Radiation and immune checkpoints blockade in metastatic NSCLC (BMS # CA209-632)

WCM IRB: 1607017434

NCT03168464

Document History

Document name	Version	Version date
Amendment 6	**7.0**	**31DEC2020**
Amendment 5	6.0	01.15.2020
Amendment 4	5.0	10.11.2019
Amendment 3	4.0	02.19.2019
Amendment 2	3.0	12.31.2018
Amendment 1	2.0	05.07.2018
Initial	1.0	07.24.2017

Protocol version 7.0 dated 31DEC2020

Radiation and immune checkpoints blockade in metastatic NSCLC (BMS # CA209-632)

IRB Protocol #: 1607017434
Version and date: V7.0 31DEC2020

Principal Investigator:

Silvia Formenti, M.D.
525 East 68th Street | Stich Radiation Center
New York, NY 10065
212-746-3608
212-746 8850
formenti@med.cornell.edu

Co-PI:

Ronald Scheff, M.D.
520 East 70th Street, Starr 341
New York, NY 10021
646-962-2066
rjs2002@med.cornell.edu

Co-Investigators:

Sandra Demaria, M.D.
525 East 68th Street | Stich Radiation Center
New York, NY 10065
212.746.6068
szd3005@med.cornell.edu

Encouse Golden, M.D.
525 East 68th Street | Stich Radiation Center
New York, NY 10065
212-746-3650
212-746 8850
eng2003@med.cornell.edu

Karla Ballman, Ph.D.
402 East 67th Street
New York, NY 10065
646-962-8023
kab2035@med.cornell.edu

Josephine Kang, M.D., Ph.D.
525 East 68th Street | Stich Radiation Center
New York, NY 10065
212.746.6600
jok9106@med.cornell.edu

Nicholas Sanfilippo, M.D.
525 East 68th Street | Stich Radiation Center
New York, NY 10065
212.746.3607
nis9129@med.cornell.edu

Ashish Saxena, M.D., Ph.D.
520 East 70th Street, Starr 341
New York, NY 10021
646-962-2066
ahs9018@med.comell.edu

Nasser Altorki, M.D.
Cardiothoracic Surgery
212-746-5156
nkaltorki@med.cornell.edu

Research Nurse:

Maria Fenton Kerimian, NP
525 East 68th Street | Stich Radiation Center
New York, NY 10065
212.746.6074
mak9186@med.cornell.edu

Study Contact:

Sharanya Chandrasekhar
525 East 68th Street | Stich Radiation Center
New York, NY 10065
646-962-2196
shc2043@med.comell.edu

Research/Data Coordinator:

Pragya Yadav
525 East 68th Street | Stich Radiation Center
New York, NY 10065
646-962-2199
pry2003@med.cornell.edu

Viji Nagaraj
525 East 68th Street | Stich Radiation Center
New York, NY 10065
646-962-3118
vin2009@med.cornell.edu

Sub Site:

Columbia University Medical Center

Principal Investigator at CUMC: Dr. Brian Henick - bh2682@cumc.columbia.edu

Co-investigators at CUMC:

Dr. Naiyer A. Rizvi - nar2144@cumc.columbia.edu

Dr. Catherine Shu - cas2145@cumc.columbia.edu

Data managers:

