A pilot randomized study of lymphocyte depletion and change in lymphocyte functionality during lung stereotactic body radiation therapy (SBRT) treatment by selectively reducing irradiation of circulating blood compared to standard of care control group

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ProtTITLE: A pilot randomized study of lymphocyte depletion and change in lymphocyte functionality during lung stereotactic body radiation therapy (SBRT) treatment by selectively reducing irradiation of circulating blood compared to standard of care control group

Study disease: Non-small cell lung cancer (NSCLC)
Corresponding Organization: University of Virginia

Key Roles and Study Governance

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INVESTIGATOR'S AGREEMENT

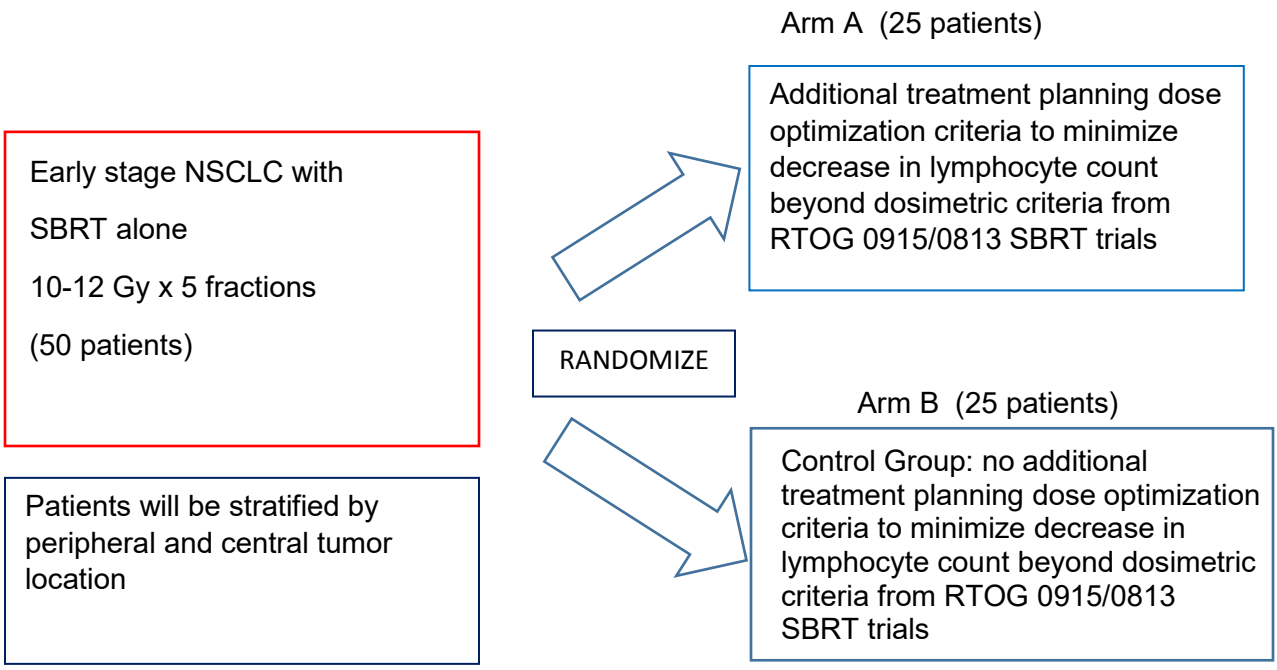
The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Principal Investigator

Krishni Wijesooriya	DocuSigned by: Krishni Wijesooriya 2ED661C3F58F48B...	11/9/2022
Name (print)	**Signature**	**Date**

SCHEMA



Abbreviations

AE	Adverse Event
AJCC	American Joint Committee on Cancer
AP/Lat	Anterior Posterior/Lateral
CBC	Complete blood count
CBCT	Cone beam computed tomography
CC DSMC	Cancer Center Data and Safety Monitoring Committee
cGy	Centigray
CI	Confidence interval
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T lymphocyte
CTV	Clinical target volume
EDC	Electronic Document Capture
ELISA	Enzyme linked immunosorbent tube assay
E/T	Effector to target ratio
FACS	Fluorescent Activated Cell Sorting
FFF	Flattening Filter Free
FSH	Follicle stimulating hormone
GBM	Glioblastoma
GCP	Good clinical practices
GTV	Gross tumor volume
Gy	Grays
HIV	Human immunodeficiency virus
HR	Hazard ratio
HRT	Hormone replacement therapy
ICH GCP	International Conference on Harmonisation Good Clinical Practice
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IGRT	Image-guided radiation therapy
IL	Interleukin
IMRT	Intensity modulated radiation therapy
IRB-HSR	Institutional Review Board-Health Sciences Research
ITV	Internal target volume
IUD	Intrauterine device
MAbs	Monoclonal antibodies
MLC	Multi Leaf Collimator
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
NTCP	Normal tissue complication probabilities
OARs	Organs-at-risk
Oncore	ON-line Clinical Oncology Research Environment
OS	Overall Survival

PBMC	Peripheral blood mononuclear cells
PET	Positron emission tomography
PFT	Pulmonary function test
PRC	Protocol Review Committee
PTV	Planning target volume
RT	Radiation therapy
RTOG	Radiation therapy oncology group
SAE	Serious adverse event
SBRT	Stereotactic body radiation therapy
SOC	Standard of care
SVC	Superior vena cava
TLC	Total lymphocyte count
TRL	Treatment related lymphopenia
UVA	University of Virginia
WOCBP	Women of childbearing potential

Table of Contents

1. SYNOPSIS

Title

A pilot randomized study of lymphocyte depletion and change in lymphocyte functionality during lung stereotactic body radiation therapy (SBRT) treatment by selectively reducing irradiation of circulating blood compared to standard of care control group

Study Description

Lymphopenia, a known consequence of radiation therapy to virtually every part of the body, was first described in the early 20th century shortly after the discovery of X-rays[1]. Radiation therapy (RT) can induce lymphopenia in the absence of concomitant chemotherapy or steroids and even when neither bone marrow nor lymphatic tissue is included in the treatment field. It is highly possible that irradiation of blood rich organs such as the great vessels, would reduce the lymphocyte count significantly. Given this known radiation-induced toxicity, circulating blood should be considered an organ at risk during irradiation, and efforts should be made to understand the toxicity from radiation to circulating blood -normal tissue complication probabilities (NTCP) so that this may be included in the optimization strategy during radiation treatment. Additionally, recent data have suggested that lymphocyte subsets exhibit differential sensitivity to radiation, with helper CD4+ T cells being more sensitive than cytotoxic CD8+ T cells in glioblastoma (GBM) treated with RT and temozolomide[2], and naïve T cells more sensitive than memory T cells in prostate cancer[3].

Based on existing data on the effects of irradiation on total lymphocyte count and the effects on subsets of T cells, we have created a lymphodepletion predictive algorithm. In this clinical trial, we will test whether optimized SBRT plans lead to lower lymphocyte depletion and whether the algorithm can accurately predict lymphocyte decreases following SBRT. Optimized SBRT plans will meet all standard of care dose-volume objectives for SBRT and for protection of organs-at-risk (OAR), but will also reduce radiation to the regional great vessels, lungs, and heart beyond what is currently optimized to reduce the integral dose to circulating blood/lymphocytes. This study will allow us to evaluate the performance of our predictive algorithm for post-SBRT decrease in lymphocyte count and to determine whether additional steps in SBRT planning will deliver lower risk of post-SBRT decreases in lymphocyte count.

Objectives and Endpoints

Table 1: Objectives and Endpoints

Objective	Endpoint(s)
Primary	
Assess if the lymphodepletion predictive algorithm developed by UVA coupled with the existing optimization algorithm in radiation treatment planning software predicts the magnitude of lymphocyte depletion in a prospective cohort of patients treated with lung SBRT.	After randomization and completion of treatment planning, the algorithm will predict the decrease in lymphocyte count for each participant (in Arms A and B) at 4 weeks after SBRT. The endpoints are:

	a) the within patient difference in the change from baseline in predicted and observed lymphocyte counts b) the within patient difference in the percent change from baseline in predicted and observed lymphocyte counts
Determine if lymphocyte-sparing SBRT planning (Arm A, investigational) results in less *in vivo* lymphocyte depletion compared to controls who receive standard SBRT planning (Arm B, control).	Observed changes in lymphocyte count from baseline and 4 weeks after completion of lung SBRT for participants in each arm. Expected lymphocyte nadir time point of 4 weeks following SBRT will be used to estimate both the actual and percentage decreases in lymphocyte counts seen in each arm.
Secondary	
Describe the adverse event profile of participants treated with and without lymphocyte-sparing SBRT planning (Arms A and B).	Frequency, severity, and duration of adverse events according to CTCAE version 5.0
Determine if our lymphodepletion predictive algorithm coupled with the existing optimization algorithm in radiation treatment planning software predicts the magnitude and trajectory of lymphocyte depletion in a prospective cohort of patients treated with lung SBRT.	After randomization and completion of treatment planning, the algorithm will predict the decrease in lymphocyte count for each participant (in Arms A and B) for each follow-up time point (at end of SBRT, and 4 weeks and 6 months after SBRT). The predicted time course of lymphocyte count will be compared with both the actual and percentage decrease in lymphocyte count observed.
Determine if lymphocyte-sparing SBRT planning (Arm A, investigational) results in less *in vivo* lymphocyte depletion after lung SBRT compared to controls who receive standard SBRT planning without extra efforts to reduce lymphocyte exposure (Arm B, control). We will quantify peripheral blood mononuclear cells collected at baseline through 6 month follow-up.	Observed changes in lymphocyte count from baseline to 6 months after completion of lung SBRT for participants in each arm. We will quantify peripheral blood mononuclear cells collected at baseline through follow-up (end of SBRT, 4 weeks and 6 months after lung SBRT).
Determine the phenotype and function of lymphocytes *in vitro* in Arm A versus Arm B patients.	1. T and B cell responses to pokeweed mitogen in polyclonal in vitro immunoglobulin synthesis; and

	2. Serum levels of immunoglobulin G (IgG) to specific recall antigens (tetanus toxoid)
Exploratory	
Determine the phenotype and function of lymphocytes *in vitro* in Arm A versus Arm B patients.	Specific anti-tumor interferon gamma (IFN-γ) EliSpot responses to lung cancer cell lines.
To evaluate overall survival (OS) after lung SBRT for up to 30 months after SBRT.	Overall survival after lung SBRT (every 6 months for the first year, then annually).
Identify correlations between lymphocyte depletion and functional impairment with dose to great vessels, lung tissue, heart, and other normal tissue containing large amounts of circulating blood.	1. Dose to circulating blood 2. TLC and functionality of T cell sub types.
Improve our existing simulation algorithm to predict the total lymphocyte loss for a given RT beam plan using the clinical trial data.	This model will take into account patient specific (i) total blood volume, (ii) initial lymphocyte count, (iii) tumor volume, (iv) the organs from the Thorax CT data set, and (v) time dependent dose map through each organ for a given delivery system. These data will be combined with general information from published literature such as (i) varying blood output to organs (ii) blood velocity for all organs (iii) random mixing of blood after each trip through the exposed dose area, with the remaining blood volume of the body.
Describe the changes in lymphocyte subtypes following SBRT in Arms A and B	Quantity of lymphocyte subtypes (e.g. CD4, CD8) prior to and 4 weeks following SBRT

Study Population

Patients who decline surgery or are considered to be medically inoperable with (American Joint Committee on Cancer (AJCC) edition 8 clinical stage NSCLC lung cancer that are clinically node negative by positron emission tomography computed tomography (PET CT) imaging and have planned treatment with SBRT as definitive therapy.

