cell lung cancer following chemotherapy based on 2-deoxy-2-[F-18]fluoro-D-glucose-positron emission tomography: a feasibility study. Mol Imaging Biol. 2007;9(5):308-17. PubMed PMID: 17623254.
33. van Baardwijk A, Bosmans G, Dekker A, van Kroonenburgh M, Boersma L, Wanders S, et al. Time trends in the maximal uptake of FDG on PET scan during thoracic radiotherapy. A prospective study in locally advanced non-small cell lung cancer (NSCLC) patients. Radiother Oncol. 2007;82(2):145-52. PubMed PMID: 17258339.
34. Feng M, Kong FM, Gross M, Fernando S, Hayman JA, Ten Haken RK. Using fluorodeoxyglucose positron emission tomography to assess tumor volume during radiotherapy for non-small-cell lung cancer and its potential impact on adaptive dose escalation and normal tissue sparing. Int J Radiat Oncol Biol Phys. 2009;73(4):1228-34. Epub 2009/03/03. doi: S0360-3016(08)03726-7 [pii]

10.1016/j.ijrobp.2008.10.054. PubMed PMID: 19251094.
35. Kong FM, Mahasittiwat P, Yuan S, Xie C, Ritter T, Shen Z, et al. Define tumor volume during radiotherapy to individualize adaptive radiation dose escalation in non-small cell lung cancer. Imaging for Treatment Assessment in Radiation Therapy(ITART) 2010;Abstract 14327.
36. Kong FP, Ten Haken RK, Hayman JA, Cease K, Ramnath N, Arenberg D, et al. Can We Deliver High Dose Radiation in 6 Weeks with Concurrent Chemotherapy in Stage III Non-small Cell Lung Cancer? Int J Radiat Oncol Biol Phys. 2010;78(3, Supplement):S35.
37. Shioyama Y, Jang SY, Liu HH, Guerrero T, Wang X, Gayed IW, et al. Preserving functional lung using perfusion imaging and intensity-modulated radiation therapy for advanced-stage non-small cell lung cancer. Int J Radiat Oncol Biol Phys. 2007;68(5):1349-58. Epub 2007/04/21. doi: S0360-3016(07)00310-0 [pii]
10.1016/j.ijrobp.2007.02.015. PubMed PMID: 17446001.
38. Lavrenkov K, Christian JA, Partridge M, Niotsikou E, Cook G, Parker M, et al. A potential to reduce pulmonary toxicity: the use of perfusion SPECT with IMRT for functional lung avoidance in radiotherapy of non-small cell lung cancer. Radiother Oncol. 2007;83(2):156-62. PubMed PMID: 17493699.
39. Seppenwoolde Y, Engelsman M, De Jaeger K, Muller SH, Baas P, McShan DL, et al. Optimizing radiation treatment plans for lung cancer using lung perfusion information. Radiother Oncol. 2002;63(2):165-77. PubMed PMID: 12063006.
40. McGuire SM, Zhou S, Marks LB, Dewhirst M, Yin FF, Das SK. A methodology for using SPECT to reduce intensity-modulated radiation therapy (IMRT) dose to functioning lung. Int J Radiat Oncol Biol Phys. 2006;66(5):1543-52. Epub 2006/11/28. doi: S0360-3016(06)02757-X [pii]
10.1016/j.ijrobp.2006.07.1377. PubMed PMID: 17126212.
41. Gayed IW, Chang J, Kim EE, Nunez R, Chasen B, Liu HH, et al. Lung perfusion imaging can risk stratify lung cancer patients for the development of pulmonary complications after chemoradiation. J Thorac Oncol. 2008;3(8):858-64. Epub 2008/08/02. doi: 10.1097/JTO.0b013e31818020d5