Melissa McAvoy – mm5088@ cumc.columbia.edu

Danial Otap – do2267@ cumc.columbia.edu

Leslie Segall – ls3429@ cumc.columbia.edu

Principal investigator	Catherine Shu (1)	cas2145@cumc.columbia.edu	212-305-3997
Sub-Investigator	Naiyer Rizvi (1)	nar2144@cumc.columbia.edu	212-305-3997
Sub-Investigator	Mark Stoopler (1)	mbs5@cumc.columbia.edu	212-305-8230
Sub-Investigator	Matthen Mathew (1)	mm4709@cumc.columbia.edu	212-305-3997
Sub-Investigator	Brian Henick (1)	bh2682@cumc.columbia.edu	212-305-3997
Sub-Investigator	Simon Cheng	sc3225@cumc.columbia.edu	
Clinical Research Manager	Natalie Busby (3)	nb2718@cumc.columbia.edu	212-305-8487
Regulatory Coordinator	Melissa McAvoy (4)	mm5088@cumc.columbia.edu	212-305-1440
Research Pharmacy Director	Elnaz Enjom (2)	chemorx@lists.cumc.columbia.edu	212-305-8684
Drug Shipments Contact	Research Pharmacy (2)	chemorx@lists.cumc.columbia.edu	212-305-8684
Research Pharmacists	Nour Trabilsy (2)	nt2511@cumc.columbia.edu	212-305-8684
Research Pharmacists	Manal Saad (2)	ms5271@cumc.columbia.edu	212-305-8684
Research Pharmacists	Anthony Galante (2)	ag4051@cumc.columbia.edu	212-305-8684
Clinical Research Coordinator	Natalie Sooksatan (3)	ns3240@cumc.columbia.edu	212-342-3747
Clinical Research Coordinator	Angelica Husni (3)	ah3574@cumc.columbia.edu	212-305-5480
Clinical Research Coordinator	Robert Garofano (3)	rfg2115@cumc.columbia.edu	212-304-5686
Clinical Research Coordinator	Priyanka Shah (3)	ps3051@cumc.columbia.edu	212-305-0787
Data Coordinator	Madeline Dansky (4)	md3446@cumc.columbia.edu	212-305-0787
Research Nurse	Kelly Murphy (1)	kam2312@cumc.columbia.edu	212-342-3142
Research Nurse	Vanessa Reed (1)	vr2405@cumc.columbia.edu	212-305-1440
Staff Assistant-Radiology	Hao Yang	Yh2580@cumc.columbia.edu	212-342-3018
Research Specimen Processor	Allison Rainey	ar3282@cumc.columbia.edu	212-342-6053

Document History

	Version	Version date
Amendment 6	**7.0**	**31DEC2020**
Amendment 5	6.0	01.15.2020
Amendment 4	5.0	10.11.2019
Amendment 3	4.0	02.19.2019
Amendment 2	3.0	12.31.2018
Amendment 1	2.0	05.07.2018
Initial	1.0	07.24.2017

Summary of changes Amendment 6 – version 7.0 dated 31DEC2020

Changes based on Dr. Wilkin's email dated 01.25.2021.

Modifying HIV exclusion criteria and Hep B and Hep C Criteria:

1. Patients with uncontrolled HIV infection. Note: participants with a plasma HIV viral load less than 200 copies/mL are eligible.
2. Patients with active Hepatitis B and Hepatitis C infection. Patients undergoing active therapy will be excluded from the trial.

Inclusion Criteria #11 modified to state the following:

1) **Criteria #11:** Patients may have brain metastases if these are stable for at least 2 weeks (including radiosurgery treated lesions) and patients are not symptomatic or steroid dependent; Baseline MRI will be required." Note these lesions are not considered primary targets for the purposes of this protocol

Exclusion Criteria: Based on amendment version 2.0 dated 07MAY2018. This amendment was already approved on 06/06/2018 (eIRB). The changes that is being made is strictly administrative and will add more clarity to the eligibility criteria.

Deleted #7 : Prior therapy with immune checkpoint blockade

Summary of changes Amendment 5 – version 6.0 Dated 15JAN2020

1. The baseline biopsy will be optional.

Summary of changes Amendment 4 – version 5.0 Dated 11OCT2019

1. Removing Dr. Eric Ko
2. Adding Dr. Encouse Golden as the Co-PI
3. Adding Dr. Giaccone to the protocol as a Co-investigator.
4. Columbia University changes: Dr. Henick will be the PI for Columbia University and Dr. Shu will be the Co-I for the study.
5. updating contact information for Study personnel.
6. Adding Viji Nagaraj as a data manager

Informed Consent:

1. The Principal investigator for Columbia University has been changed to Dr. Brian Henick.

Summary of changes Amendment 3 – version 4.0 dated 02/14/2019

1. Added Osimertinib to Inclusion Criteria #3.
2. Changed Nivolumab infusion to 360mg every 3 weeks instead of 240mg every 2 weeks, per BMS recommendations. This was not consistently changed in all sections.
3. Corrected minor typographical errors.

