NYU S14-00208 / CA184-392

PHASE II STUDY OF COMBINED IONIZING RADIATION AND IPILIMUMAB IN METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC)

Principal Investigator: Abraham Chachoua, M.D.
Abraham.Chachoua@nyumc.org
212-731-5388

Co-P.I.
Silvia Formenti, M.D.

Co-Investigators:
Sandra Demaria, M.D.
Peter Schiff, M.D., Ph.D.
Harvey Pass, M.D.
Kent Friedman, M.D.
Divya Sridhar, M.D.
Jean Lee, M.D.
Judith Goldberg, Sc.D.
Christine Hitchen, M.S.
Adriana Heguy, Ph.D.

Research Nurse: Olivier Maisonet, NP
Natalia Cherkassky, NP
Melissa Martinez, NP
Eliana Ferriera, NP
Jennifer Hull, RN

VERSION 2.3 12/04/2015

Summary of Changes	Version	Date
1. Adding Adriana Heguy, Ph.D. as a Co-Investigator on the trial. 2. Removing the following personnel no longer at NYU: Ralph Vatner, M.D., Ph.D., Keith DeWyngaert, Ph.D., Maria Fenton-Kerimian, NP	2.3	12/04/2015
1. Changing the Principal Investigator to Dr. Abraham Chachoua 2. Revising section 8.9 to include statement that anonymous data will be shared with Weill Cornell Medical College due to the re-location of personnel- Dr. Encouse Golden. Inclusion of statement that anonymous data will only be shared after a transfer agreement is executed.	2.2	10/12/2015
1. Changing the Principal Investigator to Dr. Encouse Golden 2. Changing the Principal Investigator information in the informed consent. 3. Adding risk information to the informed consent	2.0	03/23/2015
1. Adding +/- 7 days window to PET/CT/MRI scan prior to enrollment 2. Changing study calendar to reflect screening 28days (+/- 7 days) 3. Including MRI in study calendar along with PET/CT 4. Removing the phrase “within one month” from the inclusion criteria # 12. 5. Removing BHC site from the protocol 6. Appendix 2 – Lab correlatives have been updated (Rationale：Heparin may interfere with some of the planned assays. It was decided to collect blood using EDTA which will not pose this potential problem) 7. Added Jean Lee, M.D. Co-Investigator 8. No changes to the informed consent	1.4 1.4 1.4 1.4 1.4 1.4 1.3 1.5	08/21/2014 08/21/2014 08/21/2014 08/21/2014 08/21/2014 08/21/2014 10/08/2014

Added: Co-Investigator: Keith DeWyngaert, Ph.D. Christine Hitchen, M.S. Jean Lee, M.D. Research Nurse: Jennifer Hull, RN Changed: Protocol Synopsis: Inclusion Criteria: 11. Added: *"…or greater than 2 weeks post- gamma knife therapy…"* *12 Added : "*Brain Scan (CT/MRI) within a month prior to enrollment" 4 Subject Selection criteria: 11. Added *"...or greater than 2 weeks post- gamma knife therapy…"* 4.3.6.2 Treatment: Removed: *"as a reference set."* Added: *"…or orthogonals (for IMRT) on first day."* Removed: *"In addition to the portal imaging…"* *"If shifts are made based upon the CBCT images, the portal images will be repeated."* 4.4.1 Prohibited Therapies: Removed: *"…or treatments*…" Events Schedule: Removed: duplicate *Endocrine Panels* line Moved: from day 4 to day 2: Physical Examination Vital Signs AE Assessment Serum chemistry Endocrine panels Eligibility Worksheet 8. Added: *"…or greater than 2 weeks post- gamma knife therapy…"*		

"Brain Scan (CT/MRI) within a month prior to enrollment"		
Refined Inclusion Criteria (Page 9): 1. "8. Performance status ECOG 0-1, removed Karnofsky >50%" Refined Inclusion Criteria (Page 30) 1. "8. Performance Status ECOG 0-1 removed Karnofsky >50%"	1.6	10/29/2014
Corrected Version Number in header (1.6) Removed "Karnofsky >50%" from Eligibility Checklist (pg 56) Section 4.3.6.2 Treatment: 1. Removed " to an isodose surface encompassing <u>> 90% of the PTV"</u> 2. <u>Added "Part B fractionation: the PTV will receive 28.5 Gy in 3 fractions of 9.5 Gy each, delivered on alternate days within 1 week, with a minimum of 36 hours between fractions."</u> 3. Section 8.5- Laboratory correlative studies – Adding language 4. Section 8.7 – Shipping of Samples	1.7	02/13/2015

Table of Contents

LIST OF TABLES

Protocol Synopsis

Protocol Title:	**PHASE II STUDY OF COMBINED IONIZING RADIATION AND IPILIMUMAB IN METASTATIC NSCLC**
Sites	NYU Clinical Cancer Center 160 E. 34th Street New York, NY 10016 Tisch Hospital 560 First Ave
Investigator Names:	Abraham Chachoua, M.D. Sandra Demaria, M.D. Peter Schiff, M.D., Ph.D. Harvey Pass, M.D. Kent Friedman M.D. Divya Sridhar, M.D. Judith Goldberg, Sc.D. Olivier Maisonet, NP

Study Schema: **(Drugs / Doses / Length of Treatment)**	Eligible patients have metastatic NSCLC who have progressed on chemotherapy and who have at least 2 measurable sites of disease. A biopsy of one of the measurable lesions will be performed in patients who consent to this optional biopsy. Patients will receive ipilimumab (Ipi) 3mg/kg i.v. over 90 minutes, within 24 hours of starting radiotherapy (RT) to the biopsied lesion, 6 Gy x5 (conformally or by intensity modulated RT (IMRT) with image guidance to maximally spare normal tissue). Ipilimumab is repeated on days 22, 43 (for patients who consent to the first biopsy), and 64. Repeat biopsy will be performed between day 22-29, and patients will be re-imaged between day 81-88 and evaluated for response (defined as an objective response by irRC of the measurable metastatic sites outside the radiation field). This response will be evaluated assessing clinical and computed tomography (CT) responses in the non-irradiated measurable metastatic sites using the modified WHO criteria. If the initial Phase II trial is terminated after the first stage of the two stage design, new patients will be enrolled in Part B at the following dosing: 9.5 Gy x3 fractions, with the same dose and schedule for ipilimumab.
Trial Objectives: **(Primary and Secondary)**	**Objective 1: Evaluate the safety and therapeutic efficacy of anti-CTLA-4 mAb and concurrent local RT in NSCLC patients with metastatic disease.** An open label phase II trial will evaluate the preliminary efficacy of the combination of Ipi and RT, applied to a single metastatic site. Efficacy is measured with respect to systemic tumor responses (abscopal response, outside the field of therapy) defined by immune-related Response Criteria (irRC) in all non-irradiated measurable lesions, as a demonstration of an effective anti-tumor immune response. Secondary endpoints include local response in the RT treated tumor, progression free survival, and overall survival. **Objective 2: Determine the effects of RT and anti-CTLA-4 mAb on development of anti-tumor immunity.** We hypothesize that RT will convert the irradiated tumor into an *in situ* vaccine (3) and elicit an endogenous tumor-specific cellular and humoral immune response, which in the presence of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blockade will promote immune-mediated destruction of the irradiated and abscopal metastases. Pre- and post-treatment tumor biopsies will be examined for changes in immune contexture (4), and blood for evidence of emerging anti-tumor immune responses. Associations with clinical response will be explored.

Study Design:	An optimal two-stage Simon design is used to conduct this phase II study. Ten patients will be treated in Stage 1; if there are no abscopal responses, the trial will be terminated. If there are one or more abscopal responses in Stage 1, the trial will proceed to enroll an additional 19 patients. If the trial is terminated at the end of Stage 1 due to a lack of response, then a second phase II trial with an identical design will be conducted using the 9.5 Gy x3 regimen of radiotherapy and the same dose and schedule of ipilimumab.
Accrual Goal: (Total number of patients)	We expect to accrue up to a total of 29 patients (29+10=39 patients if the trial proceeds to the 9.5 Gy x3 regimen) over 2.5 years. We do not plan to enroll vulnerable populations.
Accrual Rate: (Number of patients expected per year)	15-20 patients
FPFV: (first patient first visit) **LPLV:** (last patient last visit) **Follow Up:**	March 1st, 2014 August 31st, 2016 3 years or until patient's death
Correlative Studies: (e.g., PK/PD)	The following exploratory analyses will be carried out using descriptive statistics and graphical approaches: 1. Summary of changes in antibody and T-cells from baseline to response evaluation. 2. Association between abscopal responses and antibody and T-cell changes from baseline. All patients will be followed for progression and survival. Kaplan-Meier curves for progression-free survival and survival will be provided to summarize the results.

Inclusion Criteria:	1) Ability to understand and the willingness to sign a written informed consent document; 2) Histologic diagnosis of metastatic NSCLC; 3) Any Kras or EGFR status is permitted; 4) Patients must have at least two distinct measurable metastatic sites, with one of at least 1 cm or larger in its largest diameter. Patients may have additional non-measurable metastatic lesions (e.g., bone metastases); 5) Patients must have prior treatment with at least one line of therapy for metastatic NSCLC. Any prior therapy is permitted except prior therapy with ipilimumab; 6) An interval of 2 weeks from last previous therapy is required; 7) Patients must have adequate organ and marrow function as defined by initial laboratory tests: • WBC ≥ 2000/uL • ANC ≥ 1000/uL • Platelets ≥ 50 x 10^3/uL • Hemoglobin ≥ 8 g/dL • Creatinine ≤ 3.0 x ULN • AST/ALT ≤ 2.5 x ULN, or ≤ 5 x ULN if liver metastases are present. • Bilirubin ≤ 3.0 x ULN, (except patients with Gilbert's Syndrome, who must have a total bilirubin ≤ 3.0 mg/dL; 8) Performance status ECOG 0-1; 9) Men and women, ages > 18 years of age; 10) Life expectancy > 3 months; 11) Patients may have brain metastases if these are stable for at least 4 weeks or greater than 2 weeks post gamma knife therapy and patient is not steroid dependent; 12) Brain Scan (CT/MRI) prior to enrollment 13) Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 8 weeks after the last dose of Ipi.

Exclusion Criteria:	1) Patients having no lesions outside the field of radiation thus nullifying the ability to measure an abscopal effect; 2) Autoimmune disease: Patients with a history of inflammatory bowel disease are excluded from this study as are patients with a history of symptomatic disease (e.g., rheumatoid arthritis, progressive systemic sclerosis [scleroderma]), systemic lupus erythematosus, autoimmune vasculitis [e.g., Wegener's granulomatosis]; 3) Any underlying medical or psychiatric condition, which in the opinion of the Investigator, will make the administration of study drug hazardous or obscure the interpretation of adverse events (AEs), such as a condition associated with frequent diarrhea; 4) Concomitant therapy with any of the following: IL-2, interferon or other non-study immunotherapy regimens; cytotoxic chemotherapy; immunosuppressive agents; other investigation therapies; or chronic use of systemic corticosteroids; 5) Prior therapy with ipilimumab or another anti-CTLA-4 antagonist; 6) Women who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 8 weeks after the last dose of Ipi, or have a positive pregnancy test at baseline, or are pregnant or breastfeeding; 7) Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness.

Criteria for Evaluation: (Efficacy, safety, stopping rules, etc.)	**1) CLINICAL ENDPOINTS** Tumor Response will be evaluated using the irRC best response (Partial Response (PR) + Complete Response (CR)). Modified WHO criteria will be used for measurement of tumors. The irradiated lesion will be excluded from the assessment of response. **2) IMMUNOLOGICAL ENDPOINTS** We propose to analyze immunological changes that reflect both local and systemic responses, and investigate both general immune activation as well as tumor antigen-specific T- and B-cell responses. Serial blood samples will be collected for serum and peripheral blood mononuclear cells (PBMC): at baseline (pre-treatment), 3 weeks after initiation of Ipi (end of first cycle), end of third cycle of Ipi (~9 weeks), and at evaluation of response (3 months). An additional sample will be collected at 6 months follow up. Small aliquots of PBMC (~10^6 cells) will be used *ex vivo* for preparation of DNA and RNA, and the remainder preserved frozen until evaluation by flow cytometry and/or by functional assays. Optional tissue biopsies will be obtained at baseline in all patients as well as a post-treatment biopsy, performed at 3 weeks in patients who receive RT to an accessible metastasis.
Statistics	The abscopal response rate with confidence limits will be estimated at the end of the second stage with a total of 29 patients from both stages combined. If the true response rate is 20%, a 96.7% confidence interval equal to the observed proportion +/-14.5% will be produced with 29 patients. The distribution of types of outcomes with respect to target (non-irradiated & measurable) and non-target (irradiated) lesions will be summarized.

1 INTRODUCTION

1.1 Research Hypotheses

a) Through a combination of local RT and ipilimumab an anti-tumor immune response is elicited at the irradiated site, as an *in vivo, individualized* immunization that is systemically effective, as reflected by objective responses outside the RT field (abscopal effect).

b) The immune response can be prospectively monitored among the treated patients.

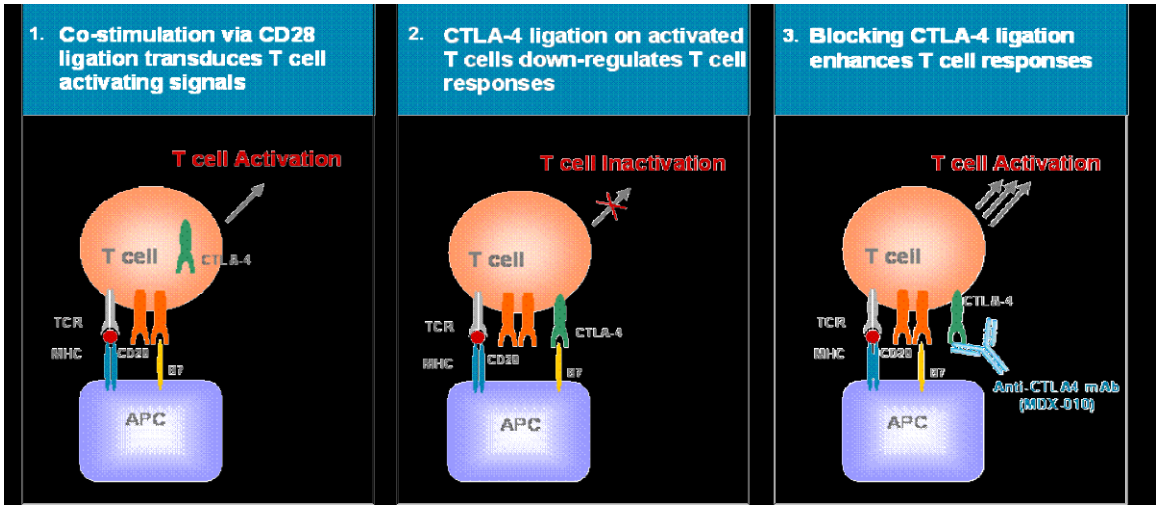
1.2 Therapeutic Rationale

Manipulation of the immune system to recover a patient's endogenous anti-tumor immune response is a strategy that has the advantages of being both natural and potentially long-lasting.(5, 6) We propose to combine immunotherapy with radiotherapy directed at a metastatic site with the goal of creating a hub for *in vivo* immunization against the tumor in order to enable immune mediated tumor rejection at other metastatic sites. This approach to *in vivo* immunization is explored as a viable alternative to an individualized vaccine. Pre-clinical data generated by us and others support the initiation of a proof of principle clinical trial that may open the field to an alternative use of radiotherapy in a novel partnership with cancer immunotherapy.(7-9)

In addition, we will perform immune monitoring of the patients accrued to the trial to generate important information for hypothesis driven laboratory research investigating the mechanisms behind the clinical findings.

1.2.1. CTLA-4 and T-Cell Activation

Figure 1.2.1 Mechanism of Action



Advances in the understanding of the mechanisms that regulate T-cell activation have allowed the rational design of new strategies for immunotherapy of tumors. Engagement of the T-cell antigen receptor by itself is not sufficient for T-cell activation; a second co-stimulatory signal is required for induction of IL-2 production, proliferation and differentiation to effector function of naive T-cells. Abundant data now indicate that the primary source of this co-stimulation is mediated by engagement of CD28 on the T-cell surface by ligands of the B7 family on the antigen presenting cell (APC).(10) Refer to Figure 1.2.1. Expression of B7 has been shown to be limited to "professional" antigen presenting cells; that is, specialized cells of the hematopoietic lineage, including dendritic cells,

activated macrophages, and activated B cells. It has been suggested that this sharply-defined restriction of B7 expression is a fail-safe mechanism for maintenance of peripheral T-cell tolerance, insuring that T-cell activation can only be stimulated by appropriate APCs.(11) The fact that tumor cells do not express B7 contributes to their poor capacity to elicit immune responses.(12, 13) The demonstration that induction of expression of B7 on many tumor cells by transfection, transduction, or other mechanisms, can heighten tumor immunogenicity led to great interest in pursuing this as an approach to tumor immunotherapy. As demonstrated *in vivo* in murine tumor models, the utility of B7 expression as a vaccination approach is limited by the following factors: (1) B7-expressing tumor cell vaccines are only effective when the tumor cells have a high degree of inherent immunogenicity; (2) while B7-expressing vaccines have been shown in many cases to be effective in inducing protective immune responses, they have demonstrated only limited utility in inducing responses to established tumors; and (3) inactivation of tumor cells by radiation has been shown to destroy the immuno-enhancing activity of the B7 gene product.(14, 15)

In the past few years it has become apparent that co-stimulation is even more complex than originally thought. After activation, T-cells express CTLA-4, a close homologue to CD28. CTLA-4 binds members of the B7 family with a much higher affinity than CD28.(16) Although there was initially some controversy as to the role of CTLA-4 in regulating T-cell activation, it has become clear that CTLA-4 down-regulates T-cell responses.(17) This was initially suggested by the following *in vitro* observations: (1) blockade of CTLA-4/B7 interactions with antibody enhanced T-cell responses; (2) cross-linking of CTLA-4 with CD3 and CD28 inhibited T-cell responses; and (3) administration of antibodies to CTLA-4 *in vivo* enhanced the immune response to peptide antigens or superantigens in mice.(18-21) Blocking CTLA-4-B7 interaction while preserving signaling via CD28 resulted in enhanced T-cell responses *in vitro*.(19)

1.2.2 CTLA-4 receptor blockade

The identification of multiple tumor-associated antigens (TAA) in recent years has provided many good potential targets for vaccination. The ability of cytotoxic T-cells (CTL) to eradicate cancers has been unequivocally demonstrated in experimental models.(22) Clinical trials have also provided the proof of principle that it is possible to treat cancer successfully by immune manipulation.(23, 24) However, significant clinical responses of patients with established vascularized tumors are difficult to achieve with most immunotherapy (IT) strategies. Major recognized obstacles are the tolerance that is established by the time a tumor becomes clinically apparent, and the immunosuppression associated with tumor progression. The inhibitory CTLA-4 receptor is a key player in tolerance maintenance.(25) Although CTLA-4 is required for normal lymphoid homeostasis, in conditions of suboptimal APC function such as in tumor-bearing hosts, the transient removal of CTLA-4-mediated inhibition (CTLA-4 blockade) has been shown to induce effective anti-tumor immunity.(26, 27) However, the efficacy of CTLA-4 blockade as a single treatment is limited to intrinsically immunogenic tumors. For poorly immunogenic tumors, like most human cancers, CTLA-4 blockade needs to be combined with a granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing vaccine that, by itself, is otherwise effective in a prophylactic but not therapeutic setting.(28, 29) Results from recent clinical trials show that CTLA-4 blockade is one of the most powerful strategies to induce active anti-tumor immunity, and emphasize the importance of integration of this modality with some form of vaccination.(30)

1.2.3 RT+ CTLA-4 blockade: pre-clinical studies

Ionizing radiation has been shown to alter the tumor milieu by enhancing trafficking of immune cells, induction of cytokines and co-stimulatory molecules and promotion of cross-priming (reviewed in ref (7)). We have applied the term “abscopal” (ab-scopus, away from the target, originally introduced by Mole et al.(31) in a different setting) to define a systemic effect elicited by radiotherapy in the presence of immunotherapy.(32)

We have shown in the poorly immunogenic 4T1 mouse mammary carcinoma model that local radiation therapy to established primary tumors elicits effective CD8+ T cell mediated anti-tumor responses when combined with CTLA-4 blockade.(33) The elicited immune response was effective against spontaneous lung metastases as well as the primary tumor. Regressing primary tumors had increased infiltration by activated CD8+ T-cells, and an expanded pool of tumor-specific memory CD8+ T-cells could be demonstrated in cured mice. These results demonstrate that RT to the primary tumor in combination with CTLA-4 blockade induces a therapeutically effective anti-tumor response, and it may provide antigenic stimulation similar to vaccination with irradiated autologous tumor cells.

