WVU Cancer Institute
MARY BABB RANDOLPH CANCER CENTER

Use of Response-Adapted Hypofractionated Radiation Therapy to Potentiate a Systemic Immune Response to Checkpoint Inhibitors in Non-Small Cell Lung Cancer

09/09/2021

NCT03035890

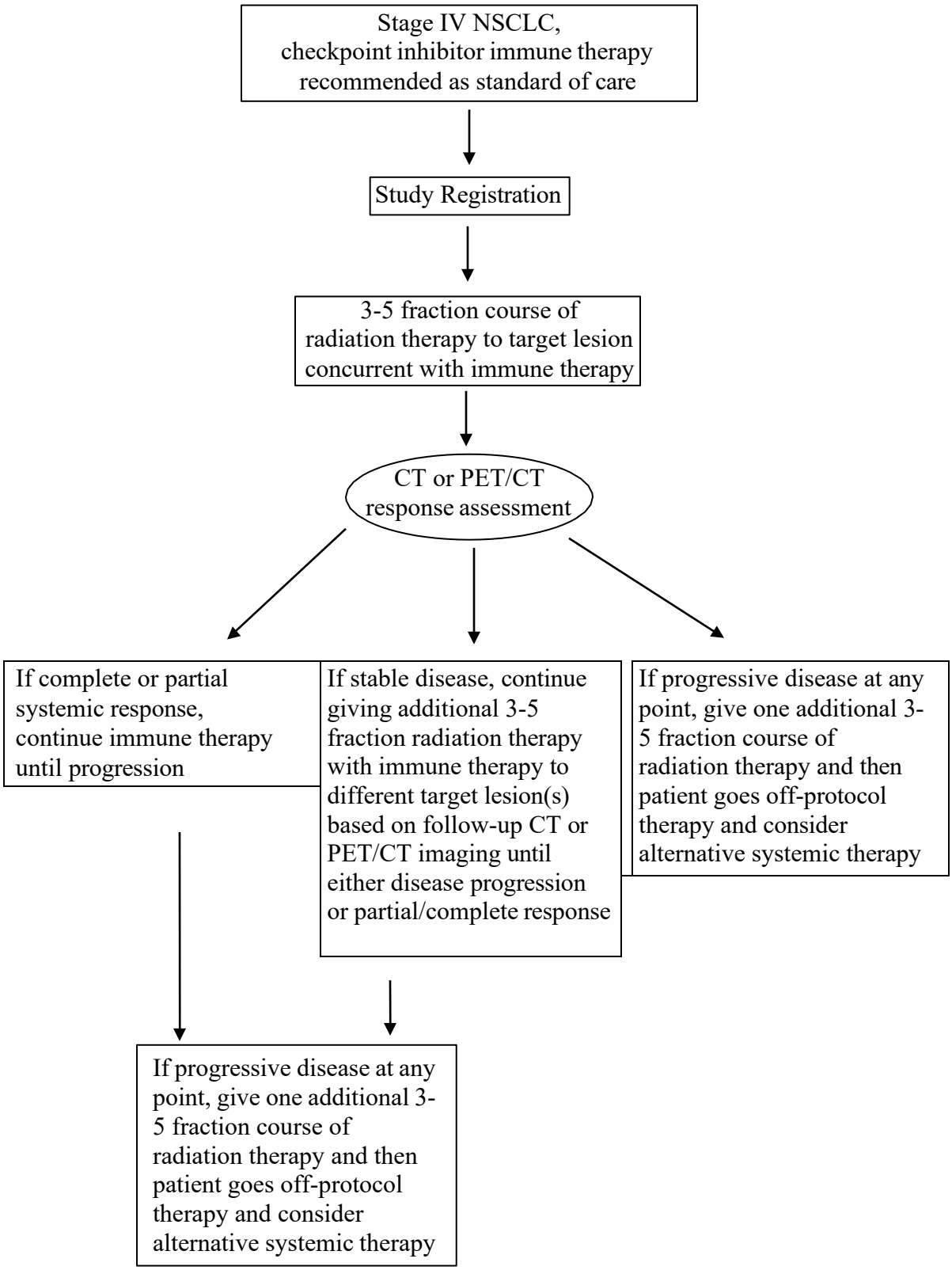
STUDY NUMBER: WVU010516
STUDY TITLE: Use of Response-Adapted Hypofractionated Radiation Therapy to Potentiate the Systemic Immune Response to Checkpoint Inhibitors in Non-Small Cell Lung Cancer

PRINCIPAL INVESTIGATOR: Mohammad Shaikh, MD Department of Radiation Oncology West Virginia University School of Medicine 1 Medical Center Drive Morgantown, WV 26506 304-293-4706 mohammad.shaikh@hsc.wvu.edu	**CO-INVESTIGATOR:** Malcolm Mattes, MD Department of Radiation Oncology West Virginia University Cancer Institute Mary Babb Randolph Cancer Center 1 Medical Center Drive Morgantown, WV 26506 304-598-4706 mdmattes@hsc.wvu.edu
CO-INVESTIGATOR: Mohammed Almubarak, MD Department of Medicine (Hem/Oncology) West Virginia University Cancer Institute Mary Babb Randolph Cancer Center 1 Medical Center Drive Morgantown, WV 26506 304-293-4229 malmubarak@hsc.wvu.edu	**CO-INVESTIGATOR:** Timothy Eubank, PhD Department of Microbiology/Immunology/ Cell Biology West Virginia University School of Medicine 1 Medical Center Drive Morgantown, WV 26506 304-293-0525 tdeubank@hsc.wvu.edu
CO-INVESTIGATOR: Matthew Smolkin, MD, HCLD Department of Pathology West Virginia University Cancer Institute Mary Babb Randolph Cancer Center 1 Medical Center Drive Morgantown, WV 26506 304-293-3404 MBSMOLKIN@hsc.wvu.edu	**CO-INVESTIGATOR:** Ann Morris, MD Department of Radiation Oncology West Virginia University Cancer Institute Mary Babb Randolph Cancer Center 1 Medical Center Drive Morgantown, WV 26506 304-293-9086 ammoris1@hsc.wvu.edu
CO-INVESTIGATOR: Geraldine Jacobson, MD, MPH, MBA Department of Radiation Oncology West Virginia University Cancer Institute Mary Babb Randolph Cancer Center 1 Medical Center Drive Morgantown, WV 26506 304-293-4706 gmjacobson@hsc.wvu.edu	**CO-INVESTIGATOR:** Todd Tenenholz, MD, PhD Department of Radiation Oncology West Virginia University Cancer Institute 1 Medical Center Drive Morgantown, WV 26506 304-293-4706 todd.tenenholz@hsc.wvu.edu
STATISTICIAN: Sijin Wen, PhD Department of Biostatistics West Virginia University Cancer Institute 1 Medical Center Drive Morgantown, WV 26506 304-581-1971 siwen@hsc.wvu.edu	**STUDY COORDINATOR:** Carla Ross West Virginia University 1206 HSC South, Floor 1 PO Box 9260, 64 Medical Center Drive Morgantown, WV 26506 304-293-2633 cjross@hsc.wvu.edu

SPONSOR: WVU Cancer Institute Mary Babb Randolph Cancer Center / WV Clinical and Translational Institute

SUPPORT/FUNDING: WV Clinical and Translational Institute

STUDY SCHEMA



PROTOCOL SYNOPSIS

Protocol Number/ Title	Protocol Number WVU010516 Use of Response-Adapted Hypofractionated Radiation Therapy to Potentiate the Systemic Immune Response to Checkpoint Inhibitors in Non-Small Cell Lung Cancer
Study Phase	Phase II
Brief Background/ Rationale	Preclinical data suggest that radiation therapy may be uniquely suited to combine with immune checkpoint inhibitors, since radiation can disrupt a tumor's physical barriers to T-cell infiltration and augment antigen presentation, thus serving as an "in situ personalized vaccine" to activate the immune system and potentially enhance the systemic response. However, the optimal way to combine immune checkpoint inhibitors and radiation therapy is poorly understood, and there is little prospective data evaluating the efficacy in patients. The rationale for this study is to determine the safety and efficacy of combined immune checkpoint inhibitors and radiation therapy in metastatic non-small cell lung cancer patients.
Primary Objective	Primary Endpoint: Best overall response rate (ORR) (complete and partial), measured on follow-up imaging as per immune-related Response Criteria (irRC) after initiation of therapy.
Secondary Objective(s)	Secondary Endpoint(s): Progression-free survival (PFS), overall survival (OS), toxicity using CTCAE v4.0, quality of life using FACT-L.
Exploratory Objective(s)	Exploratory Endpoint(s): Univariate and multivariate analysis will be used to correlate the presence of response with radiotherapy variables (biologic effective dose, number of fractions, duration of radiotherapy, sequence of radiation and immune therapy), tumor variables (irradiated tumor site, size, histology, local response, extent of prior chemotherapy use), and patient variables (age, smoking status, and biologic correlates).
Correlative Objective(s)	Correlative Endpoint(s): In consenting patients peripheral blood samples will be collected before initiation of radiation therapy, at the time of the first follow-up imaging study, and for any change in response status on reimaging to assess levels of circulating CD4+, CD8+ and Treg T-cell subsets, as well as iNOS+/CD80+ macrophages. We will also assess immune cell trafficking by determining levels of circulating cytokines (HGF, TGF-β) and chemokines (CCL2 for M1 macrophages, and CXCL9, CXCL10, and CXCL11 for T-cells). We will also determine changes of the circulating T-cells immune-repertoire (i-repertoire) by performing next generation sequencing of the CDR3 region of the T-cell receptor (TCR).
Sample Size	33
Disease Sites/ Conditions	Stage IV NSCLC (ICD-10 code C34.90)
Interventions	Radiation therapy, 3-5 fraction course over 3-21 days Immune therapy per standard of care physician's discretion

ABBREVIATIONS

3DCRT	3-Dimensional Conformal Radiation Therapy	**irAE**	Immune-related Adverse Events
BED	Biologically Effective Dose	**irRC**	Immune-related Response Criteria
CCL	Chemokine (C-C Motif) Ligand	**ITV**	Internal Target Volume
CD4	Cluster of Differentiation 4	**MBRCC**	Mary Babb Randolph Cancer Center
CD8	Cluster of Differentiation 8	**MDSC**	Myeloid Derived Suppressor Cell
CRF	Case Report Form	**MHC**	Major Histocompatibility Complex
CRP	C-reactive Protein	**MRI**	Magnetic Resonance Imaging
CT	Computed Tomography	**MV**	Megavoltage
CTCAE	Common Terminology Criteria for Adverse Events	**NGS**	Next Generation Sequencing
CTLA-4	Cytotoxic T-lymphocyte Antigen 4	**NSCLC**	Non-Small Cell Lung Cancer
CTRU	Clinical Trials Research Unit	**OAR**	Organ-at-Risk
CTV	Clinical Target Volume	**ORR**	Overall Response Rate
CXCL	Chemokine (C-X-C motif) ligand	**OS**	Overall Survival
DSTC	Data Safety Toxicity Committee	**PD-1**	Programmed Death Receptor 1
ESR	Erythrocyte Sedimentation Rate	**PD-L1**	Programmed Death-Ligand 1
ECOG	Eastern Cooperative Oncology Group	**PERCIST**	PET Response Criteria in Solid Tumors
FACIT	Functional Assessment of Chronic Illness Therapy	**PET**	Positron Emission Tomography
FACT	Functional Assessment of Cancer Therapy	**PFS**	Progression Free Survival
FDA	Food and Drug Administration	**PRMC**	Protocol Review and Monitoring Committee
Gy	Gray	**PTV**	Planning Target Volume
GTV	Gross Tumor Volume	**QOL**	Quality of Life
HGF	Hepatocyte Growth Factor	**RECIST**	Response Evaluation Criteria in Solid Tumors
ICF	Informed Consent Form	**SBRT**	Stereotactic Body Radiation Therapy
Ig	Immunoglobulin	**SOC**	Standard of Care
IMRT	Intensity Modulated Radiation Therapy	**TCR**	T-Cell Receptor
iNOS	Inducible Nitric Oxide Synthase	**TGF**	Transforming Growth Factor
		Treg	Regulatory T-cell

TABLE OF CONTENTS

1. OBJECTIVE

The overall objective of this study is to determine the safety and efficacy of combined immune checkpoint inhibitors and radiation therapy in metastatic non-small cell lung cancer patients.