Informed consent:

Made changes to Nivolumab infusion (now 360mg every 3 weeks) throughout the ICF.

Summary of changes Amendment 2 – Version 3.0 Dated 12.31.2018

1. Revised accrual goal to 44 patients.
2. Added Columbia University as a sub-site for this trial
3. Updated inclusion criteria to include first-line metastatic (treatment-naïve) NSCLC patients.
4. Changing Nivolumab dose from 240 mg q2 weeks to 360mg q3 weeks.
5. Updated statistical considerations section.
6. Added Type I Interferon assessment assay in the correlative section.

7. Added Tumor mutational burden component to the correlative section of the protocol.
8. Added information for sub sites in relevant sections.
9. Adding Columbia University investigators and contact information
10. Adding baseline MRI in the inclusion criteria.
11. Adding PET/CT or CT (CAP) for imaging modality.
12. Updating blood collection for immune monitoring studies.
13. CTCAE v5.0 will be used in this study instead of CTCAE v4.0

Summary of changes: Amendment 1 – version 2.0 dated 05.07.2018

1. Updated personnel contact information
2. Replacing Christina Castro with Pragya Yadav.
3. Clarification of ipilimumab infusion time from 90 minutes to 30 min and updating the protocol for consistency.
4. Response evaluation time has been made consistent to 70 days (+/- 7)
5. Samples will be collected at baseline, at Day 22, at Day 70 and at 6 months.
6. Clarifying the KPS to state >= 70 in the eligibility criteria.
7. Removing check point inhibitors from inclusion criteria # 5
8. Adding prohibited medication to eligibility criteria –

   Patients in this study may not use vaccines for the treatment of cancer for up to one month before the first dose of ipilimumab. Concomitant systemic or local anti-cancer medications or treatments are prohibited while receiving study treatments.
9. Updated study calendar to add CBC and COMP to Day 1 of the infusion.
10. APPENDIX 22 – adding a sub-study as part of this protocol –
    "Innovative immune monitoring in a clinical trial of radiotherapy and immune checkpoint blockade"

PROTOCOL SYNOPSIS

Protocol Title	Radiation and immune checkpoints blockade in metastatic NSCLC
Sites	Weill Cornell Medicine – New York Presbyterian Columbia University, New York Presbyterian

