Protocol Title:

Phase II trial of immune checkpoint inhibitor and novel in situ radiation "booster shot" tumor vaccination in patients with metastatic carcinoma

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1. BACKGROUND

In 2014, according to the Centers for Disease Control and Prevention there were nearly 1.5 million new cancer cases diagnosed and almost 600,000 cancer-related deaths in the United States(1). In fact, cancer is the second leading cause of death in the United States; 1 in every 4 deaths is due to cancer. Novel therapies are much needed to improve the outcomes of cancer patients.

1.1 Immunotherapy Overview

Although the medical field has seen numerous discoveries none has been as revolutionary as that of immunotherapy, which harnesses the body's immune system to more effectively eliminate cancer cells. In fact, *Science* magazine named immunotherapy as the Breakthrough of the Year in 2013(2). Immune responses directed against tumor are one of the body's natural defenses against the growth and proliferation of cancer cells. However, over time and under pressure from immune attack, cancers develop strategies to evade immune-mediated killing allowing them to develop unchecked. One such mechanism involves upregulation of surface proteins that deliver inhibitory signals to cytotoxic T cells. Programmed cell death ligand 1 (PD-L1) is one such protein, and is upregulated in a broad range of cancers with a high frequency, with up to 88% expression in some tumor types. In a number of these cancers, including lung(3), renal(4), ovarian cancer(5), and hematologic malignancies(6) tumor cell expression of PD-L1 is associated with reduced survival and an unfavorable prognosis.

1.2 Immune Checkpoint Inhibitors

PD-L1 is part of a complex system of receptors and ligands that are involved in controlling T-cell activation. PD-L1 acts at multiple sites in the body to help regulate normal immune responses and is utilized by tumors to help evade detection and elimination by the host immune system tumor response. In the lymph nodes, PD-L1 on antigen-presenting cells binds to PD-1 or CD80 on activated T cells and delivers an inhibitory signal to the T cell(7). This results in reduced T cell activation and fewer activated T cells in circulation. In the tumor microenvironment, PD-L1 expressed on tumor cells binds to PD-1 and CD80 on activated T cells reaching the tumor. This delivers an inhibitory signal to those T cells, preventing them from killing target cancer cells and protecting the tumor from immune elimination(8).

The Food and Drug Administration (FDA) has recently approved several immunotherapy drugs called immune checkpoint inhibitors (ICIs) for treatment of lung cancer, bladder cancer, and several others based on clinical trials showing dramatic responses in patients that have progressed through standard treatments like chemotherapy(9, 10). These drugs decrease inhibitory signals, effectively "releasing the brake" on the immune system so that it may better identify and eliminate cancer cells.

Despite the improved outcomes with ICIs, clinical trials of ICI monotherapy show that only approximately 20% of patients will have a significant response. Combining an ICI with other therapies seems to be a more effective strategy and several trials have shown that giving multiple ICIs together results in improved outcomes although at the expense of increased toxicity(10-12).

1.3 Radiation Therapy and Immune Effects

The published literature consistently shows that RT can initiate and promote both innate and adaptive immunity against tumors by mechanisms including: 1) enhanced expression of damage

associated molecular patterns (DAMPs) leading to stimulation of dendritic cells and subsequent increase in antigen presentation; 2) enhanced expression of MHC class I molecules, adhesion molecules and stress-induced ligands, and death receptors on tumor cells leading to increased recognition and killing by T cells; 3) induction of chemokines CXCL9, CXCL10 and CXCL16 promoting recruitment of effector CD8+ T cells; and 4) release of pro-inflammatory cytokines such as interleukin 1β, TNFα, IFNγ driving anti-tumor immunity(13-15). Because radiation therapy (RT) is a potent stimulator of the immune system and functions as an in situ tumor vaccine(13-15) by increasing tumor antigen presentation and T cell activation, preclinical studies have combined RT with an ICI that uniformly show that a robust synergism(14, 16).

Evidence of the potential for RT to generate antitumor immune responses has gained momentum in recent years. Lee et al. studied the effects of single fraction high dose RT on dendritic cells (DC) maturation and migration and noted that 5 days following a single fraction of 20 Gy, draining lymph nodes (LNs) showed an increase in tumor-specific DCs, which were associated with elevated markers of maturation(17). DCs from irradiated tissue are better able to stimulate effector T-cells, shown in mouse models of melanoma and breast cancer by Sharabi et al where hypofractionated RT of 18 Gy x 1 or 7 Gy x 3 increased the proliferation and activation of antigen specific CD8+ T-cells in the draining LNs compared to unirradiated controls(18). While limited preclinical data exist for the differential immune effects of fractionation(19), the impact of subsequent, delayed courses of radiotherapy has not been formally examined. The more dramatic implications of a systemic anti-tumor immune reaction are the occurrence of the so-called abscopal effect and an increased durability of response not seen with chemotherapy, suggesting the actions of a memory immune component.

There are not currently significant prospective data of RT and ICI combinations, although numerous clinical trials across many disease sites are currently either ongoing or planned. Many are hopeful that this treatment combination will improve outcomes based on anecdotal reports of RT causing tumor regression in lesions outside of the radiation field when given in addition to an ICI(20-22).

1.4 Rationale for Radiation Therapy and Immune Checkpoint Inhibitor Combination

Although RT is a localized treatment, directed at a specific anatomic target, it has very powerful systemic downstream effects including some that are immune system-related. First described in 1953, the term “abscopal effect” describes anti-tumor effects of RT that occur at a distant site outside of the treatment field(20). As such, RT has been described as an *in situ* tumor vaccine because it triggers a systemic anti-tumor response, in part by enhancing tumor neo-antigen exposure to antigen presenting cells (i.e. dendritic cells) that lead to robust T cell activation and migration to tumor(23, 24). These RT-induced effects may work in concert with those of systemic immunotherapy drugs, each with a unique mechanism of action. In fact, abundant preclinical data show dramatic synergy of RT given to one tumor with an ICI versus RT alone or ICI alone(16, 25, 26). For example, a group from Johns Hopkins University demonstrated in an intracranial glioma mouse model long-term survival only was achieved with combination ICI plus RT versus either ICI alone or RT alone (53 vs. 27 vs. 28 days; $p<.05$)(16).

In the absence of robust clinical data to inform whether RT plus ICI therapy should become more widely pursued in patients, a small but growing body of literature demonstrates that at least some patients do benefit from this combination. A recently published systematic review included 46 case reports of the abscopal effect identified from 1969 to 2014(27). Arguably the most well-known of these case reports described the abscopal effect in a melanoma patient who failed to

respond to an ICI alone, but after receiving palliative RT to a paraspinal mass had dramatic tumor regression in an unirradiated tumor(21). *We highlight that our proposed clinical trial intends to utilize RT in a similar manner, being to stimulate a robust immune response in combination with an ICI in the setting of suboptimal response to ICI alone.* Other studies also suggest that RT may be beneficial in addition to ICI therapy. A secondary analysis of the KEYNOTE-001 trial indicates that previous RT in patients with advanced lung cancer is associated with longer progression free survival and overall survival with pembrolizumab treatment (an ICI) compared to those who did not receive previous RT(28). A phase I study treating patients with metastatic melanoma or renal cell carcinoma with SBRT (20 Gy x 1-3 fractions) with concurrent IL-2 demonstrated a 68% (8 patients) complete (CR) or partial response (PR) rate with frequent abscopal responses among these patients(29).

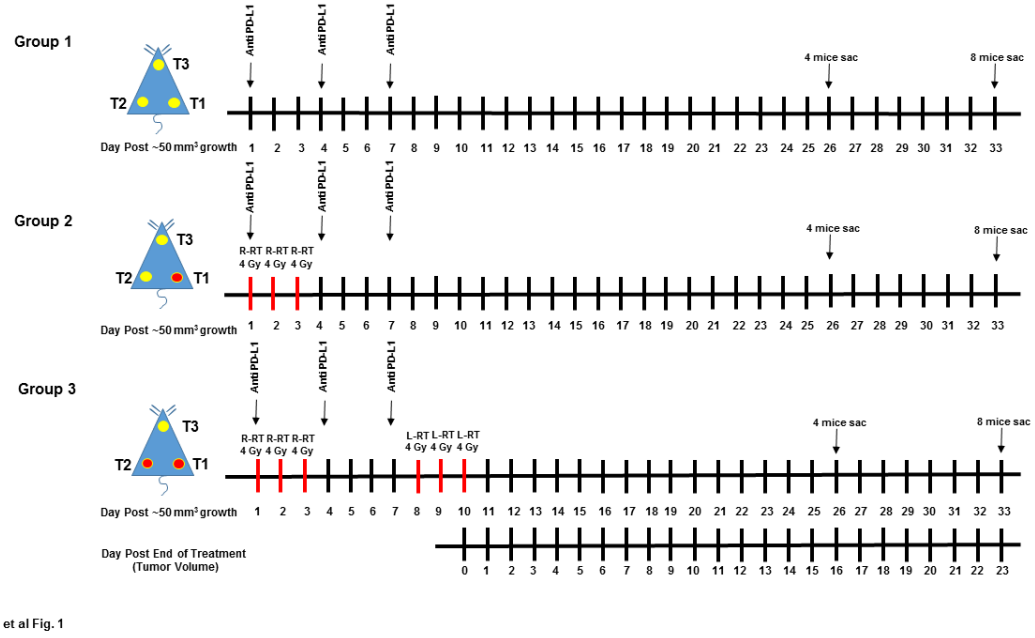
1.5 Rationale for Radiation "Revaccination" Strategy with Immune Checkpoint Inhibitor

Although there is synergism between RT and ICIs, at least in the preclinical setting, it remains unclear what the "ideal" RT parameters are to maximize an immune response and consequently lead to tumor elimination.

One of these parameters is the number of tumors that receive RT, for which there is a complete lack of data. In fact, the dominant strategy used in both preclinical and clinical studies has been treating only one tumor with RT. We propose that treating multiple tumors with RT and ICI may lead to a more robust anti-tumor response. Because RT functions as an *in situ* tumor vaccine by exposing the immune system to novel tumor antigens the rationale to treat multiple lesions with RT is borrowed from that of infectious disease prophylaxis in which a single inoculation is intentionally followed by additional inoculations (or "booster shots") out over time. As such, it has been well described that the first exposure of a foreign antigen to the immune system leads to an initial antigen-specific response; subsequent exposure to the same antigen causes a more rapid and robust antigen-specific immune response(30).