Phase of Study

Pilot

Study Sites

This is a single site study being conducted at the University of Virginia (UVA).

Intervention

Participants will receive standard of care SBRT using standard technology (treatment planning software and delivery system) that meets all requirements for tumor coverage and sparing of

dose to organs-at-risk. Participants will be randomized to one of two arms, with Arm A participants receiving lung SBRT plans that are also optimized to reduce risk and/or magnitude of decreases in lymphocyte count through reduced treatment of the great vessels beyond standard treatment objectives. Arm B participants will not receive this additional optimization (standard of care).

Study Duration

We expect it will take about 36 months to reach target accrual and data analysis/publication is expected to take an additional 12 months.

Participant Duration

Participants will be assessed prior to SBRT and then will be followed for 6 months following SBRT in order to assess changes in lymphocyte count and lymphocyte subsets. Overall survival will be assessed every 6 months for 1 year following SBRT and then annually. Results from CBC blood tests will also be collected (for participants with results available by standard of care) every 6 months.

Accrual Goal

In order to have 50 evaluable participants, as defined in *section 7.8*, up to 65 participants will be enrolled.

2. INTRODUCTION

Lymphopenia, a known consequence of radiation therapy to virtually every part of the body, was first described in the early 20th century shortly after the discovery of X-rays[1]. Radiation therapy (RT) can induce lymphopenia in the absence of concomitant chemotherapy or steroids and even when neither bone marrow nor lymphatic tissue is included in the treatment field. Studies have shown that irradiation of the brain, which contains neither bone marrow nor lymphatic tissue, can cause a greater than 60% decrease in lymphocyte count[4]. One study demonstrated that irradiation of circulating blood with cesium placed inside a shielded dialysis unit caused a 60% to 80% decrease in the number of circulating lymphocytes that persisted for many years after radiation exposure[5]. Therefore, it is highly possible that irradiation of blood rich organs, such as the great vessels, would reduce the lymphocyte count significantly. Given this known radiation-induced toxicity, circulating blood should be considered an organ at risk during irradiation, and efforts should be made to understand the toxicity from radiation to circulating blood (normal tissue complication probabilities) so that this may be included in the optimization strategy during radiation treatment.

It has also been demonstrated that in addition to radiation's direct toxicity to lymphocytes, RT also affects the immune system by altering cytokine production. In patients who have human immunodeficiency virus (HIV) or are receiving chemotherapy, there are increased levels of interleukin – 7 (IL-7). IL-7 is known to stimulate lymphocyte proliferation. However, in glioma patients who were treated with radiation and temozolomide, there was no increase in IL-7 production that would be expected given the patients' severe lymphopenia[6].

Recent studies have shown a correlation between treatment-induced lymphopenia and inferior survival in patients with glioblastoma, advanced stage non-small cell lung cancer (NSCLC), pancreatic cancer and squamous cell carcinoma of the head and neck[2,7-10]. In a study published in the *Journal of the National Comprehensive Cancer Network*, investigators collected and analyzed data from 4 independent solid tumor sites from 297 patients with newly diagnosed malignant glioma, resected and un-resected pancreatic cancer, and stage III NSCLC and recorded lymphocyte count, prognostic factors, treatment and survival. They defined treatment related lymphopenia (TRL) as <500 cells/mm^3 and found an increased risk for death attributed to TRL in each cohort (gliomas: hazard ratio [HR], 1.8; 95% confidence interval (CI), 1.13–2.87; resected pancreas: HR, 2.2; 95% CI, 1.17–4.12; unresected pancreas: HR, 2.9; 95% CI, 1.53–5.42; lung: HR, 1.7; 95% CI, 0.8–3.61) and in the entire study population regardless of pathologic findings (HR, 2.1; 95% CI, 1.54–2.78; P <.0001). They observed severe TRL in 40% of patients two months after the initiation of chemoradiation and found that it was independently associated with shorter survival from tumor progression[11]. See *Figure 1.*

RT-induced lymphopenia is likely due to the repeated exposure of radiosensitive lymphocytes to radiation, and possibly the expansion of regulatory T cells and elaboration of immunosuppressive cytokines in response to radiation. Notably, recent data have suggested that lymphocyte subsets exhibit differential sensitivity to radiation, with helper CD4+ T cells being more sensitive than cytotoxic CD8+ T cells in GBM treated with RT and temozolomide[2], and naïve T cells more sensitive than memory T cells in prostate cancer[3]. We will test the hypothesis that in optimized SBRT plans, lower lymphocyte depletion may lead to smaller decreases of lymphocyte functions.

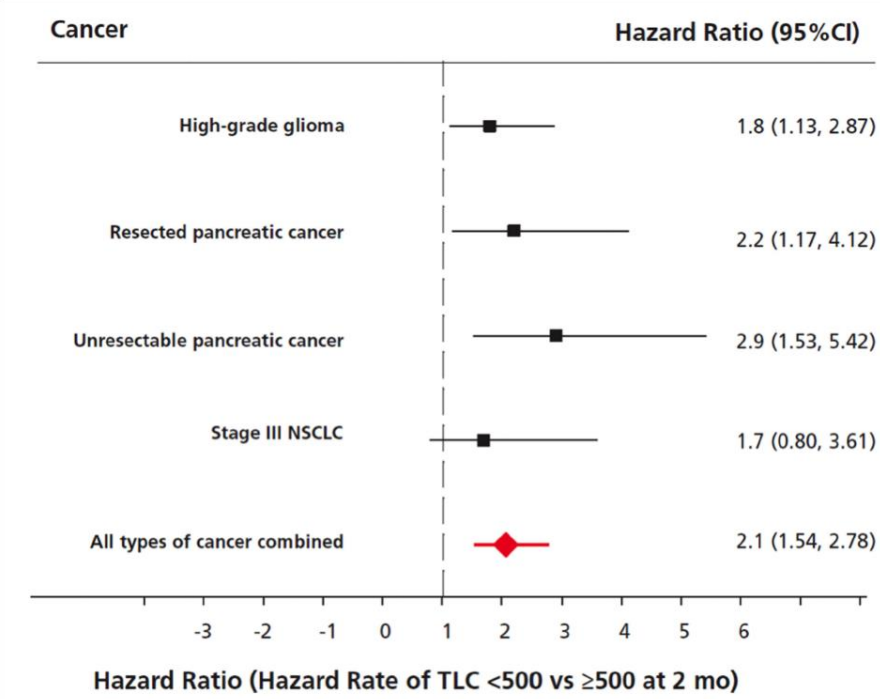


Figure 1: Relationship between survival and grade III/IV treatment-related lymphopenia in 297 patients with solid tumors.[11]

SBRT is a technique that allows high doses of radiation to be delivered to highly-focused extra-cranial targets by utilizing respiratory motion correction and daily image guidance[12]. SBRT provides an ideal setting to study radiation's effect on lymphocyte counts and function, due to the smaller target volumes which allow for flexibility in treatment planning options regarding entry and exit dose. Additionally, since this treatment does not usually include concurrent chemotherapy, it allows one to specifically evaluate radiation effects. SBRT lung optimization to reduce dose to the great vessels, lung, and heart are currently focused on maximal dose delivered to a small volume of the great vessel walls, and to the heart to reduce radiation injury to the vessel wall and intrathoracic hemorrhage. In the current study, we will continue to maintain the critical dose constraints to the heart, lung, and great vessels in both study arms, but we will also further optimize plans in the study group to reduce radiation to the regional great vessels, lungs, and heart beyond what is currently optimized to reduce the integral dose to circulating blood/lymphocytes.

While recent studies show that decreased fractionation is inversely related to total lymphocyte count (TLC) after treatment[4], there are currently no tools to predict the decrease in lymphocyte count that should be expected with different radiation treatment plans. There was no clinical data available until our recent retrospective research[13,14] showed a correlation of dose to different organs in the thorax and lymphocyte depletion. We used this data to develop an algorithm that predicts the decrease in TLC after lung SBRT treatment and we will test this model prospectively in this study. Data from this study will be used to refine the algorithm to make it more clinically robust by including functional lymphocyte effects in addition to absolute lymphocyte count effects. Ultimately, the goal will be to use the algorithm during SBRT planning to guide radiation dose optimization via utilization of predicted immune normal tissue complication probabilities in addition to dose optimization of other regional organs-at-risk (OAR) including the heart, esophagus, spinal cord, tracheobronchial system, lungs, chest wall, and brachial plexus.