01243894-200808000-00008 [pii]. PubMed PMID: 18670303.
42. Christian JA, Partridge M, Nioutsikou E, Cook G, McNair HA, Cronin B, et al. The incorporation of SPECT functional lung imaging into inverse radiotherapy planning for non-small cell lung cancer. Radiother Oncol. 2005;77(3):271-7. Epub 2005/11/09. doi: S0167-8140(05)00428-7 [pii]
10.1016/j.radonc.2005.08.008 [doi]. PubMed PMID: 16274762.
43. Lavrenkov K, Singh S, Christian JA, Partridge M, Nioutsikou E, Cook G, et al. Effective avoidance of a functional spect-perfused lung using intensity modulated radiotherapy (IMRT) for non-small cell lung cancer (NSCLC): an update of a planning study. Radiother Oncol. 2009;91(3):349-52. Epub 2008/11/11. doi: S0167-8140(08)00539-2 [pii]
10.1016/j.radonc.2008.10.005. PubMed PMID: 18995919.
44. Das SK, Miften MM, Zhou S, Bell M, Munley MT, Whiddon CS, et al. Feasibility of optimizing the dose distribution in lung tumors using fluorine-18-fluorodeoxyglucose positron emission tomography and single photon emission computed tomography guided dose prescriptions. Med Phys. 2004;31(6):1452-61. Epub 2004/07/21. PubMed PMID: 15259648.
45. Yuan S, Kirk FA, Gross MD, Morris DE, Hayes DN, Halle J, et al. Semi-quantification and classification of local pulmonary function by V/Q SPECT in patients with non-small cell lung cancer. J Thorac Oncol. 2011;6(1):pending. Epub 2011/02/20. doi: 10.1097/JTO.0b013e3181915028
01243894-200901000-00012 [pii]. PubMed PMID: 19096310.
46. Yuan S, Frey KA, Gross M, Hayman J, Arenberg D, Cai X. Changes in global function and regional ventilation and perfusion on SPECT during the course of radiotherapy in patients with non-small-cell lung cancer. Int J Radiat Oncol Biol Phys. 2012;82(4):e631-8.
47. Mascarenhas F, Silvestre ME, Sa da Costa M, Grima N, Campos C, Chaves P. Acute secondary effects in the esophagus in patients undergoing radiotherapy for carcinoma of the lung. Am J Clin Oncol. 1989;12(1):34-40. Epub 1989/02/01. PubMed PMID: 2536215.
48. Choy H, LaPorte K, Knill-Selby E, Mohr P, Shyr Y. Esophagitis in combined modality therapy for locally advanced non-small cell lung cancer. Semin Radiat Oncol. 1999;9(2 Suppl 1):90-6. Epub 1999/04/21. PubMed PMID: 10210546.
49. Belderbos J, Heemsbergen W, Hoogeman M, Pengel K, Rossi M, Lebesque J. Acute esophageal toxicity in non-small cell lung cancer patients after high dose conformal radiotherapy. Radiother Oncol. 2005;75(2):157-64. Epub 2005/05/14. doi: S0167-8140(05)00128-3 [pii]
10.1016/j.radonc.2005.03.021. PubMed PMID: 15890421.
50. Maguire PD, Sibley GS, Zhou SM, Jamieson TA, Light KL, Antoine PA, et al. Clinical and dosimetric predictors of radiation-induced esophageal toxicity. Int J Radiat Oncol Biol Phys. 1999;45(1):97-103. PubMed PMID: 10477012.