Our group was the first to explore this concept in a preclinical study.(31) Mice bearing pancreatic tumors in three different sites (left flank, right flank, upper back) were injected with an ICI and exposed to 3 daily consecutive fractions of 4 Gy each to 1 versus 2 flank tumors (Figure 1).

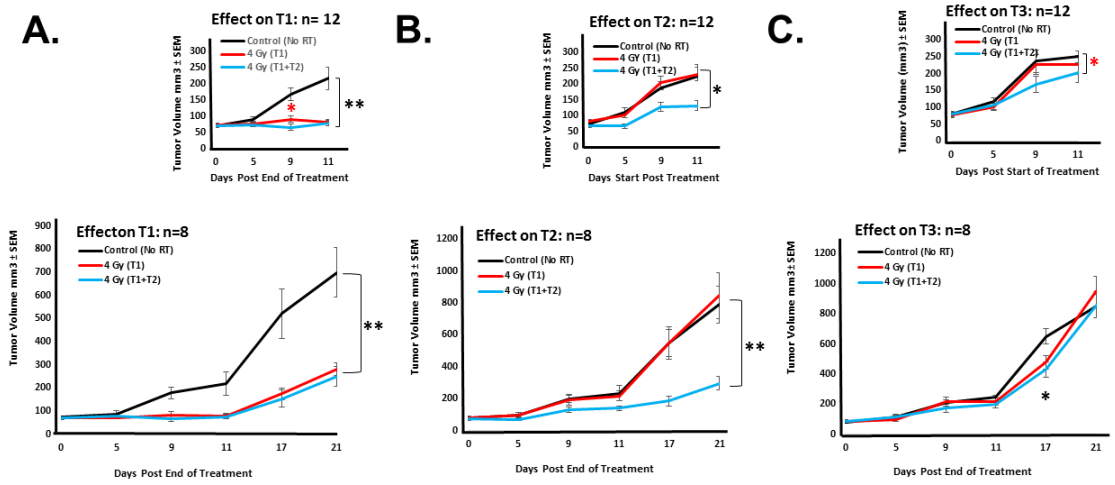
Figure 1. *Schematic representation of the treatment plan.(31) Three groups of mice were injected with mouse pancreatic cancer panc02 cells at three different sites: right flank (T1), left flank (T2), and the back (T3). Once the tumors reached ~50 mm^3, mice in each group were injected with anti PD-L1 antibodies on day 1, 4 and 7. Group 1 received no further treatments while Groups 2 and 3 were irradiated (4 Gy) to the T1 tumor on Days 1, 2 and 3. Group 3 was subsequently irradiated (4 Gy) to the T2 tumor on Days 8, 9 and 10. Day 10 is considered day 0 of post end of treatment. Yellow circles indicate location of non-irradiated tumors. Red circles indicate location of irradiated tumors.*



Chuong et al Fig. 1

Our data indicate that delivering an RT to a one flank followed by an RT "booster shot" to the contralateral flank tumor reduced tumor growth in the non-irradiated tumor in the upper back. RT-induced tumor regression in the non-irradiated site was observed earlier (day 9) in mice that received RT to two tumors compared to mice that received RT to a single tumor (day 17), demonstrating proof-of-principle that a "revaccinating" with radiation along with immunotherapy can lead to a more rapid immune response (Figure 2).

Figure 2. *Evaluation of radiation booster shot in a mouse pancreatic cancer syngeneic model.(31) Panc02 cells were injected subcutaneously in the flanks of immunocompetent C57BL/6 mice and treated as indicated in Figure 1. Tumor volumes were measured by caliper at the indicated intervals and expressed as Tumor volume ± standard error of the mean (SEM). Black line represents Group 1 (no RT), red line represents Group 2 (RT to the T1 tumor alone) and blue line represents Group 3 (RT to both T1 and T2 tumors). Measurements were performed on the T1 (A), T2 (B) and T3 (C) tumors of 8 mice up to 21 days post end of treatments. Insets: measurements performed on the initial 12 mice up to 11 days post end of treatments. Four mice per group were sacrificed on day 16 for blood, tumors and lymph nodes collections. * p<0.05, ** p<0.005 one-way ANOVA; * p<0.05 Student's paired t-test.*

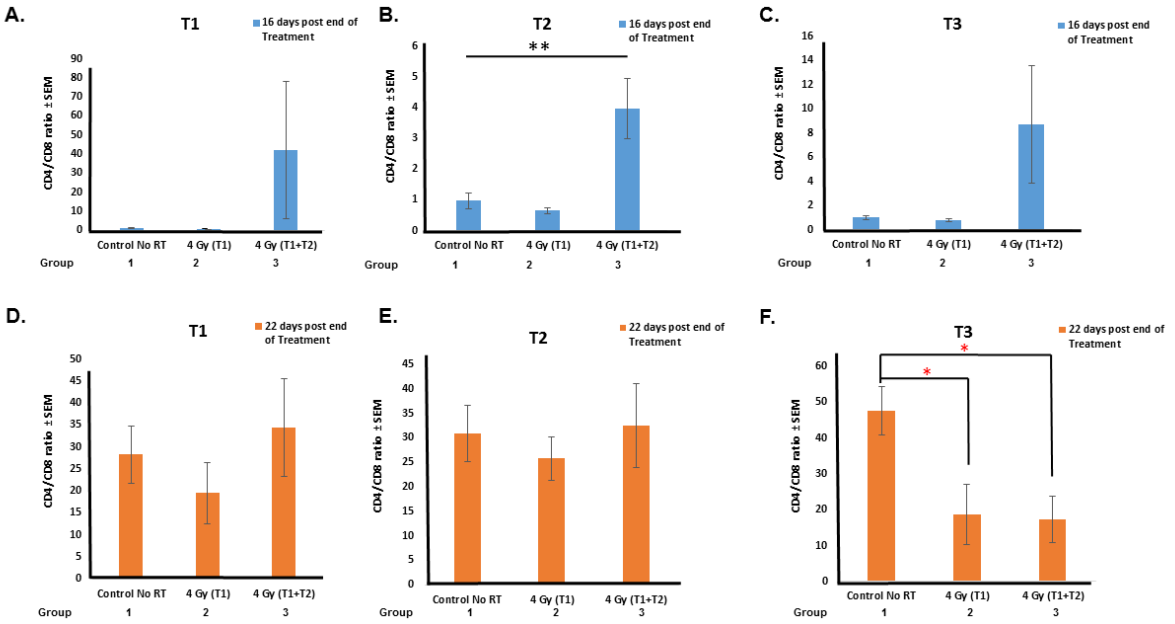


Chuong et al Fig. 2

Despite our data being the first to demonstrate that the number of tumors treated with RT influences tumor growth outside of the radiation field, we observed a modest and transient effect. There are several plausible reasons for this that have influenced the current trial design: 1) pancreatic cancer is not highly immunogenic(32) although the tumors included in this clinical trial are; 2) ICI was started concurrently with RT in the preclinical study although starting the ICI prior to RT has been suggested to be more effective(9) and therefore this sequence is included in the design of this clinical trial; 3) the radiation dose that we used in the preclinical study (4 Gy x 3) was possibly too low(9) to trigger a robust immune response and this has prompted a higher dose to be used in this clinical trial (8 Gy x 5, 6 Gy x 5).

Furthermore, we found that decreased growth of the non-irradiated tumor correlated with a transient increase of the CD4/CD8 T cell ratio in tumor and tumor associated macrophages in the draining lymph nodes (Figure 3). We are not aware of any other preclinical studies that have explored this same RT "revaccination" concept.

***Figure 3**: CD4⁺ and CD8⁺ T-cell subsets in the indicated mice tumors following indicated treatments.(31) CD4:CD8 ratios were determined for all tumors at sixteen (A-C) and twenty-two (D-F) days post end of treatment. Ratios are expressed ± standard error of the mean (SEM). Four mice per group were evaluated. ** p<0.005 one-way ANOVA;*
** p<0.05 Student's paired t-test.*



Chuong et al Fig. 3

To our knowledge there is only one published clinical report of multi-course RT and immunotherapy(33). Patients with metastatic cancer received 35 Gy in 10 fractions to 2 separate metastatic lesions with concurrent granulocyte-macrophage colony-stimulating factor (GM-CSF). Serious toxicities were uncommon. Immunomonitoring was not performed so the immunologic effect of the RT to the second tumor could not be specifically evaluated. Still, responses in non-irradiated tumors were observed in approximately 20% of patients. Note that this study was not performed with an ICI, and importantly that there are no existing prospective clinical trial data available to inform us about the probability of an abscopal response with ICI plus RT (whether to one or multiple lesions). There are numerous combination therapy trials ongoing that are targeting one lesion with RT but none that we know of that intentionally target multiple lesions.

Therefore, we propose a novel open label single arm phase 2 clinical trial to study the efficacy of RT given to 2 separate cancer lesions with concomitant ICI in generating an abscopal response within unirradiated tumor(s).

2. STUDY OBJECTIVES

Primary endpoint

- To evaluate best overall response rate (ORR) in non-irradiated lesion(s).

Secondary endpoints

- To evaluate ORR as defined by immune-related response criteria (irRC) in non-irradiated lesion(s).
- To evaluate duration of response (DOR) from the time CR or PR is first determined until the first date of documented progressive disease.
- To evaluate overall survival (OS) from the start of radiation therapy of the first lesion to the date of death for any cause.
- To evaluate progression free survival (PFS) from the start of radiation therapy of the first lesion to the date of progressive disease or death for any cause.
- To evaluate treatment-related adverse events.

3. STUDY DESIGN

This is an open label single arm phase 2 clinical trial in patients with metastatic solid malignancy of any histology who have previously experienced limited progression in at least 1 and up to 5 lesions while on ICI monotherapy.