1.2.4 Abscopal effect of radiotherapy

Originally described by R.H. Mole in 1953, the abscopal effect of radiotherapy is a remote effect of ionizing radiation on malignancy outside the radiation field.(31) The phenomenon was named the abscopal effect, from the Latin *ab* (position away from) and *scopus* (mark or target). Other investigators, over the years, have reported findings consistent with the abscopal effect definition, possibly as an occasional result of recovered anti-tumor immunity after radiotherapy.(34-36) The mechanism remains unexplained, although a variety of mechanisms can be hypothesized,(37) and recent research has re-focused on the immunological effects mediated by radiation.(32, 37, 38)

1.2.5 Harnessing the pro-immunogenic effects of radiation in cancer treatment: a new paradigm

Experimental work done in two syngeneic mouse models of Lewis lung tumors and mammary carcinoma, testing radiotherapy with FLT-3 ligand (a growth factor for dendritic cells), demonstrated the induction of an immune response that reduced tumor growth outside the field of radiation (32, 39). The findings inspired a trial testing the combination of subcutaneous GM-CSF (125 $\mu g/m^2$) with radiotherapy to a metastatic site in patients with metastatic solid tumors. GM-CSF increases the percentage of dendritic cells and their maturation, facilitating cross-presentation of newly released antigens after cell death at the site of radiotherapy. With a standard radiation fractionation of 3.5 Gy x10 fractions, an abscopal response was detected in 27% of the patients accrued to the trial(40).

Abscopal responses were also detected among 15 patients with low-grade B-cell lymphoma treated by low-dose radiotherapy to a single tumor site that was injected with a synthetic oligodeoxynucleotide (also referred to as CpG) that targets TLR9 (pf-3512676). These compounds can activate both lymphoma B-cells as well as nearby antigen-presenting cells, particularly plasmacytoid DC, as previously demonstrated in a murine lymphoma model (41).

Another combination strategy to overcome immune tolerance consists of the blockade of CTLA-4, a negative regulator of T-cell activation. Prolonged survival and some cures occurred in a syngeneic model of poorly immunogenic, metastatic breast carcinoma, a process requiring CD8+ T-cells (33). Postow et al.(42) recently reported a clinical case report with the same combination. A melanoma patient with disease progression while receiving ipilimumab, a monoclonal antibody that targets CTLA-4, was treated with hypo-fractionated radiotherapy to a pleural-based paraspinal metastasis;

several other pre–existing metastases in the spleen and in the right lung hilum, (outside the radiation field) completely regressed, and remained controlled for an additional eight months. Importantly, immuno-monitoring of several markers, including antibody response to NY-ESO-1 mirrored the clinical course. Seromic analysis detected 10 antigenic targets with enhanced antibody responses after radiotherapy. A similar effect was previously reported in a study of radiation with a recombinant cancer vaccine to prostate cancer (43). These results, although still anecdotal, support the concept that local radiotherapy and immunotherapy can synergize to produce a therapeutically effective anti-tumor immune response.

Combining radiotherapy with immunotherapy presents considerable advantages. Because of its localized nature, radiotherapy is devoid of most systemic effects commonly encountered with chemotherapy, and it limits interference with systemic immunotherapy. Moreover, a radiotherapy-focused intervention on the tumor may selectively subvert its micro-environment and in combination with the optimal immune intervention, may ideally render the cancer an *in situ*, individualized, vaccine.

The phase III randomized trial of ipilimumab (3 mg/kg) alone versus ipilimumab (3 mg/kg) with gp100 vaccine versus gp100 vaccine alone in previously treated metastatic melanoma patients demonstrated an improvement in overall survival in both ipi alone and ipi with vaccine arms of 4 months when compared to vaccine alone. The best overall response rate was 11%. 46% of ipi treated patients were alive at 1 year and 24% were alive at 2 years demonstrating prolonged disease control.(44) Because of the ability of local radiotherapy to convert the irradiated tumor in an individualized vaccine, we predict that its role as an adjuvant to ipilimumab may be more successful. To test this hypothesis we are currently conducting a trial of metastatic melanoma where patients are randomly assigned to first line ipilimumab or ipilimumab plus radiation to a selected metastatic lesion: endpoint of the trial are objective response rate and survival (NCT01689974).

Finally, the promising data on the effect of ipilimumab in melanoma and our pre-clinical data in murine models of mammary and colorectal carcinomas suggest that this effect of RT with ipilimumab is translatable to all solid tumor types. Consistently we attempted the combination in a patient with metastatic NSCLC, and confirmed the abscopal effect.

1.2.6 Abscopal effect of RT + ipilimumab in metastatic NSCLC: a case report

We recently reported the first abscopal case in a NSCLC patient who had undergone radiotherapy to a metastatic lesion followed by 4 cycles of ipilimumab (45).

A 64 year-old Caucasian male with a 70 cigarette-pack-year history presented in March 2010 with a palpable left supraclavicular node. An excisional biopsy of the mass demonstrated metastatic adenocarcinoma with an immunohistochemical profile consistent with a lung primary (CK7 and TTF-1 positive and CK20 and CDX2 negative). The patient's initial PET/CT demonstrated 2 right upper lobe nodules, a left lower lobe nodule, and right supraclavicular and bilateral hilar/mediastinal adenopathy. He was staged T1bN3M1a (stage IV), according to the American Joint Commission Cancer 7th edition cancer staging manual. The patient was initiated on pemetrexed 500 mg/m^2 and carboplatin (area under the curve, 5) given every 3 weeks for 6 cycles. After the 6th cycle, PET/CT demonstrated a decrease in size and metabolic activity of both the right supraclavicular adenopathy (from 2.8 x 1.7 cm and SUV of 10.2 to 2.0 x 1.2 cm and SUV of 3.8) and the left lower lobe nodule (from 6 mm and SUV of 2.3 to 3 mm and undetectable SUV). The 2 right upper lobe nodules and hilar/mediastinal nodal disease remained stable in size and metabolic activity.

The patient, thereafter, continued maintenance therapy with pemetrexed 500 mg/m^2 BSA alone, given every 3 weeks. However, after 3 cycles, he developed severe lower extremity cellulitis, at which point

the pemetrexed was temporarily discontinued. After antibiotic treatment and resolution of the cellulitis, the patient received an additional 3 cycles of pemetrexed and subsequently underwent a repeat PET/CT. The PET/CT revealed stable disease in the right upper lobe and left lower lobe nodules and improvement in the size and metabolic activity in the right supraclavicular (now 1.0 x 0.8 cm, SUV 3.3) and hilar/mediastinal adenopathy (now subcentimeter, SUV 3.4).

From February 2011 to April 2011, systemic chemotherapy was interrupted to start RT to the metabolically active right lung nodules and the right supraclavicular, hilar, and mediastinal adenopathy to a total dose of 59.4 Gy distributed over 33 fractions. The patient's subsequent chest CT in May and July 2011, in comparison to his RT pretreatment imaging, demonstrated a decrease in size of the irradiated pulmonary nodules and adenopathy. However, in September 2011, a surveillance PET/CT revealed increased metabolic activity and size of the right upper lobe nodule (now 1.9 x 1.0 cm, SUV 4.7, and a separate ill defined but growing mass, SUV 3.3 up from 1.7) and the previously seen left lower lobe nodule (now 0.9 x 0.7 cm, SUV 4.3). The patient resumed treatment with pemetrexed 500 mg/m^2 BSA alone, given every 3 weeks for an additional 10 cycles.

A June PET/CT revealed progression with new FDG avid hepatic lesions, new periaortic adenopathy, and a new bony lesion in the sacrum. Additionally, the right upper lobe and left lower lobe nodules and hilar/mediastinal adenopathy demonstrated an increase in metabolic activity. The patient was then treated with gemcitabine 750 mg/m^2 and vinorelbine 30 mg/m^2 given every 2 weeks. After the 4th cycle, in August 2012, a PET/CT demonstrated further disease progression, including multiple new hypermetabolic foci in the liver without CT correlate (SUV 14), growth of multiple new lytic lesions in the bony pelvis (SUV 53 on left, SUV 12.8 on right, SUV 6.6 in midline, SUV 23 in lateral left iliac wing lesion, and 5.3 in medial lesion), thoracolumbar spine (SUV 21.6 in T6, 56 in L2, and 8.5 in L4 lesions), and right humerus. In addition to the new skeletal lesions, the disease in the chest also progressed, including the left lower lobe nodule (now 1.9 x 1.2 cm, SUV 23.1), mediastinal lymph node (now 1.4 x 1.2 cm, SUV 7), right hilar lymphadenopathy (e.g., superior LN 1.1 x 1.1 cm, SUV 13.2, and inferior LN 1.8 x 1.2, SUV 23).

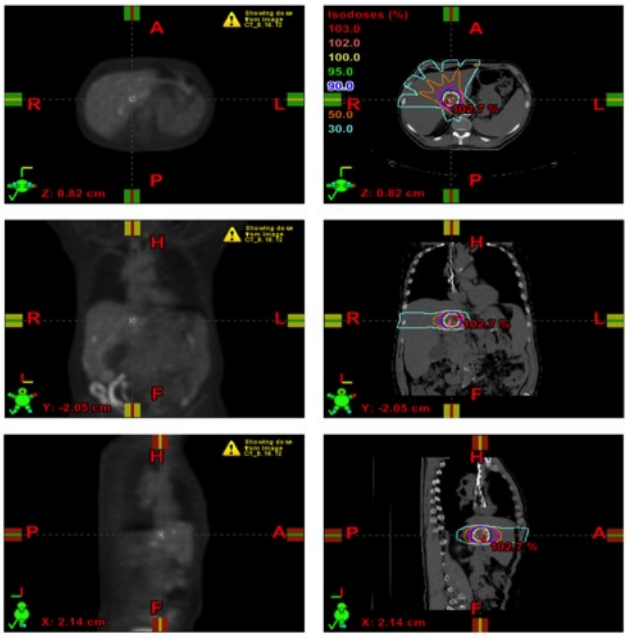


Figure 2. PET/CT images and match CT-Simulation cuts with isodose distribution for treatment of a single metastatic liver lesion.

The patient was thereafter initiated on ipilimumab (received as a compassionate exemption) and local RT to one of the hepatic metastases with the intent to generate an abscopal response. The experimental nature of this approach was extensively discussed with the patient, who was informed of the only two available reports in melanoma and the lack of available evidence for NSCLC patients. The patient was simulated in the supine position and his CT/simulation was registered to the August 2012 PET/CT for treatment planning purposes (Figure 2). The most metabolically active liver mass, located in the caudate lobe, was selected as the RT target and contoured as the gross tumor volume (GTV). An additional 0.5 cm margin was added to create a clinical target volume (CTV) and another 0.5 cm margin was added to the CTV to create a planning target volume (PTV) (Figure 1). RT to a total dose of 30 Gy distributed over 5 fractions was delivered

over a period of 10 days with 6-MV photons and a coplanar 5-field intensity-modulated technique (Figure 2). The day after the first RT fraction, the patient was infused with ipilimumab (3 mg/kg). Thereafter, the patient completed 3 more cycles of ipilimumab (3 mg/kg) infused at 3 week intervals. The patient tolerated RT and ipilimumab without any treatment related adverse events. Maintenance infusions of ipilimumab were not given afterwards.

After treatment with RT in combination with ipilimumab, a post-treatment chest CT (November 2012) and PET/CT (January 2013) demonstrated a dramatic treatment response of the patient's known disease with a near complete response. Not only was an objective response detected in the RT field, but striking responses were also observed at distant sites. The multiple hypermetabolic liver metastases completely resolved, both within and outside of the site of RT. Additionally, there was complete resolution of the myriad non-irradiated osseous metastases. Furthermore, there was a decrease in the size and metabolic activity of the left lower lobe nodule (now 0.9 x 0.7 cm with minimal FDG avidity, down from 1.9 x 1.1 cm, SUV 17.2) and complete resolution of the previously irradiated right upper lobe nodule. The one area of increased metabolic activity was in the right hilar lymph nodes, which went from SUV 4 up to 5.4.

An increase in absolute lymphocyte count (ALC) and absolute eosinophil count (AEC) were noticed after ipilimumab treatment, two biomarkers that were associated with improved survival in ipilimumab-treated melanoma patients (Figure 3A). We observed that the ALC increased after RT and ipilimumab treatment (1100/μl of whole blood at baseline, 2700/μl of whole blood at peak levels, and 2200/μl of whole blood prior to the third infusion of Ipi) and that the AEC increased between the first two infusions of Ipi (200/μl of whole blood prior to the first infusion and 470/μl of whole blood prior to the second infusion of Ipi). Additionally, the post-treatment CEA levels returned to normal after reaching a peak of 119.6 ng/ml one week following RT to the liver lesion.

A recent PET/CT on September 15, 2013 documented a persistent radiographic response with minimal systemic disease.

1.2.7 Experience of anti-CTLA-4 agents in NSCLC

There is evidence that ipilimumab combined with chemotherapy is effective and safe in patients with recurrent or stage IIIB/IV NSCLC. In a phase II study by Lynch, et al, 204 patients were randomized to treatment with carboplatin (area under the curve = 6) and paclitaxel (175 mg/m^2) infused every three weeks with or without ipilimumab (10 mg/kg). Patients receiving Ipi were divided into two groups, a concurrent group who received Ipi starting with the first dose of chemotherapy, and a phased group who started Ipi with the third cycle of chemotherapy. Patients without disease progression or adverse effects from ipilimumab continued with maintenance therapy once every 12 weeks. The study met its primary endpoint of improved immune-related PFS (irPFS, which takes into account tumor regression in the presence of new lesions) with the phased regimen (median 5.7 months, hazard ratio (HR) 0.72, p = 0.05), but not with the concurrent regimen (median 5.5 months, HR 0.81, p = 0.13), as compared to chemotherapy alone (median 4.6 months). The phased regimen was also associated with a significant improvement over chemotherapy alone in PFS (median 5.1 months vs. 4.2 months) and immune-related best overall response rate (irBORR, 32% vs. 18%), an improvement not seen in the concurrent Ipi group (median PFS 4.1 months, irBORR 21%). Of note, on subset analysis there appeared to be a greater improvement in PFS for the phased Ipi regimen for patients with squamous cell carcinoma (HR 0.4 [95% CI, 0.18 to 0.87]) as compared with patients with non-squamous cell histology (HR 0.82 [95% CI, 0.52 to 1.28]). All three regimens had tolerable safety profiles, with grade 3 or 4 treatment related AE rates of 39% for the phased regimen, 41% for the concurrent group, and 37% for the chemotherapy alone group, with more immune-related AEs

reported in the groups that received Ipi (e.g., rash, pruritus, and diarrhea) (46).

Anti-CTLA-4 treatment has also been tested as monotherapy in patients with locally advanced or metastatic NSCLC after ≥4 cycles first-line platinum based chemotherapy, albeit with less success. Zatloukal, et al, presented in abstract form the results of a smaller randomized phase II study (n = 87) comparing a humanized immunoglobulin G (IgG)2 monoclonal antibody (mAb) antagonist of CTLA-4 (tremelimumab) with best supportive care in patients without radiographic evidence of disease progression by Response Evaluation Criteria in Solid Tumors (RECIST). Tremelimumab (15 mg/kg, based on phase I and II trials in melanoma) was administered intravenously (i.v.) every 90 days until disease progression. Tremelimumab was generally well tolerated, with 20.5% of patients experiencing grade 3 or 4 AEs, and while the only 2 partial responses were in the tremelimumab arm, the treatment did not significantly improve PFS (20.9% vs. 14.3%).(47)

1.2.8 Immunological checkpoint blockade in NSCLC with anti-PD1

Based on the promising results of immunotherapy using checkpoint inhibitors such as Ipi in the malignant melanoma trials, and the early results seen with NSCLC, interest in other immune checkpoints and their inhibitors have grown rapidly. One such immune checkpoint is regulated by the programmed death receptor 1 (PD1). Engagement of PD1 on activated T-cells by its ligands PD-L1 and PD-L2 results in T-cell inactivation and functions as part of the network of immune checkpoints to prevent uncontrolled inflammation and autoimmunity. Some tumors can subvert anti-tumor immunity and induce immune tolerance by expressing PD-L1. Monoclonal antibodies that block the PD-L1 interaction with PD1 can effectively release this “break” on the anti-tumor immune response and restore T-cell activation and effector function.

BMS-936558 is a humanized IgG4 mAb with high affinity for PD1, blocking its binding to both PD-L1 and PD-L2. This antibody was recently evaluated as monotherapy in a phase IB clinical trial with an expansion cohort in NSCLC.(48) Patients (n = 122) with advanced NSCLC refractory to at least one prior chemotherapy regimen were treated with this mAb infused i.v. every 2 weeks for up to 2 years. Eligibility criteria included ECOG performance status ≤1, and patients could have no history of autoimmune conditions or any evidence of brain metastases. Overall, therapy was well tolerated with only 9% (n = 11) experiencing a grade 3 or higher toxicity, including fatigue (n = 2), pneumonitis (n = 2) and elevated AST (n = 2). Overall response rate by RECIST 1.0 criteria was 16%. Responses tended to be long lasting, although follow-up interval was insufficient to determine duration of response or OS, however, PFS at 24 weeks was 33%. There was an initial suggestion that patients with squamous cell carcinoma may be slightly more responsive to the treatment, but more recent data presented in abstract form shows no difference in response based on tumor histology (49).

More modest results were obtained in the first in human trial of a mAb antagonist of PD-L1, BMS 936559. In this phase I trial, anti-PD-1L mAb was administered i.v. (dose escalation from 0.3 to 10 mg/kg) every two weeks to 207 patients with advanced cancers, including 75 with NSCLC.(50) Of the 75 patients with NSCLC, 49 patients were evaluable for response. Objective response was seen in five of the 49 patients. Tumor response was seen in squamous (1 of 13 patients, 8%) and non-squamous (4 of 36 patients, 11%) histologies. An additional six (12%) patients had stable disease for at least six months. The most common treatment related adverse events were fatigue, infusion reactions, and diarrhea.