51. Takeda K, Nemoto K, Saito H, Ogawa Y, Takai Y, Yamada S. Dosimetric correlations of acute esophagitis in lung cancer patients treated with radiotherapy. Int J Radiat Oncol Biol Phys. 2005;62(3):626-9. Epub 2005/06/07. doi: S0360-3016(05)00605-X [pii]
10.1016/j.ijrobp.2005.04.004. PubMed PMID: 15936536.
52. Challand T, Thureau S, Dubray B, Giraud P. [Esophageal toxicity of radiation therapy: clinical risk factors and management]. Cancer Radiother. 2012;16(5-6):364-71. Epub 2012/08/29. doi: 10.1016/j.canrad.2012.07.180
S1278-3218(12)00337-X [pii]. PubMed PMID: 22925486.
53. Bradley J, Deasy JO, Bentzen S, El-Naqa I. Dosimetric correlates for acute esophagitis in patients treated with radiotherapy for lung carcinoma. Int J Radiat Oncol Biol Phys. 2004;58(4):1106-13. Epub 2004/03/06. doi: 10.1016/j.ijrobp.2003.09.080
S0360301603020236 [pii]. PubMed PMID: 15001251.
54. Wei X, Liu HH, Tucker SL, Liao Z, Hu C, Mohan R, et al. Risk factors for acute esophagitis in non-small-cell lung cancer patients treated with concurrent chemotherapy and three-dimensional conformal radiotherapy. Int J Radiat Oncol Biol Phys. 2006;66(1):100-7. Epub 2006/07/15. doi: S0360-3016(06)00701-2 [pii]
10.1016/j.ijrobp.2006.04.022. PubMed PMID: 16839700.
55. Kong FM, Ten Haken R, Eisbruch A, Lawrence TS. Non-small cell lung cancer therapy-related pulmonary toxicity: an update on radiation pneumonitis and fibrosis. Semin Oncol. 2005;32(2 Suppl 3):S42-54. PubMed PMID: 16015535.
56. Yi ES, Bedoya A, Lee H, Chin E, Saunders W, Kim SJ, et al. Radiation-induced lung injury in vivo: expression of transforming growth factor-beta precedes fibrosis. Inflammation. 1996;20(4):339-52. PubMed PMID: 8872498.
57. Anscher MS, Kong FM, Marks LB, Bentel GC, Jirtle RL. Changes in plasma transforming growth factor beta during radiotherapy and the risk of symptomatic radiation-induced pneumonitis. Int J Radiat Oncol Biol Phys. 1997;37(2):253-8. PubMed PMID: 9069294.
58. Bai YH, Wang DW, Cui XM, Long JY, Cui CB, Jia CY, et al. Expression of transforming growth factor beta in radiation interstitial pneumonitis. J Environ Pathol Toxicol Oncol. 1997;16(1):15-20. PubMed PMID: 9256927.
59. Rube CE, Uthe D, Schmid KW, Richter KD, Wessel J, Schuck A, et al. Dose-dependent induction of transforming growth factor beta (TGF-beta) in the lung tissue of fibrosis-prone mice after thoracic irradiation. Int J Radiat Oncol Biol Phys. 2000;47(4):1033-42. PubMed PMID: 10863076.