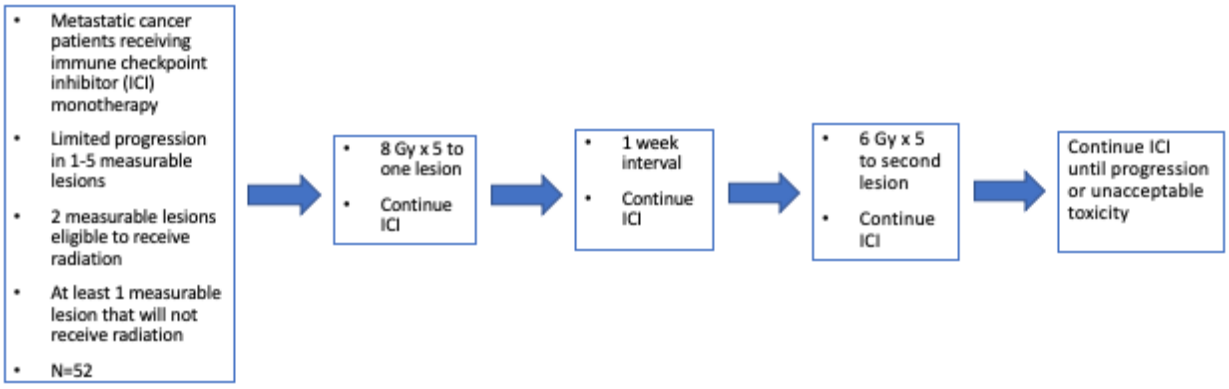
All potential subjects are required to undergo screening evaluation to determine eligibility within 28 days of study enrollment.

As illustrated in the study schema (Figure 1), eligible subjects will continue the same ICI on which they experienced limited progression and will also receive radiation therapy (RT). RT for all subjects will consist of treating one tumor of the treating physician's preference (40 Gy in 5 fractions), and after a 1-week interval during which ICI is continued alone, RT will be given to a second and separate tumor (30 Gy in 5 fractions). No additional RT will be delivered. ICI will be continued until disease progression or unacceptable toxicity.

Diagnostic imaging studies will be performed to determine treatment response at baseline/screening, 8 weeks after initiation of RT to the first lesion and every 8 weeks thereafter.

A total of 52 subjects will be enrolled on this trial. The expected rate of accrual is 2 patients per month at a single institution over 26 months.

Figure 4. *Study schema*



4. SUBJECT SELECTION

Study subjects must fulfill all of the following inclusion and exclusion criteria to be eligible for enrollment on this study.

4.1 Inclusion Criteria

1. ≥18 years of age at the time of study entry.
2. Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2.
3. Life expectancy of ≥12 weeks as estimated by the treating physician.
4. Metastatic carcinoma confirmed by biopsy or imaging study if biopsy is not deemed feasible.
5. Most recent anti-cancer therapy consists of a single ICI drug including but not limited to ipilimumab, nivolumab, pembrolizumab, atezolizumab.
6. Radiographic evidence of progression while on a single ICI drug in 1 and up to 5 lesions.
7. Eligible to continue ICI during and after radiation therapy.
8. ≥3 radiographically distinct and measurable lesions (primary and/or metastatic lesions) by RECIST 1.1 criteria, with ≥3 lesions separated from each other by ≥5 cm
9. Subjects must consent to all study procedures described in the protocol including radiographic evaluation and blood draws.
10. Immunosuppressive doses of systemic medication including steroids must be discontinued at least 14 days prior to the start of RT.
11. Adequate normal organ and marrow function as defined below:
    a. Hemoglobin ≥ 9.0 g/dL that may be achieved with transfusion
    b. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L (≧ 1500 per mm^3)
    c. Platelet count ≥ 100 x 10^9/L (≧100,000 per mm^3)
    d. Serum bilirubin ≤ 1.5 x institutional upper limit of normal (ULN).
    e. AST (SGOT)/ALT (SGPT) ≤ 2.5 x institutional upper limit of normal unless liver metastases are present, in which case it must be ≤ 5x ULN
    f. Serum creatinine CL>40 mL/min by the Cockcroft-Gault formula (Cockcroft and Gault 1976) or by 24-hour urine collection for determination of creatinine clearance:

Males:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

Females:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}} \times 0.85$$

12. Female subjects must either be of non-reproductive potential (i.e., post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy), have a negative serum or urine pregnancy test within 14 days of study enrollment, and not be breastfeeding.

4.2 Exclusion Criteria

1. Any contraindication to having an MRI scan.
2. Chemotherapy, biologic agent, investigational therapy, or RT given within 14 days of study enrollment.
3. Symptomatic or uncontrolled brain metastasis requiring treatment.
4. The need for palliative RT to a non-target lesion prior to RT to one of 2 target lesion on this study.
5. Prior RT to any lesion that would receive RT on this protocol.
6. Prior RT to a lesion located within 4 cm of previously irradiated structures: spinal cord that previously received >45 Gy; brachial plexus that previously received >45 Gy; small/large intestine or stomach that previously received >45 Gy; prior total lung V20 >30%.
7. Prior RT that could lead to an unacceptably high risk of clinically significant normal tissue injury due to high cumulative normal tissue dose as determined by the investigator.
8. History of any primary malignancy with the exception of
    a. Malignancy treated with curative intent and with no known active disease for at least 3 years before enrollment on this study.
    b. Adequately treated non-melanoma skin cancer or lentigo maligna without evidence of disease.
    c. Adequately treated carcinoma in situ without evidence of disease (i.e. cervical carcinoma in situ; superficial bladder cancer).
9. Any unresolved toxicity (Common Terminology Criteria for Adverse Events version 5.0 > grade 2) from previous anti-cancer therapy. Subjects with irreversible toxicity that is not reasonably expected to worsen by treatment on this study are permitted to enroll on this study.
10. Active or prior documented autoimmune disease within the past 2 years. Subjects with vitiligo, type I diabetes mellitus, Graves disease, or psoriasis not requiring systemic treatment (within the past 2 years) are not excluded.
11. Subjects requiring systemic corticosteroid (>10 mg daily prednisone equivalent) or other immunosuppressive medication within 14 days of study enrollment.
12. Contraindication to IV contrast despite premedication for iodine allergy, which would limit the ability to assess radiographic response to study treatment.
13. Prior allogeneic organ transplantation.

14. Presence of liver cirrhosis.
15. Active or prior documented inflammatory bowel disease (i.e. Crohn's disease, ulcerative colitis).
16. History of leptomeningeal metastases.
17. Any condition in the opinion of the investigator that would interfere with evaluation of study treatment or interpretation of patient safety or study results.
18. History of intolerance to any immune checkpoint inhibitor.
19. Female patients who are pregnant or breastfeeding.

4.3 Subject Recruitment

The patient's treatment team, the study investigator, or treating institution's research team will identify potential study participants. The site principal investigator may screen records of patients for the limited purpose of identifying patients who would be eligible for study enrollment and limited contact information may be recorded to contact the patient regarding study enrollment. However, it is expected that the patient's treatment team or investigator working in consultation with the treatment team will conduct the initial discussion of possible study enrollment with the patient. This recruitment process presents no greater than minimal risk to the privacy of screened patients. A (partial) limited waiver of authorization is requested for reviewing medical records to identify potential research participants, conversing with patients about possible enrollment, and handing of personal health information.

5. PRETREATMENT EVALUATION

The following will be assessed during a screening visit:

1. Signed informed consent
2. Inclusion and exclusion eligibility
3. Medical history within 28 days of enrollment
4. Signs and Symptoms assessment within 28 days of enrollment
5. Physical examination within 28 days of enrollment
6. Vital signs (blood pressure, heart rate, temperature, oxygen saturation via pulse oximetry, weight) within 28 days of enrollment
7. ECOG performance status within 28 days of enrollment
8. Concomitant medication collection obtained within 28 days of enrollment
9. Documentation within 28 days of enrollment of all prior anti-cancer therapy
10. Laboratory tests within 28 days of enrollment
    a. Complete blood count
    b. Comprehensive metabolic panel
    c. TSH, free T4, T3
11. Baseline tumor assessment within 28 days of enrollment
    a. CT scan of the chest, abdomen pelvis and all other known sites of disease preferably done with IV and oral contrast
    b. MRI with contrast may be performed at the discretion of the treating physician (i.e. to better visualize disease in certain organs such as the liver)
    c. PET/CT is permitted instead of CT scan alone, but is not required
    d. MRI brain should be done in patients with known brain metastasis

5.1 Immune Checkpoint Inhibitor

Subjects will continue to receive the same ICI that was previously administered at the time of documented progression at a standard schedule and dosage.

5.2 Radiation Therapy

The intent of RT in this study is to augment a cancer-specific immune response when given with ICI. As such, while RT is commonly and effectively used to treat symptomatic lesions in metastatic cancer patients, the presence of symptomatic lesions is not a requirement for RT to be given in this study.

Appropriate subjects will have at least 3 measurable lesions to be eligible for this study, 2 of which would receive RT and at least 1 that would not.

Day 1 of the study will be considered the day that the first fraction of radiation is delivered to the first lesion. This should be no more than 10 calendar days after enrollment.

5.3 Target Selection

The following will pertain to the two lesions that will receive RT:

1) Will be identified prior to starting study therapy.
2) May not be delivered to brain metastases.
3) Must be delivered to targets within the following locations:
    a. Cervical lymph node
    b. Supraclavicular lymph node
    c. Lung
    d. Mediastinal lymph node
    e. Liver
    f. Adrenal gland
    g. Abdominal/retroperitoneal lymph node
    h. Bone
4) Should preferably include any lesion(s) that has progressed on prior ICI monotherapy.
5) Should preferably be symptomatic or are expected to become symptomatic or lead to significant morbidity with further disease progression.
6) Should not include any lesion that has received RT in the past regardless of the dose delivered (even palliative doses, for example 8 Gy x 1).
7) RT to the first lesion should begin within 14 days of the previous dose of the ICI.

5.4 Dose Fractionation

There are no existing clinical data that clearly identify an ideal dose fractionation schedule to use for maximizing the likelihood of an abscopal response. However, preclinical data strongly suggest that hypofractionation should be preferred. Dewan and colleagues studied 3 different fractionation schedules (20 Gy x 1, 8 Gy x 3, and 6 Gy x 5) with concurrent ICI in breast and colorectal cancer mouse models, and their data showed exceptionally more effective tumor growth reduction in an unirradiated tumor with the 3- and 5-fraction regimens(34). As such, this protocol utilizes a 5-fraction regimen.

The prescribed dose to the first irradiated lesion will be 40 Gy in 5 daily consecutive fractions of 8 Gy each prescribed to the isodose line covering at least 95% of the planning target volume (PTV). This is a dose that is commonly used in the clinic to provide durable local control and is well tolerated.