Rationale for Clinical Trial – Based upon the background described above, our work to date has shown that our algorithm can predict decrease in lymphocyte count after lung SBRT based upon radiation doses delivered to vasculature during standard of care lung SBRT. By accounting for radiation dose to circulating lymphocytes, we have also shown that we can generate SBRT plans that both (1) meet all standard of care dose-volume objectives for lung SBRT *and* (2) result in substantially lower risk of post-SBRT lymphopenia by reducing dose to circulating lymphocytes within great vessels. We have developed this pilot clinical trial of 50 evaluable participants to evaluate prospectively the biological consequences of optimized plans that reduce integral dose to circulating blood during lung SBRT (and also meet national treatment planning constraints to all OAR) compared to controls with standard of care treatment with SBRT plans that meet national treatment planning constraints to all OAR. This study will allow us to evaluate the performance of our predictive algorithm for post-SBRT decrease in lymphocyte count and to determine whether additional steps in SBRT planning will deliver a lower risk of post-SBRT lymphopenia.

SBRT Summary - Participants in both arms (all 50 evaluable participants) will receive standard of care SBRT using standard technology (treatment planning software and delivery system) that meets all requirements for tumor coverage and sparing of dose to OAR for lung SBRT. Participants will be randomized to one of two arms. Arm A (25 evaluable participants) will receive lung SBRT plans that meet all standard of care SBRT requirements and are optimized to reduce risk of lymphopenia through reduced treatment of the great vessels further beyond standard treatment objectives. Participants randomized to Arm B (25 evaluable participants) will have standard lung SBRT without the addition of investigational planning steps to reduce exposure to circulating lymphocytes within the great vessels.

3. OBJECTIVES

3.1 Primary Objectives

3.1.1 Performance of Algorithm to Predict Decrease in Lymphocyte Count Post-SBRT

Determine if the lymphodepletion predictive algorithm developed by UVA coupled with the existing optimization algorithm in radiation treatment planning software predicts the magnitude of lymphocyte depletion in a prospective cohort of patients treated with lung SBRT.

Hypothesis: We hypothesize that our predictive algorithm, which estimates risk of post-SBRT lymphopenia based upon estimation of radiation dose to blood vessels, can accurately predict change in lymphocyte count at 4 weeks following SBRT in a prospective cohort of lung SBRT patients.

Endpoint: After randomization and completion of treatment planning, the algorithm will predict the decrease in lymphocyte count for each participant (in Arms A and B) at 4 weeks after SBRT. The predicted decrease will be compared with the actual decrease in lymphocyte count observed.

3.1.2 Impact of Lymphocyte-Sparing SBRT Planning Objectives on Post-SBRT Lymphocyte Count

Determine if lymphocyte-sparing SBRT planning (Arm A, investigational) results in less *in vivo* lymphocyte depletion compared to controls who receive standard SBRT planning (Arm B, control).

Hypothesis: We hypothesize that we can generate treatment plans that both meet standard of care objectives for organs-at-risk (OAR) and result in a smaller reduction in lymphocyte count by reducing exposure of circulating lymphocytes in great vessels.

Endpoint: Observed changes in lymphocyte count from baseline and 4 weeks after completion of lung SBRT for participants in each arm. Expected lymphocyte nadir time point of 4 weeks following SBRT will be used to assess changes in lymphocyte counts in each arm.

3.2 Secondary Objectives

3.2.1 Safety

Describe the adverse event profile of participants treated with and without lymphocyte-sparing SBRT planning (Arms A and B).

Hypothesis: We hypothesize that the adverse event profile of participants treated with and without lymphocyte-sparing SBRT planning will be similar, except that those treated in Arm Awill have less frequency and/or severity of decrease in lymphocyte count

Endpoints: Frequency, severity, and duration of adverse events according to CTCAE version 5.0

3.2.2 Performance of Algorithm to Predict Decrease in Lymphocyte Count Post-SBRT

Determine if our lymphodepletion predictive algorithm coupled with the existing optimization algorithm in radiation treatment planning software predicts the magnitude and trajectory of lymphocyte depletion in a prospective cohort of patients treated with lung SBRT.

Hypothesis: We hypothesize that our predictive algorithm, which estimates risk and magnitude of post-SBRT decrease in lymphocyte count based upon estimation of radiation dose to blood vessels, can accurately predict the time course of changes in lymphocyte counts in a prospective cohort of lung SBRT patients.

Endpoint: After randomization and completion of treatment planning, the algorithm will predict the decrease in lymphocyte count for each participant (in Arms A and B) for each follow-up time point (at end of SBRT, and 4 weeks and 6 months after SBRT). The predicted time course of lymphocyte count will be compared with the actual decrease in lymphocyte count observed.

3.2.3 Impact of Lymphocyte-Sparing SBRT Planning Objectives on Post-SBRT Lymphocyte Counts

Determine if lymphocyte-sparing SBRT planning (Arm A, investigational) results in less *in vivo* lymphocyte depletion after lung SBRT compared to controls who receive standard SBRT planning without extra efforts to reduce lymphocyte exposure (Arm B, control). We will quantify peripheral blood mononuclear cells collected at baseline through 6 month follow-up.

Hypothesis: We hypothesize that we can generate treatment plans that both meet standard of care objectives for organs-at-risk (OAR) and result in a smaller reduction in lymphocyte count by reducing exposure of circulating lymphocytes in great vessels.

Endpoint: Observed changes in lymphocyte count from baseline to 6 months after completion of lung SBRT for participants in each arm. We will quantify peripheral blood mononuclear cells collected at baseline through follow-up (end of SBRT, 4 weeks and 6 months after lung SBRT).

3.2.4 Lymphocyte Phenotype and Function

Determine the phenotype and function of lymphocytes *in vitro* in Arm A versus Arm B patients.

Endpoints:

1. T and B cell responses to pokeweed mitogen in polyclonal in vitro immunoglobulin synthesis; and
2. Serum levels of immunoglobulin G (IgG) to specific recall antigens (tetanus toxoid)

3.3 Exploratory Objectives

3.3.1 Lymphocyte Phenotype and Function (additional)

Determine the phenotype and function of lymphocytes *in vitro* in Arm A versus Arm B patients.

Endpoint: Specific anti-tumor interferon gamma (IFN-γ) EliSpot responses to lung cancer cell lines.

3.3.2 Overall Survival

To overall survival (OS) after lung SBRT for up to 30 months after SBRT.

Endpoints: Overall survival after lung SBRT (every 6 months for the first year, then annually).

3.3.3 Correlation between Lymphocyte Depletion and Function and Radiation Dosimetry

Identify correlations between lymphocyte depletion and functional impairment with dose to great vessels, lung tissue, heart, and other normal tissue containing large amounts of circulating blood.

Endpoints:

1. Dose to circulating blood
2. TLC and functionality of T cell sub types.

3.3.4 Optimization of Predictive Algorithm

Improve our existing simulation algorithm to predict the total lymphocyte loss for a given RT beam plan using the clinical trial data.

Endpoints: This model will take into account patient specific (i) total blood volume, (ii) initial lymphocyte count, (iii) tumor volume, (iv) the organs from the Thorax CT data set, and (v) time dependent dose map through each organ for a given delivery system. These data will be combined with general information from published literature such as (i) varying blood output to organs (ii) blood velocity for all organs (iii) random mixing of blood after each trip through the exposed dose area, with the remaining blood volume of the body.

3.3.5 Description of Changes in Lymphocyte Subtypes

Describe the changes in lymphocyte subtypes following SBRT in Arms A and B

Endpoint: Quantity of lymphocyte subtypes (e.g. CD4, CD8) prior to and 4 weeks following SBRT

4. PATIENT SELECTION

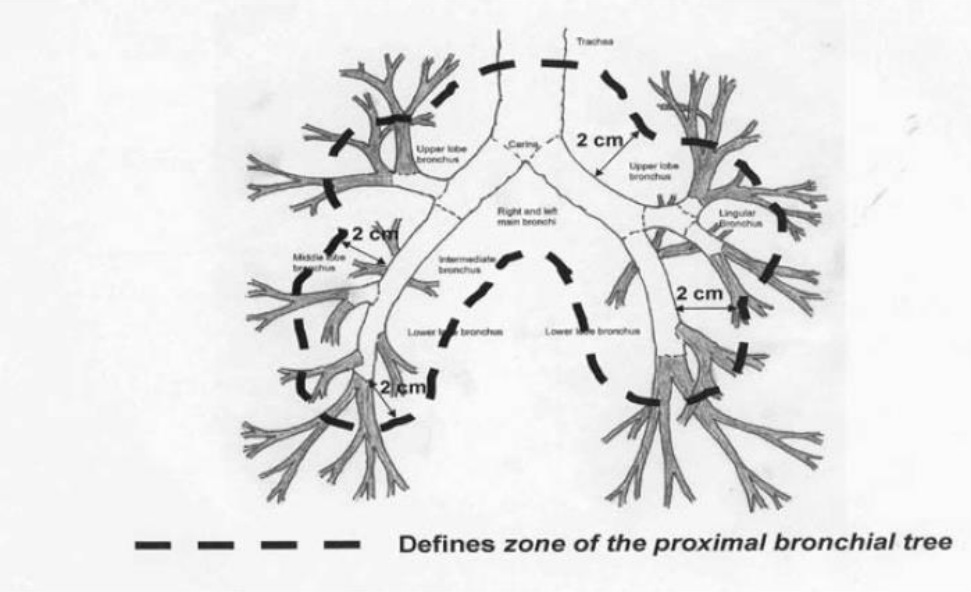


Figure 2: Definition of proximal bronchial tree, for the distinction of centrally located tumors from peripheral tumors (taken from Radiation Therapy Oncology Group (RTOG) 0915 protocol).

4.1 Eligibility

4.1.1 Inclusion Criteria

1. Willingness and ability to provide written informed consent and to comply with the study protocol.
2. Diagnosis of biopsy confirmed non-small cell lung cancer (NSCLC) with planned treatment with SBRT as definitive therapy OR imaging confirmed lung lesion for which SBRT is planned for primary lung cancer (when clinician determines biopsy is not indicated), Registration should occur within 5 business days (before or after) of planning CT.
3. Patients must decline surgery or tumor(s) must be considered to be medically inoperable
4. Location and size of tumor- Participants must have either:
    - peripherally located tumors (> 2 cm in all directions from the proximal bronchial tree; see *Figure 2* above) as defined by RTOG 0915, OR
    - centrally located tumors (tumor size ≤ 5 cm, tumors within or touching the zone of the proximal bronchial tree or adjacent to mediastinal or pericardial pleura) as defined by RTOG 0813.