60. Vujaskovic Z, Marks LB, Anscher MS. The physical parameters and molecular events associated with radiation-induced lung toxicity. Semin Radiat Oncol. 2000;10(4):296-307. PubMed PMID: 11040330.
61. Kong FM, Anscher MS, Sporn TA, Washington MK, Clough R, Barcellos-Hoff MH, et al. Loss of heterozygosity at the mannose 6-phosphate insulin-like growth factor 2 receptor (M6P/IGF2R) locus predisposes patients to radiation-induced lung injury. Int J Radiat Oncol Biol Phys. 2001;49(1):35-41. PubMed PMID: 11163495.
62. De Jaeger K, Seppenwoolde Y, Kampinga HH, Boersma LJ, Belderbos JS, Lebesque JV. Significance of plasma transforming growth factor-beta levels in radiotherapy for non-small-cell lung cancer. Int J Radiat Oncol Biol Phys. 2004;58(5):1378-87. PubMed PMID: 15050313.
63. Stenmark M, Cai X, Shedden K, Hayman J, Yuan S, Ritter T, et al. Combining Physical and Biologic Parameters to Predict Radiation-Induced Lung Toxicity in Patients With Non-Small-Cell Lung Cancer Treated With Definitive Radiotherapy. Int J Radiat Oncol Biol Phys. In press.
64. Xu H, Zhao L, Bi N, Wang J, Wang L. The Combination of Elevation of Plasma TGF-β1 During Radiation Therapy and Preradiation Cyfra21-1 Level Predicts Radiation-induced Lung Toxicity in Patients With Non-small Cell Lung Cancer int J Radiat Oncol Biol Phys. 2012;83(3 (suppl)):S611.
65. Hart JP, Broadwater G, Rabbani Z, Moeller BJ, Clough R, Huang D, et al. Cytokine profiling for prediction of symptomatic radiation-induced lung injury. Int J Radiat Oncol Biol Phys. 2005;63(5):1448-54. Epub 2005/08/24. doi: 10.1016/j.ijrobp.2005.05.032. PubMed PMID: 16115739.
66. Kong FM, Ao X, Wang L, Lawrence TS. The use of blood biomarkers to predict radiation lung toxicity: a potential strategy to individualize thoracic radiation therapy. Cancer Control. 2008;15(2):140-50. Epub 2008/04/01. PubMed PMID: 18376381.
67. Dillman RO, Herndon J, Seagren SL, Eaton WL, Jr., Green MR. Improved survival in stage III non-small-cell lung cancer: seven-year follow-up of cancer and leukemia group B (CALGB) 8433 trial. J Natl Cancer Inst. 1996;88(17):1210-5. PubMed PMID: 8780630.
68. Sause W, Kolesar P, Taylor SI, Johnson D, Livingston R, Komaki R, et al. Final results of phase III trial in regionally advanced unresectable non-small cell lung cancer: Radiation Therapy Oncology Group,