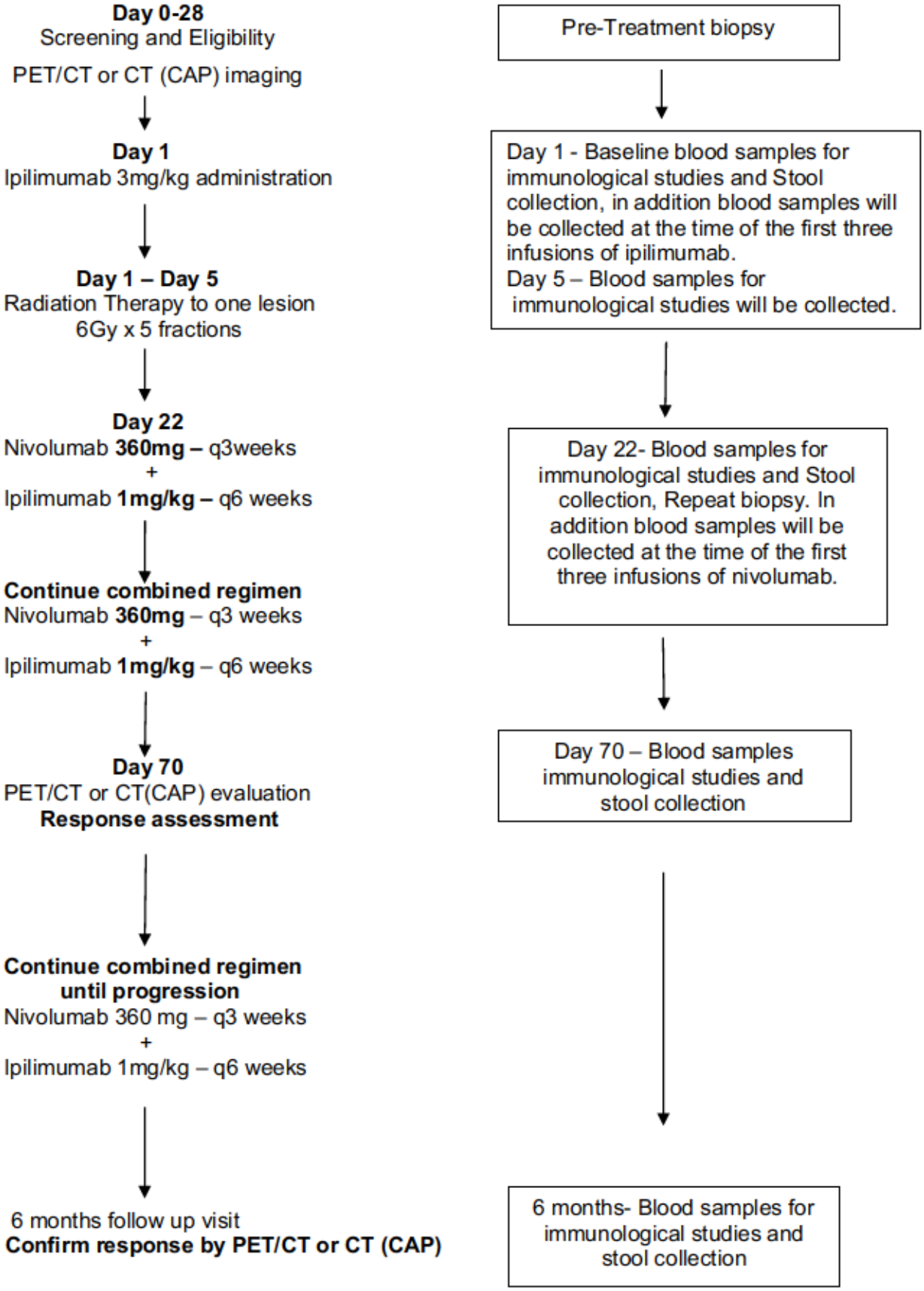
Study Schema	Metastatic NSCLC patients with a minimum of two metastatic lesions (at least one measurable) are eligible if they have an ECOG Performance Status of 0-1. Patients must be willing to undergo a required pre-treatment biopsy prior to enrolling onto the study. Non-ablative radiotherapy (6GyX5) is directed to one lesion on day 1 with Ipilimumab 3 mg/kg (± 24hrs from first RT dose). On day 22 the combined treatment of ipilimumab plus nivolumab will start (nivolumab 360mg q 3 weeks, ipilimumab 1mg/kg q 6 weeks), and administered until evidence of progression. Patients are re-imaged at Week 9 (day 70 ± 7) to evaluate for response (defined as an objective response by RECIST of the measurable metastatic sites outside the radiation field). This response will be evaluated assessing clinical and PET/CT responses in the non-irradiated measurable metastatic sites using RECIST 1.1.

Trial Objectives	**Primary Objectives:** To enhance the ORR to the combination of Ipi/Nivo in metastatic NSCLC by preceding it with a combination of Ipi/RT to convert the irradiated tumor into an *in situ* vaccine **Secondary objectives:** To explore whether early changes in TCR repertoire in peripheral blood are associated with response to treatment To explore if serum markers levels/changes are associated with response To explore associations of ORR with changes in the microbiome Progression Free survival and Overall Survival (OS) Time to progression and Duration of response (DOR)
Study Design	A Simon's two-stage optimal design is chosen for this trial. Fifteen patients are required for stage 1. If 5 or more responses are seen, then the trial will proceed to stage 2 and an additional 29 patients will be enrolled and treated. If a total of ≥ 18 objective responses are demonstrated the trial will achieve its main endpoint. We expect to accrue up to a total of 48 patients, independently from their PD-L1 status.