5. Patients with recurrence of prior surgically treated lung cancers are eligible if no further surgery is planned and they otherwise meet the eligibility criteria.
6. Measurable disease on chest CT, PET CT, CT simulation at diagnosis (must be within 8 weeks of SBRT).
7. Pre- radiation therapy total lymphocyte count > 0.5k/μL on blood count drawn within 2 weeks prior to registration.
8. In the opinion of the treating clinician, patient is medically able to tolerate the study SBRT treatment of 50-60 Gy in 5 fractions.
9. ECOG performance status of 0-2.
10. Age ≥ 18 years.
11. If participant is a woman of childbearing potential (WOCBP), agreement to adhere to contraception requirements from the time of consent through completion of SBRT (see *section 4.1.3*).

4.1.2 Exclusion Criteria

1. Prior history of radiation therapy within 2 years of registration, however radiation therapy for skin cancer is allowed
2. Systemic anti-cancer therapy within the last year prior to registration or planned use during or within 6 months post SBRT follow up timeframe (see study calendar).
3. Major surgery within the last 30 days before registration and/or planned before the completion of the 6 months post SBRT follow up timeframe (see study calendar).
4. Subject is a prisoner.
5. Subject is a pregnant woman.
6. Subject is not medically able to tolerate the study SBRT treatment of 50-60 Gy in 5 fractions or cannot comply with other aspects of the study including serial bloodwork.
7. Subject has HIV, AIDS, any type of hepatitis and/or any blood borne infectious disease for which the research lab cannot receive blood samples.

4.1.3 Contraception Requirements

Women of childbearing potential must agree to use adequate contraception prior to study entry and through completion of SBRT.

Women of childbearing potential (WOCBP) includes any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal [defined as amenorrhea ≥ 12 consecutive months]; or women on hormone replacement therapy (HRT) with documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL

[1] Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy

[2] The following birth control methods are allowed during the study:

a. Barrier methods:
   [1] Intra-uterine device (IUD)
   [2] Diaphragm with spermicide

[3] Cervical cap with spermicide
[4] Condom with spermicide

b. Abstinence (no heterosexual activity)

5. STATISTICAL CONSIDERATIONS

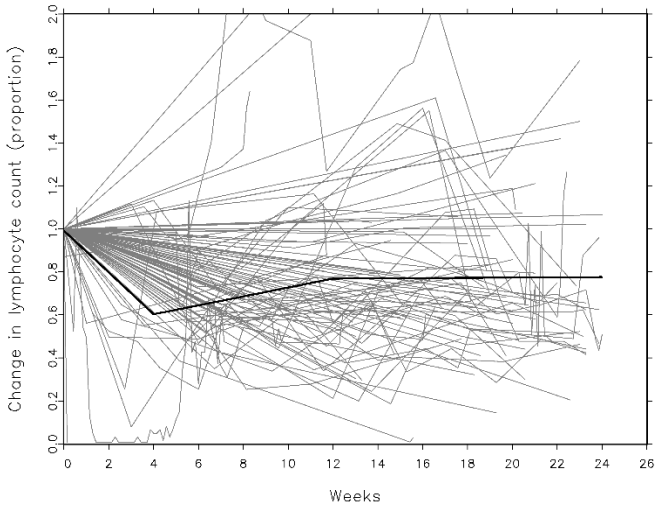
Study populations Any participant who initiates either standard or optimized SBRT will be considered evaluable for adverse events. Participants with observed changes in lymphocyte counts at any of the follow-up times will be considered evaluable for the analyses of changes in lymphocyte counts.

Randomization and stratification

Participants will be randomized to standard or optimized SBRT in a 1:1 ratio using randomly permuted blocks. Block sizes of 2 or 4 will be randomly chosen with equal probability. The randomization will be stratified by central or peripheral lung lesion (section 4.1.1)

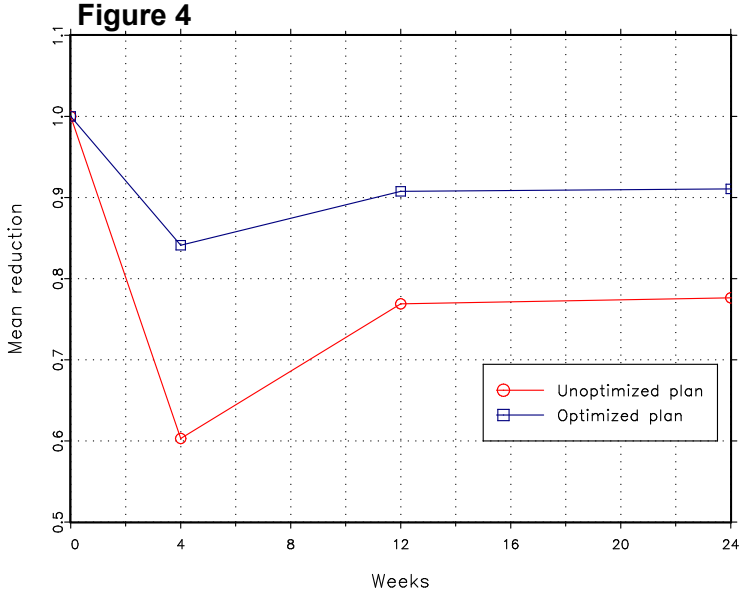
Sample size and power The first aim is to compare the actual and predicted values from the simulations. We will calculate the empirical distribution function of the difference between predicted and actual, both in terms of lymphocyte counts and percent change from pre-treatment counts. Current simulation is able to predict to 17% accuracy, with a standard deviation of 15%. The model will be considered an improvement if 80% of the predictions are within 10% of the actual counts or percent changes. With a sample of size 50 evaluable, the one-sample t-test has 95% power, with a one-sided significance level of 5%, for testing whether the mean percent difference is less than 17%, when the true mean is 10%.

Figure 3



The second aim is to compare standard versus optimized SBRT on the basis of the primary outcome, lymphocyte drop at 4 weeks. The sample size was chosen to have sufficient power for comparing the groups using a repeated measures model for comparing the groups. Estimates of effect sizes and variation were based on repeated measures models, with change points at 4 and 12 weeks, fit to retrospective data on lymphocyte drop within 24 weeks following standard SBRT. A spatial-power model was used for the covariance matrix of percentage changes from baseline in lymphocyte counts, yielding an estimated standard deviation of 10.5 percentage points and a within-subjects correlation estimate of 0.40 for observations taken 1 week apart. The fitted model, overlaid on the individual patient profiles is displayed above.

Figure 4



With n=25 patients, measured at baseline and 4 and 24 weeks months post radiation, the F-test in the repeated measures model provides 80% power for comparing the mean lymphocyte depletion profile over the follow-up period between the groups. For the power calculation, we assumed that in the optimized dose participants, mean lymphocytes relative to baseline are equal to $\Delta + (1-\Delta)\mu(t)$, where $\mu(t)$ is the mean estimated from historical controls at time t = 0, 4, or 24 weeks. With this, $\Delta = 0$ means no effect of the optimization; $\Delta = 1$ means no lymphocyte depletion with optimized SBRT treatment. *Figure 3* displays the mean profile for $\Delta = 0.60$, a profile for which n = 25 per group yields 80% power for the F-test, with a 2-sided significance level of 5%. For the specific comparison at 4 weeks, a contrast in the repeated measures model has more than 99% power for the assumed profile. We computed power for the comparing the entire trajectory, and not just at 4 weeks, because of the uncertainty in the point at which the maximum drop and the maximum difference between the groups will occur. To allow for 10% dropout, 55 patients will be enrolled in the study.

Analyses. For the first aim, which compares the actual and predicted lymphocyte counts, the empirical distribution function of the differences, both in terms of the lymphocyte count and the percentage change from baseline, will be tabulated. We will assess the proportion of cases in which the predicted values is within 10% of the actual value. In addition to computing the empirical distribution functions, exploratory analyses will assess patient characteristics associated with the magnitude of the deviation between predicted and actual values.

For the second aim, repeated measures models will be used to compare the groups with respect to the percentage drop in lymphocyte counts. The stratification factor will be used as a covariate in the model. Contrasts will be used to compare the groups at specific time points, including the 4 week post-treatment time point, where we anticipate there will be the greatest difference between the standard and optimized SBRT groups. The sample size was based on an assumed spatial power covariance structure, but we will consider other covariance matrices as well, such as random effects models. In exploratory analyses will tabulate local control as well as adverse events (see *section 8*) and estimate differences these between groups. Kaplan–Meier curves will be used to summarize overall survival in each of the standard and optimized SBRT groups.

Quantitative analysis of blood counts and radiation dose to the great vessels

We will collect blood, serum and PBMC at baseline and at end of treatment, weeks 4, and 24 after treatment for all study patients. The reference point for post-treatment blood collection will be the start date of radiation therapy to permit intergroup comparisons between the two study arms. This comparison framework was selected based on our available preliminary data, which

suggests that RT-induced lymphocyte effects occur as early as one week following radiation initiation due to the high radiosensitivity of circulating lymphocytes. We will quantify absolute neutrophils, hemoglobin, platelet, and lymphocyte counts for all patients at these time points and compare the changes between the groups and also compare the lymphocyte count results to what was predicted by the simulation algorithm. We will perform a multivariate analysis to identify the correlation between TRL and mean/integral radiation dose to the great vessels. Since substantial circulating blood is also present in the heart and lungs we will also correlate mean heart and lung dose with TRL. Measurement of the magnitude of lymphocyte depletion and correlation with great vessel, lung, and heart integral dose will be performed and compared to the predicted levels for the simulation algorithm.

6. REGISTRATION PROCEDURES

Participants cannot be registered until institutional IRB approval of the protocol and consent are obtained. Registration and randomization should occur around the time of planning CT (+/- 5 business days). SBRT must begin within 4 weeks after registration/randomization.

6.1 Patient Registration & Randomization

All participants must sign the consent form prior to determination of eligibility for this study. Patients will be screened and evaluated to meet all eligibility criteria before being registered on the trial. Once a patient is determined to be eligible for the study, study staff will register the patient in the UVA Cancer Center OnCore database in accordance with the Clinical Trial Management System Policy, via the UVA OnCore Resources link in Oncore. Participants will be associated with a UVA unique identifier, such as UVA SBRT-001, that will represent the participant in the study. This will be listed as the participant's sequence number in the OnCore database and will be on all research blood samples collected from participants.