Eastern Cooperative Oncology Group, and Southwest Oncology Group. Chest. 2000;117(2):358-64. PubMed PMID: 10669675.
69. Marino P, Preatoni A, Cantoni A. Randomized trials of radiotherapy alone versus combined chemotherapy and radiotherapy in stages IIIa and IIIb nonsmall cell lung cancer. A meta-analysis. Cancer. 1995;76(4):593-601. PubMed PMID: 8625152.
70. Pritchard RS, Anthony SP. Chemotherapy plus radiotherapy compared with radiotherapy alone in the treatment of locally advanced, unresectable, non-small-cell lung cancer. A meta-analysis. Ann Intern Med. 1996;125(9):723-9. PubMed PMID: 8929005.
71. Le Chevalier T, Arriagada R, Quoix E, Ruffie P, Martin M, Douillard JY, et al. Radiotherapy alone versus combined chemotherapy and radiotherapy in unresectable non-small cell lung carcinoma. Lung Cancer. 1994;10 Suppl 1:S239-44. PubMed PMID: 8087515.
72. Furuse K, Fukuoka M, Kawahara M, Nishikawa H, Takada Y, Kudoh S, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. J Clin Oncol. 1999;17(9):2692-9. PubMed PMID: 10561343.
73. Curran WJ SC, Langer CJ, Komaki R, Lee JS, Hauser S, et al. Long-term benefit is observed in a phase III comparison of sequential vs. concurrent chemo-radiation for patients with unresected stage III NSCLC: RTOG 9410 [abstract]. Proc Am Soc Clin Oncol. 2003; 22:621.
74. Zatloukal P, Petruzelka L, Zemanova M, Havel L, Janku F, Judas L, et al. Concurrent versus sequential chemoradiotherapy with cisplatin and vinorelbine in locally advanced non-small cell lung cancer: a randomized study. Lung Cancer. 2004;46(1):87-98. PubMed PMID: 15364136.
75. Rosenman JG, Halle JS, Socinski MA, Deschesne K, Moore DT, Johnson H, et al. High-dose conformal radiotherapy for treatment of stage IIIA/IIIB non-small-cell lung cancer: technical issues and results of a phase I/II trial. Int J Radiat Oncol Biol Phys. 2002;54(2):348-56. PubMed PMID: 12243807.
76. Vokes EE. Induction chemotherapy followed by concomitant chemoradiotherapy for non-small cell lung cancer. Oncologist. 2001;6 Suppl 1:25-7. PubMed PMID: 11182002.
77. Albain KS, Crowley JJ, Turrisi AT, 3rd, Gandara DR, Farrar WB, Clark JI, et al. Concurrent cisplatin, etoposide, and chest radiotherapy in pathologic stage IIIB non-small-cell lung cancer: a Southwest Oncology Group phase II study, SWOG 9019. J Clin Oncol. 2002;20(16):3454-60. PubMed PMID: 12177106.
78. Belani CP, Choy H, Bonomi P, Scott C, Travis P, Haluschak J, et al. Combined chemoradiotherapy regimens of paclitaxel and carboplatin for locally advanced non-small-cell lung cancer: a randomized phase II locally advanced multi-modality protocol. J Clin Oncol. 2005;23(25):5883-91. PubMed PMID: 16087941.
79. Gandara DR, Chansky K, Albain KS, Leigh BR, Gaspar LE, Lara PN, Jr., et al. Consolidation docetaxel after concurrent chemoradiotherapy in stage IIIB non-small-cell lung cancer: phase II Southwest Oncology Group Study S9504. J Clin Oncol. 2003;21(10):2004-10. PubMed PMID: 12743155.
80. Vokes EE. Optimal therapy for unresectable stage III non-small-cell lung cancer. J Clin Oncol. 2005;23(25):5853-5. PubMed PMID: 16087940.
81. Vokes E, Herndon, JE, Kelley, MJ, Watson, D, Cicchetti, MG, Green, MR. Induction chemotherapy followed by concomitant chemoradiotherapy versus chemoradiotherapy alone for regionally advanced unresectable non-small cell lung cancer: initial analysis of a randomized phase III trial. J Clin Oncol. 2004;22(14S):suppl; abstr 7005.
82. Hanna N, Neubauer M, Yiannoutsos C, McGarry R, Arseneau J, Ansari R, et al. Phase III study of cisplatin, etoposide, and concurrent chest radiation with or without consolidation docetaxel in patients with inoperable stage III non-small-cell lung cancer: the Hoosier Oncology Group and U.S. Oncology. J Clin Oncol. 2008;26(35):5755-60. Epub 2008/11/13. doi: JCO.2008.17.7840 [pii]
10.1200/JCO.2008.17.7840. PubMed PMID: 19001323.
83. Auperin A, Le Pechoux C, Rolland E, Curran WJ, Furuse K, Fournel P, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. J Clin Oncol. 2010;28(13):2181-90. Epub 2010/03/31. doi: JCO.2009.26.2543 [pii]
10.1200/JCO.2009.26.2543. PubMed PMID: 20351327.
84. Timmerman R, Paulus R, Galvin J, Michalski J, Straube W, Bradley J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. JAMA. 2010;303(11):1070-6. Epub 2010/03/18. doi: 303/11/1070 [pii]
10.1001/jama.2010.261. PubMed PMID: 20233825; PubMed Central PMCID: PMC2907644.
85. Arrieta O, Gallardo-Rincon D, Villarreal-Garza C, Michel RM, Astorga-Ramos AM, Martinez-Barrera L, et al. High frequency of radiation pneumonitis in patients with locally advanced non-small cell lung cancer treated with concurrent radiotherapy and gemcitabine after induction with gemcitabine and