These trials demonstrate that checkpoint blockade is a safe and potentially effective treatment option for patients with NSCLC, and that immunological manipulation can be successful even in patients with advanced NSCLC.

1.2.9 Choice of radiotherapy dose

We have shown in two syngeneic murine tumor models from mice of different genetic background, mammary carcinoma TSA (BALB/c) and colorectal carcinoma MCA38 (C57BL/6), that fractionated RT regimens (6 Gy x5 and 8 Gy x3) were able to convert these unresponsive tumors into tumor responsive to anti-CTLA-4 mAb (51). Conversely a single RT dose of 20 Gy failed to show efficacy. In collaboration with Drs. MJ Aryankalayil and CN Coleman (NCI Experimental Therapeutics Section, Radiation Oncology Branch), we have analyzed the gene expression profiles in TSA tumors *in vivo* immediately after RT. Data show the rapid induction of immune response genes by the fractionated but not single dose RT regimen with a dominant type I IFN response emerging, which was confirmed by qRT-PCR. Data suggest that **fractionated RT can convert an unresponsive tumor into one responsive to anti-CTLA-4 at least in part by inducing an active immune microenvironment**, an hypothesis that will be tested in the proposed studies**.**

While the pre-clinical data support the choice of fractionated radiotherapy for the proposed combination with anti-CTLA-4, the optimal radiation dose per fraction remains undetermined. Table 1 reports the currently available clinical data. Based on the linear-quadratic model (52) and with the assumption of **α/β** = 10 Gy for tumor, none of the regimens used in the 2 published clinical reports of abscopal effects during CTLA-4 blockade in melanoma and in our case of NSCLC, used biological effective doses (BEDs) for tumor control comparable to those attributed to an ablative regimen of 20 Gy x3 (equivalent to BED_{10}=180).(53) Despite a much lower BED than that predicted to result in tumor ablation, in each of the examples quoted in Table 1 a complete remission was observed at the irradiated site, supporting a direct contribution of the immune system. Interestingly, the BEDs for the regimens used in the cases of abscopal responses were within a close range. In the NSCLC case report summarized above the regimen used (6 Gy x5, BED_{10}=48) successfully and persistently eliminated the liver metastasis in the field, suggesting a contribution of the immunotherapy with anti-CTLA-4. Importantly, no complications associated with the combination in this dose range of RT and ipilimumab were reported. This limited but relevant information has informed the design of the proposed trial. Noticeably, the regimen used by Postow et al, adopted a dose per fraction of 9.5 Gy. Dose per fraction in excess of a threshold of 8 Gy have shown to cause endothelial cell death and best leverage the radiation effects on the tumor vasculature (54). Whether these effects are also required for an optimal combination of RT and immunotherapy remains to be determined.

Table1: Biological Effective Doses of Regimens

Biological effective doses (BED) of regimens used in case reports of abscopal responses from RT and ipilimumab, compare to RTOG-defined ablative dose

References	RT regimen used	BED_{10}
Postow et al(1)	9.5 Gy x3	55.57
Stamell et al(2)	8 Gy x3	43.2
Golden et al (45)	6 Gy x5	48
RTOG ablative dose	20 Gy x3	180

1.2.10 Summary of the rationale for the combination

In metastatic NSCLC, the use of radiotherapy is reserved to palliation. We have shown in different

mouse tumor models that **RT is a potent partner for anti-CTLA-4 and can convert tumors resistant to single agent anti-CTLA-4 into susceptible ones.**(51, 55, 56) Importantly, anti-tumor CD8 T-cells activated by the combination of local RT and anti-CTLA-4 not only contributed to the response within the irradiated field but also inhibited metastases outside of the RT field, and long-term protective memory responses were generated in mice achieving complete tumor rejection (51, 55-58). An analogous effect was recently reported in a case of melanoma (59) and the first reported case of NSCLC described above. Therefore, anti-tumor immune responses generated by the combination of RT and anti-CTLA-4 in pre-clinical models and clinical settings resistant to anti-CTLA-4 monotherapy (60, 61) **suggest a role for RT as a powerful immune adjuvant that can recruit into response otherwise non responsive patients to treatment with anti-CTLA-4**.

Since CTLA-4 blockade alone has demonstrated limited activity in NSCLC, abscopal responses in this trial are very likely to reflect the specific efficacy of the combination, as inferred by our pre-clinical data(62). Based on this background and rationale we are proposing a phase II study of combined ionizing radiation and ipilimumab in metastatic NSCLC.

The optimal regimen for this combination has yet to be defined: we will start with the regimen successful at inducing an abscopal effect in the NSCLC patient reported above. Within a Simon design, patient accrual will continue to the second stage only if at last one abscopal response is observed among the first 10 patients. Conversely, in the absence of an abscopal response within the first 10 patients treated with 6 Gy x5, a new group of 10 patients will be treated with the same dose and schedule of ipilimumab but at a higher dose of RT, 9.5 Gy x3, the dose-fractionation regimen from the Postow case report of abscopal response.(1) If testing at the higher dose-fractionation level proves effective at inducing abscopal effects, it will suggest a key role for endothelial cell death and will stimulate more pre-clinical work in such a direction.

1.3 Summary of Results of Ipilimumab Investigational Program

1.3.1 Pharmacology

Ipilimumab is a humanized IgG1κ anti-CTLA-4 monoclonal antibody. Studies performed *in vitro* with ipilimumab have demonstrated that it binds specifically to CTLA-4, actively inhibits CTLA-4 interactions with B7.1 and B7.2, does not show any cross-reactivity with human cell lines deficient of B7.1 and B7.2, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues, as demonstrated by immunohistochemistry. Ipilimumab does cross-react with CTLA-4 in non-human primates including cynomolgus monkeys.

Ipilimumab was originally produced and purified from a B-cell hybridoma clone. Subsequently, a transfectoma (CHO cell) was generated that is capable of producing more ipilimumab on a per cell basis than the hybridoma. Material from the transfectoma will be utilized in this and future ipilimumab clinical studies. Biochemical, immunologic, and *in vivo* pre-clinical primate assessments demonstrated similarity between hybridoma and transfectoma derived ipilimumab.

1.3.2 Pre-Clinical Toxicology

Complete information on the pre-clinical toxicology studies can be found in the ipilimumab Investigator Brochure (IB). Non-clinical toxicity assessments included *in vitro* evaluation for the potential of ipilimumab to mediate complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC), and toxicology assessments in cynomolgus monkeys alone and in the presence of vaccines.

The *in vitro* studies demonstrated that ipilimumab did not mediate CDC or ADCC of PHA- or anti-CD3-activated human T-cells. These data are consistent with the requirement of high levels of antigen expression on the surface of target cells for efficient ADCC or CDC. Since ipilimumab is a human IgG1, an isotype capable of mediating CDC and ADCC, the lack of these activities is likely due to a very low expression of CTLA-4 on activated T-cells. Therefore, these data suggest that ipilimumab treatment would not result in depletion of activated T-cells *in vivo*. Indeed, no depletion of T-cells or T-cell subsets were noted in toxicology studies in cynomolgus monkeys.

No mortality or signs of toxicity were observed in three independent 14 day intravenous toxicology studies in cynomolgus monkeys at multiple doses up to 30 mg/kg/dose. Furthermore, ipilimumab was evaluated in sub-chronic and chronic toxicology studies in cynomolgus monkeys with and without Hepatitis B vaccine and melanoma vaccine. Ipilimumab was well tolerated alone or in combination in all studies. There were no significant changes in clinical signs, body weight, clinical pathology values or T-cell activation markers. In addition, there were no significant histopathology changes in the stomach or colon.

1.3.3 Clinical Safety

Ipilimumab immunotherapy was FDA approved for the treatment of metastatic melanoma in March 2011. Randomized phase III trials demonstrated an overall survival benefit, and patients who responded had an extended duration of response with almost 50% of responders alive at one year and 25% alive at two years, as reported in the original publication of this trial.(44) Recently, updated long term survival data was presented in abstract form at the European Cancer Conference 2013 with pooled patient data from 12 studies with a total of 1861 patients treated with Ipi, including the 790 patients enrolled in the two major phase III trials, MDX010-20 and CA184-024. They reported an OS of 22% at 3 years with most of these patients surviving at 10 years (63).

Ipilimumab has been administered to over 14,000 patients with different cancers with a dose range between 0.1 mg/kg and 20 mg/kg. Most experience with ipilimumab exists at the 3 mg/kg and 10 mg/kg dose levels. Patients who received ipilimumab at 3 mg/kg were treated in clinical trials conducted early in the development program and received either a single or multiple injections. Based on a Phase III randomized trial,(44) ipilimumab was FDA approved for the treatment of metastatic melanoma in 2010 at the recommended dose of 3 mg/kg. This will be the dosage used in this trial.

1.3.3.1 Drug Related Serious Adverse Events (SAEs):

Drug-related adverse events were reported in studies with ipilimumab as monotherapy as well as in combination in which ipilimumab was administered with vaccines, cytokines or chemotherapy.

The AE profile of ipilimumab is relatively well characterized with drug-related AEs mostly being immune-related adverse events (irAEs), which are considered to be associated with the mechanism of action of ipilimumab. Most common irAEs are colitis and diarrhea, rash, pruritus, deficiencies of endocrine organs (pituitary, adrenal or thyroid), hepatitis, and uveitis. Rare complications are bowel perforations (~1%) resulting from underlying severe colitis, which have required surgical intervention.

Drug-related Grade 3 or 4 SAEs include: rash/desquamation, pruritus, uveitis, speech impairment, abdominal pain, diarrhea/colitis, nausea/vomiting, transaminase elevation, adrenal insufficiency, panhypopituitarism and atrial fibrillation. Some of these events, such as rash/desquamation, pruritus, uveitis, diarrhea/colitis, transaminase elevation, adrenal insufficiency and panhypopituitarism, may

represent drug induced irAEs (see Section 4.3.4). Please refer to the most recent version of Investigator's Brochure for the latest update on SAEs.

1.3.4 Immune-Related Adverse Events (irAEs)

Many of the adverse events considered related to ipilimumab may be immune in nature and presumably a consequence of the intrinsic biological activity of ipilimumab. An immune-related adverse event is defined as any adverse event associated with drug exposure and consistent with an immune-mediated event. Disease progression, infections and other etiologic causes are ruled out or deemed unlikely as contributing to the event. Supportive data, such as autoimmune serology tests or biopsies, are helpful but not necessary to deem an event an irAE. Events of unclear etiology which were plausibly "immune mediated" have been conservatively categorized as irAEs even if serologic or histopathology data are absent. These irAEs likely reflect a loss of tolerance to some self antigens or an unchecked immune response to gut or skin flora. Some breakthrough of immunity may be inseparably linked to the clinical antitumor activity of ipilimumab.

Approximately 60% of subjects developed any grade irAEs which predominately involved the GI tract, endocrine glands, or skin. Based on data from the safety database, the number of subjects with serious irAEs was approximately 17%, including 10% for serious GI irAEs (diarrhea and/or colitis), 3% of serious endocrinopathy (primarily hypophysitis/hypopituitarism) and 2% of serious skin irAEs. Bowel perforation was reported in less than 1% of subjects. With few exceptions these irAEs were clinically manageable and reversible with supportive care or corticosteroids. In responding patients, addition of corticosteroids does not appear to have a temporal relationship to change in objective tumor response.

Additionally, the Surgery Branch at the National Cancer Institute reported an increased rate of bowel wall perforation in some patients administered high-dose IL-2 following treatment with ipilimumab (64). Of 22 patients administered high-dose IL-2, 3 patients experienced bowel wall perforations. This is a higher rate than would be expected with high-dose IL-2 treatment alone. All 3 patients had metastatic melanoma and had previously received their last dose of ipilimumab > 77 days before the first dose of IL-2. Two of the patients had clinically significant ipilimumab-related diarrhea or colitis and the symptoms had completely resolved prior to IL-2 administration. One patient did not experience ipilimumab-related diarrhea. It is unknown whether this observation represents a true association or is mechanistically unrelated to prior ipilimumab exposure.

1.3.4.1 Drug Related Deaths

Drug related deaths are relatively rare with ipilimumab treatment (~1%). The most common cause of drug related death was GI perforation. Other causes included multiorgan failure, sepsis, hypotension, acidosis and adult respiratory distress syndrome. For details on all drug-related deaths please refer to the most recent version of the IB.

1.3.5 Clinical Efficacy

Treatment with ipilimumab has demonstrated clinically important and durable tumor responses in several malignancies including melanoma, prostate cancer, and renal cell carcinoma. There is a phase II study showing promising results from a combination of Ipi and chemotherapy in patients with recurrent or stage IIIB/IV NSCLC, which is reviewed in detail above in section 1.2.7. However, the most extensively studied tumor type has been malignant melanoma, providing objective data demonstrating durable responses in randomized clinical trials.

Specifically, there are two phase III randomized clinical trials demonstrating the efficacy of ipilimumab. The first trial, by Hodi et al, compared treatment with ipilimumab (3 mg/kg) alone versus ipilimumab (3 mg/kg) with gp100 vaccine versus gp100 vaccine alone in patients previously treated for metastatic melanoma patients. This trial demonstrated an improvement in overall survival in both groups treated with Ipi alone and Ipi with vaccine. Of the Ipi treated patients, 46% were alive at 1 year and 24% were alive at 2 years, demonstrating prolonged disease control.(44)

A second phase III randomized trial of ipilimumab in patients with metastatic melanoma compared treatment with Ipi alone versus Ipi with dacarbazine in previously untreated patients. This study demonstrated similar response and survival rates as compared to the Hodi study of patients with prior treatment, with 1 and 2 year overall survival of 47% and 29%, respectively. The higher dose of Ipi used in this trial (10 mg/kg) apparently provided no additional benefit.(65) These responses at 2 years are generally durable based on the reported 10 year follow-up data (63).

Based on the data above, this trial will use the FDA approved dosing recommendation of 3 mg/kg i.v. over 90 minutes every 3 weeks for 4 doses. Further details on clinical results can be found in the current version of the Investigator Brochure.

1.3.5.1 Relationship between Response and Immune Breakthrough Events in Subjects with Metastatic Melanoma

Approximately 51% of subjects treated with ipilimumab have developed drug-related adverse events of any grade considered to be immune-mediated in nature. These irAEs, most often Grade 1 or 2, are an expected consequence of inhibiting CTLA-4 function. Interestingly, there may be an association between objective response to ipilimumab and the occurrence of higher grade irAEs. For example, in a preliminary analysis of subjects treated at 3 mg/kg of Ipi in the MDX-010-05 study, there was a statistically significant association between subjects who responded to ipilimumab and subjects who developed severe/serious irAEs (P = 0.0219). See Table 2 for information.

While no data is available for NSCLC patients, careful clinical monitoring will be implemented for early detection and treatment of potential immune mediated events.

Table 2: Response and Immune Mediated Events - Subjects Evaluable for Efficacy in MDX-010-05 (N = 56)

	Subjects with Severe/Serious[a] Immune-mediated Events	Subjects without Severe/Serious Immune-mediated Events
Non-responders	12	37
Responders[b]	5	2

P = 0.0219 by Fisher's Exact Test

[a]Severe is defined as ≥ Grade 3

[b]Defined as subjects with a best objective response of CR or PR.

1.4 Overall Risk/Benefit Assessment

This study will test the combination of two safe, well-characterized cancer treatments, the immunomodulator ipilimumab, and RT in patients with metastatic NSCLC. In terms of the risks of treatment under this protocol, ipilimumab, although it is safe and FDA approved for the treatment of metastatic melanoma, it is associated with AEs which can cause morbidity and (rarely) even death.

Specifically, at the dose used in this protocol (3 mg/kg, the FDA approved dose) the risk of irAEs is approximately 60% based on the clinical trials in metastatic melanoma. Of these AEs, only 17% of patients had Grade 3-5 irAEs, with about 1% risk of fatal AEs, although the risk is probably lower now that an effective approach to AE management has been developed for Ipi. The most common of these SAEs were diarrhea and/or colitis (10%), endocrinopathy (usually hypophysitis/hypopituitarism, 3%), and skin reactions (2%), with bowel perforation reported in <1% of subjects, and these are almost always manageable and reversible with supportive care or corticosteroids. Radiation therapy also has its associated risks, which depend on the dose, fractionation, volume treated, and site of treatment. The volume and site of treatment will be patient dependent, but generally the volume is extremely localized, and the risk of any Grade 3-5 AE from RT is minimal (<<1%). It is true that the combination of Ipi and RT has not been utilized enough in NSCLC to determine its safety, but there have been no reported SAEs related to this combination, either in NSCLC or in melanoma, a disease for which probably over 1000 patients have been treated with RT while undergoing treatment with Ipi. In addition to the clinical risks of the treatment, there are also inherent risks associated with phlebotomy, the potential for loss of confidentiality, biopsy, and PET/CT.

On the contrary, there are reports correlating treatment with Ipi and RT with a significant response in systemic disease both in melanoma and NSCLC, an effect we hope to replicate with this combination in our study. Additionally, Ipi does have activity in metastatic NSCLC, as reported by Lynch, et al. These investigators found a significant improvement in median irPFS from 4.6 to 5.7 months in this phase 2 study. These findings, along with the rationale for this combination of RT and Ipi, the pre-clinical data supporting our hypothesis, and the case report of a near CR in a patient with metastatic NSCLC receiving RT-Ipi, all suggest there is a significant potential benefit to this combined treatment.

In addition to the potential risks and benefits of treatment outlined above, it is also important to consider the natural history of metastatic NSCLC. To state it plainly, the prognosis of this disease is grim. Upon diagnosis with stage IV disease, median survival is less than one year even with the best treatments available. Although second, third, and fourth line chemotherapy may extend survival for a minority of patients, usually on the order of months, this cannot be considered a standard of care, and new approaches to therapy are needed.

In summary, it is clear that ipilimumab is generally tolerable with little severe morbidity, as demonstrated by mature studies of its use in thousands of patients with melanoma, and more recent phase II data in patients with NSCLC. The profile of AEs has been well characterized and an effective approach to their management has been developed. Despite this, grade 3 and 4 AEs can occur, and there is an approximately 1% risk of treatment associated mortality from ipilimumab. However, any risk/benefit assessment must weigh the potential benefits of treatment not only against the potential risks of the experimental treatment, but also against the known risk of the standard of care and the natural history of the disease. In the case of patients with metastatic NSCLC that has progressed through first line chemotherapy, the prognosis is poor with a median survival on the order of months, even with the best cytotoxic chemotherapy and all of its associated morbidity. The potential benefits of this experimental treatment are therefore at least comparable to, and possibly better than, alternative options including the estimated risks of the experimental treatment as well as the known risks and limited benefit associated with the standard of care.

1.5. STUDY RATIONALE

1.5.1 Combining RT and CTLA-4 blockade

The availability of ipilimumab, the phase I and II experience already accrued with the antibody in NSCLC, and the phase III data demonstrating its impressive utility in malignant melanoma make it compelling to translate to the clinic the pre-clinical findings of an abscopal effect in poorly immunogenic syngeneic murine tumors treated by CTLA-4 blockade and RT. Additionally, the experience described above in a case of metastatic NSCLC and the emerging evidence of response to checkpoint blockade in NSCLC patients have motivated the design of the current study.

2 STUDY OBJECTIVES

Objective 1

Evaluate the safety and therapeutic efficacy of anti-CTLA-4 mAb and concurrent local RT in NSCLC patients with metastatic disease.