Randomization will be discussed with participants during the process of informed consent and informed consent must be documented prior to randomization. Participants will be randomized 1:1 to Arm A or Arm B using randomly permuted blocks, with block sizes of 2 or 4 randomly chosen with equal probability. Stratification is described below. Randomization will be performed using custom randomization software (RAND) that incorporates the randomization scheme generated by the study statistician. Clinical research staff and treating clinicians will be aware of arm assignment and participants may be made aware of their arm assignment. The labels on research blood will not include the treatment arm on which the participant is assigned and this information will not be provided to lab investigators and staff.

6.2 Patient Stratification

Participants will be stratified as having peripheral or central lung lesions and the randomization process will ensure equal numbers of participants with peripheral or central lung lesions on each arm of the study.

7. RADIATION THERAPY TREATMENT PLAN

Intensity modulated radiation therapy (IMRT) is the only acceptable form of treatment planning allowed for this protocol.

<u>SBRT radiation treatment planning overview</u>

For each participant, we will determine the tissue-specific radiation dose to vital body structures using SBRT planned treatments. SBRT treatments will be planned using normal tissue dose constraints according to national lung SBRT clinical trials (RTOG 0915 (peripheral tumors) and RTOG 0813 (central tumors)).

Prediction of lymphocyte depletion using our current simulation algorithm

Using the existing simulation algorithm, we will predict the *in vivo* lymphocyte depletion for each participant on both arms to test the robustness of the algorithm.

7.1 Dose Specifications

7.1.1 PTV Dose Prescription

The tumor will be prescribed 50-60 Gy in 5 fractions to the planning target volume (PTV).

7.1.2 Normal Tissue Organs-At-Risk (OARs)

For participants in Arm B, plans will meet standard of care tissue dose constraints as described above. For participants in Arm A, plans will meet standard of care tissue dose constraints (described above) and additional planning constraints to reduce the dose to the great vessels , including the circulating blood within them, to as low as achievable while still meeting the other RTOG 0915 and RTOG 0813 dose constraints. These constraints will be reported in the radiation planning score card and treatment plan per standard of care institutional policy.

7.1.3 Great Vessels and Heart

The great vessels and heart will be contoured using mediastinal windowing on CT to correspond to the vascular wall and all muscular layers out to the fatty adventitia. The great vessels should be contoured starting at least 5 cm above the superior extent of the PTV and continuing on every CT slice to at least 5 cm below the inferior extent of the PTV.

7.1.4 Dose Fractionation

Participants on both Arm A and Arm B will receive 50-60 Gy in 5 fractions to the PTV. Treatments should be delivered within 5-10 business days (excluding the weekend(s) and holidays when the clinic is closed).

7.2 Technical Factors

Only photon (x-ray) beams produced by linear accelerators with photon energies of 4-10 MV will be allowed.

7.3 Localization, Simulation, and Immobilization

7.3.1 Patient Positioning

Patients will be positioned in a stable position using the **BodyFix** system (Medical Intelligence, Munich, Germany), with suction to reduce mobility, allowing accurate reproducibility of the target position from treatment to treatment.

Patient immobilization must be reliable enough to ensure that, in combination with the techniques used to inhibit target motion, the gross tumor volume (GTV) does not deviate beyond the confines of the planning target volume (PTV).

7.3.2 Inhibition of Effects of Internal Organ Motion

Special considerations will be made to account for the effect of internal organ motion (e.g., breathing) on target positioning and reproducibility. Accelerator beam gating with the respiratory cycle for tumors that move >8mm, and treating the FULL_ITV (internal target volume) for others will be utilized. All systems used to account for internal organ motion will be consistent with standard of care lung SBRT at UVA.

Internal organ inhibition maneuvers must be reliable enough to ensure that the GTV does not deviate beyond the confines of the PTV as defined in *Section 7.4*.

7.3.3 Localization

Isocenter or reference point port localization images should be obtained on the treatment unit immediately before treatment to ensure proper alignment of the geometric center (i.e., isocenter) of the simulated fields. All participants in both arms will follow the exact same pre-treatment image-guided radiation therapy (IGRT) protocol. On the first day of the treatment, planar kV imaging orthogonal images will be acquired to align the bony anatomy to the planning CT bony anatomy. Secondly, a cone beam CT image will be acquired and aligned to the planning, time averaged CT image. They will be registered such that soft tissue registration for the lung tumor is applied. The deviations between bony anatomy registration and soft tissue registration should be ≤7mm. In case of deviations >7mm, an additional cone beam computed tomography (CBCT) will be acquired to verify the target localization. **OAR contours from the planning data set, such as the great vessels, and lung positioning, will be verified daily prior to radiation delivery.**

In case of gated treatment, following the above mentioned two pre-treatment imaging verifications, a gated anterior posterior/lateral (AP/Lat) image would be taken to verify the tumor location within the gated window. For all participant treatments in all machines, agreement between the treatment unit isocenter and imaging isocenter will be verified each day to be within 1mm.

7.4 Treatment Planning/Target Volumes

7.4.1 Image Acquisition

Computed tomography (CT) will be the image platform for targeting and treatment planning. Axial acquisitions with spacing = 3.0 mm should be utilized. 4-dimensional CT image-guided (4DCT) internal target volume (ITV) around the GTV will take tumor motion into consideration and will be the primary method of target volume definition. This target will also be the clinical target volume (CTV)].

The target lesion will be outlined by an appropriately trained physician, using a maximum intensity projections (MIP) image of 4DCT and designated to be the internal target volume (ITV). The target will generally be drawn using CT pulmonary windows.

The PTV will include the ITV plus an additional 0.5 cm margin uniformly applied to the ITV. Up to an 8mm margin may be applied in the craniocaudad direction per the treating radiation oncologist preference. The planning CT would be the time averaged CT image from the four dimensional CT.

7.4.2 Dosimetry

IMRT coplanar or non-coplanar beam arrangements will be custom designed for each case to deliver highly conformal prescription dose distributions. Non-opposing, non-coplanar beams are preferable. Typically, approximately 10 beams of radiation will be used with roughly equal weighting. For arc rotation techniques, partial arcs or full arcs could be utilized. In order to obtain acceptable coverage, field aperture size and shape should correspond nearly identically to the projection of the PTV along a beam's eye view (i.e., no additional "margin" for dose buildup at the edges of the blocks or multi leaf collimator (MLC) jaws beyond the PTV). Prescription lines covering the PTV will typically be the 60-90% line (where the maximum dose is 100%).

For purposes of dose planning and calculation of monitor units for actual treatment, this protocol will require tissue density heterogeneity corrections. Algorithm used for heterogeneity corrections must be credentialed by the RTOG.

For patients in Arm A, the beam energy would be 6 X –FFF (flattening filter free) to minimize overall treatment time, and for angles whether it is arcs, or step and shoot, IMRT should be carefully selected such that overall lymphocyte reduction is at the maximum dictated by the prediction algorithm. An example of a planning study is given in *Figure 5* below.

Example Treatment Plans of a Central Tumor:

Aorta integral dose went from 721.1 x 181.63 = 1.3 x10^5 cGy cc to 322.2x181.63 = 5x10^4 cGy cc superior vena cava (SVC) integral dose went from 1769.7x19.16 = 3.4 x10^4 cGy cc to 438.3x 19.16= 8.4x10^3 cGy cc.
This resulted in a reduction to the total great vessel dose from 1.6x10^5 cGy cc to 0.6x10^5 cGy cc.

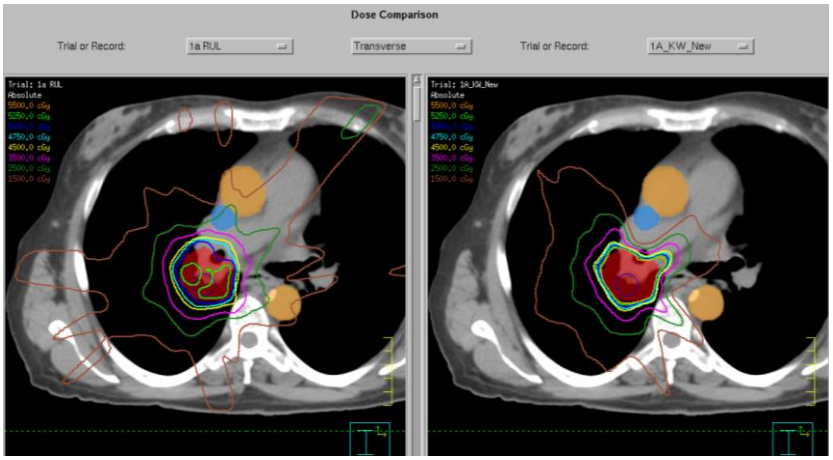


Figure 5: Axial slice of a pat*i*ent's lung SBRT sample plan. On the left is a RTOG 0813 followed plan (as used in Arm B). Note the dose through the great vessels, aorta (orange), and superior vena cava (blue), compared to the plan on the right (as used in Arm A), where additional constraints were imposed on the dose to the great vessels.

7.5 Radiation Treatment Plan Pre-treatment Quality Assurance

All institutional standard of care SBRT quality assurance measurements and calculations will be made and the plan must pass these quality assurance processes within institutional standards

of care and be approved by the treating medical physicist and radiation oncologist prior to treatment delivery.

7.6 Image Guided Treatment

During pre-treatment IGRT for each fraction, the image registration procedure should include the proximal great vessels and heart location in addition to the treated tumor. This information will be recorded in the treatment notes by the therapist.

7.7 Discontinuation/Withdrawal of Therapy

Participants may withdraw or be discontinued from the study at any time for any of the following reasons:

- Inter-current illness that prevents further treatment
- Subject decides to withdraw from the study
- Unacceptable adverse events(s)
- Requirement of medication listed in the exclusion criteria
- If the investigator feels it is no longer in the participant's interest to continue participation
- Death
- Participant does not comply with study procedures

The primary reason for discontinuation or withdrawal should be entered into OnCore. All participants that discontinue or withdraw from the study will continue to receive standard of care treatment. Participants removed from study treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Participants who have withdrawn consent will not be followed for any reason.