carboplatin. J Thorac Oncol. 2009;4(7):845-52. Epub 2009/06/03. doi: 10.1097/JTO.0b013e3181a97e17. PubMed PMID: 19487963.
86. Yom SS, Liao Z, Liu HH, Tucker SL, Hu CS, Wei X, et al. Initial evaluation of treatment-related pneumonitis in advanced-stage non-small-cell lung cancer patients treated with concurrent chemotherapy and intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys. 2007;68(1):94-102. Epub 2007/02/27. doi: S0360-3016(06)03637-6 [pii]
10.1016/j.ijrobp.2006.12.031. PubMed PMID: 17321067.
87. Chopra RR, Bogart JA. Radiation therapy-related toxicity (including pneumonitis and fibrosis). Hematol Oncol Clin North Am. 2010;24(3):625-42. Epub 2010/05/22. doi: S0889-8588(10)00060-2 [pii]
10.1016/j.hoc.2010.03.009. PubMed PMID: 20488358.
88. Albain KS, Swann RS, Rusch VW, Turrisi AT, 3rd, Shepherd FA, Smith C, et al. Radiotherapy plus chemotherapy with or without surgical resection for stage III non-small-cell lung cancer: a phase III randomised controlled trial. Lancet. 2009;374(9687):379-86. Epub 2009/07/28. doi: S0140-6736(09)60737-6 [pii]
10.1016/S0140-6736(09)60737-6. PubMed PMID: 19632716.
89. Fournel P, Robinet G, Thomas P, Souquet PJ, Lena H, Vergnenegre A, et al. Randomized phase III trial of sequential chemoradiotherapy compared with concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer: Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Francais de Pneumo-Cancerologie NPC 95-01 Study. J Clin Oncol. 2005;23(25):5910-7. Epub 2005/08/10. doi: JCO.2005.03.070 [pii]
10.1200/JCO.2005.03.070. PubMed PMID: 16087956.
90. Cox JD, Pajak TF, Asbell S, Russell AH, Pederson J, Byhardt RW, et al. Interruptions of high-dose radiation therapy decrease long-term survival of favorable patients with unresectable non-small cell carcinoma of the lung: analysis of 1244 cases from 3 Radiation Therapy Oncology Group (RTOG) trials. Int J Radiat Oncol Biol Phys. 1993;27(3):493-8. Epub 1993/10/20. PubMed PMID: 8226140.
91. Yuan ST, Frey KA, Gross MD, Hayman JA, Arenberg D, Curtis JL, et al. Semiquantification and classification of local pulmonary function by V/Q single photon emission computed tomography in patients with non-small cell lung cancer: potential indication for radiotherapy planning. J Thorac Oncol. 2011;6(1):71-8. Epub 2010/12/02. doi: 10.1097/JTO.0b013e3181f77b40. PubMed PMID: 21119546; PubMed Central PMCID: PMC3351273.
92. Nijkamp J, Rossi M, Lebesque J, Belderbos J, van den Heuvel M, Kwint M, et al. Relating acute esophagitis to radiotherapy dose using FDG-PET in concurrent chemo-radiotherapy for locally advanced non-small cell lung cancer. Radiother Oncol. 2012. Epub 2012/12/12. doi: S0167-8140(12)00490-2 [pii]
10.1016/j.radonc.2012.09.024. PubMed PMID: 23219463.
93. Fernando S, Zhao L, Brown RKJ, Gross M, Feng M, Hayman JA, et al. Changes of Esophageal FDG Activity During Fractionated Radiation May Be Associated With Radiation Esophagitis. Int J Radiat Oncol Biol Phys. 2006;66(3 (suppl)):S485.
94. Antonia SJ1, Villegas A1, Daniel D1, Vicente D1, Murakami S1, Hui R1, Yokoi T1, Chiappori A1, Lee KH1, de Wit M1, Cho BC1, Bourhaba M1, Quantin X1, Tokito T1, Mekhail T1, Planchard D1, Kim YC1, Karapetis CS1, Hiret S1, Ostoros G1, Kubota K1, Gray JE1, Paz-Ares L1, de Castro Carpeño J1, Wadsworth C1, Melillo G1, Jiang H1, Huang Y1, Dennis PA1, Özgüroğlu M1; PACIFIC Investigators. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. N Engl J Med. 2017 Nov 16;377(20):1919-1929doi: 10.1056/NEJMoa1709937. Epub 2017 Sep 8.
95. Huang, A. C. et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature 545, 60, doi:10.1038/nature22079
96. Demaria, S., Golden, E. B. & Formenti, S. C. Role of Local Radiation Therapy in Cancer Immunotherapy. JAMA Oncology 1, 1325, doi:10.1001/jamaoncol.2015.2756 (2015).
97. Galon, J. et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. The Journal of pathology 232, 199-209, doi:10.1002/path.4287 (2014).
98. Fujimoto, D. et al. Alteration of PD-L1 expression and its prognostic impact after concurrent chemoradiation therapy in non-small cell lung cancer patients. Scientific Reports 7, 11373, doi:10.1038/s41598-017-11949-9 (2017).
99. Kallinteris, N. L. et al. New method for immunoprofiling of the tumor microenvironment of cancer patients by opal multiplex quantitative