Accrual Goal (Total number of patients)	48 (44 evaluable patients and up to 4 additional patients accrued to replace those patients who withdraw or are not evaluable)
Accrual Rate (Number of patients expected per year)	20-25
FPFV (first patient first visit) **LPLV** (last patient last visit) **Follow Up**	September 1, 2017 October 31, 2022 5 years or until patient's death
Correlative Studies (e.g., PK/PD)	Tumor biopsy will be required at baseline and, whenever possible at day 22 to analyze changes in T cell infiltration and expression of PDL-1 and other markers by IHC. Tumor tissue will also be used to determine TCR repertoire and mutational load by deep sequencing. Blood for immune monitoring studies will be collected at baseline, end of radiation therapy, at the time of the first three infusions of ipilimumab, at the time of the first three infusions of nivolumab, at response evaluation (day 70), and at 6 month follow up. PBMC and serum/plasma will be stored for analysis of immune subsets by multiparameter flow cytometry and TCR repertoire, as well as levels of sCD25 and sMICA/B. Stool collection will occur before treatment, at day 22, at Day 70 and 6 months to explore changes of the microbiome during immune checkpoint blockade (ICB).

Criteria for Evaluation (Efficacy, safety, stopping rules, etc.)	**CLINICAL ENDPOINTS** Tumor Response will be evaluated using the RECIST best response (Partial Response (PR) + Complete Response (CR)). The irradiated lesion will be excluded from the assessment of response. Time to progression and duration of response are also endpoints of the study. **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, end of radiation therapy), at the time of the first three infusions of ipilimumab, at the time of the first three infusions of nivolumab, at response evaluation (Day 70 ± 7 days), and at 6 months. 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. In addition, Stool samples will be collected before treatment, at day 22, at Day 70 response (± 7 days) and 6 months to explore changes of the microbiome during ICB. Tissue biopsies will be obtained at baseline and at Day 22 (optional), post treatment from patients who consent to the optional biopsies.
Statistics	A Simon's two-stage optimal design will be used. The null hypothesis is that the true response rate is 31% will be tested against a one-sided alternative. If there are 4 or fewer responses in these 15 patients, the study will be stopped. Otherwise 29 additional patients will be accrued for a total of 44 patients. The null hypothesis will be rejected if 18 or more responses are observed in 44 patients. This design yields a type I error of 0.10 and power of 90% when the true response rate is 51%. The target sample size is 44 evaluable patients and up to 48 patients will be accrued to account for non-evaluable patients or patients who withdraw prior to the 9-week evaluation.

SCHEMA


Day 0-28
Screening and Eligibility
PET/CT or CT (CAP) imaging
Day 1
Ipilimumab 3mg/kg administration
Day 1 – Day 5
Radiation Therapy to one lesion
6Gy x 5 fractions
Day 22
Nivolumab 360mg – q3weeks
+
Ipilimumab 1mg/kg – q6 weeks
Continue combined regimen
Nivolumab 360mg – q3 weeks
+
Ipilimumab 1mg/kg – q6 weeks
Day 70
PET/CT or CT(CAP) evaluation
Response assessment
Continue combined regimen
until progression
Nivolumab 360 mg – q3 weeks
+
Ipilimumab 1mg/kg – q6 weeks
6 months follow up visit
Confirm response by PET/CT or CT (CAP)
Pre-Treatment biopsy
Day 1 - Baseline blood samples for immunological studies and Stool collection, in addition blood samples will be collected at the time of the first three infusions of ipilimumab.
Day 5 – Blood samples for immunological studies will be collected.
Day 22- Blood samples for immunological studies and Stool collection, Repeat biopsy. In addition blood samples will be collected at the time of the first three infusions of nivolumab.
Day 70 – Blood samples immunological studies and stool collection
6 months- Blood samples for immunological studies and stool collection

1.0 INTRODUCTION

1.1 Research Hypotheses

This Phase I-II study is designed to test the hypotheses that:

1) Initial local radiotherapy during anti-CTLA-4 blockade with Ipilimumab treatment is safe and it increases the objective response rate to immune checkpoint blockade (ICB) by the combination of ipilimumab and nivolumab by 20%.