1.1. Primary Objective

To determine the best overall response rate (ORR) (complete and partial), as measured on follow-up CT or PET/CT imaging after initiation of radiation therapy.

1.2. Secondary Objective(s)

To determine 1-year progression-free survival (PFS); 1-year overall survival (OS); safety/toxicity using CTCAE v4.0 until 30 days after the last dose of immune therapy or until the patient begins a new cancer-directed therapy, or death, whichever comes first; quality of life using FACT-L and FACIT Fatigue Scale at 3 month intervals until the time of systemic disease progression. In order to determine factors predictive of response to therapy, we will correlate the presence of response with radiotherapy variables (biologic effective dose, number of fractions, duration of radiotherapy, sequence of radiation and immune therapy), tumor variables (irradiated tumor site, size, histology, PD-L1 status, local response, extent of prior chemotherapy use), and patient variables (age, smoking status, and biologic correlates).

1.3. Correlative Objective(s)

Peripheral blood samples will be collected before initiation of radiation therapy, at the time of the first reimaging study, and with each subsequent change in response status to assess levels of circulating CD4+, CD8+ and Treg T-cell subsets, iNOS+/CD80+ macrophages, cytokines (HGF, TGF-β) and chemokines (CCL2, CXCL9, CXCL10, CXCL11). We will also determine changes of the circulating T-cells immune-repertoire (i-repertoire) by performing next generation sequencing of the CDR3 region of the T-cell receptor (TCR).

2. BACKGROUND

2.1. Background of Study Disease

An estimated 221,000 new cases of lung cancer were diagnosed in 2015, making it the second most common malignancy in men and women. It is also the leading cause of cancer death in the United States.[1] Non-small cell lung cancer (NSCLC) accounts for approximately three-fourths of new lung cancer diagnoses, the majority of which are locally advanced or metastatic at presentation. Patients with metastatic NSCLC often decline relatively rapidly, with one-year overall survival on the order of only 15-20%.[2]

2.2. Checkpoint Inhibitor Immune therapy for Stage IV NSCLC

The immune system normally functions to prevent and fight against invasive organisms and diseases. However, a dysregulated immune response can also be detrimental in the form of autoimmunity or excessive inflammation. As such, several immune checkpoints

have evolved in order to maintain homeostasis. Cancer has been shown to co-opt these checkpoints, leading to immune tolerance and subsequent progression of malignancy.[3-5] In recent years immune checkpoint inhibitors have emerged as a novel therapy for a variety of human malignancies.[6-11] One well characterized checkpoint being targeted in metastatic NSCLC clinical trials is PD-1/PD-L1, with monoclonal antagonist antibody pharmaceuticals, such as Nivolumab, Pembrolizumab, and Atezolizumab, resulting in response rates of 19-25% and median response duration of 12.5-17.0 months.[8-11] To date, Nivolumab, Pembrolizumab, and Atezolizumab have been approved by the U.S. Food and Drug Administration for treatment of advanced NSCLC in second line therapy and beyond. Promising clinical outcome data continue to emerge from clinical trial studies suggesting activities of PD-1 blockade in small cell lung cancer (SCLC) and mesothelioma as well.

2.3. Rationale for Combining Radiation Therapy with Immune therapy

While these findings are encouraging, the fact that only a minority of patients will respond to checkpoint inhibitors alone highlights that other barriers to immune stimulation exist. Radiation therapy has been combined with immune therapy to overcome some of these barriers in both preclinical and clinical studies.[12-14] Radiation may help to break up the dense stromal barrier and acidic microenvironment surrounding tumors that is hostile to T-cell function, thus better mobilizing antigens and immune effector cells and activating the immune system. These benefits are not only restricted to the local area of irradiation, but also have an abscopal/systemic effect on cancer outside of the irradiated area.[13-17] Radiation is in many ways uniquely suited to combine with immune therapy, since surgery removes the tumor and thus the antigenic source, and many chemotherapeutic agents are toxic to the host immune system. Radiation can upregulate targets of the immune system, augment their antigen presentation, and cause immune cell infiltration into the tumor.[15-17] Altogether, these radiation-induced changes could lead to activation and proliferation of cytotoxic T-cells in an antigen-specific fashion in draining lymph nodes.[18-21] The evolution of radiation therapy from serving as a purely locoregional therapy to a modality that can also stimulate a systemic cancer response represents an important innovation in oncology. However, the optimal way to combine immune checkpoint inhibitors and radiation therapy is poorly understood, and there is little prospective data evaluating its efficacy in patients, which is the rationale for performing this study. We hypothesize that this combination therapy will result in higher response rates, longer duration of response, and lengthened overall survival compared with previous studies utilizing immune checkpoint inhibitors alone.

2.4. Approach to Radiation Therapy Delivery

In standard clinical practice, the goal of radiotherapy in a patient with metastatic lung cancer is predominantly palliative for relief of symptoms. Quality of life is as important as longevity of life, and as such the radiation therapy administered in this trial will take into account quality of life, symptom relief, and minimizing morbidity along with the primary study goal of inducing an enhanced systemic immune response. We believe that these objectives will be best achieved by giving the treating physician discretion over the treated tumor site and choice of dose-fractionation used, within a set of guidelines. Preclinical data suggest that a shorter course of hypofractionated radiation therapy in 5

fractions or fewer is more immune-stimulatory, whereas a longer course of conventionally fractionated radiation is more immunosuppressive, through their differential effects on the up- and down-regulation of CD8 T-cells, Treg and MDSC cells, and expression of OX40, PD-L1, and MHC-1.[22-24] Fractionated courses are also superior to a single fraction treatment in stimulating an abscopal effect.[22] As such, for the purposes of this study, we will use a dose of 6-12Gy per fraction in 3 fractions (total dose 18-36 Gy) or 4-10 Gy per fraction in 5 fractions (total dose 20-50 Gy) to be completed over the course of 3-21 days. This variety of regimens offers a biologically effective dose (BED) range of 28-100 for early responding tissue (which correlates with tumor control) and 47-217 for late responding tissue (which correlates with side effects). This will give the treating physician a full range of options, such that an adequate dose can be given to the tumor while meeting all dose constraints of normal organs. The optimal hypofractionated radiation regimen is unknown, and there is some evidence that lower dose (and thus less ablative) regimens that would result in inferior local control may actually be more systemically immune-stimulating.[24] As such, regimens with a lower BED will be favored for this study, with the exception of situations in which improved local control to palliate symptoms is also a priority. Higher BED regimens may also be considered for larger size tumors and more rapidly growing tumors. This general preference for lower dose regimens will also have the favorable consequence of reducing the morbidity of therapy. Visceral metastases or metastases in organs involved in the immune system (e.g. liver) will be targeted preferentially over bone metastases due to the increased likelihood of an abscopal effect that has been previously reported for tumors in these sites.[13-14, 25] If a bone metastasis requires treatment for palliative purposes we advise also treating a visceral metastasis concurrently. Brain metastases will be treated according to standard of care if they develop during the period of trial enrollment, but because there is little evidence to suggest that treating a brain metastasis can elicit an extracranial systemic response, we advise also treating a visceral metastasis concurrently to increase the likelihood of an abscopal effect.

Finally, subsequent courses of radiation will also be delivered as part of this study in patients who are not having a systemic response to immune therapy or who are experiencing progressive disease. The rationale for these subsequent courses of radiation is to provide a frequent source of antigenic stimulation to potentially elicit an enhanced immune response. There is evidence to suggest that treatment of multiple lesions with SBRT in the metastatic setting has a survival benefit in lung cancer,[26] and scheduled radiation therapy has improved survival in other malignancies as well.[27]

2.5. Background and rationale of correlative studies

In consenting patients, peripheral blood samples will also be collected at the time of any change in response status as assessed on follow-up imaging studies in order to correlate the biological effects of treatment to response, and potentially help characterize optimal treatment delivery. Preclinical data suggests that immune checkpoint inhibitors increase the quantity of the activated cytotoxic T-cell subpopulation, with a higher CD8/Treg ratio correlating with more robust immune activation.[28-29] Additionally, M1/TH1 pro-inflammatory iNOS+ macrophages may also be recruited to a tumor and involved in cell-killing in this clinical context.[30-31] As such, we will use flow cytometry to characterize

levels of circulating CD4+, CD8+ and Treg T-cell subsets, as well as iNOS+/CD80+ macrophages. Changes in cytokine and chemokine levels specific for immune cell trafficking have not been well studied in patients receiving immune checkpoint inhibitors or radiation, so we will also evaluate for levels of circulating cytokines (HGF, TGF-β1, TGF-β2, TGF-β3) and chemokines (CCL2 for M1 macrophages, and CXCL9, CXCL10, and CXCL11 for T-cells). Finally, the clonality and diversity of the T-cell receptor (TCR) CDR3 region may help determine the specific lung cancer antigens and host immune response that was triggered by immune therapy and radiation. We will use next generation sequencing of the circulating T-cells immune-repertoire (i-repertoire) to better understand the evolution of tumor response during the course of treatment.

3. STUDY DESIGN

3.1. Study Design

This will be a Phase II prospective non-randomized clinical trial, with the primary objective being to assess the efficacy of combined checkpoint inhibitor immune therapy and hypofractionated radiation therapy in patients with Stage IV NSCLC. Toxicity is a secondary endpoint since the doses of radiotherapy to be used are within the realm of standard of care, and available medical literature suggests that the combination of radiation therapy with various immunomodulatory agents does not lead to excessive safety issues that would warrant a Phase I study. [14, 20, 23, 32-37] Since there is limited prospective clinical data on this approach in patients we felt that a randomized Phase III approach is not justifiable at present. Patients may be enrolled into this study before initiation of immune therapy (with radiation administered to one target lesion, starting at any time between the first and second cycles of immune therapy).

3.2. Number of Subjects

Approximately 33 subjects will be enrolled in this trial.

3.3. Expected Duration of Treatment and Subject Participation

Each course of radiation therapy will be 3-5 fractions delivered over 3-21 days. The first course of radiation will be initiated at any time point between the date of cycle 1 of immune therapy through the date of cycle 2 of immune therapy. The duration of immune therapy administration and the number of courses of radiation therapy that a subject receives will be dependent upon the subject's response to prior treatment. There is no specific maximum number of cycles of immune therapy if a patient continues to respond to treatment, or courses of radiation therapy if a patient has persistent stable disease on imaging. Progressive disease will be defined per irRC criteria as assessed by two imaging studies $\geq$ 4 weeks apart. Because abscopal responses have been reported even when the radiation is given after the immune therapy was completed, a final course of radiation therapy will be offered to patients at the time of progressive disease on imaging.[38-39] Subsequent therapy decisions will be at the discretion of the treating physicians, including the option of continuing the immune therapy past progression if clinically indicated.

The radiation therapy or immune therapy may be cancelled or discontinued at any point if no longer medically advisable due to intercurrent illness, toxicity/adverse event, decline in performance status, pregnancy, the best interests of the patient, or death. Subjects may also withdraw consent from treatment (partial consent) or from the study (full consent).