A participant's participation is considered completed when the subject has received all study treatments and has completed all follow-up procedures (or other milestone to define a completed subject).

7.8 Definition of Evaluable and Replacement of Study Participants

Participants that discontinue from the study prior to the 4-week post-SBRT blood draw will be considered unevaluable for the analyses of lymphocyte counts and will be replaced. These participants will be evaluable for the tabulation of adverse events.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Clinical Assessments

Some data will be accessed via the participant's medical chart or results of diagnostic tests performed as part of the participant's regular medical care. These data may be used for screening or as part of collection of trial data.

8.1.1 Assessment of Adverse Events

Each participant will be evaluated by a licensed clinician at each study visit. The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5 will be used for the characterization and grading of adverse events.

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8.1.2 Complete Blood Count (CBC) with Differential

A CBC with differential should be collected and processed at all time-points as designated in the Study Calendar of Assessments in *section 9.*

8.1.3 Concomitant Medications

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications, and herbal supplements. Whenever possible, corticosteroids/immune modulators should be avoided, especially around the time of CBC or research blood draws, as they can affect the lymphocyte count. If a participant requires any medication listed in the exclusion criteria during or in the 6 months following SBRT, he or she will no longer be followed for the study and no additional blood will be drawn for research purposes. If this occurs within the 4 weeks following SBRT, the patient will be replaced. These medications have been shown to affect the way white blood cells respond to radiation. There are no other prohibited medications related to study participation. Medications should be collected from 28 calendar days before registration through 6 months following SBRT.

8.1.4 Review of Medical Record

In this patient population, the following assessments are frequently completed as part of standard clinical care. If they are completed during the timeframes described below, accompanying results should be entered in the designated EDC system as study data.

Assessment	Estimated Timeframe(s)
PET with FDG	Within 8 wks prior to SBRT and if there is a suspicion of tumor recurrence (prior to first recurrence following study participation)
CT scan with contrast	Within 8 wks prior to SBRT and within 24 months after SBRT
Tumor response eval	At SOC intervals until first recurrence during 30 months of follow-up
Hospitalization history	Within 12 wks prior to SBRT and at SOC intervals until first recurrence during 30 months of follow-up

8.2 Research Blood

Research blood will be collected according to the study calendar of assessments in *section 9*. 40 ml of blood will be collected at two time points (pre SBRT and 1 month after SBRT) in green top tubes. Following collection, the blood will be transported at room temperature to Dr. Lawrence Lum's laboratory for processing and analysis.

Blood will be analyzed in Dr. Lum's lab. Research tubes will be labeled directly with a unique identifier ID and associated with the UVA IRB HSR number. All leftover research blood will be stored in Dr. Lum's lab at UVA once analysis described in the sections below is completed.

8.3 Correlative Studies

In addition to the following, additional assays may be performed for proliferation, function and subtypes depending on study findings.

8.3.1 Lymphocyte functionality *in vitro* assays

These assays will be run on all participants in both arms at each blood draw time-point.

8.3.2 Lymphocyte Subsets and Activation Phenotypes

The T cell subsets and activation will be measured by flow cytometry using multiple markers to identify T cell subpopulations. The PBMC will be stained for central memory (including but not limited to CD4+/CD45RO+/CCR7-/CD62L+; CD8+/CD45RO+/CCR7-/CD62L+), memory (including but not limited to CD4+/CD45/RO+/ CCR7-/CD62L-; CD8+/CD45RO+/CCR7-/CD62L-), and effector (including but not limited to CD4+/CD45RO+/CCR7-/CD27+; CD8+/ CD45 RO+/CCR7-/CD27+) CD4+ and CD8+ T cells, CD68 for monocyte, CD33+/CD11b+ for MDSC. Briefly, PBMC will be stained for 30 minutes on ice with the cocktails of fluorescently conjugated monoclonal antibodies (mAbs) or isotype-matched controls, washed twice with Fluorescent Activated Cell Sorting (FACS) buffer. Cells will be analyzed on a FACScalibur (BD Biosciences) and data will be acquired using CellQuest software (BD Biosciences).

8.3.3 Evaluation of Specific Antibody Response Against Recall Antigens in Serum

To determine whether SBRT affects humoral immune responses, serum samples will be analyzed for IgG anti-tetanus toxoid antibody. Briefly, antibodies against pooled peptides of tetanus toxoid will be used to capture specific IgG anti-tetanus toxoid antibodies in participant serum, followed by detection of bound antibodies using goat-anti-human IgG by enzyme linked immunosorbent type assay (ELISA). In addition, we will also test the T and B cell responses to pokeweed mitogen in a polyclonal T-dependent in vitro B cell antibody synthesis assay. The IgG antibody titers to tetanus toxoid at different time points after radiation will be compared to baseline measurements.

8.3.4 Evaluation of IFN-ɣ secreting T cells specific to tumor using an EliSpot assay

To determine whether SBRT affects the tumor specific cytotoxic T cells, PBMC will be isolated from whole heparinized blood by Ficoll-hypaque density gradient centrifugation and cryopreserved until use. PBMC will be exposed to a cocktail of stimulating tumor cells (either lung cancer cell lines-A549, H292 and H520 or liver cancer cell lines-SK-HEP-1, SNU-423 and SNU-475) in plates coated with anti-human IFN-γ antibody. The interferon gamma (IFN-γ) EliSpot assay measures the CD8-mediated cytotoxic T lymphocyte (CTL) activity and CD4-mediated helper responses. IFN-γ EliSpots produced by PBMC will be assessed after 18 hours of stimulation with the above listed cocktail of cell lines as well as spontaneous IFN-γ EliSpots (non-stimulated PBMC) produced by PBMC at an effector to target ratio (E/T) of 1:1. This will allow for detection of tumor specific IFN-γ cytokine producing activated T cells. The quantification of IFN-γ secreting specific T cells will be performed by EliSpot CTL software.

The results of the lymphocyte functionality *in vitro* analyses will be compared between Arms A and B and correlated to integral or mean great vessel radiation dose, mean heart dose, and mean lung dose.

9. STUDY CALENDAR OF ASSESSMENTS

Procedures	Screening/Baseline[1]	SBRT (5 clinic days)	Completion of SBRT[5]	4 weeks +/-2 weeks following SBRT	3 months +/-2 weeks following SBRT	6 to 9 months following SBRT	Overall Survival (every 6 months for one year and then annually, by phone or medical record review)
Informed consent	X						
Demographics	X						
Medical history	X						
Randomization	X[7]						
Concomitant medication review	X[8]		X----------	----------	----------	----------X	
Physical exam	X		X		X		
COVID-19 Questions	X		X		X	X	
Vital signs	X		X		X		
Height	X (last recorded for standard clinical care)						
Weight	X		X[6] ----------	----------	----------	---------- X[6]	
Performance status	X		X			X	
Serum or urine pregnancy test (Women of Childbearing Potential Only)	X						
Overall Survival (OS)							X
Hematology- CBC with Diff	X		X[4]	X		X	X[3]
Research Blood	X[2]			X			
Planning/Chest CT scan with contrast	X (w/in 8 wks of SBRT)[7]				X[6]		
Review of Medical Record (see *section 8.1.4*)	X		X		X	X	X
Adverse event review and evaluation	X----------	----------	----------	----------	----------	----------X	
SBRT		X					

[1] All screening procedures should occur up to 1 month prior to registration unless otherwise specified
[2] Research blood should be completed following confirmation of eligibility/registration and before beginning SBRT.
[3] If the participant is followed at UVA and CBC results are available in Epic, these should be entered in the designated EDC system (q 6 months +/- 1 month).
[4] Complete within 3 days after completion of SBRT (can be immediately following completion of SBRT)
[5] Complete within 5 business days (before or after) of completion of SBRT unless otherwise stated
[6]Only collected if part of standard clinical care. Not collecting this information during/following SBRT is not considered a deviation
[7]Registration and randomization should occur around the time of planning CT (+/- 5 business days). SBRT must begin within 4 weeks after registration/randomization.
[8] Medications should be collected from 28 calendar days before registration

10. DATA AND SAFETY MONITORING PLAN

10.1.1 DSMC Meetings and Audits

The UVA CC DSMC will meet every month for aggregate review of data. Tracking reports of the meetings are available to the PI for review. Issues of immediate concern by the DSMC are brought to the attention of the sponsor (and if appropriate to the PRC and IRB) and a formal

response from the sponsor is requested. Per the UVA Cancer Center NIH approved institutional plan, this study will be audited approximately every year. The audit may include direct access to source data/documents.

10.2 Adverse Events and Serious Adverse Events

10.2.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated (at least possibly related) with the use of a medical intervention in humans.

10.2.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A planned medical or surgical procedure is not, in itself, an SAE.

10.2.3 Classification of an Adverse Event

10.2.3.1 Severity of Event

For adverse events (AEs) not included in CTCAE version 5, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

10.2.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention (both SBRT and blood draws that are completed solely for the research study) assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician

10.2.3.3 Expectedness

The treating clinician will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

The following side effects are considered expected from a standard blood draw, in order of likelihood: pain, bruising, fainting or passing out, or infection. SBRT radiation side effects should be consistent with standard clinical care. The likely SAEs from SBRT are described in Bezjak et al from RTOG 0813, which included patients receiving SBRT radiation for centrally located NSCLC[15]. In addition to these SAEs from SBRT, participants may experience a drop in lymphocyte count, lung fibrosis, fatigue and/or skin irritation/skin changes.