An open label phase II trial will evaluate the preliminary efficacy of the combination of Ipi and RT, applied to a single metastatic site. Efficacy is measured with respect to systemic tumor responses (abscopal response, outside the field of therapy) defined by irRC in all non-irradiated measurable lesions, as a demonstration of an effective anti-tumor immune response. Secondary endpoints include local response in the RT treated tumor, progression free survival, and overall survival.

Objective 2

Determine the effects of RT and anti-CTLA-4 mAb on development of anti-tumor immunity.

We hypothesize that RT will convert the irradiated tumor into an *in situ* vaccine (3) and elicit an endogenous tumor-specific cellular and humoral immune response, which in the presence of CTLA-4 blockade will achieve immune-mediated destruction of the irradiated and abscopal metastases. Pre- and post-treatment tumor biopsies will be examined for changes in immune contexture (4), and blood for evidence of emerging anti-tumor immune responses. Associations with clinical response will be explored.

3 STUDY DESIGN

3.1 Objective 1

To estimate, in metastatic NSCLC patients, the response rate (as defined by irRC) in all measurable abscopal metastatic sites after ipilimumab and local RT directed to a single metastatic site.

A phase II study of combined RT and Ipi (RT-Ipi) in metastatic NSCLC is proposed. Bristol-Myers Squibb (BMS) will provide drug support (ipilimumab) to patients on study (see letter of commitment from BMS). The clinical protocol has received approval by the NYU Cancer Institute Lung Cancer Disease Management Program and by the Protocol Monitoring and Review Committee, and is

currently undergoing review by the NYU Institutional Review Board. The protocol is attached and is summarized below.

Eligibility criteria: Patients >18 years of age with histologically proven metastatic NSCLC, at least two measurable sites of disease, either relapsed or progressive after one or more lines of systemic therapy, adequate organ and bone marrow function, ECOG PS 0-1 or Karnofsky > 50%, and informed consent for participation. Systemic or local anti-cancer therapies must be completed at least 2 weeks before the start of treatment. Baseline PET/CT is required within 4 weeks from accrual.

Treatment: At study entry all measurable metastatic lesions identified clinically or at PET/CT are recorded. Choice of the metastasis to be biopsied before and after RT-Ipi is based on ease of access (lymph nodes and subcutaneous metastases are preferred) and the estimated lowest risk for complications associated with tissue acquisition. The treatment starts a week after biopsy (during this week patients undergo CT simulation for radiation therapy). A regimen of 6 Gy x5 daily fractions will be delivered to the biopsied lesion. Patients will receive Ipi 3 mg/kg i.v. over 90 minutes initiated within 24 hours of the first dose of RT to the biopsied measurable lesion, conformally or by IMRT, with image guidance to maximally spare normal tissue. Ipi infusion is repeated on days 22, 43, and 64. Patients will be re-imaged by PET/CT between days 81-88 and evaluated for response. If the initial phase II trial is terminated after the first stage of the two stage Simon design due to lack of response, additional patients will be enrolled in a second identical two stage Simon design trial (Part B) which will test RT at 9.5 Gy x3 fractions with the same dose and schedule for Ipi.

Assessment of response: Response is defined as an objective radiographic response of the measurable metastatic site(s) outside the radiation field evaluated by PET/CT at 3 months after the start of treatment. Lesions will be measured using the modified WHO criteria, and immune-related Response Criteria will be used, as described (66), and best response (PR + CR) based on these criteria will be recorded. To reduce confounding, the irradiated lesion will be excluded from baseline measurement and measurement for the assessment of in-field response will be recorded separately.

Sample size considerations. The optimal Simon two-stage design to test the null hypothesis that, at the 6 Gy x5 fractions RT regimen, the probability of abscopal response to RT-Ipi is <0.05 versus the alternative that the probability of abscopal response is of >0.20 has an expected sample size of 17.62 and a probability of early termination of 0.599. If RT-Ipi is actually not effective in inducing an abscopal effect, there is a 0.047 probability of concluding that it is effective (the target for this value was 0.05). If RT-Ipi is actually effective in inducing an abscopal effect, there is a 0.199 probability of concluding that it is not effective (the target for this value was 0.20, power =0.80). After treating 10 patients in the first stage, the trial will be terminated if no patients have an abscopal response (and a new cohort of 10 patients will be studied with RT doses of 9.5 Gy x3). If there is a response and the trial goes on to the second stage, a total of 29 patients will be studied. If the total number of patients responding with an abscopal effect is ≤ 3 then the RT-Ipi dose will be rejected (PASS 2008, NCSS, J. Hintze, Kaysville, UT).

Accrual Rate. An accrual rate of 10-15 patients/year is planned. By the end of the first year 10 patients will be accrued, and the study will either progress to the second stage or be terminated. If it continues to the end of the second stage, we will accrue a total of 29 patients. If the trial concludes at the end of the first stage with no observed abscopal CR/PR after the 12 week evaluation, the trial will be restarted with the same design but with a higher dose of radiation, 9.5 Gy x3.

3.2 OBJECTIVE 2:

Determine the effects of RT and anti-CTLA-4 mAb on development of anti-tumor immunity.

An important goal of our study is to gather evidence in patients in support of the hypothesis that RT plays a unique role as an immune adjuvant that, when combined with anti-CTLA-4 mAb, enhances the response to anti-CTLA-4.

A comprehensive approach is proposed to analyze immunological changes that reflect both local and systemic responses, and investigate both general immune activation as well as tumor antigen-specific T- and B-cell responses. To this end, serial blood samples will be collected for serum and peripheral blood mononuclear cells (PBMC): at baseline (pre-treatment), 3 weeks after initiation of Ipi (end of first cycle), end of third cycle of Ipi (~9 weeks), and at evaluation of response (3 months). An additional sample will be collected at 6 months follow up. Small aliquots of PBMC (~10^6 cells) will be used *ex vivo* for preparation of DNA and RNA, and the remainder preserved frozen until evaluation by flow cytometry and/or by functional assays. Tissue biopsies will be obtained at baseline in all patients. An initial biopsy is obtained under Standards of Care practices to establish a diagnosis; a second optional pre-treatment biopsy will be performed in patients who consent to at an accessible lesion, purely for research purposes. An optional post-treatment biopsy will be performed at 3 weeks in patients who receive RT to an accessible metastasis. We expect approximately 50% of the patients to comply with the second tissue acquisition. We believe that even a limited number of biopsy sets (before and after RT) can provide invaluable information about the mechanism of action of the combination. The specimens acquired will enable the following studies:

1) Evaluation of systemic T-cell populations and Myeloid-derived Suppressor Cells (MDSC). The activity of anti-CTLA-4 mAb is though to be mainly dependent on modulating the quantity and quality of T-cells in cancer patients, and the correlation of some parameters such as ALC and ICOS+ T-cells with response supports this notion (67). Therefore, CD4 and CD8 T-cell subsets will be characterized by multi-color flow cytometry for the expression of ICOS, CD27 (marker of activation and memory T-cells), CD134 (marker of acutely activated T-cells and involved in maintenance of response), CD137 (expressed mainly on CD8 T-cells and involved in T-cell proliferative responses post-activation), CD154/CD40L (acutely activated CD4 T-cells), CD152/CTLA-4, CD272/BTLA, CD279/PD-1 and TIM-3, which are expressed post-activation and associated with suppression/T-cell exhaustion (68). Additionally, T-cells will be stained for the proliferation/cycling marker Ki67 (67, 69), and for the transcription factor EOMES, since changes in these markers have been reported to be associated with outcome and irAEs (69). CD8 T and NK cells will also be stained for NKG2D expression, a receptor implicated in functionality of these effector cells and response to anti-CTLA-4 (58, 70). Regulatory T-cells will be characterized for expression of cell surface markers (CD4, CD25, CD127, CD45RA) and transcription factors (FoxP3, Helios) (71). Finally, MDSC ($Lin^-CD14^+HLA\text{-}DR^{neg/low}$) will be evaluated since their levels were shown to correlate with response, and to decrease after RT (59, 72).

2) Evaluation of tumor antigen-specific cellular and humoral responses. Tumor tissue (archived at study entry) will be tested for the expression of seven antigens frequently expressed in NSCLC (Table 3), and for which reagents are available (recombinant proteins, tetramers and/or overlapping peptide pools (OLP) to allow us to test for the presence of specific antibodies and/or T-cells across one or more HLA-haplotypes. Immunohistochemistry (IHC) procedures follow standard techniques as previously

published by our group in other cancers (73).

Table 3: List of NSCLC antigens

C-T Antigen	Expression in NSCLC (%)
Mage-A1	46
Mage-A3	45
Mage-B2	25
Mage-C1	18
Bage	17
Gage	41
NY-ESO-1	27
KK-LC-1	34

Antigen-specific T-cell responses will be studied *ex vivo* by MHC tetramer staining to a sub-panel of 5 antigens including NY-ESO-1, using high grade purified tetramer reagents from the Ludwig Institute for Cancer Research by Serametrix (Carlsbad, CA) (74). The epitopes used are confidential but the service covers most HLA haplotypes and detection of $CD4^+$ as well as $CD8^+$ T-cells. In addition, T-cell responses to NY-ESO-1, MAGE-A3 and survivin will be monitored by intracellular cytokine staining utilizing OLP pools, as previously described (75, 76). This method is suited for analysis of cryopreserved PBMCs, allows the detection of CD4 and CD8 T-cell responses (optimized by use of 15-mer peptides with an overlap by 11 amino acid residues) and an assessment if multiple cytokines are being secreted by single cells. These assays are performed routinely by the NYUCI Immunomonitoring Core Lab according to standardized SOPs. OLP ProMixes, purchased from Proimmune, will include two controls, CEF and MOG. Samples are acquired on a BD LSR II flow cytometer. Seven color compensation (parallel controls using cells singly stained for each color) and data analysis are done with FlowJo flow cytometry analysis software (TreeStar). Boolean analysis is used determine the percentage of single, double and triple cytokine-producing CD4 and CD8 T-cells. The induction or boosting of tumor antigen-specific T-cell immunity is defined as a post-treatment value of at least 3-fold higher than baseline that is also 3-fold or greater than parallel negative controls (and at least 0.03). If necessary, *in vitro* stimulated T-cell responses will be assessed, as described (75).

Antigen-specific antibody responses will be evaluated using two methods. First, antibody titers for antigens expressed by each patient tumor as evaluated by IHC will be measured by ELISA. At least 5 of the most common NSCLC antigens, including MAGE-A3, NY-ESO-1, and survivin, will be tested for all sample to capture cases with low expression in the tumor that may be undetectable by IHC, and because an integrated antibody and CD8 T-cell response can be evaluated for these antigens, as described (74). Changes will be considered significant if seroconversion occurs or pre-existing antibody titers will increase (or decrease) by 5-fold between two time points. In patients with antibodies to NY-ESO-1 we will consider evaluating antibodies against different domain of the protein (N-terminal, central and C-terminal), since changes in reactivity against different epitopes were described after RT in a melanoma patient (59).

In addition, a comprehensive evaluation of the repertoire of antibody responses post-RT and Ipi, will be performed by seromics, an antibody profiling method utilizing protein microarrays with the capacity to detect >9000 antigens induced by a vaccine. Assays will be performed as described previously (77, 78). Plasma/serum collected pre-treatment and at time of disease evaluation (post) will be plated onto ProtoArrays (v4.0; Invitrogen) and incubated with fluorescent antibodies. Arrays will be scanned at 10uM resolution using microarray scanner (Axon 4200AL with GenePix Pro Software; Molecular Devices) to detect fluorescence. After normalization, performed as described (78), the ratio of the pre/post values for each antigen will be calculated and considered to be a response if it exceeds 5-fold difference (59).

3) Evaluation of the tumor immune environment. Evaluation of tumor tissue obtained pre-treatment and at 3 weeks post-treatment will be performed in two ways: **(a)** IHC on tumor sections to assess density, location and functional orientation of tumor infiltrating lymphocytes (TILs) (4). Sections will be stained for CD3, CD8, and CD45RO, TIA-1 and FoxP3. These markers provide limited information about functional orientation of the T-cells but have been shown to strongly correlate with prognosis and response to treatment in other malignancies (4, 79-81). In fact, a change in density and location of TILs and ratio of CD8/FoxP3+ cells was seen in our patient post-treatment

(45). TILs will be evaluated by counting T-cells positive for each marker in 10 high power fields using a semi-automated system as we have previously described (82). Distribution of TILs will be categorized as restricted to perivascular location or diffuse. Median and mean number of TILs positive for each marker will be determined and cases with values above or below the mean by at least 2 standard deviations (SD) classified as high or low, respectively. Association of total TILs numbers and distribution, expression of each marker, and ratio of CD8/FoxP3+ with response will be explored.

(b) Gene expression profiling will be performed at the NYU Genome Technology Center using U133A 2.0 Affymetrix microarrays as described (83). To identify genes differentially expressed between pre- and post-treatment samples a minimum 1.5-fold difference will be used, and changes in expression of key genes will be confirmed by qRT-PCR. Pathway analysis will be performed using the IPA tool. Gene expression in pre-treatment tumor samples of responders and non-responders will be compared to explore its association with response.

4) Evaluation of T-cell repertoire changes in blood and tumor following treatment. A remarkable breadth of diversity in T-cell receptors (TCRs) is generated by combinatoric shuffling of gene segments in somatic cells. The TCR is composed of two peptide chains, encoded by the TCRA and TCRB genes respectively. The antigenic specificity of αβ T lymphocytes is in large part determined by the amino acid sequence in the hypervariable complementarity-determining region 3 (CDR3) regions of TCR (84). The existence of multiple V_α and J_α gene segments in the TCRA locus and multiple V_β, D_β, and J_β gene segments in the TCRB locus permits a large combinatorial diversity in TCR composition, and template-independent deletion or insertion of nucleotides at the V_α-J_α, V_β-D_β, and D_β-J_β junctions further adds to the potential diversity (85). A healthy adult has approximately 10 million different TCRB chains contained within their 10^{12} circulating T-cells (86). Adaptive Biotechnologies has developed a novel method that amplifies rearranged TCR CDR3 sequences (87) and exploits the capacity of high-throughput sequencing technology to sequence tens of thousands of TCR CDR3 chains simultaneously. Because the technology utilizes genomic DNA, the frequency of sequenced CDR3 chains is representative of the relative frequency of each CDR3 sequence in the starting population of T-cells. Here we will use this technology to determine: (a) whether TIL repertoire is distinct from the repertoire in peripheral blood at baseline; (b) whether TIL and peripheral blood repertoire is significantly altered after treatment with RT and Ipi. To this end, we will sequence 40,000 genomes at each time point, for each sample. Genomic DNA (2 □g) will be prepared from snap frozen tumor tissue and PBMC before treatment and at 3 weeks. If <2□g are obtained, all available extracted DNA will be used. Amplification and sequencing of TCRB CDR3 regions will be carried out using the ImmunoSEQ platform at Apative Biotechnologies. For each tumor sample, percentage of T-cell infiltration in the tumor will be estimated using a droplet digital PCR technique (88). TCRB overlap between tumor and blood, and between each site before and after treatment, will be calculated for each patient as described (88), using Adaptive Biotechnologies bioinformatics software.

(5) Measuring tumor and serum MICA and NKG2D expression on Natural Killer (NK) and T-cells during treatment. We have shown that mAb-mediated blockade of NKG2D on CD8 T-cells abrogated the formation of immune synapses between CD8 TILs and tumor cells and tumor rejection in mice treated with RT and anti-CTLA-4 mAb (58). In humans, a similar block of NKG2D is mediated by soluble MICA (sMICA), which is released by some tumors and reaches high levels in the serum (89). To determine if this occurs in the patients treated with RT + Ipi, serially collected serum will be tested for levels of sMICA, and expression levels of NKG2D in CD8 T-cells and NK cells will be assessed by flow cytometry. Importantly, there are reports that sMICA levels can drop after initiation of Ipi due to increased clearance from the generation of anti-sMICA antibodies (90). Decreased levels

of sMICA were associated with increased expression of NKG2D in T and NK cells, and with response to treatment. Anti-sMICA antibodies and sMICA levels will be measured in serum with ELISA by using recombinant MICA protein and anti-human MICA monoclonal antibodies, as described (90). Because RT is known to upregulate MICA on the surface of tumor cells (91), pre and post-treatment tumor sections will be tested for expression of MICA.

4 SUBJECT SELECTION CRITERIA

For entry into the study, the following criteria MUST be met:

4.1 Inclusion Criteria

1) Ability to understand and the willingness to sign a written informed consent document;
2) Histologic diagnosis of metastatic NSCLC;
3) Any Kras or EGFR status is permitted;
4) Patients must have at least two distinct measurable metastatic sites, with one of at least 1 cm or larger in its largest diameter. Patients may have additional non-measurable metastatic lesions (e.g., bone metastases);
5) Patients must have prior treatment with at least one line of therapy for metastatic NSCLC. Any prior therapy is permitted except prior therapy with ipilimumab;
6) An interval of 2 weeks from last previous therapy is required;
7) Patients must have adequate organ and marrow function as defined by initial laboratory tests:
   - WBC $\geq$ 2000/uL
   - ANC $\geq$ 1000/uL
   - Platelets $\geq 50 \times 10^3$/uL
   - Hemoglobin $\geq$ 8 g/dL
   - Creatinine $\leq$ 3.0 x ULN
   - AST/ALT $\leq$ 2.5 x ULN, or $\leq$ 5 x ULN if liver metastases are present.
   - Bilirubin $\leq$ 3.0 x ULN, (except patients with Gilbert's Syndrome, who must have a total bilirubin $\leq$ 3.0 mg/dL;
8) Performance status ECOG 0-1;
9) Men and women, ages > 18 years of age;
10) Life expectancy > 3 months;
11) Patients may have brain metastases if these are stable for at least 4 weeks, or greater than 2 weeks post- gamma knife therapy and patients are not steroid dependent;
12) Brain Scan (CT/MRI) prior to enrollment
13) Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 8 weeks after the study.

WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal [defined as amenorrhea $\geq$ 12 consecutive months; or women on hormone replacement therapy (HRT) with documented serum follicle stimulating hormone (FSH) level

> 35 mIU/mL]. Even women who are using oral, implanted or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin (hCG)) within 72 hours prior to the start of study medication.

4.2 Exclusion Criteria

1. Patients having no lesions outside the field of radiation thus nullifying the ability to measure an abscopal effect;
2. Autoimmune disease: Patients with a history of inflammatory bowel disease are excluded from this study as are patients with a history of symptomatic disease (e.g., rheumatoid arthritis, progressive systemic sclerosis [scleroderma]), systemic lupus erythematosus, autoimmune vasculitis [e.g., Wegener's granulomatosis];
3. Any underlying medical or psychiatric condition, which in the opinion of the Investigator, will make the administration of study drug hazardous or obscure the interpretation of adverse events (AEs), such as a condition associated with frequent diarrhea;
4. Concomitant therapy with any of the following: IL-2, interferon or other non-study immunotherapy regimens; cytotoxic chemotherapy; immunosuppressive agents; other investigation therapies; or chronic use of systemic corticosteroids;
5. Prior therapy with ipilimumab or another anti-CTLA-4 antagonist;
6. Women who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 8 weeks after cessation of study drug, or have a positive pregnancy test at baseline, or are pregnant or breastfeeding;
7. Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness.