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

3) Modifications in the stool microbiome of these patients correlates with response to RT+ ICB.

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 [1, 2]. 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 [3-5]. Pre-clinical data generated by us and others[6] supported the initiation of a proof of principle clinical trial of Ipilimumab and localized Radiation in metastatic NSCLC: a 33% ORR in pretreated NSCLC patients who completed 4 cycles of Ipilimumab after localized radiotherapy was demonstrated[7].

Nivolumab is an established therapy for metastatic NSCLC, based on 2 Phase III trials showing OS benefit when compared to SOC [8, 9].

Since a high response rate in the same patient population was reported when adding nivolumab (anti-PD-1) to ipilimumab [10] we propose to converge these promising results and test the use of radiotherapy before the combination of Ipi/nivo in metastatic NSCLC, with the rationale of converting the primary tumor into an in situ vaccine.

1.2.1 CTLA-4 and T-Cell Activation

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 (APCs)[11]. 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[12]. The fact that tumor cells do not express B7 contributes to their poor capacity to elicit immune responses [13, 14]. 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 [15, 16].

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 [17]. 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 [18]. 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 super-antigens in mice [19-22]. 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 [23]. Clinical trials have also provided the proof of principle that it is possible to treat cancer successfully by immune manipulation [24, 25]. 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 [26]. 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[27, 28]. 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[29, 30]. 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 [31].

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. 3). We have applied the term “abscopal” (ab-scopus, away from the target) originally introduced by Mole et al. [32] to define a systemic effect elicited by radiotherapy in the presence of immunotherapy[6]. 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 was 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, as 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[32]. 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 [37-39].

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 [37, 40]. The findings inspired a trial testing the combination of subcutaneous GM-CSF (125 µg/m2) 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 [41].

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 [42].

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 [7] . Postow et al.[43] 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 [44]. 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 median 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 [45]. 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 originally reported the first abscopal case in a NSCLC patient who had undergone radiotherapy to a metastatic lesion followed by 4 cycles of ipilimumab [46].

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/m2 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. Thereafter, continued maintenance therapy with pemetrexed 500 mg/m2 BSA alone, given every 3 weeks. 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. After an initial response to RT in September 2011, PET/CT revealed increased metabolic activity and size of the right upper lobe nodule. The patient resumed treatment with pemetrexed 500 mg/m2 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/m2 and vinorelbine 30 mg/m2 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: the left lower lobe nodule (1.9 x 1.2 cm, SUV 23.1), mediastinal lymph node (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).

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. The most metabolically active liver mass, located in the caudate lobe, was selected as the RT target (Figure 1). RT to a total dose of 30 Gy distributed over 5 fractions of 6 Gy each 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, well tolerated without any treatment related adverse events. 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. Two additional cycles of ipilimumab were given, but maintenance infusions of ipilimumab were not given afterwards. This patient remains disease-free at 43 months follow-up, without any additional cancer treatment. This case report triggered the design and conduction of NYU S14-00208, "PHASE II STUDY OF COMBINED IONIZING RADIATION AND IPILIMUMAB IN METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC)" with ipilimumab provided by Bristol-Myers Squibb.

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/m2) 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). 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 adverse events reported in Ipi groups (e.g., rash, pruritus, and diarrhea) [47].

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 3/4 AEs, and 2 partial responses in the tremelimumab arm, but the treatment did not significantly improve PFS (20.9% vs. 14.3%)[48]. Table 1 summarizes the results of two randomized studies on CTLA-4 blockade in metastatic NSCLC: modest response rates were demonstrated, with no patient achieving a complete response.