10.2.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All reportable AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition (including a laboratory abnormality) that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. For the purposes of this study, laboratory abnormalities (at baseline or during the study) will not be recorded and are not reportable, except for hematological laboratory abnormalities in a complete blood count (with or without differential), as these are the only laboratory results that have any likelihood of being related to SBRT/study intervention.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study staff will record all reportable events with start dates occurring any time after informed consent is obtained until the 6 month visit following SBRT (for non-serious AEs) or anytime (for SAEs considered related to the study intervention). At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

10.2.5 Adverse Event Reporting

AEs considered related to either a research-only blood draw or SBRT must be recorded into the University of Virginia Cancer Center's designated EDC database in accordance with the Clinical Trial Management System Policy Note that the AE Reporting Table below has been revised in the following ways from the standard AE Reporting table for Low Risk Studies:

- Reporting for grade 4-5 unrelated AEs was updated to "Not Required" because this study includes participants that may have severe/serious AEs due to their disease that are completely unrelated to SBRT or blood draws. All treatment meets standard of care guidelines.
- Reporting for Grade 1-2 (expected and unexpected) was updated to "30 days", as investigators are interested in reviewing ALL related AEs for feasibility/equivalence of safety.
- Reporting for laboratory AEs is limited to complete blood count (with or without differential), as these are the only laboratory adverse events that would have any possibility of relationship with SBRT

Table C: Low Risk Studies

Reporting requirements for AEs that occur within 6 months of the last protocol specified treatment/intervention					
	Grade 1-2	Grade 1-2		Grade 3	Grade 4-5
	Expected	Unexpected		Expected or Unexpected	Expected or Unexpected
		Without hospitalization	With hospitalization		
Unrelated	Not required	Not required	Not required	Not required	Not required
Related	30 days**	30 days**	30 days**	15 days**	(24-hrs)* 15 days**
*Enter into Cancer Center database within 24 hours if unexpected and definitely related to protocol specified treatment Hospitalization defined as an inpatient hospital stay or prolongation of a hospital stay equal to or greater than 24 hours ** The only laboratory AEs that need to be reported are hematological studies, as SBRT will not affect other laboratory results					

10.2.6 Serious Adverse Event Reporting

The study clinician will record any serious adverse event, whether or not considered study intervention related, including those listed in the protocol, and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

- Internal Events Resulting in death that are deemed DEFINITELY related to (caused by) study participation
    - Report to the UVA IRB-HSR within 24 hours. Report within 24 hours using IRB Online and a phone call.

- Internal, Serious, Unexpected, Related to Study Intervention
    - Report to the UVA IRB-HSR within 7 days from the time the study team receives knowledge of the event. Timeline includes submission of signed hardcopy of AE form. Report using IRB Online.

10.3 Reporting Events to Participants

If there is any new information relevant to the participant's willingness to continue to participate in the study, such as if there are new risks of the study treatment identified that were not included on the consent form that the participant signed, the study team will contact the participant to discuss this information. If the participant is still receiving study treatment, the study team will present the participant with an updated consent and confirm that he or she wants to continue receiving study treatment. The PI will determine whether new risks are applicable to participants who are in follow-up, whether participants need to be notified, and whether re-consenting is required.

10.4 Unanticipated Problems

10.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems (UPs)(may include a data breach) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.4.2 Unanticipated Problem Reporting

- Report UPs that are not adverse events, protocol deviations, or data breaches (see *section 10.5.2* ***Error! Reference source not found.*** for reporting for data breaches) to the UVA IRB-HSR within 7 calendar days from the time the study team receives knowledge of the event. Report using the Unanticipated Problem Report form.
- Report UPs that are SAEs in accordance with the guidelines for SAE reporting.

10.4.3 Reporting Unanticipated Problems to Participants

If during the course of the study there is an unanticipated problem that affects current or past participants, affected participants will be contacted if needed.

10.5 Data Breach

10.5.1 Definition of Data Breach

An unauthorized acquisition, access, or use of protected health information (PHI) that compromises the security or privacy of such information.

10.5.2 Reporting a Data Breach

- Report to the UVA Corporate Compliance and Privacy Office as soon as possible and no later than 24 hours from the time the incident is identified. Report by telephone.

- Report to InfoSec if the breach involves electronic data. Report as soon as possible and no later than 24 hours from the time the incident is identified. Refer to the following for details: http://security.virginia.edu/report-information-security-incident.

- Report to UVA police if the breach includes such things as stolen computers. Report by telephone.

10.6 Protocol Deviation

10.6.1 Definition of Protocol Deviation

A protocol deviation is defined as any change, deviation, or departure from the study design or procedures of a research project that is NOT approved by the institution's IRB prior to its initiation or implementation, OR deviation from standard operating procedures, Good Clinical Practices (GCPs), federal, state or local regulations. Protocol violations may or may not be under the control of the study team or UVa staff. These protocol violations may be major or minor violations.

10.6.2 Reporting of a Protocol Deviation

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations. All deviations must be addressed in study source documents and reported as follows:

- Report to the UVA IRB-HSR major deviations within 7 calendar days from the time the study team received knowledge of the event. Report using the Protocol Deviation and Protocol Exception Reporting Form.

- For minor deviations, please reference the IRB-HSR for tips for recording minor deviations

10.7 Participant Withdrawals/Dropouts Prior to Study Completion

Participants who withdraw consent and those dropping out of the study secondary to an AE will be reported to the UVA IRB yearly on the IRB continuation form.

11. REGULATORY AND OPERATIONAL CONSIDERATIONS

11.1 Regulatory and Ethical Considerations

11.1.1 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. A member of the study team will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Results from procedures completed prior to consent for standard of care purposes may be used for research purposes. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.1.2 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. Consents will be maintained in a confidential manner in accordance with the code of federal regulations and HIPAA. When possible, specimens will be coded with IDs (not MRN or name). No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the University of Virginia. The study data entry and study management systems used by research staff will be secured and password protected.

11.1.3 Future Use of Stored Specimens and Data

With the participant's approval and as approved by local Institutional Review Boards (IRBs), coded and linked biological samples will be stored in Dr. Lawrence Lum's Tissue Bank for Future Cell Therapy Studies biorepository in Dr. Lum's lab at UVA. These samples could be used to research the causes of cancer, its complications and other conditions for which individuals with cancer are at increased risk, and to improve treatment.

During or after the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research.

When the study is completed, access to study data and/or samples will be provided through the Tissue Bank for Future Cell Therapy Studies. At the end of the current study, all study data/specimens will be maintained according to institutional policies.

11.1.4 Safety Oversight

The University of Virginia Cancer Center Data and Safety Monitoring Committee (CC DSMC) will provide oversight of the conduct of this study. The CC DSMC will report to the UVA Protocol Review Committee (PRC).

The UVA CC DSMC will review the following:

- All adverse events
- Audit results
- Application of study designed stopping/decision rules
- Whether the study accrual pattern warrants continuation/action
- Protocol violations

The UVA CC DSMC will meet according to their approved plan as described in *section 10.1.1*.

Any study under the purview of the University of Virginia HSR-IRB is subject to review. Studies are chosen for Post-approval Monitoring either a) at random or b) requested by a study team member or any member of the IRB-HSR.

The purpose of Post-approval Monitoring audits is to ensure that documentation of clinical research studies is of the highest quality, verify protocol adherence, and ensure that all Federal and local rules concerning clinical research are being fulfilled. Post-approval monitoring is done by staff within the office of the Vice President for Research (VPR) in accordance with their Standard Operating Procedures. The conduct of an on-site review may include but is not limited to:

- requests for progress reports from investigators,
- examinations of research records, including signed informed consent documents, protocol modifications, and unexpected, serious, and/or related adverse experience reports,
- contacts with research participants, or
- observation of the consent process and/or research procedures. Examples of when observation of the consent process could occur are:
    - Full board IRB determines during review of a project that a conflict of interest exists such that the informed consent process should be observed by a neutral party;
    - IRB is made aware of a complaint or concern with regard to the informed consent process; or
    - IRB determines as a result of the monitoring process that the consent process is insufficient and education/training is required for conduct of consent.

11.2 Data Handling and Record Keeping

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data will be collected using a password-protected, centralized electronic case report form called **ON**-line **C**linical **O**ncology **R**esearch **E**nvironment = **Oncore** and/or ForteEDC. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

11.3 UVA Institutional Review Board for Health Sciences Research

The UVA Institutional Review Board for Health Sciences Research (UVA IRB-HSR) will approve all aspects of this study, including the clinical trial protocol, informed consent documents, and patient materials. Modifications to the protocol or consent form will be reviewed and approved by the UVA IRB-HSR prior to implementation, except when necessary to eliminate apparent immediate hazards to the study participants. The study will undergo continuing IRB review based on the level of risk as assessed by the IRB. This review will take place no less than annually. Reporting to the UVA IRB-HSR will occur as specified above.

12. REFERENCES

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13. APPENDICES

Appendix 1: ECOG Performance Status

Appendix 2: RECIST 1.1 Criteria

Appendix 3: Summary of Changes

13.1 Appendix 1: ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade 10.2	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

13.2 Appendix 2: RECIST 1.1 Criteria

Please refer to the following publication for evaluation of clinical response by RECIST 1.1.

E.A. Eisenhauer et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer, 2009, 45: 228-247. PMID: 19097774

13.3 Appendix 3: Summary of Changes to Protocol

Changes from protocol version date 10/20/21 to 4/25/2022

Section(s)	Description
9.Study Calendar of Assessments	Change the timeframe for one of the collections of CBC with differential from 6 months +/-2 weeks following SBRT to 6 to 9 months following SBRT.
Rationale: Timeframe was adjusted to account for subject scheduling changes.	

Changes from protocol version date 4/9/2021 to 10/20/2021

Section(s)	Description
Accrual Goal	Increased the accrual goal to 65 subjects from 53 subjects.
Rationale: To better account for screen failures and replacements needed for subjects who are discontinued before the 4 week blood collection, in order to obtain 50 evaluable subjects.	
4.1.2 Eligibility Criteria- Exclusion Criteria	Updated exclusion criteria #1 to include a 2 year timeframe for prior radiation and to include subjects who've had prior radiation therapy for skin cancer (at any time) are eligible
Rationale: Radiation therapy performed 2 year prior to lung SBRT and skin cancer radiation therapy is not expected to impact the objectives of the study.	
4.1.2 Eligibility Criteria- Exclusion Criteria	Added exclusion criteria #7 in which subjects with HIV, AIDS, any type of Hepatitis, and/or any blood borne infectious disease Dr. Lum's research lab cannot process blood samples for are ineligible for the study.
Rationale: Dr. Lum's research lab is a BSL-2 level laboratory however, the lab cannot accept samples from individuals with a known diagnosis of HIV, AIDS, and/or any type of Hepatitis. Since the research blood from these subjects would not be analyzed, these subjects are ineligible for the study. This exclusion criteria will be assessed via review of the subject's medical record and/or subject acknowledgement. No diagnostic tests will be required to determine/confirm diagnosis of these diseases.	
9 Study Calendar of Assessments	Adjusted 4 week Follow up timeframe allowance to +/- 2 weeks from +/- 1 week.