3.4. Duration of Follow-Up

Follow-up of subjects should continue for 30 days after systemic disease progression or initiation of an alternate systemic therapy (whichever comes first). After the study period, the sponsor-investigator may still obtain data from a subject's medical records or by phone call in order to document overall survival per patient agreement. Any adverse events experienced after a patient has discontinued the study treatment will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

3.5. Overall Duration of the Study

The study begins when the first subject signs the ICF. The primary analysis will occur when all subjects have completed the surveillance period and the last subject experiences progressive disease. Complete enrollment is expected over a 2 year period.

3.6. Study Termination

The investigator and sponsor retain the right to temporarily suspend or terminate study participation at any time. The date and reason for discontinuation must be documented. The investigator is to notify the institutional review board (IRB)/independent ethics committee (IEC) in writing of the study's completion or early termination, send a copy of the notification to the sponsor or sponsor's designee, and retain 1 copy for the site study regulatory file. Every effort should be made to complete the appropriate assessments.

4. SUBJECT SELECTION AND REGISTRATION

Each of the criteria in the sections that follow must be met in order for a subject to be considered eligible for this study. Use the eligibility criteria to confirm a subject's eligibility.

Patient's Name _____

Medical Record # _____

Research Nurse /
Study Coordinator Signature:_____**Date** _____

Treating Physician [Print] _____

Treating Physician Signature:_____Date_____

4.1. Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment:

____ 4.1.1 Subjects must have Stage IV (metastatic) histology- or cytology-confirmed NSCLC. The tumor type must be confirmed by biopsy but the stage may be based on biopsy or imaging.

____ 4.1.2 Subjects must have measurable disease of at least 1.0 cm in greatest dimension in at least 2 previously non-irradiated sites (except for lymph nodes, in which the short-axis dimension must be at least 1.5cm).

____ 4.1.3 Subjects must be planned to receive standard of care checkpoint inhibitor immune therapy.

____ 4.1.4 Subjects must be age ≥ 18 years since lung cancer is uncommon in children and likely to have different biologic properties and response to therapy in this age group.

____ 4.1.5 Subjects must have ECOG Performance status 0-2 [See Appendix A].

____ 4.1.6 Subjects must have life expectancy greater than 3 months

___ 4.1.7 Subjects must have adequate organ and marrow function as defined below within one month of study entry:

- Hemoglobin ≥ 8.0 g/dl
- Leukocytes ≥ 2,000/mcL
- Absolute neutrophil count ≥ 1,000/mcL
- Platelet count ≥ 50,000/mcL
- Total bilirubin ≤ 3.0 X institutional upper limit of normal, with the exception that patients with Gilbert disease may have a serum bilirubin ≤ 6 X upper limit of normal
- AST (SGOT) and ALT (SGPT) ≤ 3 X institutional upper limit of normal (or ≤ 5 X institutional upper limit of normal if liver metastases are present)
- Serum Creatinine ≤ 3.0 X institutional upper limit of normal

____ 4.1.9 Subjects must have the ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrollment.

____ 4.2.1 Subjects with active autoimmune disease (e.g. inflammatory bowel disease, rheumatoid arthritis, scleroderma, systemic lupus erythematosus, or autoimmune vasculitis), primary immunodeficiency syndrome, HIV/AIDS, or hepatitis B or C. Acknowledgement of any of these conditions is sufficient to exclude the patient from the study and no specific testing to exclude these conditions is required unless the patient expresses uncertainty about their presence.

____ 4.2.2 Subjects with oral corticosteroid dependency. Use of immunosuppressive doses of systemic medications within 7 days prior to starting study drug. Subjects are permitted to enroll if lower doses of systemic corticosteroids (< 10 mg/day

prednisone equivalents) are used within 7 days prior to study drug administration. Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Short-term steroids used to enable the use of IV contrast for an imaging study are permitted.

____ 4.2.3 Prior treatment with any antibody or drug targeting T-cell costimulation or immune checkpoint pathways (anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4, etc), or subjects actively receiving any other investigational cancer-directed agent, Interleukin-2, or immunosuppressive agents.

____ 4.2.4 Subjects with uncontrolled or untreated active brain metastases/CNS disease will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with previously treated brain metastases (by whole brain radiation and/or stereotactic radiosurgery) are eligible if the treatment was greater than 7 days from enrollment. In this case, imaging to confirm intracranial response to therapy is not required as long as the patient does not have any worsening neurologic symptoms. Of note, if brain metastases develop while the patient is enrolled on this study and they are treated, the patient may continue participation in this study at the discretion of the treating physicians.

____ 4.2.5 Subjects with uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

____ 4.2.6 Subjects who are pregnant are excluded from this study because radiation therapy has the potential for teratogenic or abortifacient effects. Women of childbearing potential must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 8 weeks after the last treatment.

4.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. It is the policy of the Mary Babb Randolph Cancer Center to strive for gender and minority patient participation that represents the population of West Virginia in all clinical investigations. Between January 2015 and December 2015, 233 patients were enrolled onto clinical trials at the Mary Babb Randolph Cancer Center. Of these patients 55% percent were female and 3% percent were members of minority ethnic groups. It is anticipated that a similar or greater proportion of patients on this study will be female and/or members of ethnic minorities. It is important to recognize that according to the 2014 US Census Bureau (available at http://quickfacts.census.gov) that the State of West Virginia minority ethnic group (e.g., not limited to African American and Hispanic) population is 3.6% Black (national average 13.2%) and 1.5% Hispanic (national average 17.4%). The majority of Black West Virginians live in the central and southern part of the state and Gilmer County is the only county in WV whose Black population approaches the national average.

4.4 Registration

At the point of registration, the study coordinator will complete registration in the OnCore database, including demographic, consent and on-study information.

The patient will be assigned a unique sequence number for the study prior to the initiation of the study treatment.

For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded.

All source documents that support eligibility, signed informed consent/HIPAA and signed eligibility checklist must be available for review and verification by the quality coordinator prior to starting therapy.

Patient Travel Reimbursement

Travel reimbursement may be offered to patients on trial. Due to the limited travel funds available, travel reimbursement will be reviewed on case by case basis by the Principal Investigator.

5. TREATMENT PLAN

5.1. Initial Radiation Therapy

5.1.1 Dose-fractionation: The initial course of radiation therapy will be administered in 3 or 5 fractions over 3-21 days, at a recommended dose of 6-12 Gy per fraction for 3 total fractions (total dose 18-36 Gy) or 4-10 Gy per fraction for 5 total fractions (total dose 20-50 Gy). Dose-fractionation schemes with a lower BED will be favored, with the exception of situations in which improved local control to palliate symptoms is also a priority for a given patient. Higher BED regimens may also be considered for larger size tumors and more rapidly growing tumors.

5.1.2 Targeted Site: Visceral metastases or metastases in organs involved in the immune system (e.g. liver, lymph nodes) will be targeted preferentially. If a bone or brain metastasis develops while the patient is enrolled in this trial and requires treatment for palliative purposes, we advise also treating a visceral metastasis.

5.1.3 Radiotherapy Technique: Stereotactic body radiation therapy (SBRT) will be the favored treatment delivery technique due to its high conformality to a target volume and avoidance of organs-at-risk (OAR) of toxicity. Other techniques, like 3-dimensional conformal radiotherapy (3DCRT) and intensity modulated radiation therapy (IMRT), will be permitted with the caveat that the patient will, irrespective of technique, undergo CT simulation for treatment planning using SBRT immobilization devices, undergo daily image guidance to confirm the positioning of the tumor prior to treatment, and the plans must meet standard 3 or 5 fraction SBRT dose constraints to normal tissues. The use of 4D-CT and respiratory motion management for treatment planning and delivery are encouraged as necessary.

5.1.4 Treatment Planning: All patients will undergo CT simulation using SBRT immobilization devices. Target volumes (i.e. GTV, CTV, ITV, PTV) will be delineated at the discretion of the treating physician. In general, minimal or no margin should be added

for microscopic disease extension in this setting, with the focus on covering gross disease plus an appropriate margin to encompass tumor motion and any physical setup uncertainties. OARs should be contoured according to standard guidelines provided by NRG Oncology for clinical trials.[40] Megavoltage photons of at least 6MV will be used for all treatment plans. Ideally 95% of the planning target volume (PTV) should receive 100% of the prescribed dose, and all OAR constraints detailed in Appendix B must be achieved in order to minimize the risk of toxicity. In patients receiving multiple courses of radiation therapy, there will be an assumption of no tissue recovery, and the total dose to any organ from the sum of all radiotherapy courses will need to achieve the dose limits for a single course of hypofractionated radiation therapy as described in Appendix B.

5.1.5 Treatment Delivery: The thoracic radiation oncologist or his/her designated physician is required to be present at the console to review the daily CT and projection images prior to each treatment. A brief documentation of the patient set up is required in the treatment monitoring system of each institution by the treating physician.

5.1.6 Coordination of Radiation with Immune Therapy: The first course of radiation will be initiated at any time point between the date of cycle 1 of immune therapy through the date of cycle 2 of immune therapy.

5.2. Standard of Care Immune Checkpoint Inhibitors

Immune checkpoint inhibitors that are FDA approved for use in patients with metastatic NSCLC will be acceptable for use concurrently with radiotherapy in this trial. The choice of agents will be at the treating medical oncologist's discretion, and include:

- Nivolumab 240 mg once every 2 weeks (14 day cycle) or 480 mg once every 4 weeks (28 day cycle)
- Pembrolizumab 200 mg once every 3 weeks (21 day cycle)
- Atezolizumab 1200 mg once every 3 weeks (21 day cycle)

These agents should be continued per standard of care until either disease progression or unacceptable toxicity. In the case of Pembrolizumab, it is also recommended that patients should be on the drug for no more than 24 months even if there is no evidence of disease progression. Pembrolizumab may also be used in combination with Pemetrexed and Carboplatin for the first-line treatment of metastatic non-squamous NSCLC. If any of the immune therapy agents are permanently discontinued at any point, a final course of radiation therapy should be offered to the patient before switching to an alternative therapy. While the patient is receiving immune therapy, no other investigational or commercial agents or therapies may be administered with the intent to treat the subject's malignancy.

5.3. Subsequent Response-Adapted Radiation Therapy

Any patient who completes the initial course of radiation therapy and continues on one of the immune checkpoint inhibitors described above will be eligible for subsequent course(s) of radiation therapy to a different lesion that was not previously irradiated based on the results of follow-up imaging studies detailed below and in section 11.

- Patients with a partial or complete response per irRC criteria will be observed without any further radiation therapy until progression.
- Patients with stable disease per irRC criteria will undergo an additional course of radiation therapy given concurrently with the next cycle of immune checkpoint

therapy, and then continue the immune therapy as above. Each subsequent course of radiation therapy should ideally be at least 2 months after completion of the prior course of radiation therapy, but is left at the discretion of the treating physician depending on the patient's palliative needs. Day 1 of radiation should be on Day 1 of the next cycle of immune therapy.

- Patients with progressive disease at any time per irRC criteria will undergo a final course of radiation therapy. This may be given concurrently with a final cycle of immune therapy or as stand-alone treatment within 6 weeks after discontinuation of immune therapy but before an alternative systemic therapy is initiated.

Please see Section 5.5 below regarding situations in which these subsequent courses of radiation therapy may be withheld. Each subsequent course of radiation therapy should be given in a similar style as detailed for the initial course of radiation therapy above. In patients with a mixed response to therapy, preference will be given to an enlarging tumor (rather than one that is stable or decreasing in size) as the site of treatment. Reimaging will again be obtained after completion of the radiation therapy with timing at the discretion of the treating physicians, and the same response-adapted approach to decision making for further courses of radiation therapy will be carried out as described above.

5.4. General Concomitant Medications and Supportive Care Guidelines

Subjects should receive full supportive care, including transfusions of blood and blood products, antibiotics, antiemetics, corticosteroids (see section 6.2), etc when appropriate.