After a 1 week interval, a second lesion will be prescribed 30 Gy in 5 daily consecutive fractions of 6 Gy each prescribed to the isodose line covering at least 95% of the PTV. This dose fractionation has been shown to invoke systemic immune response.(34) There is an intentional lowering of the prescription dose to the second lesion to explore the hypothesis that RT given to the first lesion may "prime" the immune system with a lower dose to the second lesion being sufficient to propagate that response. An analogy is that it takes a larger amount of energy to accelerate an object from standstill compared to after it has already been put into motion. Another rationale for prescribing a lower dose to the second target lesion would lower the probability of radiation-induced toxicity while still providing a dose that is likely to provide local control. Furthermore, a lower dose prescribed to the second target lesion would decrease the probability of radiation-induced toxicity while still providing a dose that can provide local control.

5.5 Simulation

All patients will have CT-based treatment planning; a custom-made immobilization device prior to radiation therapy should be considered but is not mandatory. Patients should be positioned in stable position that would facilitate daily setup reproducibility. It is anticipated that most patients will be immobilized in the supine position although the prone position may be considered, for example to displace the small bowel away from the target. CT simulation should include both planned lesions. The CT simulation scan should be obtained with uniform slice thickness of ≤3 mm and should include the targeted lesions in addition to any relevant organs at risk (OARs), defined below. Both lesions to be treated in addition to relevant OARs must be included in the simulation scan. Treatment planning for both lesions will be done prior to the start of radiation therapy. A second CT simulation scan will not be done specifically for treatment of the second lesion because there is only a short interval between treating both lesions although this may be done at the discretion of the investigator. While it is ideal to treat both lesions in the same treatment position, if both lesions are not in the same region it is acceptable to use different treatment positions (and immobilization devices if needed) for each lesion if this would decrease OAR dose and/or improve daily treatment setup reproducibility.

The use of oral and/or IV contrast should be considered depending on the treatment site. For example, contrast should be omitted for treatment of a peripheral lung metastasis although both IV and oral contrast may be considered for treatment of a liver metastasis.

Simulation must be performed no later than 3 calendar days from study enrollment and ideally within 1 calendar day. This is to expedite the start of radiation therapy, especially considering that the patient population in this study has progressive metastatic disease on an immune checkpoint inhibitor alone.

5.6 Motion Management

Because the organs in the thorax (i.e. lung) and upper abdomen (i.e. liver) are common sites of distant metastasis, it is important to account for tumor motion related to respiration. This is to improve targeting accuracy as well as reduce dose to OARs. A 4D-CT simulation scan and/or

breath hold scan, should be used at the time of simulation for any targeted lesion that is expected to move at least 5 mm as a result of respiration.

5.7 Daily MR image-guidance

The use of daily image-guidance is mandatory. A volumetric MRI using a TRUFI sequence will be obtained prior to delivery of each fraction. Alignment should be to soft tissue within the treatment field.

5.8 Target Volumes

The gross target volume (GTV) is defined as disease visible on CT and/or MRI scans. Contrast-enhanced MRI, when available, may assist GTV delineation and may be especially helpful for treatment of liver metastases.

For targets that move with respiration, an internal GTV (IGTV) should be created with margins not exceeding 5 mm in the direction of maximum breathing motion if treating in free breathing. An IGTV will not be used if treatment is in breath hold.

A clinical target volume (CTV) will be not used for any patient.

Planning target volume (PTV) margins will be created as a 3-5 mm expansion from the GTV/IGTV.

5.9 Technical Factors

All patients will be treated on a ViewRay MRIdian MR-linac using 6 MV photons.

Every patient will receive radiation therapy according to the respective physician approved treatment plan using the MRIdian Linac system for alignment (MR image-guidance), dose prediction, tracking, gating and on-table adaptive planning when clinically indicated.

For each delivered fraction, a volumetric MRI data set will be obtained using system integrated sequences; the preferred sequence is a balanced gradient echo most similar to Siemens' True FISP scan with T2*/T1 weighted image-characteristics with at least a 1.5 x 1.5 x 3.0 mm image matrix resolution. The external contour of the patient should be inside the field of view.

The target volume will be rigidly aligned to a reference position by virtual couch movement in cranio-caudal, left-right and anterior-posterior direction to optimally align with the planned treatment isocenter. Depending on operator assessment, these shifts are then executed and treatment is initiated.

If optimal target to isocenter alignment cannot be achieved, system integrated deformable image registration between the primary treatment image data (used for simulation and treatment planning) and the respective fraction MRI dataset is performed. Original plan contours are propagated onto the respective fraction MRI dataset. If on-table adaptive planning is clinically indicated, then all tumor volumes and critical structures within a 3 cm distance from the surface of the original PTV will be re-contoured on the volumetric high-quality MRI data set.

An estimated delivered dose will be calculated and saved using the software on the console (dose prediction). An adapted radiation therapy plan is created based on physician assessment of medical necessity. General guidelines to establish medical necessity include but are not limited to the following:

i) Any OAR constraint is violated at 1.0 cc of the structure or more exceeding the allowed maximum dose (note that this is a more lenient constraint than per the initial plan, allowing for the day-to-day segmentation inaccuracies and estimated varying position of at-risk structures).
ii) Coverage of the GTV/IGTV is less than 85% by the prescribed dose.
iii) There is a favorable shift in the relation between GTV/IGTV and dose-limiting organs-at-risk, such that adaptive planning would likely improve the coverage of the GTV by the prescribed dose by 10% or more.

Adaptive dose re-planning does not need to be performed if the dose prediction shows compliance with minimum dose coverage of the GTV/IGTV and adherence to upper dose/volume limits of all OARs. If both GTV/IGTV and OAR dose criteria are met, attention should be directed toward dose/volume changes of other OAR, specifically kidneys and spinal canal as the surrogate for the spinal cord. Major violation of dose/volume limits to these structures may also warrant adaptive dose re-planning according to the discretion of the treating physician.

During radiation dose delivery, continuous MR image acquisition in at least one principal plane (suggested sagittal, but at the discretion of the treating physician) in cine mode is mandatory for soft tissue tracking and radiation beam gating. To this end, a tracking slice of 7-10 mm thickness is positioned to include a cross-sectional cut of the target for intra-fractional soft tissue tracking. The tracking/gating volume is delineated based on either the GTV or the PTV. The software should be set so that if 5% or more of the tracked target leaves the boundary the beam will gate off. At the discretion of the treating physician the threshold can be set to less than 5%. For soft tissue tracking and gating, a minimum cine frame rate of 4 frames/second is mandatory throughout dose delivery.

Based on institution preference, breathing motion management is to be employed. Allowable breathing management includes shallow breathing (with or without abdominal compression) or breath hold. Breath hold may be patient directed or based on staff coaching. Breath hold assistance devices such as use of mirrors to visualize a wall mounted monitor, MR compatible goggles or image projection into the bore for target positional visualization are allowable and encouraged for use. Abdominal compression should be done with an MR compatible compression belt.

All MRI setup images, MRI images used for plan dose prediction, adaptive re-planning and all cine images are to be saved and stored in the MRIdian associated software. All image data is to be backed up for permanent storage and later image analysis.

Image data storage will be based on institutional protocols but need to allow for later anonymized image data export to a central, HIPAA and other regulation compliant image repository approved by the ViewRay Clinical Cooperative Think Tank (C2T2) Scientific Advisory Committee.

All clinically approved plans, structures delineated for dose prediction, predicted doses as well as all adapted radiation therapy plans will need to be saved and stored in the MRIdian associated software. All initial plans, structures delineated initially and on-table, predicted dose, and adapted

plan data is to be backed up for permanent storage and potential later institutional or centralized analysis.

Structure, radiation dose and radiation plan data storage will be based on institutional protocols but need to allow for anonymized data export to a central, HIPAA and other regulation compliant image and plan data repository approved by the ViewRay Clinical Cooperative Think Tank (C2T2) Scientific Advisory Committee.

5.10 Radiation Delivery Schedule

It is strongly encouraged that radiation therapy for each lesion begin on a Monday. However, it is permissible for treatment to start on a Tuesday or Wednesday. Radiation will be delivered daily on Days 1-5 and Days 15-19.

5.11 Missed Treatments

Missed treatments and treatment breaks should be entered into the treatment record. Any missed treatments should be made up so that the total prescribed radiation dose is delivered to each lesion. This should be done as expeditiously as possible and must respect time needed off treatment for any patients who experience significant toxicity. More than one fraction should not be delivered per day to make up treatments although treatment on Saturday/Sunday is permitted.

5.12 Treatment Planning

3D conformal radiation therapy (3DCRT) is preferred although intensity modulated radiation therapy (IMRT) may be used as required to meet organ at risk constraints.

Because 2 lesions will sequentially receive radiation, 2 separate treatment plans will need to be generated.

Composite plans should be generated to account for normal tissue dose from each lesion that is treated. Dose summation from multiple treatment sites should be determined on a single CT scan that encompasses the relevant anatomic region. A planning CT dataset that includes all targets and relevant critical structures in the imaging study should be obtained when possible.

The prescription isodose will be chosen such that at least 95% of the PTV is conformally encompassed by the prescription isodose. Any dose >105% of the prescription dose should be located within the PTV and not within OARs.

IV contrast from the planning dataset, when present, should be converted to water equivalent density for planning.

It is required that treatment planning be completed no later than 8 calendar days from simulation. It is strongly encouraged to complete treatment planning as soon as possible to expedite the start of radiation therapy, especially considering that the patient population in this study has progressive metastatic disease.

5.13 Organs at Risk

OARs must be contoured and the specific OAR will depend on the location of the lesion that is treated with radiation therapy. For example, the larynx is not a pertinent OAR for a target in the liver. However, the larynx would be a pertinent OAR for a target in the neck. For each of the two treated lesions any OAR within 3 cm of the PTV should be contoured and considered during treatment planning. OAR dose constraints are listed in Table 1.

OAR contours should be generated as follows:

- Larynx: The larynx will be contoured according to published guidelines(35). The contour should begin just inferior to the hyoid bone and include the inner surface of the thyroid cartilage, lateral surfaces of the aryepiglottic folds, posterior surface of the arytenoids excluding the pyriform sinus, the posterior surface of the cricoid cartilage, and the inferior extent of the cricoid cartilage.

- Spinal cord: The spinal cord will be contoured starting at least 10 cm above the superior extent of the PTV and then on all slices to at least 10 cm below the inferior extent of the PTV.

- Cauda equina: The cauda equina starts at the inferior extent of the spinal cord (~L1/L2) and includes the spinal canal into the sacrum to the filum terminale.