Rationale: Timeframe was adjusted to account for scheduling changes. Widening this timeframe is not expected to have a significant impact on the lymphocyte count data.	
9 Study Calendar of Assessments	Removed Hemoglobin A1C test
Rationale: Subjects with diabetes (even uncontrolled diabetes) are not excluded from the study. In addition, the values are not being recorded as data. Collecting Hemoglobin A1C data only at baseline is not necessary for the study objectives.	
9 Study Calendar of Assessments	Adjusted study calendar for AE collection timeframe to include Screening/Baseline and SBRT.
Rationale: To accurately reflect that AE capture starts at the time of informed consent	
8.1.4 Adverse Events and Serious Adverse Events	Updated definition of an adverse event to "Adverse event means any untoward medical occurrence associated (at least possibly related) with the use of a medical intervention in humans"
Rationale: The new definition streamlines the capture of AEs without underreporting. This definition captures pertinent standard clinical care and intervention related AEs of any category.	

Changes from protocol version date 4/5/2021 to 4/9/2021

Section(s)	**Description**
Abbreviation Table 9 Study Calendar of Assessments 10.2.5 AE Reporting 8.1.4 Review of Medical Record 11.2 Data Handling and Record Keeping	updated "Oncore" references to " designated EDC system"
Rationale: Oncore is no longer the exclusive data entry system for the study. ForteEDC will also be used to capture data and adverse events.	

Changes from protocol version date 10/2/2020 to 4/5/2021

Section(s)	**Description**
4.1.2 Exclusion Criteria	Updated Exclusion Criteria 2 & 3 to include "planned before the completion of the 6 month post SBRT follow up timeframe

Rationale: Systemic cancer therapies and surgery can affect the lymphocyte counts. Blood draws to record the lymphocyte counts occur up until the 6 month follow up timeframe. The goal is to minimize the impact to the counts from procedures not a part of the study's treatment.	
9 Study Calendar of Assessments	Added 3 month Follow up visit to the protocol and included corresponding assessments
Rationale: To align with SOC scheduling. Patients usually have a 3 month follow up for SOC. Since an office visit with their physician will not be required at 1 month, assessments that were being collected for the 1 month will now be collected at the 3 month visit. Blood draws will still be collected at the 1 month.	
8.2 Research Blood 9 Study Calendar of Assessments	Updated the assessments required for 1 month Follow up visit to just the blood draws
Rationale: To align with SOC scheduling. The 1 month follow up visit is only SOC when the patient experiences side effects. The 1 month study goal is just to collect the blood draws.	
9 Study Calendar of Assessments	Added the collection of COVID screening questions.
Rationale: Infections and vaccines could affect the lymphocyte counts. Information for COVID is being collected to assess potential impact to the study data.	
10.2.5 Adverse Event Reporting	AE reporting table was updated from 30 days to 6 months
Rationale: To align with section 10.2.4 Time period and Frequency for Event Assessment and Follow up	
8.1.3 Concomitant Medications	Reworded the Con Meds section to include "immune modulators"
Rationale: For clarity to indicate that corticosteroids are not the only immune modulating medications	

Changes from protocol version date 1/15/2020 to 10/02/2020

Page(s)	Section(s)	Description
1	Title page	Personnel were updated to include additional key investigators and remove some of the contact information

Rationale: Additional investigators will take a greater role in the project, and contact information (address, etc) is not necessary for all investigators, as this is a single site study		
Throughout	1 Synopsis, Schema, 2 Introduction, 3.1.2 Impact of Lymphocyte-Sparing SBRT Planning Objectives on Post-SBRT Lymphocyte Count, 3.2.1 Safety, 3.2.2 Performance of Algorithm to Predict Decrease in Lymphocyte Count Post-SBRT, 3.2.3 Impact of Lymphocyte-Sparing SBRT Planning Objectives on Post-SBRT Lymphocyte Counts, 3.2.4 Lymphocyte Phenotype and Function, 3.3.1 Lymphocyte Phenotype and Function (Additional), 3.3.5 Description of Changes in Lymphocyte Subtypes, 6.1 Patient Registration and Randomization, 7.1.2 Normal Tissue Organs-At-Risk (OARs), 7.1.4 Dose Fractionation, 7.4.2 Dosimetry, 8.3.4 Evaluation of IFN-ɣ secreting T cells specific to tumor using an EliSpot assay	Arm 1 has been updated to Arm A and Arm 2 has been updated to Arm B
Rationale: To be consistent with the electronic case report form system (OnCore)		
18	4.1.1 Inclusion Criteria	Added “business” to “business days” Revised #6 to clarify that chest CT, PET CT and CT simulation are all acceptable scans Revised #7 to make it consistent with lab results in the medical record
Rationale: To allow a little more flexibility, clarity, and consistency		
20	4.1.2 Exclusion Criteria	Exclusion criterion # 2 was removed.
Rationale: The concerns related to exposures that may significantly affect lymphocyte count or function are addressed by the remaining criteria.		
22	6.1 Patient Registration & Randomization	Revised sentence regarding blinding
Rationale: Participants may be told about their arm assignment, but this is not required		

26	8.1.1 Assessment of Adverse Events	Removed “Toxicity diaries will be distributed to participants and reviewed by study personnel”
Rationale: Toxicity diaries are not used in this study; participants are assessed for side effects and symptoms by clinicians according to standard clinical care		
29	9 Study Calendar of Assessments	Physical exam, vitals, and performance status at time of SBRT may be within 5 days before/after completion of SBRT Weight during SBRT and following SBRT is not essential for the endpoints, except for the purposes of AEs. Weight should be collected according to standard clinical care from SBRT and during follow-up, and not collecting weight during this period is not a deviation. Footnotes were revised Overall survival may be collected by phone or from the medical record
Rationale: For clarity, flexibility and to be consistent with standard clinical care		
31	10.2.3.3 Expectedness	Revised wording and organization
Rationale: For improved clarity		
32	10.2.4 Time Period and Frequency for Event Assessment and Follow-Up	Added “reportable”
Rationale: For internal consistency with reporting standards		
31-32	10.2.4 Time Period and Frequency for Event Assessment and Follow-Up 10.2.5 Adverse Event Reporting	Added a sentence clarifying that non-hematological laboratory abnormalities are not reportable and do not need to be reported as adverse events
Rationale: The only laboratory AEs that need to be reported are hematological studies, as SBRT will not affect other laboratory results		

Changes from protocol version date 1/10/2020 to 1/15/2020

Page(s)	Section(s)	Description
19	4.1.2 Exclusion Criteria	Exclusion Criteria #3: “Chemotherapy” was revised to “Systemic anti-cancer therapy” and “or planned

26 27	7.7 Discontinuation/ Withdrawal of Therapy 8.1.3 Concomitant Medications	use during or within 6 months following SBRT" was added Exclusion Criteria #4 and #6 were removed The concomitant medications section and the Discontinuation section were revised to indicate that if drugs listed in the exclusion criteria were necessary for a participant within the 6 months following SBRT, the participant would no longer be followed for the study and no additional blood would be drawn for research.
Rationale: Certain medications have been shown to affect lymphocyte count and would affect the accuracy of the designed algorithm		
22	6.1 Patient Registration & Randomization	Language regarding blinding was revised to clarify that investigators involved with lab research will be blinded to treatment arm
Rationale: Clarification		
29	9 Study Calendar of Assessments	Removed recurrence free survival (RFS) from the calendar
Rationale: This was mistakenly missed in the 1/10/2020 version		

Changes from protocol version date 6/24/19 to 1/10/2020

Page(s)	**Section(s)**	**Description**
3 9 15 32	Schema 1 Synopsis 2 Introduction 3 Objectives 10.2.3.3 Expectedness	The term "lymphopenia" was replaced with "decrease in lymphocyte count" or other similar language.
Rationale: Lymphopenia is a clinical term for particular levels of lymphocytes. In this study, the amount of decrease in lymphocytes is under study.		
3	Schema	"so there are equal numbers of patients in each arm" was deleted
Rationale: This repetitive with the information already available in the schema		
9-10 15-16	1 Synopsis 3.1 Primary Objectives	The objectives and endpoints have been re-worded. "Local control" and "Recurrence free survival" were removed from the objectives.

16 17	3.2 Secondary Objectives 3.3.2 Local Control and Overall Survival	
Rationale: For clarification and because local control/DFS/RFS are beyond the scope of this study.		
19	4.1.2 Exclusion Criteria	Exclusion criteria were added and revised
Rationale: To more specifically identify the population that the algorithm is likely to predict decrease in lymphocyte count. Recent use of certain drugs or recent diagnoses or procedures can affect the lymphocyte count.		
20-21 23 27	5 Statistical Considerations 6.1 Patient Registration & Randomization 7.8 Definition of Evaluable and Replacement of Study Participants	Additional information was added to clarify the Study Population and process of Randomization and stratification. Additional clarifications were added to these sections to provide additional information.
Rationale: For clarification		
26	7.6 Image Guided Treatment	This section was added to clarify that the image registration procedure should include the proximal great vessels and heart location in addition to the treated tumor and that this should be recorded in the treatment notes.
Rationale: The algorithm is based on treating the great vessels and heart as organs at risk.		
27	8.1.3 Concomitant Medications	“restricted” was changed to “prohibited” regarding the fact that there are no prohibited medications related to study participation. A sentence was added to discourage use of corticosteroids, especially around the time of CBC or research blood draws
Rationale: Use of corticosteroids can affect the lymphocyte count, especially from about 4 hours after administration through about 48 hours after administration		
27-28 30	8.1.4 Review of Medical Record 9 Study Calendar of Assessments	Removed chest x ray, lung-specific assessments, and Charlson Index from the assessments that will be recorded from the medical record. Rows were added to the study calendar to list “Review of Medical Record” and “HbA1c”

Rationale: The removed assessments are beyond the scope of this study but “Review of Medical Record” was added to the study calendar for clarification. “HbA1c” was added to the calendar, as uncontrolled diabetes can affect lymphocyte count		
28	8.3 Correlative Studies	A sentence was added to clarify that additional assessments related to proliferation, function, and subtypes may be completed
Rationale: To better describe the potential studies that may be completed based on study findings		