6. DOSE DELAYS/DOSE MODIFICATIONS

6.1. Radiation Therapy

A range of dose-fractionation schemes is available to the treating radiation oncologist as part of this study. Most treated lesions will not require deviations in dose from the stated ranges, with some exceptions:

- Brain metastases that develop while the patient is enrolled in this study may be treated with single fraction stereotactic radiosurgery or 3 Gy X 10 fractions as indicated.
- If only less conformal techniques are available to a patient for the treatment of vertebral lesions and the spinal cord will receive the full prescription dose, lower dose regimens of 4 Gy in 5 fractions or 3 Gy in 10 fractions are permissible in order to avoid toxicity.

For every patient, the total dose, dose per fraction, and number of fractions in every course of radiation therapy that the subject receives will be recorded.

6.2. Immune therapy

Appropriate dose modifications for immune therapy agents are at the discretion of the treating medical oncologist, and will be managed according to standard of care. All dose modifications or missed treatments should be recorded for analysis but no specific dose modification parameters will be used since the immune therapy is not the experimental component in this study. For treatment of moderate or severe immune related adverse

events (irAEs), we advise interruption of the checkpoint inhibitor and the use of corticosteroid immunosuppression. For grade 2 (moderate) immune-mediated toxicities, treatment with the checkpoint inhibitor should be withheld and should not be resumed until symptoms or toxicity is grade 1 or less. Prednisone 0.5 mg/kg/day or equivalent should be started if symptoms do not resolve within a week. For grade 3 or 4 (severe or life-threatening) immune-mediated toxicities, treatment with the checkpoint inhibitor should be permanently discontinued. Prednisone 1-2 mg/kg/day or equivalent should be given immediately. When symptoms subside to grade 1 or less, steroids can be gradually tapered over at least one month. If symptoms do not improve within 3 days of starting steroids, administer infliximab 5 mg/kg (may repeat 2 weeks later if no improvement in symptoms). If the immune therapy is withheld for a period of greater than 14 days at a time when a course of radiation therapy was planned to be given, the course of radiation therapy may be initiated prior to resuming the immune therapy.

7. ADVERSE EVENTS

7.1. Definitions

7.1.1. Adverse Event

An adverse event (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject.

7.1.2. Serious Adverse Events

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

- **Death**

- A **life-threatening** adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the subject was at immediate risk of death at the time of the event. It does not refer to an adverse event which in a more severe form might have caused death.

- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 24 hours OR
    - The admission is pre-planned (e.g., elective or scheduled surgery arranged prior to the start of the study) OR
    - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

  However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependent on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.

- Results in **persistent or significant disability/incapacity or** substantial disruption of a person's ability to conduct life's normal functions.

- Is a **congenital anomaly/birth defect**

- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the subject 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 disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. The development of a new cancer is always considered an important medical event.

7.2. Adverse Event Evaluation

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has started immune therapy are fully recorded in the subject's medical records. Source documentation must be available to support all adverse events. A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, result in a delay or dose modification of study treatment, or judged relevant by the investigator), should be reported as an adverse event.
The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious):

- Event term (as per CTCAE version 4.0)
- Description of the event

- Date of onset and resolution
- **Expectedness of the toxicity**
- **Grade of toxicity**
- **Attribution of relatedness to the investigational agent- (this must be assigned by an investigator, sub-investigator, or treating physician)**
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received con-med or other intervention, etc.
- Outcome of event

The most common adverse event from the short courses of focal radiation therapy used in this study will be fatigue. This may be accompanied by decreased appetite, changes in taste, or weight loss. Since radiation is a focal, localized treatment to a specific targeted tumor, the likelihood of all other side effects is dependent on the part of the body that is treated, as described in the table below. Unknown side effects may also arise due to the combination of radiation therapy and immunotherapy in this study.

	Acute/Short-Term/Temporary	**Chronic/Long-Term/Permanent**
Skin	redness, dryness, darkening, itching, peeling, tenderness, hair loss	thickening, firmness, discoloration, hair loss
Bone	decreased blood counts	fracture, pain
Nerves	electrical sensation	pain, loss of strength, numbness, tingling, paralysis
Lymphatics	Swelling	lymphedema
Brain	fatigue, brain swelling, headaches, nausea, vomiting, dizziness, drowsiness, altered taste or smell	loss of strength, numbness, tingling, paralysis
Lungs	cough, shortness of breath, bleeding	cough, shortness of breath, bleeding, decreased lung capacity
Heart	chest pain, pericarditis, abnormal rhythm	chest pain, heart attack, heart failure, pericarditis
Esophagus	difficulty swallowing, painful swallowing, food sticking, heartburn, nausea, vomiting, decreased appetite,	stricture, obstruction, perforation, fistula, bleeding, chest pain
Stomach/ Bowel	nausea, vomiting, fatigue, diarrhea, cramping, decreased appetite	stricture, obstruction, perforation, bleeding, abdominal pain, ulceration
Liver	nausea, vomiting, fatigue, diarrhea	decreased liver function, ascites, cirrhosis, encephalopathy, bleeding
Rectum	spasm, frequent bowel movements	ulceration, bleeding
Bladder	burning/pain with urination, increased frequency, urinary urgency	decreased bladder capacity, bladder spasms, bleeding

Immune therapy agents have their own set of expected side effects, as detailed below.

	Nivolumab	**Pembrolizumab**	**Atezolizumab**
Common	Fatigue Decreased appetite	Fatigue Decreased appetite	Fatigue Decreased appetite

	Generalized weakness	Nausea Rash	Lung inflammation Fever
Occasional	Nausea Diarrhea Joint inflammation Fever Lung inflammation Rash	Diarrhea Decreased thyroid hormone levels Increased thyroid hormone levels Lung inflammation Generalized weakness Anemia Mouth/Lip Sores	Nausea Diarrhea Joint inflammation Insomnia Lung Infection Generalized weakness
Rare	Mouth/Lip Sores Muscle inflammation Colon inflammation Anemia Peripheral Neuropathy Decreased white blood cell count Hair Loss Liver inflammation	Hair loss Decreased white blood cell count Colon inflammation Severe rash (skin blistering/ulceration) Pancreas inflammation Decreased adrenal hormone levels Muscle inflammation Liver inflammation Diabetes Mellitus	Myalgia Hair loss Pneumonia Decreased thyroid hormone levels Peripheral neuropathy Neutropenia Muscle inflammation

7.2.1. Adverse Event Grading

Descriptions and **grading scales** found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version **4** will be utilized for AE reporting.

Grade	Description
0	**No AE** (or within normal limits).
1	**Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	**Moderate**; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	**Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	**Life-threatening** consequences; urgent intervention indicated.
5	**Death** related to AE

7.2.2. Adverse Event Attribution

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. For the purposes of this study, adverse events may be differentiated as a radiation-induced AE or an immune-related AE (irAE). Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

Protocol must specify if attribution is required for individual components of the treatment regimen or the treatment regimen as a whole.

7.3. Adverse Event Reporting Procedures

All patients will be evaluable for toxicity from the time of their first treatment with immune therapy. It is the responsibility of all investigators to assess AEs during the subject's participation in the study. Subjects will be followed for ***30*** days after treatment has stopped or until an alternative systemic therapy has been started or until death, whichever comes first. Each AE should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Adverse events that occur after the subject has started immune therapy must be documented in the study database/case report forms (OnCore or other PRMC approved database), subject's medical records, and as required per additional institutional standards. Source documentation must be available to support all reported adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

This protocol will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at *http://ctep.cancer.gov* for adverse event reporting.

7.4. Serious Adverse Event Reporting Procedures

Serious Adverse Events (SAE) requiring expedited reporting within 24 hours will also be reviewed by DSTC at the next scheduled meeting. For immune-related SAE's attributed to FDA approved standard of care immune therapy, FDA MedWatch will be used only for unexpected related events (not on package insert). All serious adverse events due to radiation therapy will be reported into OnCore and internally reviewed. All subsequent SAE documentation will be submitted to the DSTC once available. The DSTC will determine if further action is required.

7.5. SAEs and OnCore

- All SAEs will be entered into OnCore.
- A copy of the SAE form(s) submitted to the sponsor-investigator is also uploaded into Oncore.

7.6. Data Safety and Toxicity Committee

It is the responsibility of each site PI to ensure that ALL SAEs occurring on this trial (internal or external) are reported to the MBRCC DSTC, Data and Safety Toxicity Committee. This submission is simultaneous with their submission to the sponsor and/or other regulatory bodies.

The sponsor-investigator is responsible for submitting an annual report to the DSTC as per MBRCC Data and Safety Monitoring Plan.

7.7. Data and Safety Monitoring Plan (DSMP)

This protocol will adhere to the policies of the Mary Babb Randolph Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7. Immune checkpoint inhibitors that are FDA approved for use in patients with metastatic NSCLC will be acceptable for use concurrently with radiotherapy in this trial.

8.1. Commercial Agent #1

8.1.1. Name of Agent Nivolumab

Other Names: Opdivo

Product description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate, sodium chloride, mannitol, diethylenetriaminepentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80), pH 6.0.

Solution preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to drug concentrations no less than 0.35 mg/mL. Note: Mix gently. Do not shake.

Compatibility: no incompatibilities between nivolumab and polyvinyl chlorie (PVC), nonPVC/non DEHP (di(2-ethylhexyl)phthalate) IV components, or glass bottles have been observed.

Attach a low protein binding filter 0.2-1.2 micron

Storage requirements: Vials of nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light, freezing and shaking.

Stability: The administration of undiluted and diluted solutions of nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 4 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 4-hour period under room temperature and room light conditions includes the product administration period.

Route of administration: Nivolumab injection is to be administered as a 30 minute IV infusion through a 0.2 micron to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter. DO NOT administer as IV push or bolus injection.

Drug Procurement: Nivolumab is considered standard of care and will be utilized from commercially available supply. Nivolumab is available as 40 mg/4 mL single-dose vial and 100 mg/10 mL single-dose vial.

The cost of this agent will be the subject's responsibility.

Drug Accountability: N/A; this is standard of care treatment.

Drug Destruction: Commercially available supply. Institutional standards apply

8.2. Commercial Agent #2

8.2.1. Name of Agent ____Pembrolizumab_____

Other Names: ____Keytruda_____

Product description: Pembrolizumab Solution for Infusion is a sterile, non-pyrogenic aqueous solution supplied in single-use Type I glass vial containing 100 mg/4 mL of pembrolizumab (MK-3475). The product is preservative-free solution which is essentially free of extraneous particulates.

Solution preparation: Pembrolizumab infusion solutions should be prepared in **0.9% Sodium Chloride Injection, USP** (normal saline) or regional equivalent or 5% Dextrose

Injection, USP (5% dextrose) or regional equivalent and the final concentration of pembrolizumab in the infusion solutions should be between 1 mg/mL and 10 mg/mL.

Please note, the preferred diluent is 0.9% Sodium Chloride and 5% dextrose is only permissible if normal saline is not available.

Storage requirements: Pembrolizumab Solution for Infusion, 100 mg/ 4 mL vial for Infusion should be stored at refrigerated conditions (2 – 8 °C) and protected from light.

Note: vials should be stored in the original box to ensure the drug product is protected from light.

Stability: Following reconstitution with sterile water for injection, Pembrolizumab infusion solutions should be prepared in **0.9% Sodium Chloride Injection, USP** (normal saline) and the final concentration of pembrolizumab in the infusion solutions should be between 1 mg/mL and 10 mg/mL.

If normal saline is not available, 5% Dextrose Injection, USP or regional equivalent (5% dextrose) is permissible.
Please note, the preferred diluent is 0.9% Sodium Chloride and 5% dextrose is only permissible if normal saline is not available.

Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion

In addition, IV bags may be stored under refrigeration at 2 °C to 8 °C (36 °F to 46 °F) for up to 24 hours. If refrigerated, allow the IV bags to come to room temperature prior to use.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if extraneous particulate matter other than translucent to white proteinaceous particles is observed.

Route of administration: Administer infusion solution intravenously over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.

Drug Procurement: Pembrolizumab is considered standard of care in this trial and will be utilized from commercially available supply. Pembrolizumab injection (solution): carton containing one 100 mg/4 mL (25 mg/mL), single-use vial

The cost of this agent will be the subject's responsibility.

Drug Accountability: N/A; this is standard of care treatment.

Drug Destruction: Commercially available supply. Institutional standards apply

Other Information:
• DO NOT USE PEMBROLIZUMAB IF DISCOLORATION IS OBSERVED.

• DO NOT SHAKE OR FREEZE THE VIAL(S).

• DO NOT ADMINISTER THE PRODUCT AS AN (INTRAVENOUS (IV) PUSH OR BOLUS).

• DO NOT COMBINE, DILUTE OR ADMINISTER IT AS AN INFUSION WITH OTHER MEDICINAL PRODUCTS.

• DO NOT CO-ADMINISTER OTHER DRUGS THROUGH THE SAME INFUSION LINE.

• Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if extraneous particulate matter other than translucent to white proteinaceous particles is observed.

8.3. Commercial Agent #3

8.3.1. Name of Agent Atezolizumab

Other Names: Tecentriq

Product description: Atezolizumab Injection is a colorless to slightly yellow solution. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in glacial acetic acid, L-histidine, sucrose, polysorbate 20. pH 5.8.

Solution preparation: Withdraw 20 mL of atezolizumab from the vial; dilute into a 250 mL bag containing 0.9% Sodium Chloride Injection, USP. Dilute dose in NS only; gently invert to mix without shaking. Discard vial if solution is cloudy, is discolored, or contains visible particles.

Compatibility: Use infusion bag made of polyvinyl chloride (PVC), polyethylene (PE), or polyolefin (PO).

Storage requirements: Diluted infusion solution may exist at room temperature for no more than 6 hours (including infusion time), or under refrigeration (2 to 8°C or 36 to 46°F) for no more than 24 hours. Protect from light. Do not shake. Do not freeze.

Stability: The administration of undiluted and diluted solutions of atezolizumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2° to 8°C (36° to 46°F) and a maximum of 6 hours of the total 24 hours can be at room temperature (20° to 25°C, 68° to 77°F) and room light. The maximum 6-hour period under room temperature and room light conditions includes the product administration period.

Route of administration: Atezolizumab injection is to be administered as a 60 minute infusion for the initial dose. If first dose is well tolerated, may infuse subsequent doses over 30 minutes. Infuse with or without a 0.2- to 0.22-micron sterile, non-pyrogenic, low-protein binding in-line filter. Do not administer as an IV push or bolus. Do not administer other medications at the same time through the same IV line. Monitor for infusion reactions. Atezolizumab is compatible in NS.

Drug Procurement: Atezolizumab will be obtained from commercially available supply. Atezolizumab is available as a 60 mg/1 mL (1200 mg/20 mL) PF single dose vial.

The cost of this agent will be the subject's responsibility.

Drug Accountability: FDA labelled indication non-small cell lung cancer (NSCLC), Metastatic, with progression during or after platinum-based chemotherapy; patients with ALK or EGFR genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving atezolizumab. Pre-clinical and clinical studies have established a safety and efficacy profile for this drug beyond that of its FDA labelled indication.

Drug Destruction: Commercially available supply. Institutional standards apply.

9. CORRELATIVE STUDIES

9.1. Correlative Study #1

Use flow cytometry to characterize levels of circulating CD4+, CD8+ and Treg T-cell subsets, as well as iNOS+/CD80+ macrophages in peripheral blood samples.

9.1.1. Background

Preclinical data suggests that immune checkpoint inhibitors increase the quantity of the activated cytotoxic T-cell subpopulation, with a higher CD8/Treg ratio correlating with more robust immune activation.[28-29] Additionally, M1/TH1 pro-inflammatory iNOS+/CD80+ macrophages may also be recruited to a tumor and involved in cell-killing in this clinical context.[30-31]

9.1.2. Rationale for Analysis

Biomarkers predictive of response to immune therapy are currently not well developed. Levels of circulating immune cells before and during therapy have the potential to help

better determine which patients will benefit from therapy. The data from these correlative studies will be used as part of a univariate and multivariate logistic regression model to assess the association between response to treatment and several variables related to the radiotherapy, tumor, and patient characteristics.

9.1.3. Collection of Specimens

After consenting patients, peripheral blood samples will be collected at baseline before initiation of treatment and at the time of any change in response status as assessed on follow-up imaging studies. All attempts will be made to draw the blood with currently scheduled venipuncture, but it will also be permissible to draw specifically for this study if necessary.

9.1.4. Handling of Specimens

Approximately 10-16 mL of blood will be obtained from the patient in two 10 ml EDTA (purple top) tube. These tubes of blood will serve as the specimen for both correlative study #1 and #2. In order to facilitate the subsequent analyses all research samples will be collected on Monday-Thursday from 8:00am-3:00pm. Specimens will be put on ice after collection and transported to the Biospecimen Processing Core Laboratory (Room 1826 MBRCC) within 30 minutes of acquisition. All subsamples will be labeled with only the following: patient research ID (obtained from the PI/study coordinator), date/time of collection, type of sample. The labeled sample for correlative study #1 and #2 will then be transported on ice to the laboratory of Dr. Tim Eubank (HSC 5602) for further processing and analysis.

9.1.5. Analytical Laboratory

The specimens will be processed and analyzed in Health Sciences Center (HSC) laboratory 5602, under the direction of Dr. Tim Eubank. Dr. Eubank and his personnel will provide technical support for the isolation of both the serum and cellular component of the samples, and provide overall guidance in experimental design and interpretation of results.

9.1.6. Methods

Need IB buffer (0.5 g BSA in 100 ml MACS buffer, filter). Keep at 4^0C.

1. Put pad in hood to work on. Mix the tube with blood.
2. Pipet x 250 ul blood in 4 yellow cap BD microtainer tubes (SST – serum separator). Centrifuge Hermle 15000 rpm/4^0C/5min. While centrifuging, prepare 4 Eppendorf tubes, with label on top – patient ID, initials, and date.
3. Collect the serum in the 4 Eppendorf tubes and freeze at -80^0C (left freezer, box “Tim Eubank Clinical Trials Plasma Samples”.
4. The remaining blood, ~ 8 ml – dilute 1:1 with PBS. Prepare 2 Falcon 15 tubes with 4 ml PBS in each. Divide the blood between the two tubes and add additional PBS depending on the volume of blood. Mix well.
5. Prepare two Falcon 15 tubes with x 4 ml Ficoll. Layer the diluted blood on top of the Ficoll – put the pipettor on “LOW” and “GRAVITY”.
6. Centrifuge at 2000 rpm/25^0C/20 min with no brake (brake=0, 0). Program Buffy. Go to next step as soon as the centrifuge stops.
7. Collect the plasma from the 2 Falcons in a new Falcon tube. [When incubating at a later step, aliquot the plasma in 10 Eppendorf tubes, labelled with patient ID/initials/date and keep in right

-80^{0}C freezer, second shelf from bottom, box "Diluted Plasma Eubank Clinical Samples"]. Leave ~3-4 mm of plasma on top of the PBMC layer. Next, collect the PBMC layer in a new Falcon tube. Volume is ~4-5ml.

8. Add 5 ml PBS to the PBMCs (10 ml, if some of the Ficoll has been transferred). Mix by inverting 3-4 times. Centrifuge 1500 rpm/5min/4^{0}C (Program No1 Cell spin).
9. Label 10 Eppendorf tubes 1-10. Take out supernatant and resuspend in 1 ml IB buffer. 10 min ice.
10. Aliquot the cells in the Eppendorf tubes:

1. Unstained
2. 70 ul cells + 5ul CD4 Ab
3. 70 ul cells + 5ul CD25 Ab
4. 70 ul cells + 5ul FoxP3 Ab
5. 70 ul cells + 2ul iNOS Ab
6. 70 ul cells + 5ul CD80 Ab
7. 190 ul cells + 5ul CD4 Ab + 5ul CD25 Ab + 5 ul FOXP3 Ab
8. 190 ul cells + 5ul CD8 Ab
9. 190 ul cells + 5ul CD80 Ab + 2 ul iNOS Ab

11. Incubate for 1h at 4^{0}C covered with foil.
12. Add x 1 ml MACS buffer and centrifuge 1500 rpm 5min/4^{0}C. Repeat.
13. Resuspend in x 100 ul MACS buffer. Add x 900 ul Methanol (keep at -20^{0}C). Incubate for 30 min on ice, covered with foil.
14. Centrifuge 2000 rpm/5min/4^{0}C. Resuspend in 1 ml MACS buffer. Centrifuge again.
15. Samples 1, 2, 3, 4, 7, and 9 – resuspend in x 300 ul MACS buffer, transfer to FACS tubes and keep at 4^{0}C covered with foil. Label the FACS tubes with the name of the antibody.
16. Samples 5, 6, 8, and 10 – add antibodies for intracellular staining.
17. Incubate on ice for 1h, covered with foil.
18. Add x 1 ml MACS buffer to the tubes. Centrifuge 2000 rpm/5min/4^{0}C. Repeat.
19. Resuspend samples in x 300 ul MACS buffer. Transfer to FACS tubes, keep at 4^{0}C covered with foil.
20. Bring tubes to the Flow facility.

9.2. Correlative Study #2

Evaluate levels of circulating cytokines (HGF, TGF-β1, TGF-β2, TGF-β3) and chemokines (CCL2 for M1 macrophages, and CXCL9, CXCL10, and CXCL11 for T-cells).

9.2.1. Background

Immune cell infiltration into malignant tumors is thought to be an important component of a patient having a response immune checkpoint inhibitors and radiation, however, mechanisms of T-cell and macrophage trafficking have not been well studied in patients receiving immune checkpoint inhibitors or radiation. Changes in cytokine and chemokine levels specific for T-cells and macrophages may correlate with levels of circulating immune cells and tumor response to therapy.

9.2.2. Rationale for Analysis

As above, biomarkers predictive of response to immune therapy are currently not well developed. Levels of circulating cytokines and chemokines for T-cells and macrophages before and during therapy have the potential to help better determine which patients will benefit from therapy. The data from these correlative studies will be used as part of a univariate and multivariate logistic regression model to assess the association between

response to treatment and several variables related to the radiotherapy, tumor, and patient characteristics

9.2.3. Collection of Specimens

After consenting patients, peripheral blood samples will be collected at baseline before initiation of treatment and at the time of any change in response status as assessed on follow-up imaging studies. All attempts will be made to draw the blood with currently scheduled venipuncture, but it will also be permissible to draw specifically for this study if necessary.

9.2.4. Handling of Specimens

Approximately 10-16 mL of blood will be obtained from the patient in two 10 ml EDTA (purple top) tubes. These tubes of blood will serve as the specimen for both correlative study #1 and #2. In order to facilitate the subsequent analyses all research samples will be collected on Monday-Thursday from 8:00am-3:00pm. Specimens will be put on ice after collection and transported to the Biospecimen Processing Core Laboratory (Room 1826 MBRCC) within 30 minutes of acquisition. All subsamples will be labeled with only the following: patient research ID (obtained from the PI/study coordinator), date/time of collection, type of sample. The labeled sample for correlative study #1 and #2 will then be transported on ice to the laboratory of Dr. Tim Eubank (HSC 5602) for further processing and analysis.