- Esophagus: The esophagus will be contoured from esophageal inlet to the gastroesophageal junction. All layers from the mucosa to the adventitia should be included.

- Heart: The heart will be contoured along with the pericardial sac starting superiorly at the level of the inferior aspect of the aortic arch and inferiorly to the apex of the heart.

- Ipsilateral brachial plexus: The ipsilateral brachial plexus includes the spinal nerves exiting the neuroforaminae on the involved side from around C5-T2. However, for the purposes of this protocol, only the major trunks of the brachial plexus will be contoured using the subclavian and axillary vessels as a surrogate for identifying the location of the brachial plexus. This neurovascular complex will be contoured starting proximally at the bifurcation of the brachiocephalic trunk into the jugular/subclavian veins (or carotid/subclavian arteries) and following along the route of the subclavian vein to the axillary vein ending after the neurovascular structures cross the second rib. If the PTVs of all irradiated lesions are >10 cm away from the brachial plexus, this structure does not need to be contoured.

- Total lung: The right and left lungs will be contoured as one structure. Contouring should be done using lung windows. If treating a lung lesion, then the GTV/IGTV should be subtracted from this contour.

- Stomach: The entire stomach and its contents should be contoured as a continuation of the esophagus and terminating at the first part of the duodenum.

- Duodenum: The duodenum should be contoured as a continuation of the stomach and will end where the superior mesenteric artery crosses over the 3rd part of the duodenum.

- Small bowel: The small bowel will be contoured as identified by oral contrast, if present. The duodenum will be excluded.

- Large bowel: The large bowel contour will extend from the ileocecal region and include the ascending, transverse, descending, and sigmoid colon.

- Liver: The entire liver should be contoured. If treating a liver lesion, then the GTV/IGTV should be subtracted from this contour.

- Kidney: Both right and left kidneys excluding the renal pelvis/collecting system will be contoured.

Table 1. Organ at Risk (OAR) dose constraints.

Organ At Risk	Volume Limit	Dose (Gy) Limit
Larynx	0.03 cc	Prescription dose
Spinal cord	0.03 cc	30
Ipsilateral brachial plexus	0.03 cc	32
Cauda equina	0.03 cc	32
Esophagus	0.03 cc	30
Heart	0.03 cc	38
Stomach	0.5 cc 0.03 cc	33 40
Duodenum	0.5 cc 0.03 cc	33 40
Small bowel	0.5 cc 0.03 cc	33 40
Large bowel	0.5 cc 0.03 cc	36 43
Total lung (minus GTV/IGTV)	10% Mean	20 6
Total kidney	Mean	10
Liver (minus GTV/IGTV)	700 cc Mean	21 18

Additional Radiation Therapy To Non-Target Lesions

Additional lesions other than the 2 planned to receive radiation may be treated with palliative radiation therapy based on the investigator's discretion when medically necessary. It is strongly encouraged to maximize medical management for symptoms, if medically appropriate, prior to proceeding with additional palliative radiation therapy. Any subject who receives non-target radiation for palliation will be explicitly considered to have had progression of disease regardless of imaging study results or any other tumor assessment method.

Patients who require palliative radiation therapy to a non-target lesion prior to treatment of the first of 2 target lesions should not be enrolled to this study. However, if new symptoms arise between treatment of lesions 1 and 2, or at any time after completion of 2 courses of SBRT under study conditions, then it will be appropriate to classify as progression.

6. PATIENT ASSESSMENT

Subjects will be formally assessed as follows:

- Once during radiation therapy to the first lesion (Day 1, 2, 3, 4, or 5)
- Once during radiation therapy to the second lesion (Day 15, 16, 17, 18, or 19)
- 8 weeks (+/-2 weeks) after completion of radiation therapy to the first lesion and then Q8 weeks.

The following will be performed at each assessment:

- Comprehensive physical examination

- Vital signs including at a minimum blood pressure, pulse, respirations, weight, oxygen saturation by pulse oximetry
- Adverse event assessment
- Review of concomitant medications

Outside this schedule, subjects should be evaluated by appropriate medical personnel at any other time that is deemed clinically appropriate.

Patients will be followed for survival status at the time of each follow up visit and by telephone every 6 months for up to 24 months after end of treatment.

Table 2. Study Schedule

	SCREENING	TREATMENT				FOLLOWUP
	BASELINE (WITHIN 28 DAYS OF ENROLLMENT)	DAY 1, 2, 3, 4, OR 5 (DURING RT TO 1ST LESION)	DAY 8 (+/-3 days)	DAY 15 (+/-3 days), 16, 17, 18, OR 19 (DURING RT TO 2ND LESION)	DAY 23 (+/-3 days)	8 (+/- 2 weeks) WEEKS AFTER RT START TO 1ST LESION, THEN Q8 WEEKS
INFORMED CONSENT	X					
VITAL SIGNS[1]	X	X		X		X
COMPREHENSIVE PHYSICAL EXAMINATION	X	X		X		X
ECOG PERFORMANCE STATUS	X	X		X		X
ADVERSE EFFECT ASSESSMENT	X	X	X	X	X	X
CONCOMITANT MEDICATION	X	X		X		X
LABORATORY TESTS	X					X[4]
PREGNANCY TEST[2]	X					
DIAGNOSTIC IMAGING STUDY	X					X[3]

1. Vital signs: systolic and diastolic blood pressure, respiration, pulse, oral temperature
2. Pregnancy Test (serum or urine in women of childbearing potential) are performed at baseline within 48 hours of start of treatment.
3. Every 8 weeks (+/-2 weeks) thereafter for the first 26 weeks after initiation of radiation therapy
4. Complete blood count and complete metabolic panel only

7. TREATMENT RESPONSE EVALUATION

7.1 Imaging Assessment

All subjects will have a CT scan of the chest, abdomen, and pelvis with IV and oral contrast to assess treatment response every 2 months. Patients with iodine/contrast allergy should receive premedication according to institutional standard of care prior to receiving contrast. Patients with known brain metastases should have an MRI of the brain with contrast every 2 months or sooner

as medically indicated. Diagnostic scans should be performed with no larger than 5 mm slice thickness.

Diagnostic imaging studies as described above should be obtained at each of the following intervals, or sooner if clinically indicated:

- Baseline/screening
- 8 weeks (+/-2 weeks) after initiation of radiation therapy to the first lesion
- Every 8 weeks (+/-2 weeks) thereafter for the first 26 weeks after initiation of radiation therapy

Additional sites of disease not encompassed by the studies described above should be imaged according to the schedule above using the most appropriate diagnostic imaging modality according to the treating physician's discretion.

7.2 Definitions for Disease Assessment

- **Measurable lesions:** Lesions that can be accurately measured in at least one dimension with longest diameter at least 10 mm on CT scan; lymph nodes must measure at least 15 mm in short axis
- **Non-measurable lesions:** All other lesions <10 mm on CT scan, or lymph nodes <15 mm in short axis. Other non-measurable lesions include: ascites, pleural or pericardial effusion, lymphangitic involvement of skin or lung, abdominal masses not measurable by reproducible imaging techniques.
- **Target lesions:** Target lesions should be identified and measured at baseline. Target lesions include measurable lesions up to a maximum of two lesions per organ and up to five lesions in total that are representative of all involved organs. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and are to undergo reproducible repeated measurements. If the largest lesion does not lend itself to reproducible measurement it should not be considered a target lesion and instead the next largest lesion that can be measured reproducibly should be selected. A sum of the longest diameters for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.
- **Non-target Lesions:** All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

7.3 Definitions of Disease Response

Definition of response in target lesions:

- **Complete Response (CR):** Disappearance of all target lesions
- **Partial Response (PR):** At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
- **Progressive Disease (PD):** At least a 20% increase in the sum of the diameters of target lesions compared to baseline sum of target lesions, or any new lesions
- **Stable Disease (SD):** Neither sufficient decrease to qualify as a PR or sufficient increase to qualify as PD

Response should be confirmed by repeat CT scan that may be performed at the next regularly scheduled scan and no earlier than 4 weeks after imaging showing a particular response (unless clinically indicated).

Definition of response in non-target lesions:

- **Complete Response (CR):** Disappearance of all target lesions
- **Progressive Disease (PD):** Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions
- **Incomplete Response/Stable Disease (SD):** Persistence of one or more non-target lesions

The response to immunotherapy may differ from the typical responses observed with cytotoxic chemotherapy including the following:

- Response to immunotherapy may be delayed
- Response to immunotherapy may occur after PD by conventional criteria
- The appearance of new lesions may not represent PD with immunotherapy
- SD while on immunotherapy may be durable and represent clinical benefit.

Modification of RECIST as described will discourage the early discontinuation of an immune checkpoint inhibitor and provide a more complete evaluation of its anti-tumor activity than would be seen with conventional response criteria.

7.4 Evaluation of Overall Response

The primary endpoint of this study is best Overall Response Rate (ORR) that is determined from the start of treatment until 6 months later (Table 2). Best ORR will depend on the status of both target and non-target disease as well as whether new lesions appear. Furthermore, it is defined as the best response at all time points. When SD is the best response it must also meet protocol specified minimum time from baseline of 8 weeks. If the minimum time is not met when SD is the best time point response, then the subject's best response depends on the subsequent assessments. For example, if SD is the best response at first assessment, and has PD at the second assessment but does not meet the minimum duration of SD will have best response of PD.

Table 3. Best Overall Response Criteria

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	PR/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

7.5 Verification Scans

Confirmation of CR or PR is required (Table 3). Confirmed CR or PR will be claimed only if the criteria for each are met at a subsequent time point (minimum 4 weeks after criteria for an objective response are first met).

Confirmation of PD is required if PD is equivocal. If repeat scan confirms PD, then PD will be claimed using the date of the initial scan. If repeat scan does not confirm PD, then PD will not be claimed.

Table 4. Best Overall Response When Confirmation of CR and PR is Required.

Overall Response At First Time Point	Overall Response At Second Time Point	Best Overall Response
CR	CR	CR
CR	PR	SD, PD, OR PR*
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise, NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise, NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, NE= not evaluable.

*If CR is achieved at first time point, but any disease including PR at subsequent time point will result in PD. Best response would depend on whether minimum duration for SD was met. Sometimes CR may be claimed when subsequent scan suggests small lesions were likely still present and the patient had PR, not CR at first time point. If this is the case, then the original CR should be changed to PR and the best response is PR.