4.3 Study Therapy

4.3.1 Ipilimumab

Each patient will receive ipilimumab intravenously at 3 mg/kg every three weeks. Infusions will be given over 90 minutes (<u>not</u> bolus or i.v. push).

Ipilimumab will be administered on Days 1, 22, 43, and 64.

Radiation will be delivered daily, Monday-Friday, for five consecutive days, either at the NYU Clinical Cancer Center or Tisch Hospital. A dose of 6 Gy x5 days will be tested. All patients will be re-imaged by PET/CT between Days 81 and 88.

<u>Ipilimumab (Yervoy)</u>

Ipilimumab is a humanized IgG1κ anti-CTLA-4 mAb. *In vitro* studies were performed with ipilimumab to demonstrate that it is specific for CTLA-4, actively inhibits CTLA-4 interactions with B7.1 and B7.2,

does not show any cross-reactivity with human B7.1, B7.2 negative cell lines, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues, as demonstrated by immunohistochemistry. Ipilimumab does cross-react with CTLA-4 in non-human primates including cynomolgus monkeys.

Dose Calculations

Total dose should be calculated as follows:

Subject body weight in kg x 3 mg/kg = total dose, mg

Total infusion volume should be calculated as follows:

Total dose in mg ÷ 5 mg/mL = infusion volume, mL

Rate of infusion should be calculated as follows:

Infusion volume in mL ÷ 90 minutes = rate of infusion, mL/min

For example, a patient weighing 114 kg (250 lb) would be administered 342 mg of ipilimumab (114 kg x 3 mg/kg = 342 mg). If the patient weighs more than 125.0 kg, the treating physician needs to be contacted to discuss the total infusion volume, infusion rate, and infusion duration.

4.3.1.1 Preparation and Administration of Ipilimumab

The study drug will be administered in the NYU Clinical Cancer Center. Ipilimumab will be stored, handled, and prepared by the NYU Investigational Pharmacy. The supplies needed for ipilimumab preparation and administration include the following items:

- Calibrated syringes
- Infusion containers

Note: Filtration of the infusate is not required.

3) As ipilimumab is stored at refrigerated temperatures (2-8ºC), allow the appropriate number of vials of ipilimumab to stand at room temperature for approximately five minutes.
4) Aseptically withdraw the required volume of ipilimumab solution into a syringe. Insert the needle at an angle into the ipilimumab vial by placing the needle – bevel side down – against the glass, with the tip touching the neck of the vial. The initial solution concentration is 5 mg/mL. [Note: A sufficient excess of ipilimumab is incorporated into each vial to account for withdrawal losses].
5) Ensure that the ipilimumab solution is clear colorless and essentially free from particulate matter on visual inspection. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall, etc.
6) Inject ipilimumab solution withdrawn into an appropriate size evacuated infusion bag to produce a final infusion volume that has been calculated from the weight of the patient.
7) If the total dose calculates to less than 90 mL of solution then the total dose needed should be diluted to a total volume of 90 mL in 0.9% sodium chloride. An in-line filter is not required.
8) Mix by GENTLY inverting several times. DO NOT shake.
9) Visually inspect the final solution. If the initial diluted solution or final dilution for infusion is not clear or contents appear to contain precipitate, the solution should be discarded.
10) Do not draw into each vial more than once. Any partial vials should be safely discarded and should not be stored for reuse.

Ipilimumab should be administered under the supervision of a physician experienced in the use of intravenous agents. Ipilimumab is administered as an i.v. infusion only.

4.3.1.2 Packaging and Labeling

Ipilimumab injection, 50 mg/10 mL (5 mg/mL) or 200 mg/40 mL (5 mg/mL), is formulated as a clear to slightly opalescent, colorless to pale yellow, sterile, non-pyrogenic, single-use, isotonic aqueous solution that may contain particles. Ipilimumab Injection, 50 mg/10 mL and 200 mg/40 mL, is supplied in 10-cc or 50-cc Type I flint glass vials, respectively, stoppered with gray butyl stoppers and sealed with aluminum seals. The drug product is formulated at a concentration of 5 mg/mL at a pH of 7.0. The label text for the study drug will include the contents of the vial (i.e., ipilimumab 50 mg/10 mL), lot number, appropriate caution statement, and storage conditions.

4.3.1.3 Drug Shipment and Storage

BMS will arrange the shipment of the study drug to the Investigational Pharmacy at NYU. The study drug will be shipped overnight for next day delivery. Shipments will be made on Monday through Thursday only. No shipments will be made on Friday, Saturday, or Sunday. Upon receipt by the Investigator or designee, the study drug must be stored at 2 to 8°C, in a limited-access area until preparation for infusion.

4.3.1.4 Agent Accountability

All investigational drugs are stored with the Investigational Pharmacy. A separate binder is issued to each study as well as a bin for storage. The bins and binder are labeled with the protocol number and drug name. The Investigational Pharmacy is located in the NYU Clinical Cancer Center. There is limited access, and only the pharmacists and support personnel working in the Investigational Pharmacy have access. After normal hours of operation, the room is locked and only accessible by authorized personnel.

4.3.2 Dose Modifications

Ipilimumab related toxicities must be resolved to baseline or ≤ Grade 1 prior to administering the next dose. No dose reductions for ipilimumab are allowed. Study drug treatment may be delayed up to 28 days if the reason for the delay is toxicity/AE. Subjects will have the opportunity to receive all 4 doses of Induction Phase ipilimumab. Treatment modifications will be made based on safety criteria specified below:

At the discretion of the investigator, it **may** be necessary to delay ipilimumab administration for the following AE(s):

- Any Grade 2 non-skin related AE, except for laboratory abnormalities;
- Any Grade 3 laboratory abnormality;

It **is** necessary to delay ipilimumab dosing for the following AE(s):

- Any Grade 3 skin-related AE, regardless of causality.

- Any AE, laboratory abnormality or inter-current illness which, in the judgment of the investigator, warrants delaying the dose of study medication.
- Elevated liver function tests ≥ 3 fold above baseline.

4.3.3 Discontinuation of Therapy

Subjects MUST be discontinued from study therapy AND withdrawn from the study for the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the Investigator, indicates that continued treatment with study therapy is not in the best interest of the subject
- Pregnancy: All WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on study pregnancy tests for WOCBP enrolled in the study.
- Imprisonment or the compulsory detention for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

4.3.3.1 Permanent Discontinuation of Ipilimumab

Ipilimumab must be discontinued if at least 1 of the following related adverse event(s) occurs:

- Grade 3 or 4 motor neuropathy, regardless of causality.
- Any ≥ Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of starting therapy OR requires systemic treatment.
- Any other ≥ Grade 3 non-skin adverse event with the exception of laboratory abnormalities, Grade 3 nausea and vomiting, Grade 3 and 4 neutropenia, Grade 3 and 4 thrombocytopenia
- Any Grade 4 laboratory abnormalities, except AST, ALT, or total bilirubin:
    - AST or ALT > 8 x ULN
    - Total bilirubin > 5 x ULN
- Any other Grade 4 AE.
- Any AE, laboratory abnormality or inter-current illness which, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued administration of the study drug.
- Subject experiences an allergic/infusion reaction while receiving study drug at a slower infusion rate due to a prior allergic/infusion reaction.

Patients with endocrinopathy related to the study drug may resume treatment once asymptomatic with or without hormone substitution. Experience is limited for treatment continuation in patients who experienced a ≥ Grade 3 endocrine event (e.g., adrenal crisis).

Please contact the medical monitor, Franco M. Muggia, M.D., before continuing treatment with ipilimumab in these cases.

4.3.4 Immune Related Adverse Events (irAEs): Definition, Monitoring, Treatment

Blocking CTLA-4 function may permit the emergence of auto-reactive T-cells and resultant clinical autoimmunity. Rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis and hypopituitarism were drug-related, presumptive autoimmune events, now termed irAEs, noted in previous ipilimumab studies.

For the purposes of this study, an irAE is defined as an AE of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an AE an irAE. Serological, immunological, and histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Suspected irAEs must be documented on an AE or SAE form.

Patients should be informed of and carefully monitored for evidence of clinically significant systemic irAE (e.g., systemic lupus erythematosus-like diseases) or organ-specific irAE (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an irAE is noted, appropriate work-up (including biopsy if possible) should be performed, and steroid therapy may be considered if clinically necessary (see Appendix 3 through 8 for suggested work-up and treatment of irAEs).

It is unknown if systemic corticosteroid therapy has an attenuating effect on ipilimumab activity. However, clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from ipilimumab. If utilized, corticosteroid therapy should be individualized for each patient. Prior experience suggests that colitis manifested as ≥ Grade 3 diarrhea requires corticosteroid treatment. See Appendix 4 for additional details.

4.3.5 Other Guidance

4.3.5.1 Infusion Reactions Associated with Ipilimumab

Infusion reactions should be graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 Allergic reaction/hypersensitivity criteria. Severe infusion reactions require the immediate interruption of study drug therapy and permanent discontinuation from further treatment. Appropriate medical therapy including epinephrine, corticosteroids, intravenous antihistamines, bronchodilators, and oxygen should be available for use in the treatment of such reactions. Subjects should be carefully observed until the complete resolution of all signs and symptoms. In each case of an infusion reaction, the investigator should institute treatment measures according to the best available medical practice. The following treatment guidelines are suggested:

- **CTCAE Grade 1 Allergic reaction/hypersensitivity (transient flushing or rash, drug fever < 38°C).**
    - Treatment: Decrease the study drug infusion rate by 50% and monitor closely for any worsening.
- **CTCAE Grade 1 or Grade 2 Allergic reaction/hypersensitivity manifesting only as delayed drug fever (starting after the completion of the study drug infusion).**
    - Treatment: Maintain blinded study drug dose and infusion rate for future infusions.
    - Consideration could be given to administration of acetaminophen or a non-steroidal anti-inflammatory drug (NSAID) prior to the subsequent study drug infusion, if not otherwise contraindicated in subjects. Dose and schedule of these agents is entirely at the investigator's discretion.
- **CTCAE Grade 2 Allergic reaction/hypersensitivity (Rash, flushing urticaria, dyspnea, drug fever ≥ 38°C).**

- Treatment: Interrupt blinded study drug infusion. Administer bronchodilators, oxygen, etc. as medically indicated. Resume infusion at 50% of previous rate once infusion reaction has resolved or decreased to Grade 1 in severity, and monitor closely for any worsening.

- **CTCAE Grade 3 or Grade 4 Allergic Reaction/Hypersensitivity: A CTCAE Grade 3 hypersensitivity reaction (symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema; hypotension) or a Grade 4 hypersensitivity reaction (anaphylaxis).**
  - Treatment: Stop the study drug infusion immediately and disconnect infusion tubing from the subject. Administer epinephrine, bronchodilators, antihistamines, glucocorticoids, intravenous fluids, vasopressor agents, oxygen, etc., as medically indicated. Contact the Medical Monitor and document as a serious adverse event (**Section 6.1**). No further study drug treatment to be administered.

4.3.5.2 Treatment of Ipilimumab Related Isolated Drug Fever

In the event of isolated drug fever, the Investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology.

If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (Investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion should be administered. The infusion rate will remain unchanged for future doses.

If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the Investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

4.3.6 Radiation Therapy Guidelines

4.3.6.1 Planning

After informed consent is obtained, a measurable lesion that is accessible for biopsy is selected by the treating physician as the site for local radiotherapy under Standard of Care practices to establish a diagnosis. The area of interest is imaged at CT planning for conformal treatment. There is no contrast media used in the CT planning. There is an exposure to small amounts of radiation with the use of CT scan. CT scan thickness should be ≤0.5 cm through the tumor bed region. These images will be used in 3D treatment planning in accordance with the dose specification constraints.

The CTV is defined as the lesion of interest with the expected motion changes, while the PTV is the CTV plus a margin ≤ 1 cm, dependent on the anatomical location, to account for setup uncertainty. While gating is not part of the current conformal setting at NYU, efforts are made to consistently treat chest and abdominal lesions with the patient maintaining shallow breathing, to limit the movement of the volume treated during respiration.

4.3.6.2 Treatment

Radiotherapy is delivered by external beam using linear accelerators capable of delivering ≥ 4 MV x-rays. The PTV will encompass all or part of the biopsied NSCLC lesion, and will be defined by the

treating physician. The PTV will be treated daily, Monday-Friday. A dose of 30 Gy in 5 fractions of 6 Gy each is delivered, daily. Part B fractionation: the PTV will receive 28.5 Gy in 3 fractions of 9.5 Gy each, delivered on alternate days within week 1, with a minimum of 36 hours between fractions.

Radiation Dose specification: The planning target volume receives a minimum of 90% of the prescription dose.

Treatment Machine: A linear accelerator with ≥ 4 MV x-rays is required.

Immobilization Technique: Patients will be set-up for treatment and CT scanning, and planned for treatment. The specific immobilization technique will be determined at the discretion of the treating physician.

Target Positioning Verification: Digitally acquired radiographic images will be used to verify the position of the target with respect to the treatment machine's isocenter using digitally reconstructed radiographs (DRRs) or orthogonals (for IMRT) on first day. Both kV and MV images may be used to verify setup.

IGRT Target Localization: Cone-beam CT (CBCT) images will be acquired prior to treatment for each fraction. By using IGRT to image the tumor bed in "real-time", the operator may automatically align the tumor bed with the treatment machine on each day of treatment.

Treatment Planning: 3D-Conformal or IMRT treatment planning is allowed. This includes "field-in-field" beams as well as the use of dynamic multi-leaf collimator (MLC) derived using inverse planning or electronic compensator techniques. Field arrangements and technique should be chosen that satisfy the PTV dose coverage constraints and normal tissue dose constraints using Dose-Volume Histogram (DVH) analysis. By carefully selecting the gantry and table angle combinations that do not enter or exit through other organs of the body, the dose can be confined to the traditional treatment volumes. Non-coplanar beam arrangements are encouraged, but not required. Dose calculations with tissue inhomogeneity correction must be used.

In view of the GI pattern of toxicity of ipilimumab, particular attention will be applied to limit exposure to the bowel. To mitigate this risk, normal tissue constraints of abdominal and pelvic organs will be employed from recommendations from AAPM Task Group 101 (92) and the H. Lee Moffitt Cancer Center experience using this fractionation schedule (93).

After completion of the first radiation + ipilimumab dose on Day 1 (or within 24 hours of RT), the patient is followed and treated with ipilimumab on Days 22, 43, and 64. Re-imaging with PET/CT will be performed during Days 81-88.

4.3.6.3 Treatment Modifications for Radiation Adverse Events:

Dosing delay: Except for radiation-associated mucositis and skin toxicity, the patient should have resolution or return to pre-treatment baseline of all grade 3-4 toxicities prior to the start of the next immunotherapy treatment.

4.3.7.2 On Study Evaluations

As summarized in the Study Calendar, patients are evaluated pre-treatment for definition of metastatic sites with PET/CT. At least two measurable metastatic sites are identified, and one of these lesions will be selected by the investigator for RT treatment with the goal of minimizing potential toxicity from RT. All lesions are followed per modified WHO criteria.

Off Study Criteria

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable toxicity (defined in Section 4.3.3.1),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

Authorized Physicians must notify the data manager and Principle Investigator when a patient is taken off study.

4.4. Prohibited and Restricted Therapies during the Study

4.4.1 Prohibited Therapies

Patients in this study may not use vaccines for the treatment of cancer for up to one month pre and post dosing with ipilimumab. Concomitant systemic or local anti-cancer medications are prohibited in this study while receiving ipilimumab treatments.

Patients may not use any of the following therapies during the study:

- Any non-study anti-cancer agent (investigational or non-investigational);
- Any other investigational agents;
- Any other (non-CA184024 related) CTLA-4 inhibitors or agonists;
- Immunosuppressive agents;
- Chronic systemic corticosteroids;

5 STUDY PROCEDURES AND OBSERVATIONS (TABLE 4)

Flow Chart/Time and Events Schedule

Procedure	Screening Visit (within 28 days (+/- 7 days)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 22	Day 43	Day 64	EOT (Day 81-88)	F/U q 12 wks for 3 yrs (+/7days)
Informed Consent	X										
Inclusion/Exclusion Criteria	X										
Medical History	X										
Pregnancy Test	X[a]						X[a]	X[a]	X[a]		
Physical Examination	X		X				X	X	X	X	X
Vital Signs	X		X				X	X	X	X	X
Adverse Events Assessment			X				X	X	X	X	X
Serum Chemistry[b]	X		X				X	X	X	X	X
Hematology[b]	X		X				X	X	X	X	X
Urinalysis	X										
Endocrine Panels[c]	X		X				X	X	X		
Radiation Therapy[e]		X	X	X	X	X					
Ipilimumab Treatment		X					X	X	X		
Bloods/T-cell Response	X						X[g]		X[g]		X[h]
Tumor biopsy	X[i]						X[j]				
PET/CT Imaging	X									X[d]	Q 12 W
Efficacy assessment										X	

a. Only for pre-menopausal female patients
b. SOC labs (COMP, CBC w/diff,)
c. TSH, T3, T4
d. Scans day 81-88.
e. 6 Gy X5, daily
f. See Appendix 2 for details.
g. to be collected PRIOR to ipilimumab dose
h. only at 6 month follow up visit
i. Anytime prior to Day 1.
j. Day 22-29.

5.1. Procedures by Visit

5.1.1 Study Completion or Early Discontinuation Visit

At the time of study early withdrawal, the reason for early withdrawal and any new or continuing AEs should be documented.

5.1.2 Study Drug Discontinuation

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable toxicity (defined in Section 4.3.3.1),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.2 Details of Procedures

5.2.1 Study Materials

Ipilimumab is an FDA approved therapy for metastatic melanoma, and is not FDA approved for NSCLC. Study drug will be provided by BMS, the manufacturer.

The radiotherapy component of this study is a standard procedure for palliation of metastatic NSCLC lesions and will be covered by the patients' insurance.

Patients will not incur costs associated with the study drug.

5.2.2 Safety Assessments

All patients who receive at least one dose of ipilimumab will be considered evaluable for safety parameters. Additionally, any occurrence of a SAE from time of consent forward, up to and including follow-up visits will be reported. Refer to safety reporting Section 6.2

Safety will be evaluated for all treated patients using the NCI CTCAE version 4.0 (http://ctep.cancer.gov). Safety assessments will be based on medical review of AE reports and the results of vital sign measurements, physical examinations and clinical laboratory tests.

5.2.3 Cost to Subjects

Each subject or their insurance company will be charged and held responsible for the costs of care provided as part of this study. The study drug (Ipi) will be provided free of charge by BMS. Radiotherapy is a standard treatment for metastatic NSCLC and will be billed to subjects and their insurance companies.

5.3 Criteria for Evaluation

5.3.1 Safety Evaluation

This protocol will be monitored by the NYUCI Data Safety Monitoring Committee which oversees the safety of all NYUCI investigator initiated clinical trials. The trial will be monitored for all adverse events, laboratory abnormalities, and serious adverse events at least once every 12 months and at the time of the completion of the first 10 patients in stage 1 of the two-stage design. In addition, serious adverse events are monitored for all studies on a monthly basis.