9.2.5. Analytical Laboratory

The specimens will be processed and analyzed in Health Sciences Center (HSC) laboratory 5602, under the direction of Dr. Tim Eubank. Dr. Eubank and his personnel will provide technical support for the isolation of both the serum and cellular component of the samples, and provide overall guidance in experimental design and interpretation of results.

9.2.6. Methods

In our sterile biosafety cabinet in lab 5602 HSC, patient whole blood will be diluted 1:2 with sterile phosphate buffered saline (PBS). The mixture will be layered atop 15 mL Ficoll and centrifuged at 1200 rpm for 20 minutes (no break). After, the serum will be collected from the top layer and aliquoted into 1.5 mL Eppendorf tubes and frozen at -80C until further analysis for cytokines/chemokines by ELISA or Multiplex analysis.

Determination of chemokines in plasma:

An aliquot (100 µL in duplicate) of patient serum will be thawed form -80C storage and subjected to ELISA analysis specific for human CCL2, CXCL9, CXCL10, and CXCL11 (R&D Systems, Duoset Kits) as described by the manufacturer. Validity of the assay: We will be using standard “sandwich” ELISA kits sold by R&D Systems, Inc. Each kit is built specifically for the detection of human CCL2, CXCL9, CXCL10, and CXCL11 from cell supernatants, homogenized tissue extract, plasma, and serum. The kit is composed of a specific monoclonal “capture” antibodies for the protein of interest. After incubation of the sample and binding, a polyclonal “detection” antibody is incubated with the sample followed by a signaling molecule that allows for detection. A standard curve is generated using recombinant protein for quantitative comparison. These samples will be run in duplicate and averaged to minimize error. This is a well-accepted and highly-published method in the literature.

9.3. Correlative Study #3

Immune-Repertoire next generation sequencing (NGS) analysis.

9.3.1. Background

The clonality and diversity of the T-cell receptor (TCR) CDR3 region may help determine the specific lung cancer antigens and host immune response that was triggered by immune therapy and radiation. We will use next generation sequencing of the circulating T-cells immune-repertoire (i-repertoire) to better understand the evolution of tumor response during the course of treatment.

9.3.2. Rationale for Analysis

Assessing the dynamic changes of the immune-repertoire of a patient under checkpoint anti-PD-1 immunotherapy, with the adjunctive addition of local radiotherapy, would allow us to gain insight into the determinant of immunotherapy response. Such knowledge would have significant diagnostic potential when validated in the future in the arena of cancer immunotherapy.

9.3.3. Collection of Specimens

After consenting patients, peripheral blood samples will be collected at baseline before initiation of treatment and at the time of any change in response status as assessed on follow-up imaging studies. All attempts will be made to draw the blood with currently scheduled venipuncture, but it will also be permissible to draw specifically for this study if necessary.

9.3.4. Handling of Specimens

Separately from the blood collected for correlative studies #1 and #2, approximately 7-8 mL of blood will be obtained from the patient in one 10 ml EDTA (purple top) tube for correlative study #3. Specimens will be put on ice after collection and transported to the Biospecimen Processing Core Laboratory (Room 1826 MBRCC) within 30 minutes of acquisition. All subsamples will be labeled with only the following: patient research ID (obtained from the PI/study coordinator), date/time of collection, type of sample. The labeled sample for correlative study #3 will then be transported on ice to the laboratory of Dr. Tim Eubank (HSC 5602) for further processing and analysis. In earlier versions of this protocol these samples were processed in the laboratory of Dr. Patrick Ma (HSC 1815). However, since he has left WVU Dr. Eubank has taken over the role of initial processing before sending the samples to Dr. Ma at Penn State University for Next Generation Sequencing.

9.3.5. Analytical Laboratory

The specimens will be processed and analyzed in Health Sciences Center (HSC) laboratory 5602, under the direction of Dr. Tim Eubank. Dr. Eubank and his personnel will provide technical support for the isolation of both the plasma and cellular component of the samples, and provide overall guidance in experimental design and interpretation of results.

9.3.6. Methods

Step one: Obtain Buffy Coat

- Receive EDTA purple top 7-8ml (usually 10ml) patient blood sample on ice
- Centrifuge with 4 C 3000g/10mins, with acceleration 9 and brake 9
- Storage plasma in 1.5 EP tube -80C
- Qiagen EL Lysis Buffer cat# 79217 10ml to lysis on ice, vortex well, ice 5 mins
- Centrifuge with 4C 3000g/5mins, with acceleration 9 and brake 9
- Remove supernatant (make sure to get rid of reddish portion)
- Qiagen EL Lysis Buffer cat# 79217 5ml to lysis on ice, vortex well, ice 3 mins
- Centrifuge with 4C 3000g/5mins, with acceleration 9 and brake 9
- Remove supernatant (make sure to get rid of reddish portion)
- Use Autoclaved 1ml ice cold PBS wash the pellet 1-2 times. Count cells.
- Cell counting: _____cell/ml, volume _____ml, total cell #_____

Step two: Extraction of RNA

- With each 3-5x10^7 cells, use Qiazol 1ml mix with buffy coat, vortexing 1 min until no visible lump
- Ice waiting 5 mins
- Add chloroform 200ul, vortexing 3 sec
- Ice waiting 2 mins
- Centrifuge 4C 13000rmp/5mins
- Take 350ul supernatant clear portion, mix with isopranolol 350ul in a 1.5 EP tube, mixing well
- Ice waiting 5mins
- Centrifuge 4C 13000rmp/5 mins
- Remove supernatant, be careful do not remove the RNA pellet
- Use 500ul 75% ethanol to wash, vortex each time 3-5 sec,
- Centrifuge 4C 13000rpm/3mins
- Repeat red steps for 3 times
- Remove as much as possible the remaining ethanol, open lid, dry up RNA EP tube in the fume hood for 5 mins til all portion of the inner tube is dried
- Add appropriate amount of Rnase-free H2O, starting from low volume, you can always add more later, the key is to have good concentration level.
- Nanodrop measurement
- Concertation _____ng/ul, 260/280_____;260/230_____;total vol_____ul; yield _____ug

Step 3: DNA Extraction—using Qiagen Blood DNA kit CAT# 51104

- The spin column's' capacity is 5x10^6 cells for buffy coat ONLY, use Qiagen protocol

Step 4: buffy coat in the LN (consider start adding this step as this new step would be helpful in future if we want to perform live immune cell assays)

- Store buffy coat with 90%FBS+10% DMSO in LN If have more cells

<u>Next Generation Sequencing</u>
Processed samples will be sent to Dr. Patrick Ma at Penn State University for this analysis.

RNA extraction and purification would be performed as outlined above. Purified RNA is then amplified using iRepertoire's HTBI-M Reagent System (barcodes 01-10) per the manufacturer's instructions using Qiagen's One-step RT-PCR and Multiplex Master kit. The amplified band of interest (~500 bp) is extracted and gel purified using Qiagen's Qiaquick Gel Extraction kit. Ten libraries will be pooled for each Illumina MiSeq Flow Cell using equimolar quantities of each amplified library. The pooled libraries will be subjected to a QC and quantification process including Qubit quantification, bioanalysis, and quantification with Kappa qPCR. The libraries will then be sequenced on an Illumina MiSeq capable of 250 paired-end reads. Bioinformatics analysis is performed in conjunction with MBRCC Bioinformatics and Biostatistics Core (Dr. Sijin Wen). When samples are sent out for NGS and bioinformatics analysis in vendors or collaborators outside of WVU Cancer Institute, MBRCC, samples or data would be fully deidentified and strict HIPAA regulation compliance will be followed.

10. STUDY PARAMETERS AND CALENDAR

10.1. Study Parameters

10.1.1. Screening Evaluation

All evaluations must be completed ≤ *28* days prior to administration of protocol therapy with documentation of the following elements:

- Informed Consent
- Demographics:
  - Patient age & gender
  - Full oncologic history including all prior cancer-directed therapies and their dates of administration.
  - Smoking history (active, past, or nonsmoker; number of pack-years)
  - History of weight loss or oral corticosteroid use in the past 3 months, autoimmune conditions, dialysis, cirrhosis, splenectomy, mental illness, diabetes mellitus, statin use, metformin use, and recurrent infections in the past 6 months (including location, frequency, severity, duration, complications, and infections with unusual organisms).
  - For females, assessment of childbearing potential
  - Baseline Symptoms Assessment and Review of Systems
- History & physical exam
- Height, weight, ECOG performance status, and vital signs (including blood pressure, pulse, respiratory rate, and temperature)
- Concomitant medications assessment
- PD-L1 status of any pre-treatment biopsy specimen
- Laboratory Studies:
  - Non-fasting:
    - Complete Blood Count (CBC) with differential and smear
    - Serum Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, phosphorous, albumin
    - Liver Function Tests: alkaline phosphatase, total bilirubin, total protein, SGOT [AST], SGPT [ALT]
    - Thyroid Function Tests (TSH)

        - Urinalysis (macroscopic and microscopic) with culture reflex
    - Special circumstances:
        - Serum ß-HCG for women of childbearing potential
        - Hemoglobin A1c for diabetic patients
- Imaging:
    - Full body PET/CT scan (preferred) or CT chest, abdomen, and pelvis with IV contrast.
    - CT brain or MRI brain (preferred) with IV contrast
- Quality of Life Questionnaires (Appendix C):
    - The validated Functional Assessment of Cancer Therapy (FACT) instrument will be used to assess physical, social/family, emotional and functional well-being, and additional questions specific for lung cancer patients (FACT-L) and fatigue (FACIT Fatigue Scale). These validated instrument is user friendly (can be completed in less than 10 minutes) and has been used extensively to measure QOL in lung cancer patients.[41-42]

10.1.2. Treatment Period & Follow-up

All medical care provided on the days of immune checkpoint therapy administration will be at the discretion of the treating medical oncologist according to standard of care. No specific data will be recorded from these visits, but at the subsequent study follow-up visit all prior administrations of immune therapy (date and dose) will be recorded. All treatment data related to the radiotherapy will be recorded on the first and last day of each course, and will include the start and end date of the radiotherapy course, total dose, dose per fraction and number of fractions of radiation received during the course. Data from follow-up studies will be recorded as per the study calendar in section 10.2.