17.0 APPENDICES

Appendix A: SOPs for blood and urine collection

1) Obtaining, handling, and delivery of blood specimens in Red Top tube for blood cells and tumor DNA/RNA analysis, collected at clinic

1. To avoid skin cell contamination, collect samples for this study after at least 2 mL of blood is collected into a separate "discard" tube (or alternatively after collecting tube(s) of blood for a clinical draw).
2. Draw aseptically 1 x 10 mL of venous blood into one red top serum tube, and immediately mix by gentle inversion 10 times (do not shake).
3. Tubes should be labeled with study number, subject de-identified study number, date, and time of collection.
4. Store and transport samples at **ambient, indoor temperature** (typically 18-25 °C) until delivery.

2) Obtaining, handling, and delivery of blood specimens in EDTA for blood cells and tumor DNA analysis, collected at clinic

1. To avoid skin cell contamination, collect samples for this study after at least 2 mL of blood is collected into a separate "discard" tube (or alternatively after collecting tube(s) of blood for a clinical draw).
2. Draw aseptically 2 x 10 mL of venous blood into 10 mL BD Vacutainer purple top EDTA Blood Collection tubes and gently invert 5 times (<u>do not shake</u>).
3. Tubes should be labeled with study number, subject de-identified study number, date, and time of collection.
4. Store and transport EDTA tubes at **ambient, indoor temperature** (typically 18-25 °C).

3) Obtaining, handling, and delivery of blood specimens in Streck tubes for blood cells and tumor DNA/RNA analysis, collected at clinic

1. Draw aseptically 1 x 10 mL of venous blood into one Streck brown top cell-free DNA BCT tube, and immediately mix by gentle inversion 10 times (do not shake).
2. Tubes should be labeled with study number, subject de-identified study number, date, and time of collection.
3. Store and transport samples at **ambient, indoor temperature** (typically 18-25 °C) until delivery.

Deliver to Dr. Muneesh Tewari's lab as soon as possible (**within 60 min of collection**).

4) Obtaining, handling, and delivery of urine specimens at clinic

1. Ask subject to provide urine specimen in a urine hat or urinal, using the Simple Urine Collection subject instructions. (Please see below for Subject Instructions for urine collection at clinic.)

2. Preferably within 10 minutes of urination, pour urine into one urine collection container that has EDTA solution in it. Urine should be added to the collection container no more than 30 minutes after urination.
3. Screw cap on tightly and invert container twice to mix urine and EDTA.
4. Fill in the provided ID Labels (study number, patient de-identified study number, date, time of collection, and method of collection) and affix to the container with the preserved urine samples.
5. Store and deliver the urine specimens with ambient temperature packs in a Styrofoam container in light protected conditions, to Dr. Muneesh Tewari's lab within 1 hr of urine collection.

Appendix B: <u>Instructions for Collecting a Urine Sample</u>

1. Read the instructions carefully, and follow each of the steps to ensure you collect the correct specimen for the study.

2. Use the urine hat or urinal provided to you for collection.

3. Lift toilet seat and place hat on top of the toilet rim, then lower toilet seat. Make sure the hat is in the correct orientation to collect urine, not stool. Urinate into the hat. If you need to have a bowel movement, collect the urine separately.

<u>Important</u>:
Do not allow bowel movement to fall into the hat.
Do not place toilet paper into the hat.

4. A study team member will receive the urine sample from you and provide additional instructions if needed.