7.6 Evaluation of Immune-Related Overall Response

Systematic criteria designated immune-related response criteria were defined based on four distinct response patterns seen after administration of an ICI: (a) shrinkage in baseline lesions,

without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); (c) response after an increase in total tumor burden; and (d) response in the presence of new lesions. These four patterns were associated with favorable survival (30).

One secondary objective of this study is objective response rate by immune-related response criteria (irRC). Immune-related objective response rate (irORR) will be determined as published by Wolchok et al(36). irORR is the proportion of patients with best overall response of irCR or irPR from the start of the study until 6 months later. Response is defined as irCR, irPR, or irSD over at least 4 weeks.

Anti-tumor response is based on total measurable tumor burden, which is determined as follows. Only index and measurable new lesions are taken into account. At baseline tumor assessment, the sum of the products of the 2 largest perpendicular diameters (SPD) of all index lesions (5 lesions per organ, up to 10 visceral lesions and 5 cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new measurable lesions (at least 5 x 5 mm; up to 5 new lesions per organ: 5 new cutaneous and 10 visceral lesions) are added together to provide the total tumor burden.

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new measurable lesions as they appear. At each tumor assessment the response in index and new measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e. the SPD of all index lesions at screening).

Table 5. Immune-related response criteria.

irCR	Disappearance of all lesions in 2 consecutive observations not less than 4 weeks apart.
irPR	At least 50% decrease in tumor burden compared with baseline in 2 observations at least 4 weeks apart.
irSD	Neither 50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir (at any time point).
irPD	At least 25% increase in tumor burden compared with nadir (at any time point) in 2 consecutive observations at least 4 weeks apart.

Major differences with other tumor response criteria include: PR or SD in the presence of new lesions, as long as they met the respective threshold of response based on total differences in tumor burden; an SD does not require confirmation; however PD requires confirmation by another scan at least 4 weeks apart in the absence of rapid clinical deterioration.

8. SAFETY ASSESSMENT

8.1 Adverse Event Assessment

Adverse events (AEs) will be defined using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 criteria.

A causal relationship between radiation therapy/immune checkpoint inhibitor and the AE should be determined for all AEs as follows:

- Related: There is a reasonable causal relationship between radiation therapy/immune checkpoint inhibitor and the AE
- Not related: There is not a reasonable causal relationship between radiation therapy/immune checkpoint inhibitor and the AE

8.2 Definition of Adverse Event

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE includes but is not limited to any clinically significant worsening of a subject's pre-existing condition. An abnormal laboratory finding (including ECG finding) that requires an action or intervention by the investigator, or a finding judged by the investigator to represent a change beyond the range of normal physiologic fluctuation, should be reported as an AE.

Adverse events may be treatment emergent (ie, occurring after initial receipt of investigational product) or non-treatment emergent. A non-treatment emergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the subject has received investigational product.

Elective treatment or surgery or preplanned treatment or surgery (that was scheduled prior to the subject being enrolled into the study) for a documented pre-existing condition that did not worsen from baseline is not considered an AE (serious or non-serious). An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or SAE.

The term AE is used to include both serious and non-serious AEs.

8.3 Serious Adverse Event Reporting

A serious adverse event (SAE) is an AE occurring during any study phase (i.e., screening, run-in, treatment, wash-out, follow-up), at any dose of the study drugs that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Is a congenital abnormality or birth defect in offspring of the subject
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above

It is important to note that hospital admission for a planned procedure or disease treatment is not considered a SAE.

SAE reporting is required once the participant signs consent and is registered on the study.

SAE reporting is required for 30 days after the participant's last treatment administration on study. Any events that occur after this 30 day period that may be related to study treatment must be reported.

An SAE is required to be reported to the IRB within 5 calendar days of the event occurrence. A Clinical Research Database (CRDB) SAE report should be submitted to the SAE Office including the following:

- Subject's name (use initials only if the report will be sent outside of MCI)
- Medical record number
- Disease/histology
- Protocol number and title
- Date of the AE occurrence
- Describe the nature of the AE (i.e. dermatitis)
- The grade of the AE
- Relationship of the AE to treatment
- Whether the AE was expected or unexpected
- The AE severity
- The intervention to address the AE
- A description of the subject's condition
- Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form

The PI's signature and date are required on this report.

8.4 Non-Serious Adverse Event Reporting

Non-serious AE reporting will begin upon initiation of treatment and for a minimum of 100 days after completion of radiation therapy.

Non-serious AEs should be followed until resolution of stabilization.

All identified non-serious AEs must be recorded and described on the non-serious AE page of the CRF.

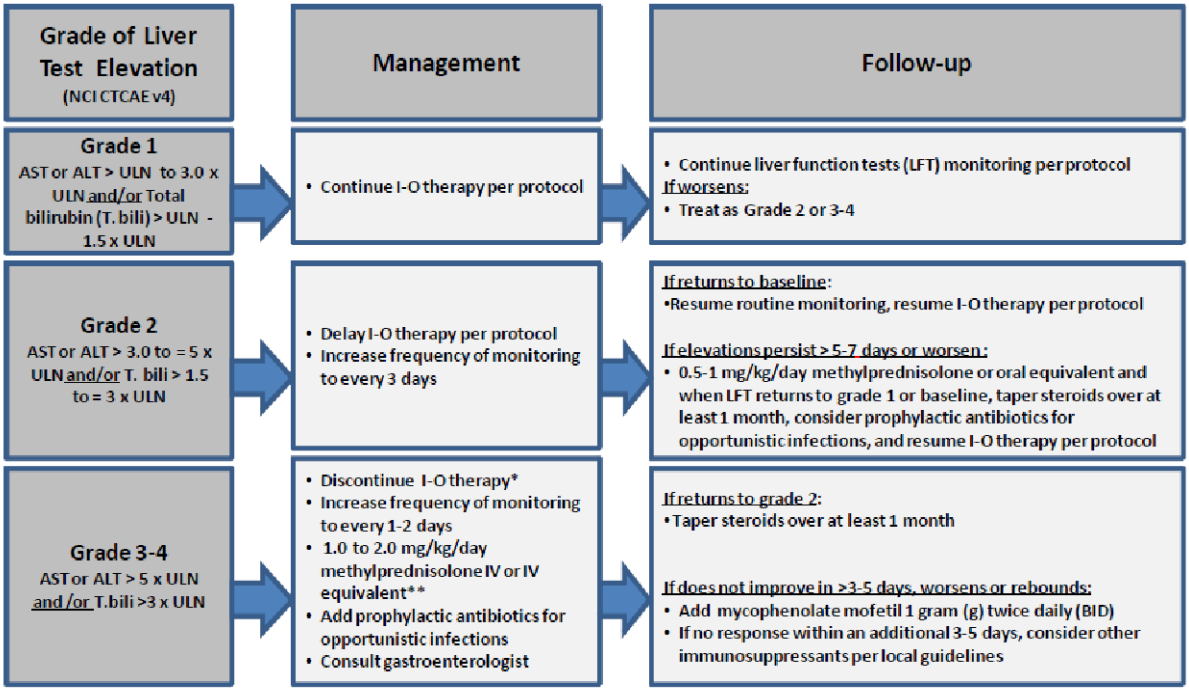
8.5 Immune checkpoint inhibitor toxicity management

The safety profile of immune checkpoint inhibitors given as monotherapy has been well established. Management of immune related adverse events (irAEs) should be done according to standard guidelines and includes dose adjustment or delay according to the treating medical oncologist.

Management algorithms have been developed to assess and manage the following groups of irAEs:

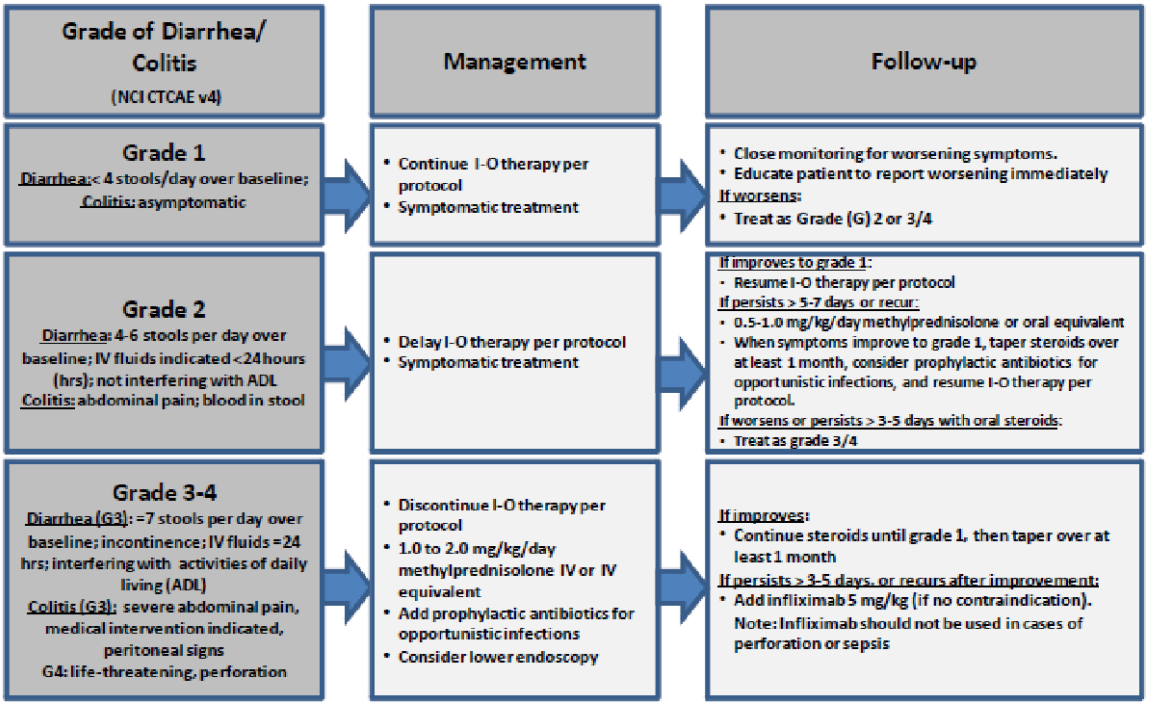
- Pulmonary
- Renal
- Gastrointestinal
- Hepatic
- Endocrine
- Skin
- Neurological