10.2. Study Calendar

Parameters	≤28 days prior to registration	Day 1 of immune therapy	Last day of first course of RT	Follow-up before documented progressive disease, at 3, 6, 9, and 12 months after initial cycle of immune therapy[1]	Follow-up before documented progressive disease, beyond 12 months after initial cycle of immune therapy is timed per standard of care at treating physicians' discretion	Follow-up after progressive disease or switch to alternative systemic therapy[2]
Informed Consent	X					
Demographics	X					
History and Physical Exam	X[3]			X[15]	X	
Wt/Vitals/ ECOG PS	X			X[15]	X	
Concomitant Med Assessment	X			X[15]		
FACT/FACIT Questionnaire[4]		X		X[15]		
Serum CBC/diff/platelets[5]	X			X[2]		
Serum chemistries[6]	X			X[2]		
Serum liver function tests[7]	X			X[2]		
Serum TSH	X			X[2]		
Urinalysis with reflex culture	X					
Serum β-hCG[8]	X					
Hemoglobin A1c[9]	X					
MRI (preferred) or CT brain with IV contrast	X					
FDG-PET/CT (preferred) or CT chest/abdomen/pelvis with IV contrast[10]	X			X[16]	X	
Research blood sample[11]		X		X[17]	X[17]	
Immunotherapy Administration Assessment[12]		X	X	X	X	
Radiation Therapy Administration Assessment[13]			X	X	X	
Survival assessment[14]						X
AE/SAE Assessment[19]	X	X	X	X	X	
PD-L1 status[20]	X					
Rebiopsy (optional)				X[18]	X[18]	X[18]

[1]The final formal assessment will be at time of documented disease progression.
[2] To be performed according to standard of care.
[3]To be documented by treating radiation oncologist. Vital signs to include blood pressure, pulse, respiratory rate, and temperature
[4] See Appendix C for questionnaires, which will be administered electronically using REDCap. A research assistant will open the questionnaire weblink (http://j.mp/2eLzfjE) on a password protected tablet computer, enter the patient's study ID into the survey instrument, and then the patient will complete the rest of the electronic form and submit it. The research assistant will be available to answer any questions that the patients have, if necessary. Details on REDCap can be found in Section 12.1 below. The initial questionnaire may be done anytime between signing the consent and the first immune therapy treatment. The questionnaires may be completed using paper forms if REDCap is unable to be accessed or based on patient preference.
[5]CBC with differential includes hemoglobin, hematocrit, white blood cell count, absolute neutrophil count, and platelets
[6]Serum chemistries include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, phosphorous, albumin
[7]Serum liver function tests include alkaline phosphatase, total bilirubin, total protein, SGOT [AST], SGPT [ALT]
[8]Only for women of childbearing potential (within 14 days of registration)
[9]Only for patients with diabetes mellitus
[10]In patients with contrast allergy a short course of steroids may be given as needed. In patients with poor kidney function in whom contrast is contraindicated a non-contrast imaging study is permissible.
[11]Please refer to protocol section 9 for details. The initial research blood sample may be drawn anytime between signing the consent and the first immune therapy treatment.
[12]Record all past and current administrations of immune therapy (sections 5.2 and 6.2), including date and dose of each administration.
[13]Record all past and current administrations of radiation therapy (sections 5.1, 5.3, and 6.1), including for each course: the start and end date, total dose, dose per fraction, number of fractions, and location of treated lesion.
[14]Survival assessment may be carried out via chart review or phone call only.
[15]A window of ± 14 days around the standard time point every 3 months is allowed as necessary
[16]A minimum of one scan every 4 months is required, however, scans may be completed at more frequent intervals as clinically indicated.
[17]Performed in coordination with imaging studies, at baseline prior to treatment, and thereafter only for a change in extracranial response on follow-up imaging. The blood will be drawn within 30 days of any imaging study showing a change in extracranial response status.
[18]Optional rebiopsy of an accessible non-irradiated tumor may be obtained at the treating physicians' discretion if it is deemed clinically warranted and safe to perform, for diagnostic purpose in order to confirm progression of disease and underlying histopathology. If indicated, it should be carried out within 30 days of the confirmatory imaging study showing disease progression, and prior to initiating any further systemic therapy.
[19]AEs and SAEs will be collected on a continuous basis starting with the first cycle of immune therapy. Both AEs and SAEs will be collected up until 30 days after the last dose of immune therapy or until the patient begins a new cancer-directed therapy, or death, whichever comes first.
[20] PD-L1 status (percentage staining by immunohistochemistry) of any pre-treatment biopsy specimen is not required for eligibility, but if information is available at any time point prior to or after enrolling in the trial, this data will be collected in the EDC.

11. MEASUREMENT OF EFFECT

11.1. Antitumor Effect

11.1.1. Imaging Modalities

At baseline and follow-up, systemic response (for unirradiated lesions) and local response (for irradiated lesions) will be assessed using FDG-PET/CT (preferred) or CT scan of the chest/abdomen/pelvis. Ideally IV contrast and CT slice thickness ≤ 5mm will be used for either type of study. Images will be acquired according to the standard of care schedule used for patients receiving immune checkpoint therapy, at intervals of 4 months or less after initiation of immune therapy. In the event of an imaging study showing disease progression, a confirmatory scan is recommended at least 4 weeks later to confirm progression. The same method of assessment and technique should be used to characterize each index lesion at baseline and during follow-up. All measurements should be recorded in metric notation and specified to the nearest millimeter. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

11.1.2. Definitions

Evaluable for objective response: Only those patients who have received at least one course of radiation therapy, and have had their disease re-evaluated by imaging at least 4 weeks after initiation of therapy will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

Immune-Related Response Criteria (irRC)[43-44]: Novel criteria for the evaluation of antitumor responses with immunotherapeutic agents, as described in Wolchok *et al*. The patterns of response to immune therapy agents differ from those of cytotoxic therapy in several ways: There may be a transient worsening of disease before stabilization or regression, responses can take months to become apparent and may continue long after completion of the initial therapy, and prolonged periods of stable disease are common. For the purposes of this study, irRC will be used for evaluation of all **non-irradiated** index lesions and the unit of measure will be the sum of the products of the two largest perpendicular diameters (SPD) for all index lesions.

Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1[45]: Method for the evaluation of antitumor responses to cytotoxic agents, as described by Eisenhauer *et al*. For the purposes of this study, RECIST 1.1 criteria will be used only for the evaluation of **irradiated** lesions and the unit of measure will be the single largest diameter.

Measurable lesions on initial imaging: Measurable malignant lesions on *initial* imaging are defined as those that can be accurately measured in at least one dimension as ≥10 mm (≥1.0 cm) with CT or PET/CT scan. Lymph nodes will be considered malignant (and thus measurable lesions) if their short axis is ≥15 mm (≥1.5 cm) by CT scan. All other lesions will be considered non-measureable disease. Bone lesions without a soft

tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered non-measurable. Simple cysts are by definition non-malignant and non-measureable, but cystic metastases can be considered as measurable lesions, if they meet the size cutoff of measurability described above. Tumor lesions that are situated in a previously irradiated areas are not considered measurable.

Measureable lesions on follow-up imaging: Per irRC specifications, new measureable lesions on follow-up imaging will be defined as being ≥ 5 x 5 mm in size

Index lesions: All measurable lesions up to a maximum of 10 visceral lesions (≤ 5 per organ) and 5 cutaneous lesions, should be identified as index lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. It is preferable to exclude cystic metastases as index lesions if other non-cystic lesions are available. Prior to selecting index lesions a decision should be made on which lesion will receive an initial course of radiation therapy, as this lesion is not eligible to be considered as an index lesion to assess response to immune therapy. If any subsequent course(s) of radiation therapy are given to what was previously considered an index lesion, this lesion will no longer be considered an index lesion for subsequent systemic response assessment. The rationale for this is that including irradiated lesions in response assessment would bias the assessment of a systemic effect.
Tumor Burden: At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions are added together to provide the total tumor burden: Tumor Burden = $SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$.

11.2. Response Criteria

The irRC criteria for assessing systemic disease response (including only non-irradiated lesions) can be seen in the following table:

New, measurable lesions (ie, ≥ 5 x 5 mm)	Incorporated into tumor burden
New, non-measurable lesions (ie, < 5 x 5 mm)	Do not define progression (but preclude irCR)
Non-index lesions	Contribute to defining irCR (complete disappearance required)
irCR	Disappearance of all lesions in two consecutive observations at least 4 weeks apart
irPR	≥ 50% decrease in tumor burden compared with baseline in two observations at least 4 weeks apart
irSD	Neither sufficient decrease in tumor burden to qualify as irPR

	nor sufficient increase in tumor burden to qualify as irPD.
irPD	≥ 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart.

Examples of irRC overall response[37]:

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),* %	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/Stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25?	Any	Any	irPD†

*Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).
†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

RECIST version 1.1 will be used to assess the local response of irradiated lesions only, taking as reference the baseline largest diameter:

Complete Response (CR)	Disappearance of the irradiated lesion
Partial Response (PR)	≥ 30% decrease in the single largest diameter of the irradiated lesion
Stable Disease (SD)	Neither sufficient decrease in diameter to qualify as PR nor sufficient increase in diameter to qualify as PD
Progressive Disease (PD)	≥ 20% increase in the single largest diameter of the irradiated lesion. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm).

11.2.1. Best Overall Response

The best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

11.2.2. Progression-Free Survival

The duration of time from start of treatment to time of progression or death, whichever occurs first. For those patients with a CR or PR, the reference for progressive disease the smallest measurements recorded since the treatment started).

11.2.3. Response Review

All responses will be reviewed by an expert radiologist and/or radiation oncologist at the study's completion, with simultaneous review of the patients' files and radiological images.

12. DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1. Data Reporting

The OnCore Database will be utilized, as required by the WVU MBRCC, to provide data collection for both accrual entry and trial data management. OnCore is a Clinical Trials Management System housed on secure servers maintained at West Virginia University. OnCore properly used is compliant with Title 21 CFR Part 11. Access to data through OnCore is restricted by user accounts and assigned roles. Once logged into the OnCore system with a user ID and password, OnCore defines roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore Administrator at lwallace@hsc.wvu.edu.

OnCore is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic Case Report Form completion in the OnCore database. A calendar of events and required forms are available in OnCore.

REDCap will be utilized to collect quality of life data directly from the patients, using the following weblink: http://j.mp/2eLzfjE. This link will be accessed on a password protected tablet computer by the clinical trials nurse on the day when quality of life data is to be collected. The clinical trials nurse will enter the patient's study ID into the survey instrument and then the patient will complete the rest of the electronic form and submit it. REDCap is a secure, web-based application for building and managing online surveys and databases. REDCap is an NIH-sponsored application and follows HIPAA guidelines. The WVCTSI instance of REDCap is housed at the Ruby Office Complex (ROC) which was built in 2008 and designed by IBM. It a fully redundant building with generator backup that is manned and monitored 24/7 with onsite staff, closed-circuit camera, automated reporting systems. It's secure by magnetic locks and only accessible via card access. Systems are secured through multiple layers of security including encryption, firewalls, reverse proxy and tested regularly for vulnerabilities. The OnCore Administrator lwallace@hsc.wvu.edu will be able to access the data reported from all patients at the time of analysis.

12.1.1. Method

Data will submitted on a real-time basis, within 2 weeks of the protocol-related visit via OnCore electronic case report forms.

12.1.2. Responsibility for Data Submission

It is the responsibility of the PI(s) at the site to ensure that all investigators understand the procedures for data submission and that protocol specified data are submitted accurately and in a timely manner via the electronic data capture system, OnCore.

Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

12.2. Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

12.2.1. Written Informed Consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and be allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject. Additionally, documentation of the consenting process should be noted in the medical record.

12.2.2. Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

12.2.3. Retention of records

The Principal Investigator supervises the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

12.2.4. Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

12.2.5. Data Safety and Monitoring Plan

This protocol will adhere to the policies of the WVU Mary Babb Randolph Cancer Center Data and Safety Monitoring Plan.

13. STATISTICAL CONSIDERATIONS

13.1. Study Design/Endpoints

The primary endpoint is the best overall response rate (complete and partial) after the initial course of radiation therapy with concurrent immune therapy. The secondary objectives include determination of the progression-free and overall survival at 1 year, toxicity, and quality of life. Correlative objectives include an assessment of levels of circulating CD4+, CD8+ and Treg T-cell subsets, iNOS+ macrophages, and the chemokines CCL2, CXCL9, CXCL10, and CXCL11 in relation to clinical response.

13.2. Sample Size/Accrual Rate

The primary analysis will be for patients enrolled at time-points (A) and (B) above, whereas patients enrolled at time-point (C) will be included in observational analysis only (see section 3.1). The primary endpoint of this study is the overall response rate (complete + partial response) and the efficacy will be assessed using the Simon's two-stage Minmax design. A sample size of 33 patients is chosen to differentiate between the response rate of 20% and 40% with 80% power at a significance level of 0.05. In particular, 18 patients will be enrolled at the first stage. If 15 or more of the first 18 patients have disease progression prior to any response, the trial will be terminated; otherwise an additional 15 patients will be treated for a total of 33 patients. In the final data analysis, if 10 or fewer patients respond among 33 patients, the treatment will be concluded ineffective. The probability of early termination due to futility is 0.72.