5.3.2 Efficacy Evaluation

Definition of Tumor Responses using irRC

5.3.2.1 Definition of Measurable/Non-Measurable and Index/Non-Index Lesions

Definitions of lesions in this study are based on irRC. **For the purpose of this study, the irradiated lesion will be excluded from baseline measurement and measurement for the assessment of response. In field response will be recorded separately.**

5.3.2.2 Definition of Measurable/Non-Measurable Lesions

- Measurable lesions are lesions that can be accurately measured in 2 perpendicular diameters, each measuring at least 10 mm x 10 mm. The area will be defined as the product of the largest diameter with its perpendicular. Skin lesions can be considered measurable.
- Non-measurable (evaluable) lesions are all other lesions, including unidimensionally measurable disease and small lesions measuring less than at least 10 mm x 10 mm., and any of the following:

Lesions occurring in a previously irradiated area (unless they are documented as new lesions since the completion of radiation therapy), bone lesions, leptomeningeal disease, ascites, pleural or pericardial effusion, lymphangitis cutis/pulmonis, abdominal masses that are not pathologically/cytologically confirmed and followed by imaging techniques and cystic lesions.

All measurable and non-measurable lesions should be measured at Screening and at the defined tumor assessment time-points (see Section 5, Table 4). Extra assessments may be performed as clinically indicated if there is a suspicion of progression.

5.3.2.3 Definition of Index/Non-Index Lesions

All measurable lesions (defined in Section 5.3.2.2), up to a maximum of **five lesions per organ** and **ten lesions in total,** should be identified as *index* lesions to be measured and recorded on the medical record at Screening. The *index* lesions should be representative of all involved organs. In addition, *index* lesions should be selected based on their size (lesions with the longest diameters), their suitability for accurate repeat assessment by imaging techniques, and how representative they are of the patient's tumor burden. At Screening, the sum of the products of diameters (SPD) for all *index* lesions will be calculated and considered the baseline sum of the products of diameters. Response criteria to be followed are listed below. The

baseline sum will be used as the reference point to determine the objective tumor response of the *index* lesions at tumor assessment (TA).

Measurable lesions, other than *index* lesions, and all sites of non-measurable disease, will be identified as *non-index* lesions. *Non-index* lesions will be recorded on the medical record and should be evaluated at the same assessment time points as the *index* lesions. In subsequent assessments, *non-index* lesions will be recorded as "stable or decreased disease", "absent", or "progression".

5.3.3 Definition of Tumor Response using irRC

The SPD at tumor assessment using the irRC for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Index Lesions Response using irRC

- irComplete Response (irCR): Complete disappearance of all *index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- irPartial Response (irPR): Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all *index* and all new measurable lesions (i.e., Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir.
- irStable Disease (irSD): Does not meet criteria for irCR or irPR, in the absence of progressive disease.
- irProgressive Disease (irPD): At least 25% increase Percentage Change in Tumor Burden (i.e., taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

Definition of Non-Index Lesions Response using irRC

- irComplete Response (irCR): Complete disappearance of all *non-index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- irPartial Response (irPR) or irStable Disease (irSD): *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.
- irProgressive Disease (irPD): Increases in number or size of *non-index* lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the index lesions increases by the required amount).

Impact of New Lesions on irRC

New lesions in and by themselves do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRC for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

Definition of Overall Response Using irRC Will Be Based on the Following Criteria:

- Immune-related Complete Response (irCR): Complete disappearance of *all* tumor lesions (index and nonindex together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.
- Immune-related Partial Response (irPR): The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).
- Immune-related Stable Disease (irSD): irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
- Immune-related Progressive Disease (irPD): It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
  - At least 25% increase in the sum of the products of all index lesions over baseline SPD calculated for the index lesions.
  - At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesions.

Table 5: irRC Definitions

Index Lesion Definition	Non-Index Lesion Definition	New measurable Lesions	New unmeasurable Lesions	% change in tumor burden (including measurable new lesions when present)	Overall irWHO Response
Complete Response	Complete Response	No	No	-100%	irCR
Partial Response	Any	Any	Any	≥ -50%	irPR
				<-50% to <+25%	irSD

Index Lesion Definition	Non-Index Lesion Definition	New measurable Lesions	New unmeasurable Lesions	% change in tumor burden (including measurable new lesions when present)	Overall irWHO Response
				>+25%	irPD
Stable Disease	Any	Any	Any	<-50% to <+25%	irSD
				>+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

Immune Related Best Overall Response Using irRC (irBOR)

irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last TA prior to subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

5.3.4 Response Endpoints

Ipilimumab is expected to trigger immune-mediated responses, which require activation of the immune system prior to the observation of clinical responses. Such immune activation may take weeks to months to be evident. Some patients may have objective volume increase of tumor lesions or other disease parameters (based on study indication, i.e., hematologic malignancies) within 12 weeks following start of ipilimumab dosing. Such patients may not have had sufficient time to develop the required immune activation or, in some patients, tumor volume or other disease parameter increases may represent infiltration of lymphocytes into the original tumor or blood. In conventional studies, such tumor volume or relevant laboratory parameter increases during the first 12 weeks of the study would constitute PD and lead to discontinuation of imaging to detect response, thus disregarding the potential for subsequent immune-mediated clinical response.

Therefore, patients with tumor volume increase detected or lack of laboratory parameter response documentation prior to week 12 but without rapid clinical deterioration should continue to be treated with ipilimumab and clinically observed with a stringent imaging schedule to allow detection of a subsequent tumor response. This will improve the overall assessment of the clinical activity or ipilimumab and more likely capture its true potential to induce clinical responses. Tumor assessments will be made using modified WHO criteria.

Radiotherapy in the dose and fractionation proposed in this study is a standard palliation tool for metastatic lesions. It is expected that approximately 50% of the irradiated lesions will achieve an objective response (PR+CR). For the purpose of this study, the irradiated lesion will be excluded from baseline measurement and measurement for the assessment of response. In field response will be recorded separately.

5.3.5 Study Suspension Criteria

The study will be terminated after Stage 1 (first 10 subjects) if there are no abscopal responses. If the trial is terminated at the end of Stage 1 due to a lack of response, then a second phase II trial with an identical design will be conducted using the 9.5 Gy x3 regimen of radiotherapy and the same dose and schedule of ipilimumab. If there are no abscopal responses in Stage 1 of this second trial, the study will be terminated.

6 ADVERSE EVENT REPORTING

6.1 Collection of Safety Information

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

During clinical trials, adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.)

A ***serious AE*** **or reaction** is any untoward medical occurrence that at <u>any dose</u>:

- results in death,
- is life-threatening (defined as an event in which the patient or subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization, (refer to note for exceptions),
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient/subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above).

NOTE:

- Pregnancy: Incidence of pregnancy is not considered a SAE; pregnancy must, however, be reported immediately to investigator. Cancer/Overdose: An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. Hospitalizations (exceptions): Criteria for hospitalizations not reported as SAEs include admissions for:
  - Planned as per protocol medical/surgical procedure
  - Routine health assessment requiring admission for baseline/trending of health status documentation (e.g., routine colonoscopy)
  - Medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial; appropriate documentation required)
  - Admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)

An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

6.2 Reporting of Serious Adverse Events (SAEs)

Following the subject's written consent to participate in the study, all SAEs should be collected and reported, including those thought to be associated with clinical trial procedures. SAE terminology and severity grading will be based on *CTCAEv4*.

This is an NYULMC investigator-sponsored study in which the study drug will be provided by BMS. The principal investigators are responsible for reporting SAEs to the IRB and the FDA or other applicable regulatory authority. The principal investigator is responsible for submitting follow-up reports for all SAEs regarding the patient's subsequent course until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment), or the patient dies. Reports of SAEs will be submitted to the NYULMC IRB through the institution's web-based portal, Research Navigator.

The following categories and definitions of causal relationship to study drug should be used for all clinical studies:

- Certain: There is a reasonable causal relationship between the study drug and the AE. The event responds to withdrawal of study drug (dechallenge), and recurs with rechallenge when clinically feasible.
- Probable: There is a reasonable causal relationship between the study drug and the AE. The event responds to dechallenge. Rechallenge is not required.
- Possible: There is reasonable causal relationship between the study drug and the AE. Dechallenge information is lacking or unclear.
- Not likely: There is a temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the AE.

- Not related: There is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.
- Adverse events classified as "serious" require expeditious handling and reporting to NYU to comply with regulatory requirements.
  - All SAEs whether related or unrelated to the ipilimumab, must be immediately reported to NYU and BMS (by the investigator or designee) within 24 hours of becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site. All SAEs should be reported to NYU and BMS via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:

    SAE Email Address: Worldwide.Safety@BMS.com
    SAE Fax Number: 609-818-3804

- Collection of complete information concerning SAEs is extremely important. Full descriptions of each event will be followed by NYU. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.
- An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. For reporting purposes, NYU considers an overdose, regardless of adverse outcome, as an important medical event.
- AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to subjects experiencing AEs that cause interruption or discontinuation of ipilimumab, or those experiencing AEs that are present at the end of their participation in the study; such subjects should receive post-treatment follow-up as appropriate.
- All SAEs must be collected which occur within 90 days of discontinuation of dosing or completion of the patient’s participation in the study if the last scheduled visit occurs at a later time. In addition, the Investigator should notify NYU of any SAE that may occur after this time period which they believe to be certainly, probably, or possibly related to ipilimumab.

6.3 Pregnancy

Sexually active women of childbearing potential must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized.

Before enrolling women of childbearing potential in this clinical trial, Investigators must review the guideline about study participation for WOCBP which can be found in the GCP Manual for Investigators. The topics include the following:

- General Information
- Informed Consent Form
- Pregnancy Prevention Information Sheet
- Drug Interactions with Hormonal Contraceptives
- Contraceptives in Current Use
- Guidelines for the Follow-up of a Reported Pregnancy

Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.

All WOCBP MUST have a **negative** pregnancy test within 72 hours **prior** to receiving ipilimumab. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of hCG. If the pregnancy test is positive, the subject must not receive ipilimumab and must not be enrolled in the study.

In addition, all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Additionally, all pregnancies must be reported to BMS via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:

SAE Email Address: Worldwide.Safety@BMS.com
SAE Fax Number: 609-818-3804

If following initiation of study treatment, it is subsequently discovered that a trial subject is pregnant or may have been pregnant at the time of ipilimumab exposure, including during at least 6 half-lives after product administration, the ipilimumab will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). Exceptions to ipilimumab discontinuation may be considered for life-threatening conditions only after consultation with the Principal Investigator or as otherwise specified in this protocol. Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of eight weeks.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information can be reported on a Pregnancy Surveillance Form provided by BMS. Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS.

7 STATISTICAL METHODOLOGY

OBJECTIVE 1:

Assessment of response: Response is defined as an objective response of metastatic site(s) outside the radiation field evaluated at 3 months after the start of treatment, assessing clinical

and CT responses. Immune-related Response Criteria will be used, as described (66), and best response (PR + CR) based on these criteria will be recorded. To reduce confounding, the irradiated lesion will be excluded from baseline measurement and measurement for the assessment of response. In field response will be recorded separately.

Sample size considerations. The optimal Simon two-stage design to test the null hypothesis that, at the 6 Gy x5 fractions RT regimen, the probability of abscopal response to RT-Ipi is <0.05 versus the alternative that the probability of abscopal response is of >0.20 has an expected sample size of 17.62 and a probability of early termination of 0.599. If RT-Ipi is actually not effective in inducing an abscopal effect, there is a 0.047 probability of concluding that it is effective (the target for this value was 0.05). If RT-Ipi is actually effective in inducing an abscopal effect, there is a 0.199 probability of concluding that it is not effective (the target for this value was 0.20, power =0.80). After treating 10 patients in the first stage, the trial will be terminated if no patients have an abscopal response (and a new cohort of 10 patients will be studied with RT doses of 9.5 Gy x3). If the trial goes on to the second stage, a total of 29 patients will be studied. If the total number of patients responding with an abscopal effect is ≤3 then RT-Ipi dose will be rejected (PASS 2008, NCSS, J. Hintze, Kaysville, UT).

Accrual Rate. An accrual rate of 10-15 patients/year is planned. By the end of the first year 10 patients will be accrued, and the study will either progress to the second stage or be terminated. If it continues to the end of the second stage, we will accrue a total of 29 patients. If the trial concludes at the end of the first stage with no observed abscopal CR/PR after the 12 week evaluation, Part B of the trial will commence with the same design, with newly accrued patients with the only modification regarding the dose of radiation, 9.5 Gy x3.

Statistical Analysis: Patient and disease characteristics will be summarized at baseline using descriptive statistics and graphical displays. The abscopal response rate will be estimated at the end of the second stage with 29 patients along with exact 95% confidence limits. If the observed response rate is 20%, an exact 95% confidence interval for the true rate of 7.6% to 38.9% will be obtained with 29 patients. If the first trial is halted at the end of stage 1, the second trial analysis will proceed in the same manner. The distribution of types of outcomes with respect to non irradiated & measurable and irradiated lesions will be summarized. All patients will be followed for progression and survival for up to 2 years following the entry of the last patient. Kaplan-Meier curves for progression-free survival and survival will be provided to summarize the results.

OBJECTIVE 2:

An important goal of our study is to gather evidence in patients in support of the hypothesis that RT plays a unique role as an immune adjuvant that, when combined with anti-CTLA-4 mAb, enhances the response to anti-CTLA-4. To this end, serial blood samples will be collected for serum and PBMCs: at baseline (pre-treatment), 3 weeks after initiation of Ipi (end of first cycle), end of third cycle of Ipi (~9 weeks), and at evaluation of response (3 months). An additional sample will be collected at 6 months follow up. Small aliquots of PBMC (~10^6 cells) will be used ex vivo for preparation of DNA and RNA, and the remainder preserved frozen until evaluation by flow cytometry and/or by functional assays. A diagnostic tissue biopsy will be obtained at baseline in all patients as a standard of care practice to confirm metastatic diagnosis. A second

optional pre-treatment biopsy will be performed in patients who consent to at an accessible lesion, purely for research purposes. Post-treatment biopsy will be performed at 3 weeks in patients who receive RT to an accessible metastasis. We expect approximately 50% of the patients to comply with the second tissue acquisition. We believe that even a limited number of biopsy sets (before and after RT) can provide invaluable information about the mechanism of action of the combination.

Statistical considerations. The distributions of baseline levels of each of the cell subsets/markers analyzed (pretreatment) will be summarized using descriptive statistics (means, SD, medians, percentiles, etc.) and graphical displays including boxplots. Bivariate scatterplots at baseline will also be provided along with correlation coefficients. Distributions of these markers at each of the subsequent repeated time points and of changes from pre-treatment levels at each time point will be summarized similarly. These serial measurements will be summarized over time using mixed effects regression models for longitudinal data that incorporate the repeated observations over time within the same patient and incorporate missing observations over time. In these models, we will incorporate a random effect for each patient with fixed effects for RT dose and time. Models will be used to test whether changes from baseline vary over time (94). Appropriate transformations of marker expression levels will be carried out to meet the assumptions of the models (e.g., log transformations). Subject specific effects will be examined in relation to response status at 3 months; however, we recognize that these analyses are exploratory with the small number of expected responders. With 29 patients in the Phase II trial, we can detect a difference of |0.54| SD in the change from baseline for a single marker at a single time point with 2-sided alpha of 0.05 and power of 80% based on a paired t-test (no adjustment for multiplicity). With 10 patients in the first stage of the initial trial (should it not continue), we can detect changes from baseline of |0.99| SD. [Calculations from PASS 2008, cited above].

For each of the tested antigens, changes over time from baseline in the percentage of antigen-specific T-cells or antibody titers will be summarized with descriptive statistics and graphical displays. Each patient will be classified at baseline and at each post treatment observation by the presence of T-cell and/or antibody responses to each of the antigens and by pattern of antibody responses to one or more of the identified antigens; responses will be summarized in frequency tables. The proportion of patients with responses to each antigen and patterns of responses including at least one antibody response (as defined above) will be estimated with 95% confidence intervals at each time point. Time to immune response (at least one antibody response) will also be summarized with life table methods for censored data. Sample size considerations for the changes from baseline (T-cells % or Ab titers) are as above for 29 patients.

All patients who consent, will have a pre-treatment biopsy of the lesion to be irradiated. However, only patients with accessible biopsy sites are likely to have a post-treatment biopsy. At baseline, TIL markers based on IHC will be summarized using descriptive statistics and graphical displays. Changes from baseline will be estimated in the approximately 50% of patients who are expected to have a repeat biopsy. With 5-15 patients, based on a paired t-test with 2-sided alpha of 0.05 and power of 80%, for a single marker, a change from baseline to post treatment of |1.68| - |0.78| SD is detectable. Appropriate transformations of marker levels

will be carried out to meet the assumptions of the t-test. We will also compare the patient and disease characteristics as well as baseline levels for each of these markers in the group of patients with a repeat sample with those who do not have a repeat sample to identify potential sources of bias in the group with repeat samples. For the analysis of changes from baseline to post-treatment biopsy based on the microarray gene expression profiling, with a false discovery rate (FDR) of 10%, with 15 (5) pairs of biopsies, we can detect a change in a single gene of |1.3| (|4.9|) SD in expression level with overall power of 80% assuming 10% of the genes have a true expression level greater than that specified. Genes that exceed the threshold for a single gene alpha of 0.0009 (2–sided) will be ranked and the highest identified changes will be validated with qRT-PCR.

The changes in serum samples for sMICA concentration and anti-sMICA antibody titers, and expression levels of NKG2D in circulating CD8 and NK cells will be plotted over time for each of these measurements to identify the nature of the changes and summarized over time using the same statistical approaches that are described in 2.1 and 2.2. Baseline levels and changes from baseline at the time of the post-treatment biopsy will be plotted for each of these markers and correlation coefficients with 95% confidence intervals will be estimated to evaluate the association between the serum and tumor marker levels.

8 ADMINISTRATIVE SECTION

8.1 Compliance with the Protocol and Protocol Revisions

The study will be conducted as described in the final approved protocol. Documentation of approval signed by the chairperson or designee of the IRB(s) will be sent to the NYU protocol manager.

All revisions (protocol amendments, administrative letters, and changes to the informed consent) will be submitted to the NYU protocol manager. The Investigator will not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

8.2 Informed Consent

The Investigator will ensure that patients are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and will include all elements required by the Code of Federal Regulations 21 Part 50.25 and the local IRB. Written informed consent will be obtained by physicians and nurse practitioners listed on the title page of this protocol. The informed consent form will be signed by the subject and the registering physician. Once signed, a copy will be given to the subject and one will be maintained with the subject’s medical record. Once eligibility is confirmed and informed consent is documented, the patient will be registered by the study coordinator/data manager. In this protocol, blood and biopsies will be procured and stored with barcoded identifiers. The study

samples obtained will be for the purpose of immune-monitoring study when funds become available.

8.3 Records and Reports

Adequate and accurate case histories designed to record all observations and other data pertinent to the investigation (e.g., case report form) will be prepared and maintained on each individual treated with ipilimumab. The investigator will retain, in a confidential manner, the data pertinent to the study.

8.4 Records Retention

The Investigator will retain source documents, and case histories designed to record all observations and other data pertinent to the investigation (e.g., case report form) for the maximum period required by applicable regulations and guidelines, or Institution procedures.

If the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB). Documentation of such transfer will be provided to NYU.

8.5 Laboratory Correlative Studies

Serial blood samples for immune monitoring, Genomics and T-cell response will be collected at baseline, and at selected time points as outlined in Section 3.1 and Appendix 2.

Approximately 4-6 mm of tissue will be obtained from four Core punch biopsies will be performed at baseline and at end of treatment.

Depending on the availability of novel assays that can improve our ability to analyze the anti-tumor immune response, samples of blood (PBMC and/or plasma fractions) may also be sent for analysis to other collaborators at sites outside of NYU. Samples will be de-identified and no other information except for the unique code will be provided.