Hepatic Adverse Event Management Algorithm



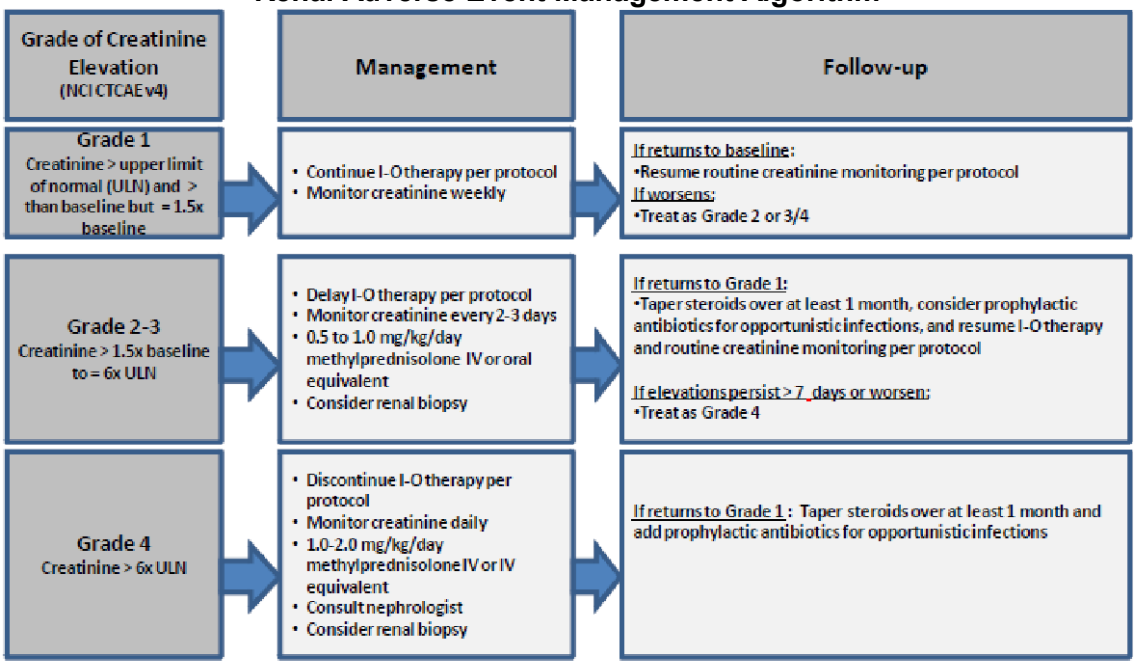
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*I-O therapy may be delayed rather than discontinued if AST/ALT = 8 x ULN and T.bili = 5 x ULN.
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

GI Adverse Event Management Algorithm



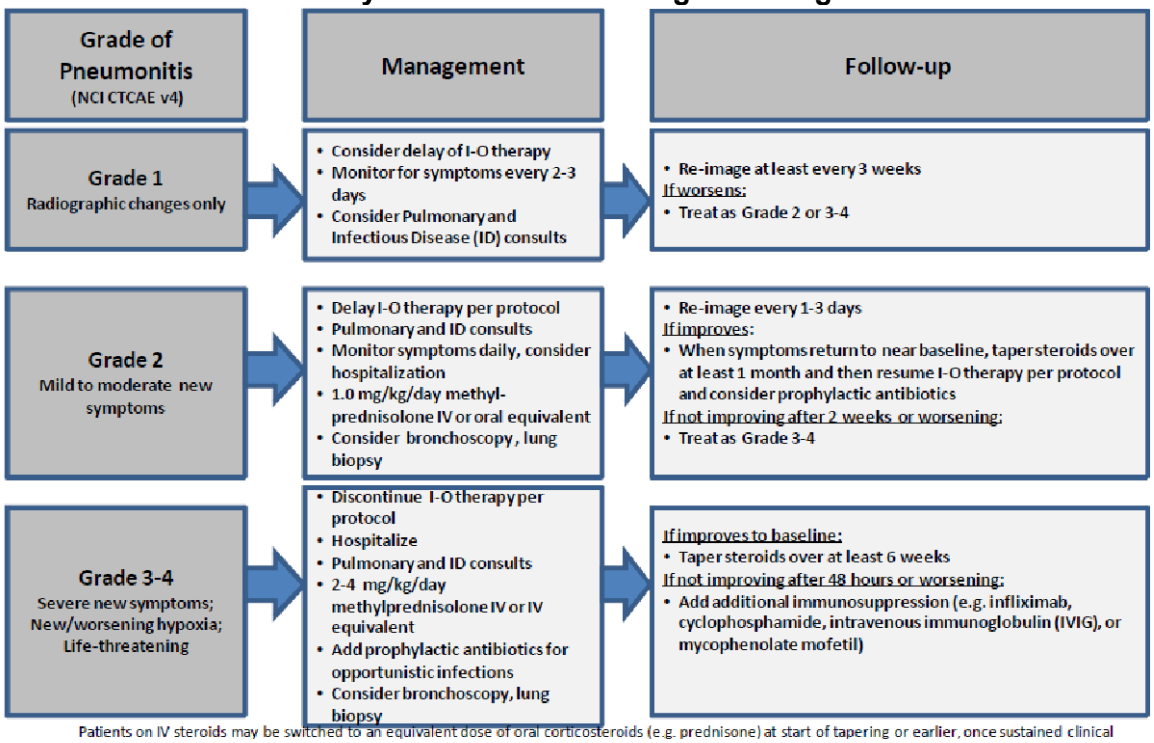
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm



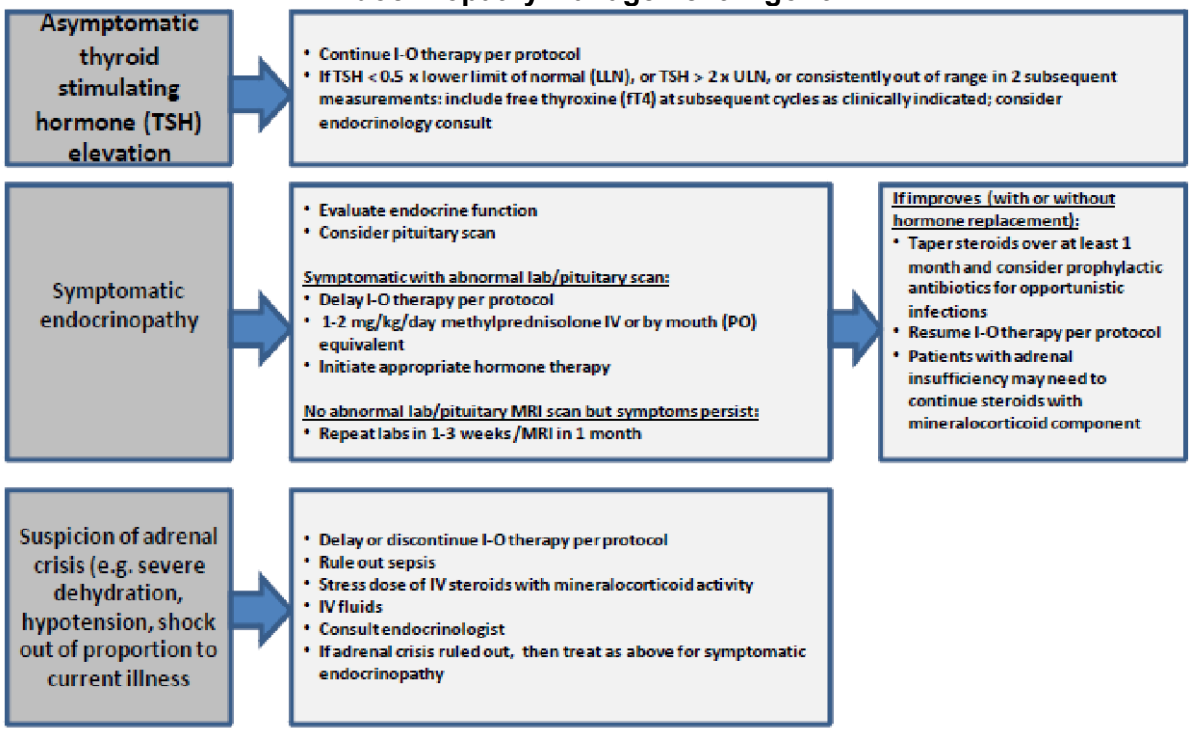
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm



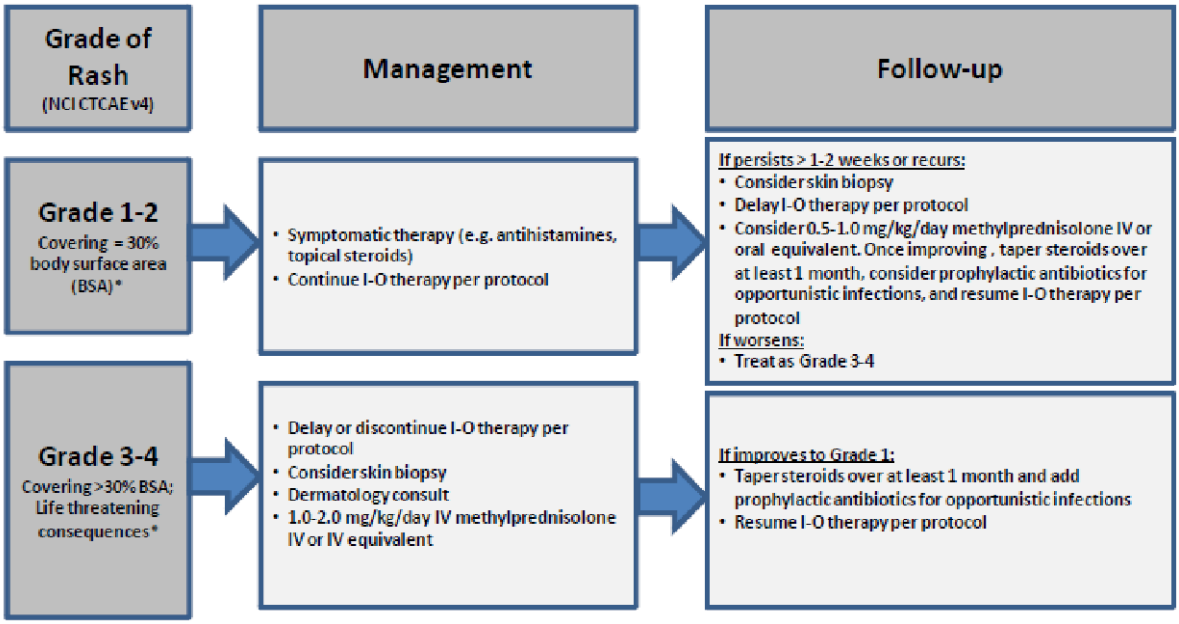
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Endocrinopathy Management Algorithm