For correlative study between biomarkers and response, this sample size will have at least 80% power to detect a 50% change/difference on biomarker data between response and non-response groups using a two-sided two-sample t-test at 0.05 significance level, assuming that the response rate is 35% or higher and the coefficient of variation (CV) of biomarkers is 0.45.

Approximately 100 patients with metastatic NSCLC are diagnosed and treated each year at our institution, many of whom will be candidates for immune checkpoint therapy. As such, we expect to accrue 3 patients per month to this trial for an estimated accrual period of one year. The full estimated duration of the study is two years.

13.3. Methods for Analysis

Data will be summarized with respect to baseline characteristics and safety measurements using descriptive statistics (quantitative data) and contingency tables (qualitative data). The safety analysis will consist of AE summaries (frequency tables based on CTCAE grades) and laboratory abnormalities summary. For the primary endpoint, the response rate and its 95% confidence interval will be estimated with a binomial distribution. Univariate and multivariate logistic regression model will be used to assess the association between response and several radiotherapy variables (biologic effective dose,

number of lesions treated, sequence of radiation and immune therapy), tumor variables (irradiated tumor site, size, growth rate, histology, PD-L1 status, local response, extent of prior chemotherapy use, and biologic correlates), and patient variables (age, smoking status, and other factors associated with secondary immunodeficiency). A predictive nomogram for response will be developed based on the outcome of the multivariate analysis. In the data analysis on the secondary endpoints, the Kaplan-Meier method will be used to estimate overall survival and progression-free survival. Wilcoxon signed-rank test for paired data will be used to assess the change of quality of life scores between two visits. Since the lung cancer score (LCS) of the FACT-L focuses on lung cancer symptoms, this will be used for the primary QOL endpoint; however, the more general subscales of physical and functional well-being on the Trial Outcome Index (FACT-TOI) will also be collected. A difference of 3 LCS points will be considered clinically significant.[12-13] Where applicable, comparison will be made to previous randomized trials assessing immune checkpoint inhibitors alone in a comparable patient population.

13.4. REFERENCES

1. American Cancer Society. Cancer Facts and Figures 2016. http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2016/index Accessed July 31, 2016
2. National Cancer Institute. Surveillance, Epidemiology, and End Results Program. Cancer Statistics: Lung and Bronchus Cancer. http://seer.cancer.gov/. Accessed July 31, 2016.
3. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.
4. Ishida M, Iwai Y, Tanaka Y, et al. Differential expression of PD-L1 and PD-L2, ligands for an inhibitory receptor PD-1, in the cells of lymphohematopoietic tissues. Immunol Lett 2002;84:57-62.
5. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer 2005;5:263-74.
6. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
7. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366:2443-54.
8. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:1627-39.
9. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:123-35.
10. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2015.
11. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018-28.

12. Ma Y, Kepp O, Ghiringhelli F, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. Semin Immunol 2010;22:113-24.
13. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst 2013;105:256-65.
14. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012;366:925-31.
15. Sharabi AB, Lim M, DeWeese TL, et al. Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy. Lancet Oncol 2015;16:e498-509.
16. Demaria S, Golden EB, Formenti SC. Role of Local Radiation Therapy in Cancer Immunotherapy. JAMA Oncol 2015;1:1325-32.
17. Reynders K, Illidge T, Siva S, et al. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. Cancer Treat Rev 2015;41:503-10.
18.. Lee Y, Auh SL, Wang Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood 2009;114:589-95.
19. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 2014;124:687-95.
20. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015;520:373-7.
21. Vanpouille-Box C, Diamond JM, Pilones KA, et al. TGFbeta Is a Master Regulator of Radiation Therapy-Induced Antitumor Immunity. Cancer Res 2015;75:2232-42.
22. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res 2009;15:5379-88.
23. Golden EB, Demaria S, Schiff PB, et al. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res 2013;1:365-72.
24. Demaria S, Formenti SC. Radiation as an immunological adjuvant: current evidence on dose and fractionation. Front Oncol 2012;2:153.
25. Demaria S, Pilones KA, Vanpouille-Box C, et al. The optimal partnership of radiation and immunotherapy: from preclinical studies to clinical translation. Radiat Res 2014;182:170-81.
26. Iyengar P, Kavanagh BD, Wardak Z, et al. Phase II trial of stereotactic body radiation therapy combined with erlotinib for patients with limited but progressive metastatic non-small-cell lung cancer. J Clin Oncol 2014;32:3824-30.
27. Parker C, Nilsson S, Heinrich D, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med 2013;369:213-23.
28. Zegers CM, Rekers NH, Quaden DH, et al. Radiotherapy combined with the immunocytokine L19-IL2 provides long-lasting antitumor effects. Clin Cancer Res 2015;21:1151-60.

29. Poleszczuk JT, Luddy KA, Prokopiou S, et al. Abscopal Benefits of Localized Radiotherapy Depend on Activated T-cell Trafficking and Distribution between Metastatic Lesions. Cancer Res 2016;76:1009-18.
30. Klug F, Prakash H, Huber PE, et al. Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 2013;24:589-602.
31. De Palma M, Coukos G, Hanahan D. A new twist on radiation oncology: low-dose irradiation elicits immunostimulatory macrophages that unlock barriers to tumor immunotherapy. Cancer Cell 2013;24:559-61.
32. Hiniker SM, Chen DS, Reddy S, Chang DT, Jones JC, Mollick JA, Swetter SM, Knox SJ.A systemic complete response of metastatic melanoma to local radiation and immunotherapy. Transl Oncol. 2012;5(6):404-7
33. Slovin SF, Hamid O, Tejwani S, et al. Ipilimumab (IPI) in metastatic castrate-resistant prostate cancer (mCRPC): results from an open-label, multicenter phase I/II study. *J Clin Oncol.* 2012;30(5 Suppl):25.
34. Silk AW, Bassetti MF, West BT, Tsien CI, Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. Cancer Med. 2013;2(6):899–906.
35. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 2014;15(7):700-12.
36. Ahmed K.A., Creelan B.C., Kim S., Dilling T.J., Perez B.A. Safety and Tolerability of Extracranial Radiation Therapy and Immune Checkpoint Inhibitors Among Patients With Metastatic Non-Small Cell Lung Cancer. 2016;96(2 Suppl):S201
37. Hiniker SM, Reddy SA, Maecker HT, Subrahmanyam PB, Rosenberg-Hasson Y, Swetter SM, Saha S, Shura L, Knox SJ. A Prospective Clinical Trial Combining Radiation Therapy With Systemic Immunotherapy in Metastatic Melanoma. Int J Radiat Oncol Biol Phys. 2016;96(3):578-88.
38. Golden EB, Frances D, Pellicciotta I, et al. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. Oncoimmunology 2014;3:e28518.
39. Simpson TR, Li F, Montalvo-Ortiz W, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med 2013;210:1695-710.
40. NRG Oncology. Center for Innovation in Radiation Oncology: Contouring atlases. https://www.nrgoncology.org/Scientific-Program/Center-for-Innovation-in-Radiation-Oncology. Accessed 7/31/16.
41. Cella D, Bonomi AE, Lloyd SR et al. Reliability and validity of the Functional Assessment of Cancer Therapy-Lung (FACT-L) quality of life instrument. Lung Cancer. 12(3): 199-220, 1995.
42. Cella D, Eton DT, Fairclough DL, et al. What is a clinically meaningful change in the Functional Assessment of Cancer Therapy- Lung (FACT-L) questionnaire? Results from Eastern Cooperative Oncology Group (ECOG) Study 5592. J Clin Epidem. 55(3): 285-295, 2002.

43. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412-20.
44. Hoos A, Eggermont AM, Janetzki S, et al. Improved endpoints for cancer immunotherapy trials. J Natl Cancer Inst 2010;102:1388-97.
45. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

14. APPENDICES

Appendix A

Eastern Oncology Cooperative Group (ECOG) Performance Status

ECOG	Description
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 selfcare. Totally confined to bed or chair

Appendix B

Dose-Volume Guidelines for Critical Structures for Hypofractionated Radiation Therapy

Structure	3 Fractions	5 Fractions
Cauda Equina	D0.03cc < 25.5Gy, D5cc < 21.9Gy	D0.03cc < 32Gy, D5cc < 30Gy
Spinal Cord	D0.03cc <22.5 Gy, D1.2cc < 13Gy	Max <28 Gy, D0.35cc < 22.Gy, D1.2cc < 15.6Gy
Total Lung *(-CTV)*	V20<15% , V11<37% , D1500cc < 10.5Gy, D1000cc < 11.4Gy	V13.5Gy<37%, D1500cc < 12.5Gy, D1000cc < 13.5 Gy
Heart	D0.03cc < 30Gy, D15cc < 24Gy	D0.03cc < 38 Gy, D15cc < 32 Gy
Esophagus	D0.03cc < 27Gy, D5cc < 17.7Gy	D0.03cc < 35Gy, D5cc < 27.5 Gy
Brachial Plexus	D0.03cc < 26 Gy, D3cc < 22 Gy	D0.03cc < 32 Gy, D3cc < 30 Gy
Trachea & Ipslat Bronchus	D0.03cc < 30Gy, D5cc < 25.8Gy	D0.3cc< 40 Gy, D5cc < 32 Gy
Sacral Plexus	D0.03cc < 24Gy, D5cc < 22.5Gy	D0.03cc < 32Gy, D5cc < 30 Gy
Great Vessels	D0.03cc < 45Gy, D10cc < 39Gy	D0.03cc < 53 Gy, D10cc < 47Gy
Skin	D0.3cc < 33Gy; D10cc<31Gy	D0.03cc < 38.5 Gy, D10cc < 36.5 Gy
Stomach	D0.03cc < 30Gy, D10cc < 22.5Gy	D0.5cc < 35 Gy, D5cc < 26.5 Gy
Duodenum	D0.03cc < 24Gy, D10cc < 15Gy	D0.5cc < 30Gy, D5cc < 18.3Gy
Bowel	D0.03cc < 34.5Gy, D20cc < 24Gy	D0.03cc < 40 Gy, D20cc < 28.5Gy
Rectum	D0.03cc < 49.5Gy, D3.5cc < 45Gy, D20cc < 27.5Gy	D0.03cc < 55Gy, D3.5cc < 50Gy, D20cc < 32.5Gy
Bladder	D0.03cc < 33Gy, D15cc < 16.8Gy	D0.03cc < 38 Gy, D15cc < 20 Gy
Ureter	D0.03cc < 40Gy	D0.03cc < 45Gy
Penile Bulb	D3cc < 25Gy	D3cc < 30 Gy
Femoral Heads	D10cc < 24Gy	D10cc < 30 Gy
Bile Duct	D0.03cc < 36Gy	D0.03cc < 41Gy
Renal hilum	D15cc < 19.5Gy	D15cc < 23Gy
Rib	D0.03cc < 50Gy, D5cc < 40Gy	D0.03cc < 57 Gy, D5cc < 45Gy
Ipsilateral Kidney	D130cc < 12.3Gy	D130cc < 14.5Gy
Total Kidney	D200cc < 15Gy	D200cc < 18Gy
Liver	D700cc < 17.1Gy	D700cc < 21Gy

Appendix C

FACT-L (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-L (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-L (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
B1	I have been short of breath	0	1	2	3	4
C2	I am losing weight	0	1	2	3	4
L1	My thinking is clear	0	1	2	3	4
L2	I have been coughing	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
L3	I feel tightness in my chest	0	1	2	3	4
L4	Breathing is easy for me	0	1	2	3	4
Q3	Have you ever smoked? No ___ Yes ___ If yes:					
L5	I regret my smoking	0	1	2	3	4

FACIT Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

		Not at all	A little bit	Some-what	Quite a bit	Very much
H7	I feel fatigued	0	1	2	3	4
H12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4