8.6 Storage of Samples

All blood and tissue samples will be processed immediately and stored indefinitely for later analysis in a locked -80°C freezer in the NYU Biorepository for research purposes only. These specimens will not be linked to any clinical data and will be de-identified in the clinical research database, Velos. Only the data manager will have access to the master list with the patient name and an identification number. This master list will be secured in a locked cabinet at the NYU Clinical Cancer Center. Only the investigators listed on this protocol will have access to these samples. After both blood and tissue samples are analyzed at a later date, any unutilized samples will be preserved indefinitely in the NYU Biorepository for potential future research.

All patients enrolled will be given a unique identifier (study ID number). Only the data manager will know the code linking patient and study ID number. Patients will be assigned a unique code number. All specimens collected will be deidentified and assigned the same unique study number of the corresponding patient and will also be marked with the collection time point.

Clinical information regarding toxicities and response will likewise be stored in a deidentified database using only the unique identifier (study ID number).

Serial blood samples will be collected for serum and PBMCs: at baseline (pre-treatment), 3 weeks after initiation of Ipi (end of first cycle), end of third cycle of Ipi (~9 weeks), and at evaluation of response (3 months). An additional sample will be collected at 6 months follow up. Small aliquots of PBMC (~10^6 cells) will be used ex vivo for preparation of DNA and RNA, and the remainder preserved frozen until evaluation by flow cytometry and/or by functional assays.

A diagnostic tissue biopsies is obtained at baseline in all patients, as per Standard of Care. Access to this histological specimen is required for the study to establish pre-treatment immunological infiltrate. In patients who consent to a pre and post-treatment biopsy of any irradiated lesions tissue will be acquired before the first radiotherapy and first Ipilimumab treatment and again at 3 weeks in patients who receive RT to an accessible metastasis and consent to these OPTIONAL research biopsy. These blood and tissue samples will be utilized for correlative studies as outlined above (section 3.2, page 24).

All blood and tissue samples will be stored indefinitely until appropriate funding has been obtained to perform correlative studies or until subject withdraws consent for banking of study specimens. If consent is withdrawn by the study subject, samples will be destroyed as per standard practices.

The storage of your blood and tissue is optional and you may withdraw your consent for the banking of these specimens at any time. You may make this request by writing to the Principal Investigator Abraham Chachoua, M.D. at NYU Cancer Institute 160 East 34th Street New York, NY 10016.

8.7 Shipping of Samples

Frozen blood specimens de-identified as descriibed above may be shipped at -80C to other sites for novel analyses that become available to better study the immune response.

8.8 Genetic Testing

No genetic research will be performed on the study samples acquired in this clinical trial.

8.9 Confidentiality

The medical, hospital and research records associated with this study are considered confidential. Members of the treating team and designated study assistants will have access to the records as required to administer treatment and comply with the protocol. Neither the name nor any other identifying information for an individual will be used for reporting or publication regarding this study. All laboratory and baseline data will be de-identified and transferred via secure links at NYU School of Medicine. Patient records will be made available for inspection to auditing agencies to satisfy regulatory requirements. Anonymous data will be shared with Weill Cornell Medical College due to the relocation of study personnel so that a paper may be

published on the findings of this study. The anonymous data will only be shared with Weill Cornell Medical College after a transfer agreement is fully executed.

9.0 Research Conflict of Interest

There are no conflicts of interest to report.

Appendix 1: Eligibility and Registration Worksheets

Patient Initial _____ Patient ID _____

REGISTRATION WORKSHEET

NYU S14-00208

Patient Name _____ **Patient ID** _____

Birth Date *(mm/dd/yyyy)* _____

SS # _____

Gender *(circle one)* Male Female

Race *(circle the most appropriate choice)*
White
Black or African American
Native Hawaiian/Pacific Islander/Filipino
Asian
Native American or Alaska Native
Other
If other, please specify_____

Ethnicity *(circle the most appropriate choice)*
Hispanic or Latino
Non-Hispanic
Other
If other, please specify _____

Address _____

(City) (State) (Zip)

Phone # _____

Referring MD _____

RT Medical Record # _____

Patient Initial _____ Patient ID _____

ELIGIBILITY WORKSHEET

Inclusion Criteria:

	YES	NO	Procedure Date	
1.	___	___	_____	Ability to understand and the willingness to sign a written informed consent document.
2.	___	___	_____	Histologically confirmed metastatic NSCLC
3.	___	___	_____	No prior therapy with ipilimumab or another anti-CTLA-4 monoclonal antibody
4.	___	___	_____	Have at least 2 distinct measurable metastatic sites which are at least 1 cm in their largest diameter.
5.	___	___	_____	Age >18 years
6.	___	___	_____	ECOG performance status 0-1
7.	___	___	_____	Life expectancy > 3 months
8.	___	___	_____	Any brain metastases must be stable for at least 4 weeks or greater than 2 weeks post- gamma knife therapy and not steroid dependent.
9.	____	_____	_____	Brain Scan (CT/MRI) within a month prior to enrollment
10.	Have adequate organ and marrow function as defined below:			
	___	___	_____	Leukocyte count ≥ 2000/uL

___ ___ _____ Absolute neutrophil count ≥ 1000/uL

___ ___ _____ Platelets ≥ 50,000/uL

___ ___ _____ Hemoglobin ≥ 8 g/dL

___ ___ _____ Total bilirubin ≤ 3.0 x ULN (or ≤ 3.0 mg/dL for patients with Gilbert's Syndrome)

___ ___ _____ AST(SGOT)/ALT(SGPT) ≤ 2.5 x ULN (or ≤ 5 x ULN if liver metastases are present)

___ ___ _____ Serum creatinine ≤ 3.0 x ULN

11. ___ ___ _____ Women of childbearing potential must agree to use adequate contraception (barrier method of birth control) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or a participating spouse are enrolled in this study, she should inform her treating physician immediately.

Exclusion Criteria:

12. ___ ___ _____ No lesions outside the field of radiation.

13. ___ ___ _____ Presence of autoimmune disease, including inflammatory bowel disease, rheumatoid arthritis, progressive systemic sclerosis (scleroderma), systemic lupus erythematosus, and autoimmune vasculitis (e.g., Wegener's granulomatosis).

14. ___ ___ _____ Treatment with cytotoxic chemotherapy within 2 weeks prior to entering the study.

15. ___ ___ _____ Treatment with IL-2, interferon, or other non-study immunotherapy within 2 weeks prior to entering the study.

16. ___ ___ _____ On steroid therapy or other immunosuppressive therapy.

17. ___ ___ _____ Undergoing therapy with other investigational agents.

18. ___ ___ _____ Any underlying medical or psychiatric condition, which in the opinion of the Investigator, will make the administration of study drug hazardous or obscure the interpretation of adverse events (AEs), such as a condition associated with frequent diarrhea.

19. ___ ___ _____ Prior therapy with ipilimumab or another anti-CTLA-4 antagonist.

20. ___ ___ _____ Unstable brain metastases or steroid dependent.

21. ___ ___ _____ Uncontrolled inter-current illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, myocardial infarction within the past 6 months, unstable angina pectoris, or unstable cardiac arrhythmia requiring assessment for clinical intervention.

22. ___ ___ _____ Women who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 8 weeks after cessation of study drug, or have a positive pregnancy test at baseline.

23. ___ ___ _____ Pregnant or nursing.

24. ___ ___ _____ Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness.

If the answer to all Inclusion criteria is "Yes" and all Exclusion criteria is "No" then the patient is considered to be protocol eligible.

Is the patient protocol eligible? YES NO

Signature (Dr.Chachoua)

APPENDIX 2: LABORATORY CORRELATE MANUAL VERSION 1.4 8.21.2014

At Baseline: BIOPSIES: a core biopsy from the lesion selected to be irradiated is obtained placed in a dry clean container (plastic jar) and immediately placed on ice and transported by BioRepository Center (BRC) personnel to pathology. Before transport call Dr. Demaria's lab (212-263-7306/8). Dr. Demaria or her designee will come immediately to collect half of the tissue. Tissue collected will be placed in Trizol-containing tube and stored frozen in Demaria's lab for later RNA/DNA isolation. The other half will be fixed in formalin and paraffin-embedded.

and delivered to Dr. Demaria's lab.
Half core will be assigned for histology and half for RNA/DNA.

Blood: Six 10 mL purple top (EDTA) tubes—to Immune Monitoring Core

One 10 mL red top (serum) tube—serum to be processed and frozen by Immune Monitoring Core (or BioRepository Center (BRC))

(Total research blood draw this day 70 mL)

Day 22: RE-BIOPSY original irradiated lesion: a core biopsy from the lesion selected to be irradiated is obtained and delivered at Dr. Demaria's lab
Half core will be assigned for histology and half for RNA/DNA.

Blood: Six 10 mL purple top (EDTA) tubes—to Immune Monitoring Core

One 10 mL red top (serum) tube—serum to be processed and frozen by Immune Monitoring Core (or BRC)

(Total research blood draw this day 70 mL)

Day 64: Blood: Three 10 mL purple top (EDTA) tubes—to Immune Monitoring Core

(Total research blood draw this day 30 mL)

Day 88: Blood: Six 10 mL purple top (EDTA) tubes—to Immune Monitoring Core

One 10 mL red top (serum) tube—serum to be processed and frozen by Immune Monitoring Core (or BRC)

(Total research blood draw this day 70 mL)

Follow up visit (at 6 months from starting treatment):

Blood: Six 10 mL purple top (EDTA) tubes—to Immune Monitoring Core

One 10 mL red top (serum) tube—serum to be processed and frozen by Immune Monitoring Core (or BRC)

(Total research blood draw this day 70 mL)

BLOOD PROCESSING:

PBMC:

aliquot obtained PBMC as follows:

- 2 aliquots of $2x10^6$ cells, pellet and freeze dry (Store in liquid nitrogen)
- The remainder preserved frozen for flow cytometry/functional assays in $10x10^6$ cell aliquots

PLASMA: collect from EDTA-blood and freeze 5 aliquots of 1-ml each.

Rationale for using EDTA tubes instead of heparin :

Heparin may interfere with some of the planned assays. It was decided to collect blood using EDTA which will not pose this potential problem

APPENDIX 3: SUGGESTED WORK-UP AND TREATMENT FOR IMMUNE RELATED ADVERSE EVENTS (IRAE)S

As discussed in Section 4.3.4, an irAE is defined as an adverse event of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event a non-dermatologic, immune-mediated event. Serological, immunological, and histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Documentation of test results should be included in the patient's medical record.

Gastrointestinal (diarrhea) and skin (rash)-related toxicities have been the most common irAEs noted in prior studies with ipilimumab. Suggested work-up procedures for suspected irAEs of the gastrointestinal tract, liver, skin, eye, pituitary, and adrenal gland are listed below. When symptomatic therapy is inadequate or inappropriate, an irAE should be treated with steroids followed by a slow taper.

Gastrointestinal tract: Diarrhea (defined as either first watery stool, or increase in frequency 50% above baseline with urgency or nocturnal bowel movement, or bloody stool) should be further evaluated and infectious or alternate etiologies ruled out. Patients should be advised to inform the Investigator if any diarrhea occurs, even if it is mild. An algorithm for working up patients with diarrhea or suspected colitis is provided in Appendix 4.

If the event is of significant duration or magnitude or is associated with signs of systemic inflammation or acute phase reactants (e.g., increased CRP or platelet count; or bandemia), it is recommended that sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy with 3 to 5 specimens for standard paraffin block be performed. If possible, 1 to 2 biopsy specimens should be snap frozen and stored. All patients with confirmed colitis should also have an ophthalmological examination, including a slit-lamp exam, to rule out uveitis. Tests should also be performed for WBCs and for stool calprotectin.

Patients with colitis should discontinue any non-steroidal anti-inflammatory medications or any other medications known to exacerbate colitis symptoms. Investigators should use their clinical judgment as to whether corticosteroids are necessary to treat colitis associated with ipilimumab therapy and as to what dose should be used. As guidance prior experience suggests that colitis manifested as ≥ Grade 3 diarrhea requires corticosteroid treatment. For severe symptoms, prednisone 60 mg or equivalent may be required to control initial symptoms and the dose should be gradually tapered over at least one month in duration. Lower doses of prednisone may be considered for less severe cases of colitis. It is suggested that prednisone (for oral administration) or solumedrol (for intravenous administration) be corticosteroid of choice in the treatment of colitis.

Liver: Elevation of LFTs ≥ 3 fold from baseline should instigate an investigation into the underlying etiology for suspected irAEs. Neoplastic, concurrent medications, viral hepatitis, and toxic etiologies should be considered and addressed, as appropriate. Imaging of the liver, gall bladder, and bile duct should be performed to rule out neoplastic or other causes for the increased LFTs. An ANA, pANCA, and anti-smooth muscle antibody test should be performed if

an autoimmune etiology is considered. Consultation with a hepatologist is appropriate for a suspected liver irAE and a biopsy should be considered.

Patients presenting with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have LFTs performed immediately and reviewed before administering the next dose of study drug. Treating physicians should discuss, with the CRO Medical Monitor, unexplained increases in LFTs $\geq$ 3 fold from baseline prior to any additional study drug administration.

Pancreas: Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, may rarely be associated with anti-CTLA-4 monoclonal antibody administration. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include serum amylase and lipase tests.

Skin: A dermatologist should evaluate persistent and/or severe rash or pruritus. A biopsy should be performed if appropriate and if possible, photos of the rash should also be obtained. Low-grade ipilimumab mediated rash and pruritus irAEs have been treated with symptomatic therapy (e.g., antihistamines). Topical or parenteral corticosteroids may be required for more severe symptoms.

Eye: An ophthalmologist should evaluate visual complaints with examination of the conjunctiva, anterior and posterior chambers and retina; visual field testing and an electroretinogram should also be performed. Patients with ipilimumab related uveitis or episcleritis have been treated with topical corticosteroid eye drops.

Endocrine: Patients with unexplained symptoms such as fatigue, myalgias, impotence, mental status changes, or constipation should be investigated for the presence of thyroid, pituitary or adrenal endocrinopathies. An endocrinologist should be consulted if an endocrinopathy is suspected. TSH and free T4 levels should be obtained to determine if thyroid abnormalities are present. TSH, prolactin and a morning cortisol level will help to differentiate primary adrenal insufficiency from primary pituitary insufficiency. Appropriate hormone replacement therapy should be instituted if an endocrinopathy is documented.

Suspected irAEs should be documented in the patient's medical record.

APPENDIX 4:

GI Toxicity Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.

Opiates/narcotics mask symptoms of perforation! Infliximab should not be used in case of perforation/sepsis!

Severity of diarrhea/ colitis	Management	Follow-up
Grade 1 Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic	• Continue ipilimumab • Symptomatic treatment	• Close monitoring for worsening symptoms • Educate patient to report any worsening immediately
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; not interfering with ADL Colitis: abdominal pain; blood in stool	• Withhold/delay ipilimumab • Symptomatic treatment	**Symptoms improve/resolve** (grade 0/1): Resume ipilimumab **Symptoms persist for > 5-7 days, worsen, or recur:** • Moderate to high dose steroids PO (e.g., prednisone 0.5 - 1 mg/kg/day) • Continue to hold/delay ipilimumab until grade 1 • When symptoms are grade 1 or less slowly taper steroids over at least 1 month and resume ipilimumab. **Symptoms worsen:** Treat as grade 3/4
Grade 3-4 Diarrhea (G3*): ≥ 7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs;interfering with ADL Colitis (G3*): fever, ileus, peritoneal signs	• Permanently discontinue ipilimumab • High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day) • Consider endoscopy	**Symptoms improve:** • Continue steroids (same dose) until grade 1 • Then taper over at least 1 month **Symptoms persist 3-5 days, or recur after improvement:** • 1 dose* of infliximab 5 mg/kg (if no contraindication)

*G4 = life-threatening, perforation

*Some patients have required a second dose of infliximab

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose 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 PO corticosteroids.

APPENDIX 5:

Skin Toxicity Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.

Severity of skin toxicity	Management	Follow-up
Grade 1-2	• Symptomatic therapy, e.g. antihistamines, topical steroids • Continue ipilimumab	**Symptoms resolve:** Continue ipilimumab **Symptoms persist > 1-2 weeks or recur:** • Continue ipilimumab • Moderate to high dose steroids PO (e.g., prednisone 0.5 - 1 mg/kg/day) • Once controlled, taper steroids over at least 1 month
Grade 3-4	• Hold/delay ipilimumab (regardless of relationship) • Take photos of rash • Consider skin biopsy • Dermatology consult • High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day)	**Symptoms resolve / return to grade 1:** • Taper steroids over at least 1 month • Resume ipilimumab • Discontinue if grade 4 toxicity considered related

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose 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 PO corticosteroids.

APPENDIX 6:

APPENDIX 7:

Neurological Toxicity Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab. Discontinue ipilimumab for any grade 3-4 motor neuropathy, regardless of relationship.

Severity of neurological toxicity	Management	Follow-up
Grade 1	• Continue ipilimumab	Continue monitoring the patient. If symptoms worsen, treat as below.
Grade 2	• Hold/delay ipilimumab • Treat symptoms per local guidelines	Resume ipilimumab when resolved / grade 1 If symptoms worsen, treat as below.
Grade 3-4 sensory	• Discontinue ipilmumab if considered related • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month
Grade 3-4 motor	• Discontinue ipilimumab **regardless of relationship** • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month **Symptoms do not resolve or progress; atypical presention:** • Consider IVIg or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose 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 PO corticosteroids.

APPENDIX 8:

APPENDIX 9: LIST OF ABBREVIATIONS

Abbreviation	Term
ADCC	Antibody Dependent Cellular Cytotoxicity
AE	Adverse Event
AEC	Absolute Neutrophil Count
ALC	Absolute Lymphocyte Count
ANC	Absolute Neutrophil Count
APC	Antigen Presenting Cell
BED	Biologically Effective Dose
BMS	Bristol-Myers Squibb
CBC	Complete Blood Count
CBCT	Cone-Beam CT
CDC	Complement Dependent Cytotoxicity
CDR3	Complementarity Determining Region 3
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T-Lymphocyte
CTV	Clinical Target Volume
DRR	Digitally Reconstructed Radiograph
DVH	Dose-Volume Histogram
ECOG	Eastern Cooperative Oncology Group

Abbreviation	Term
FDR	False Discovery Rate
FSH	Follicle Stimulating Hormone
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GTV	Gross Tumor Volume
hCG	Human Chorionic Gonadotropin
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
i.v.	Intravenous
IB	Investigator Brochure
IgG	Immunoglobulin G
IGRT	Image Guided Radiation Therapy
IHC	Immunohistochemistry
IMRT	Intensity Modulated Radiation Therapy
IP	Investigational Product
Ipi	Ipilimumab
IRB	Institutional Review Board
irBOR(R)	Immune-Related Best Overall Response (Rate)
irCR	Immune-Related Complete Response
irPD	Immune-Related Progression of Disease
irPFS	Immune-Related Progression Free Survival
irPR	Immune-Related Partial Response

Abbreviation	Term
irRC	Immune-Related Response Criteria
irSD	Immune-Related Stable Disease
irSPD	Immune-Related Sum of the Products of Diameters
IT	Immunotherapy
mAb	Monoclonal Antibody
MDSC	Myeloid-Derived Suppressor Cell
MLC	Multi-Leaf Collimator
MRI	Magnetic Resonance Imaging
NK	Natural Killer
NSAID	Non-Steroidal Anti-Inflammatory Drug
NSCLC	Non-Small Cell Lung Cancer
OLP	Overlapping Peptide
PBMC	Peripheral Blood Mononuclear Cell
PD	Progressive Disease
PD1	Programmed Death Receptor 1
PFS	Progression Free Survival
PR	Partial Response
PS	Performance Status
PTV	Planning Target Volume
RCOIC	Research Conflict of Interest Committee
RECIST	Response Evaluation Criteria In Solid Tumors

Abbreviation	Term
RT	Radiotherapy or Radiation Therapy
RT-Ipi	Radiotherapy and Ipilimumab
SAE	Serious Adverse Event
SD	Standard Deviation
sMICA	Soluble MICA
SPD	Sum of the Products of Diameters
TA	Tumor Assessment
TAA	Tumor-Associated Antigen
TCR	T-Cell Receptor
TIL	Tumor Infiltrating Lymphocyte
TNM Staging	Tumor, Node and Metastasis Staging
WOCBP	Women of Child Bearing Potential

REFERENCES

1. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. The New England journal of medicine. 2012;366(10):925-31. Epub 2012/03/09. doi: 10.1056/NEJMoa1112824. PubMed PMID: 22397654; PubMed Central PMCID: PMC3345206.