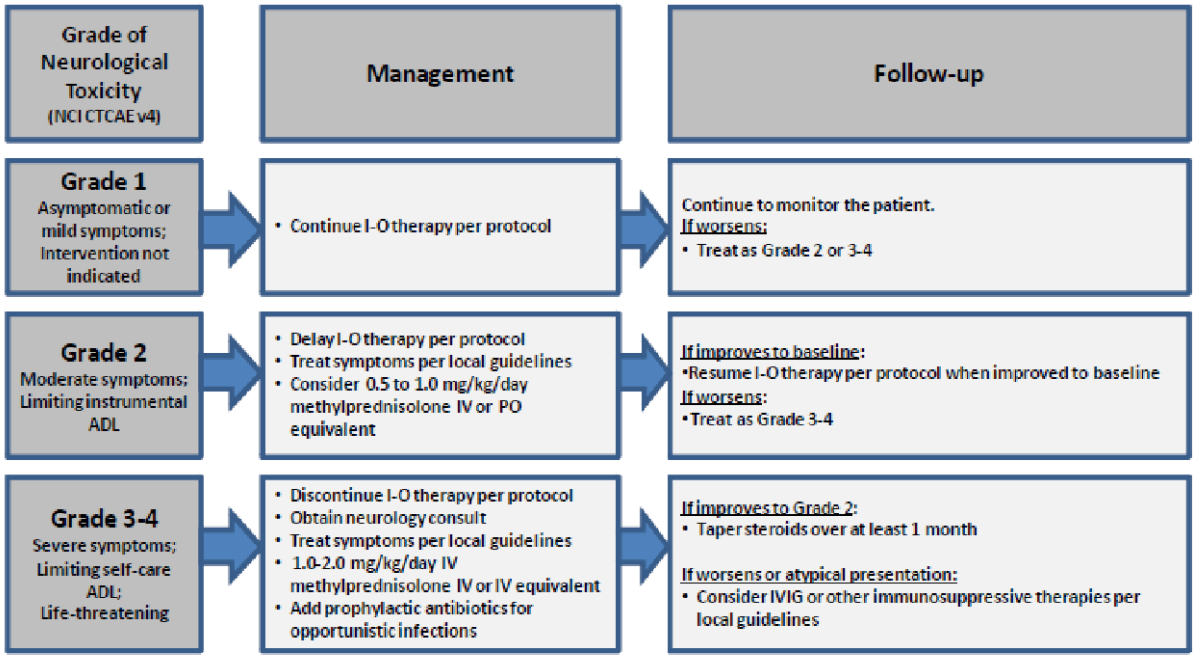
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Neurological Adverse Event Management Algorithm



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

8.6 Criteria to Resume Treatment

Subjects may resume receiving an immune checkpoint inhibitor when drug-related AEs resolved to no greater than Grade 1.

There are some exceptions to this, however. Subjects may resume treatment with an immune checkpoint inhibitor in the following situations:

- Grade 2 fatigue is present
- No greater than Grade 2 skin toxicity is present
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT or total bilirubin
- Subjects with combined Grade 2 AST/ALT and total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued.
- Drug-related pulmonary toxicity, diarrhea, colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.

Subjects should resume treatment at the next scheduled time point per the study protocol if criteria to resume treatment are met. If the treatment is delayed past the next scheduled time point per the study protocol, then the next scheduled time point will be delayed until dosing resumes.

Treatment may be delayed for up to 6 weeks from the last dose. If treatment is delayed by more than 6 weeks the subject will be discontinued from study therapy, except as specified.

8.7 Management of radiation therapy adverse events

Radiation therapy given as monotherapy may cause AEs specific to the location in the body being treated. For example, AEs as a result of liver treatment will be distinct from treatment of a lung lesion. Supportive care is given based on the specific AE when radiation therapy is given as monotherapy, and the same should be done when radiation is given with concurrent immune checkpoint inhibitor.

8.8 Pregnancy

If it becomes known that a subject is pregnant or may have been pregnant at the time of study participation, the investigator must immediately notify the PI (Michael Chuong, MD at (786) 527-8140) and the Baptist Health South Florida Institutional Review Board (BHSF-IRB at (786) 596-9280) and the patient will be discontinued from study participation.

Overdose or Misadministration of Radiation
Overdose or misadministration of radiation is defined as the accidental or intentional administration of any dose of radiation considered excessive and medically important. All occurrences of overdose or misadministration must be reported as an SAE.

9. SUBJECT DISCONTINUATION AND REPLACEMENT

9.1 Subject Discontinuation

Permanent withdrawal of a subject from the study will occur when any of the following occur pertaining to the subject in question:

- Subject withdraws consent or is lost to follow-up.
- Patient becomes pregnant or has the intent to become pregnant.
- Subject is non-compliant with requirements of this study (i.e. refusal to adhere to scheduled visits) that in the opinion of the investigator warrants study withdrawal.
- Confirmation of progressive disease and determination by the investigator that the subject is not receiving benefit from study therapy.
- Subject starts anti-cancer therapy with any agent not specifically included within this study's protocol.
- AE occurs that in the opinion of the investigator is a contraindication to further treatment on study.
- Any Grade 2 drug-related uveitis or eye pain or blurred vision that doesn't respond to topic therapy and doesn't improve to Grade 1 severity within the re-treatment period or requires systemic therapy.
- Grade 3 drug-related uveitis, pneumonitis, bronchospasm, neurologic toxicity, hypersensitivity reaction, or infusion reaction of any duration
- Grade 3 drug-related thrombocytopenia lasting >7 days or is associated with bleeding
- Drug-related liver dysfunction that meets the following criteria
    a. AST or ALT >8x ULN
    b. Total bilirubin >5x ULN
    c. Concurrent AST or ALT >3x ULN and total bilirubin >2x ULN

- Any Grade 4 drug-related AE or laboratory abnormality with the exception of the following:
  - Grade 4 electrolyte imbalance not clinically significant and corrected with appropriate medical management within 72 hours of onset
- Any dosing delays lasting >6 weeks with the following exceptions:
  - Dosing delays to allow for prolonged steroid taper to manage drug-related AE. Tumor assessments should continue as per protocol despite dosing delay.
  - Dosing delays >6 weeks for non-drug-related reasons if approved by the primary investigator. Tumor assessments should continue as per protocol despite dosing delay.

Any subject that is permanently discontinued from study participation will still be followed for safety and will also continue to have protocol-specified blood specimens drawn and imaging studies obtained, unless the subject is lost to follow-up or enrolled in another clinical study. All subjects will be followed for survival analyses. Subjects who do not return to the treating institution for evaluations required by the study protocol will be offered telephone follow up every 6 months for up to 24 months.

If consent is withdrawn, then the subject will receive no further investigational therapy or undergo any study-specific observations or examinations.

9.2 Subject Replacement

Any subject that discontinues participation in this study for any reason other than unacceptable toxicity or progressive disease before the initial efficacy evaluation (CT scan 1 month after initiation of radiation therapy to first lesion) may be replaced. These cases will be recorded and accounted for in the report of the trial.

10. STATISTICAL METHODS

10.1 Study Endpoints

The primary objective of this trial is to evaluate the best overall response rate according to RECIST 1.1 criteria of non-irradiated lesions in metastatic cancer patients receiving immune checkpoint inhibitor and radiation therapy during a 6-month period. The primary objective will be evaluated by Simon's two-stage design as described below.

For the secondary objectives, ORR defined by irRC for non-irradiated lesions will be reported as sample proportion and confidence intervals. OS and PFS will be evaluated by the Kaplan-Meier method. DOR will be calculated among patients with response from the time of response to progression or death from any cause and analyzed using the Kaplan-Meier method. Treatment-related AEs will be tabulated and summarized by grade.

10.2 Sample Size Determination

Clinical trials of ICI monotherapy including patients with locally advanced and/or metastatic disease of various tumor types have reported ORR ranging from 13-31% (37-41). To our knowledge the only prospective data of RT combined with an ICI consisted of a phase I/II trial including 10 patients with various tumor types who received durvalumab (an ICI) and RT delivered to a single target(42). Although there was a high rate of response in *irradiated* tumors (60% complete response, 20% partial response, 40% stable disease), there was no response within

un-irradiated tumors. Therefore, there are not robust data of RT to at least one tumor with concomitant ICI to guide the statistical design of this study of RT to two tumors with concomitant ICI.

The Simon's two-stage design will be used(43). A desirable response will be considered as 15% or higher while an undesirable response will be 5% or less. The null hypothesis that the true response rate is 5% will be tested against a one-sided alternative. In the first stage, 30 patients will be accrued. If there are 1 or fewer responses within 6 months of treatment in these 30 patients, the study will be stopped. Otherwise, if there are 2 or more patients with response within 6 months then 22 additional patients will be accrued for a total of 52. The null hypothesis will be rejected if 6 or more responses are observed in 52 patients. This design yields a type I error rate of 0.05 and power of 0.80 when the true response rate is 15%. Probability of early stopping is 0.55.

10.3 Accrual and Study Duration

We expect to enroll 2 subjects per month. Therefore, we expect trial accrual to be completed in 26 months.

11. ETHICAL AND REGULATORY REQUIREMENTS

11.1 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements.

Study procedures may begin once IRB approval is secured and other details (e.g. study supplies, clinical trial agreements) are in place.

11.2 Informed Consent

All subjects must sign informed consent to participate in and register for this study. The written informed consent must be signed and personally dated by the subject or the subject's legally acceptable representative.

All subjects will be informed about the following:

- Study aims
- Possible adverse effects
- Study procedures
- Confidentiality of patient data
- Medical records potentially being reviewed by authorized individuals other than the treating physician

It will be explained to each subject that participation is voluntary and that subjects have the right to refuse participation at any time during the study. If a subject refuses to participate in this study, it will not affect the quality of the patient's subsequent care.

12. DATA MANAGEMENT

Data will be managed by a designated Clinical Research Coordinator (CRC) from the Department of Radiation Oncology at Miami Cancer Institute. All data will be managed in compliance with institutional policy and kept in a CRDB. The ForteEDC will be used for data collection and management.

All plan and daily MRI image data will be submitted to a central repository (ProKnow) in anonymized form for centralized plan review and dosimetric analysis.

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