2. Stamell EF, Wolchok JD, Gnjatic S, Lee NY, Brownell I. The abscopal effect associated with a systemic anti-melanoma immune response. International journal of radiation oncology, biology, physics. 2013;85(2):293-5. Epub 2012/05/09. doi: 10.1016/j.ijrobp.2012.03.017. PubMed PMID: 22560555; PubMed Central PMCID: PMC3415596.

3. Formenti SC, Demaria S. Radiotherapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys. 2012;84(4):879-80.

4. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity. 2013;39(1):11-26.

5. Bishop MR, Fowler DH, Marchigiani D, Castro K, Kasten-Sportes C, Steinberg SM, et al. Allogeneic lymphocytes induce tumor regression of advanced metastatic breast cancer. J Clin Oncol. 2004;22(19):3886-92. PubMed PMID: 15314059.

6. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105(4):256-65. doi: 10.1093/jnci/djs629. PubMed PMID: 23291374; PubMed Central PMCID: PMC3576324.

7. Demaria S, Bhardwaj N, McBride WH, Formenti SC. Combining radiotherapy and immunotherapy: a revived partnership. Int J Radiat Oncol Biol Phys. 2005;63(3):655-66. doi: 10.1016/j.ijrobp.2005.06.032. PubMed PMID: 16199306; PubMed Central PMCID: PMC1489884.

8. Formenti SC, Demaria S. Radiation therapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys. 2012;84(4):879-80. Epub 2012/10/20. doi: 10.1016/j.ijrobp.2012.06.020. PubMed PMID: 23078897.

9. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105(4):256-65. Epub 2013/01/08. doi: 10.1093/jnci/djs629. PubMed PMID: 23291374; PubMed Central PMCID: PMC3576324.

10. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. Annu Rev Immunol. 1996;14:233-58. PubMed PMID: 8717514.

11. Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell. 1992;71(7):1065-8. PubMed PMID: 1335362.

12. Chen L, Ashe S, Brady WA, Hellstrom I, Hellstrom KE, Ledbetter JA, et al. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. Cell. 1992;71(7):1093-102. PubMed PMID: 1335364.

13. Townsend SE, Allison JP. Tumor rejection after direct costimulation of CD8+ T cells by

B7-transfected melanoma cells. Science. 1993;259(5093):368-70. PubMed PMID: 7678351.

14. Townsend SE, Su FW, Atherton JM, Allison JP. Specificity and longevity of antitumor immune responses induced by B7-transfected tumors. Cancer Res. 1994;54(24):6477-83. PubMed PMID: 7527298.

15. Allison JP, Hurwitz AA, Leach DR. Manipulation of costimulatory signals to enhance antitumor T-cell responses. Curr Opin Immunol. 1995;7(5):682-6. PubMed PMID: 8573312.

16. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991;174(3):561-9. PubMed PMID: 1714933.

17. Thompson CB, Allison JP. The emerging role of CTLA-4 as an immune attenuator. Immunity. 1997;7(4):445-50. PubMed PMID: 9354465.

18. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity. 1994;1(5):405-13. Epub 1994/08/01. PubMed PMID: 7882171.

19. Kearney ER, Walunas TL, Karr RW, Morton PA, Loh DY, Bluestone JA, et al. Antigen-dependent clonal expansion of a trace population of antigen-specific CD4+ T cells in vivo is dependent on CD28 costimulation and inhibited by CTLA-4. J Immunol. 1995;155(3):1032-6. PubMed PMID: 7543510.

20. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182(2):459-65. PubMed PMID: 7543139.

21. Krummel MF, Sullivan TJ, Allison JP. Superantigen responses and co-stimulation: CD28 and CTLA-4 have opposing effects on T cell expansion in vitro and in vivo. Int Immunol. 1996;8(4):519-23. PubMed PMID: 8671638.

22. Melief CJ. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. Adv Cancer Res. 1992;58:143-75. PubMed PMID: 1532109.

23. Rosenberg SA. Progress in human tumour immunology and immunotherapy. Nature. 2001;411(6835):380-4. PubMed PMID: 11357146.

24. Antonia S, Mule JJ, Weber JS. Current developments of immunotherapy in the clinic. Curr Opin Immunol. 2004;16(2):130-6. PubMed PMID: 15023403.

25. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol. 2002;3(7):611-8. PubMed PMID: 12087419.

26. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271(5256):1734-6. PubMed PMID: 8596936.

27. Yang YF, Zou JP, Mu J, Wijesuriya R, Ono S, Walunas T, et al. Enhanced induction of antitumor T-cell responses by cytotoxic T lymphocyte-associated molecule-4 blockade: the effect is manifested only at the restricted tumor-bearing stages. Cancer Res. 1997;57(18):4036-41. PubMed PMID: 9307290.

28. Hurwitz AA, Yu TF, Leach DR, Allison JP. CTLA-4 blockade synergizes with tumor-

derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. Proc Natl Acad Sci U S A. 1998;95(17):10067-71. PubMed PMID: 9707601.

29. van Elsas A, Sutmuller RP, Hurwitz AA, Ziskin J, Villasenor J, Medema JP, et al. Elucidating the autoimmune and antitumor effector mechanisms of a treatment based on cytotoxic T lymphocyte antigen-4 blockade in combination with a B16 melanoma vaccine: comparison of prophylaxis and therapy. J Exp Med. 2001;194(4):481-9. PubMed PMID: 11514604.

30. Dranoff G. CTLA-4 blockade: unveiling immune regulation. J Clin Oncol. 2005;23(4):662-4. PubMed PMID: 15613692.

31. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol. 1953;26(305):234-41. PubMed PMID: 13042090.

32. Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys. 2004;58(3):862-70. PubMed PMID: 14967443.

33. Demaria S, Kawashima N, Yang AM, Devitt ML, Babb JS, Allison JP, et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res. 2005;11(2 Pt 1):728-34. PubMed PMID: 15701862.

34. Kingsley DP. An interesting case of possible abscopal effect in malignant melanoma. Br J Radiol. 1975;48(574):863-6. PubMed PMID: 811297.

35. Ehlers G, Fridman M. Abscopal effect of radiation in papillary adenocarcinoma. Br J Radiol. 1973;46(543):220-2. PubMed PMID: 4706791.

36. Ohba K, Omagari K, Nakamura T, Ikuno N, Saeki S, Matsuo I, et al. Abscopal regression of hepatocellular carcinoma after radiotherapy for bone metastasis. Gut. 1998;43(4):575-7.

37. Nikitina EY, Gabrilovich DI. Combination of gamma-irradiation and dendritic cell administration induces a potent antitumor response in tumor-bearing mice: approach to treatment of advanced stage cancer. Int J Cancer. 2001;94(6):825-33. PubMed PMID: 11745485.

38. Ganss R, Ryschich E, Klar E, Arnold B, Hammerling GJ. Combination of T-cell therapy and trigger of inflammation induces remodeling of the vasculature and tumor eradication. Cancer Res. 2002;62(5):1462-70. PubMed PMID: 11888921.

39. Chakravarty PK, Alfieri A, Thomas EK, Beri V, Tanaka KE, Vikram B, et al. Flt3-ligand administration after radiation therapy prolongs survival in a murine model of metastatic lung cancer. Cancer Res. 1999;59(24):6028-32. Epub 2000/01/08. PubMed PMID: 10626784.

40. Formenti SC, Demaria S. Systemic effects of local radiotherapy. Lancet Oncol. 2009;10(7):718-26. Epub 2009/07/04. doi: S1470-2045(09)70082-8 [pii]

10.1016/S1470-2045(09)70082-8. PubMed PMID: 19573801; PubMed Central PMCID: PMC2782943.

41. Brody JD, Ai WZ, Czerwinski DK, Torchia JA, Levy M, Advani RH, et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. J Clin Oncol. 2010;28(28):4324-32. doi: 10.1200/JCO.2010.28.9793. PubMed PMID: 20697067; PubMed Central PMCID: PMC2954133.

42. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366(10):925-31. Epub 2012/03/09. doi: 10.1056/NEJMoa1112824. PubMed PMID: 22397654; PubMed Central PMCID: PMC3345206.

43. Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res. 2005;11(9):3353-62. doi: 10.1158/1078-0432.CCR-04-2062. PubMed PMID: 15867235.

44. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23. Epub 2010/06/08. doi: NEJMoa1003466 [pii]

10.1056/NEJMoa1003466. PubMed PMID: 20525992.

45. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SF. An Abscopal Response to Radiation and Ipilimumab in a Patient with Metastatic Non-Small Cell Lung Cancer Cancer Immunol Res 2013;Published OnlineFirst October 9:doi:10.1158/2326-6066.CIR-13-0115 doi: 10.1158/2326-6066.CIR-13-0115

46. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol 2012;30:2046-54.

47. Zatloukal P, Heo DS, Park K, Kang J, Butts C, Bradford D, et al. Randomized phase II clinical trial comparing tremelimumab (CP-675,206) with best supportive care (BSC) following first-line platinum-based therapy in patients (pts) with advanced non-small cell lung cancer (NSCLC). J Clin Oncol 27:15s, 2009 (suppl; abstr 8071).

48. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-54. Epub 2012/06/05. doi: 10.1056/NEJMoa1200690. PubMed PMID: 22658127; PubMed Central PMCID: PMC3544539.

49. Brahmer JR, Horn L, Antonia SJ, Spigel DR, Gandhi L, Sequist LV, et al. NIVOLUMAB (ANTI-PD-1; BMS-936558; ONO-4538) IN PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC): OVERALL SURVIVAL AND LONG-TERM SAFETY IN A PHASE 1 TRIAL. J Thorac Oncol. 2013;8(Sup 2).

50. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455-65. Epub 2012/06/05. doi: 10.1056/NEJMoa1200694. PubMed PMID: 22658128; PubMed Central PMCID: PMC3563263.

51. Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, 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(17):5379-88.

52. Fowler JF. The linear-quadratic formula and progress in fractionated radiotherapy. The British journal of radiology. 1989;62(740):679-94. Epub 1989/08/01. PubMed PMID: 2670032.

53. Yasumoto K, Hanagiri T, Takenoyama M. Lung cancer-associated tumor antigens and the present status of immunotherapy against non-small-cell lung cancer. Gen Thorac Cardiovasc Surg. 2009;57(9):449-57.

54. Fuks Z, Kolesnick R. Engaging the vascular component of the tumor response. Cancer Cell. 2005;8(2):89-91.

55. Demaria S, Kawashima N, Yang AM, Devitt M-L, Babb JS, Allison JP, et al. Immune-mediated inhibition of metastases following treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res. 2005;11:728-34.

56. Pilones KA, Kawashima N, Yang AM, Babb JS, Formenti SC, Demaria S. Invariant natural killer T cells regulate breast cancer response to radiation and CTLA-4 blockade. . Clin Cancer Res. 2009;15(2):597-606.

57. Matsumura S, Wang B, Kawashima N, Braunstein S, Badura M, Cameron TO, et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol. 2008;181:3099-107.

58. Ruocco MG, Pilones KA, Kawashima N, Cammer M, Huang J, Babb JS, et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves anti-tumor effects. J Clin Invest. 2012;122(10):3718-30. doi: doi: 10.1172/JCI61931.

59. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366(10):925-31.

60. Postow MA, Callahan MK, Wolchok JD. The antitumor immunity of ipilimumab: (T-cell) memories to last a lifetime? Clin Cancer Res. 2012;18(7):1821-3.

61. Peggs KS, Quezada SA, Allison JP. Cell intrinsic mechanisms of T-cell inhibition and application to cancer therapy. Immunol Rev. 2008;224:141-65.

62. Ruocco MG, Pilones KA, Kawashima N, Cammer M, Huang J, Babb JS, et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. J Clin Invest. 2012;122(10):3718-30. Epub 2012/09/05. doi: 10.1172/JCI61931. PubMed PMID: 22945631; PubMed Central PMCID: PMC3461908.

63. Schadendorf D, Hodi FS, Robert C, al. e. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in metastatic or locally advanced, unresectable melanoma. European Cancer Congress 2013 (ECCO-ESMO-ESTRO); September 27-October 1; Amsterdam, The Netherlands2013.

64. Smith FO, Goff SL, Klapper JA, Levy C, Allen T, Mavroukakis SA, et al. Risk of bowel perforation in patients receiving interleukin-2 after therapy with anti-CTLA 4 monoclonal

antibody. J Immunother. 2007;30(1):130. doi: 10.1097/01.cji.0000211334.06762.89. PubMed PMID: 17198092; PubMed Central PMCID: PMC2151199.

65. Robert C, Thomas L, Bondarenko I, O'Day S, M.D. JW, Garbe C, et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. New England Journal of Medicine. 2011;364(26):2517-26. doi: doi:10.1056/NEJMoa1104621.

66. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412-20.

67. Callahan MK, Wolchok JD, Allison JP. Anti-CTLA-4 antibody therapy: immune monitoring during clinical development of a novel immunotherapy. Semin Oncol. 2010;37(5):473-84.

68. Maecker HT. Multiparameter flow cytometry monitoring of T cell responses. Methods Mol Biol. 2009;485:375-91.

69. Wang W, Yu D, Sarnaik AA, Yu B, Hall M, Morelli D, et al. Biomarkers on melanoma patient T cells associated with ipilimumab treatment. J Transl Med 2012;10:146. doi: doi: 10.1186/1479-5876-10-146.

70. Jinushi M, Hodi FS, Dranoff G. Enhancing the clinical activity of granulocyte-macrophage colony-stimulating factor-secreting tumor cell vaccines. Immunol Rev. 2008;222:287-98. PubMed PMID: 18364009.

71. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol. 2010;184(7):3433-41.

72. Kitano S, Postow MA, Cortez C, Rasalan T, Gallardo HF, Panageas KS, et al. Myeloid-derived suppressor cell quantity prior to treatment with ipilimumab at 10mg/kg to predict for overall survival in patients with metastatic melanoma. . J Clin Oncol. 2012;30:(suppl; abstr 2518).

73. Adams S, Greeder L, Reich E, Shao Y, Fosina D, Hanson N, et al. Expression of cancer testis antigens in human BRCA-associated breast cancers: potential targets for immunoprevention? Cancer Immunol Immunother. 2011;60(7):999-1007.

74. Yuan J, Adamow M, Ginsberg BA, Rasalan TS, Ritter E, Gallardo HF, et al. Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab. Proc Natl Acad Sci U S A. 2011;108(40):16723-8.

75. Adams S, O'Neill DW, Nonaka D, Hardin E, Chiriboga L, Siu K, et al. Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. J Immunol. 2008;181(1):776-84.

76. Atanackovic D, Altorki NK, Stockert E, Williamson B, Jungbluth AA, Ritter E, et al. Vaccine-induced CD4+ T cell responses to MAGE-3 protein in lung cancer patients. J Immunol. 2004;172(5):3289-96.

77. Gnjatic S, Ritter E, Büchler MW, Giese NA, Brors B, Frei C, et al. Seromic profiling of ovarian and pancreatic cancer. Proc Natl Acad Sci U S A. 2010;107(11):5088-93.

78. Gnjatic S, Wheeler C, Ebner M, Ritter E, Murray A, Altorki NK, et al. Seromic analysis of antibody responses in non-small cell lung cancer patients and healthy donors using conformational protein arrays. J Immunol Methods. 2009;341(1-2):50-8.

79. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960-4.

80. Hodi FS, Butler M, Oble DA, Seiden MV, Haluska FG, Kruse A, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci U S A. 2008;105(8):3005-10.

81. Ribas A, Comin-Anduix B, Economou JS, Donahue TR, de la Rocha P, Morris LF, et al. Intratumoral immune cell infiltrates, FoxP3, and indoleamine 2,3-dioxygenase in patients with melanoma undergoing CTLA4 blockade. Clin Cancer Res. 2009;15(1):390-9.

82. Gupta R, Babb JS, Singh B, Chiriboga L, Liebes L, Adams S, et al. The Numbers of FoxP3+ Lymphocytes in Sentinel Lymph Nodes of Breast Cancer Patients Correlate With Primary Tumor Size but Not Nodal Status. Cancer Invest. 2011;29(6):419-25.

83. Ji R-R, Chasalow SD, Wang L, Hamid O, Schmidt H, Cogswell J, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother. 2012;61(7):1019-31.

84. Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human alphabeta T cell receptor diversity. Science. 1999;286(5441):958-61.

85. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature. 1988;334(6181):395-402.

86. Robins HS, Srivastava SK, Campregher PV, Turtle CJ, Andriesen J, Riddell SR, et al. Overlap and effective size of the human CD8+ T cell receptor repertoire. Sci Transl Med. 2010;2(47):47ra64.

87. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood. 2009;114(19):4099-107.

88. Emerson RO, Sherwood AM, Rieder MJ, Guenthoer J, Williamson DW, Carlson CS, et al. High-throughput sequencing of T cell receptors reveals a homogeneous repertoire of tumor-infiltrating lymphocytes in ovarian cancer. J Pathol. 2013;Sep 11. doi: 10.1002/path.4260.: [Epub ahead of print].

89. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature. 2002;419(6908):734-8.

90. Jinushi M, Hodi FS, Dranoff G. Therapy-induced antibodies to MHC class I chain-related protein A antagonize immune suppression and stimulate antitumor cytotoxicity. Proc Natl Acad Sci U S A. 2008;103(24):9190-5.

91. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature. 2005;436(7054):1186-90. Epub

2005/07/05. doi: nature03884 [pii]

10.1038/nature03884. PubMed PMID: 15995699; PubMed Central PMCID: PMC1352168.

92. Benedict SH, Yenice KM, Followill D, Galvin JM, Hinson W, Kavanagh B, et al. Stereotactic body radiation therapy: the report of AAPM Task Group 101. Med Phys. 2010;37(8):4078-101.

93. Chuong MD, Springett GM, Freilich JM, Park CK, Weber JM, Mellon EA, et al. Stereotactic body radiation therapy for locally advanced and borderline resectable pancreatic cancer is effective and well tolerated. Int J Radiat Oncol Biol Phys. 2013;86(3):516-22.

94. Fitzmaurice G, Laird N, Ware J. Applied Longitudinal Data Analysis. Sons JWa, editor2004.