Table 1

Prospective studies of CTLA-4 blockade in locally advanced and metastatic NSCLC

Reference	stage	RANDOMIZATION	# PTS
Zatloukal et al ASCO 2009 [48]	LOCALLY ADV /METS	-TREMELIMUMAB (15 mg/kg) VERSUS BSC	87
Lynch et al JCO 2012 [47]	Stage III/IV	Carbo/Taxol vs Carbo/T with Ipi (10mg/kg) Carbo/T and Ipi sequential (10mg/kg)	204

1.2.8 NYU S14-00208/ NCT02221739: Ipilimumab and local RT in chemo-refractory metastatic NSCLC

NYU S14-00208/NCT02221739 was a single institution prospective trial that tested the hypothesis that the addition of local radiotherapy to a metastatic site in metastatic chemo-resistant NSCLC could enhance the ORR of CTLA-4 blockade by ipilimumab. Patients needed to have progressed after at least one cycle of chemotherapy. Additional eligibility criteria included measurable disease in at least 2 sites, controlled brain metastases and ECOG PS 0-2. The study was a Phase II, optimal two-stage Simon design; 10 patients were to be treated in stage 1 at 6Gy x5; if no grade ≥3 toxicities, 10 additional patients treated at 9.5Gy x3; ipilimumab 3mg/kg q3 weeks x4 infusions. If ≥1 abscopal responses in stage 1, the trial was to proceed to enroll an additional 19 patients. Primary endpoint was response per RECIST v1.1 (baseline and post treatment PET/CT) Secondary endpoint was best abscopal response, as well as immunologic biomarkers. Thirty-nine patients accrued to the trial: only 18 were able to complete treatment of radiotherapy plus 4 cycles of Ipilimumab, mostly due to the advanced ECOG score inclusion of that trial (ECOG eligibility up to 2). Based on intent to treat ORR was 18%(7/39). In addition, ORR was 33% among the 18 patients who completed treatment: there were 2 CR, 5 Pr. 5 SD and 9 PD. Figure 1 shows the waterfall plot of response for the study, as measured in lesions

outside the irradiated field (abscopal response). Figure 3 demonstrates at a 1-year median follow up a survival difference between the patients who progressed versus the responders. Median survival is still not reached for CR/PR/SD versus 9 months for patients with PD (log-rank test:p = 0.016; HR = 9.174).

Figure 1

Best abscopal response of patients who completed treatment in NYU S14-00208

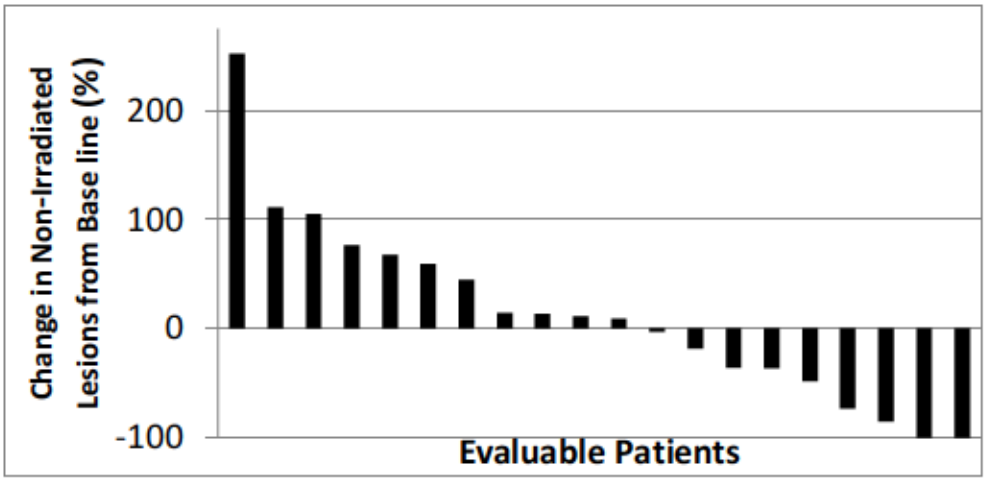
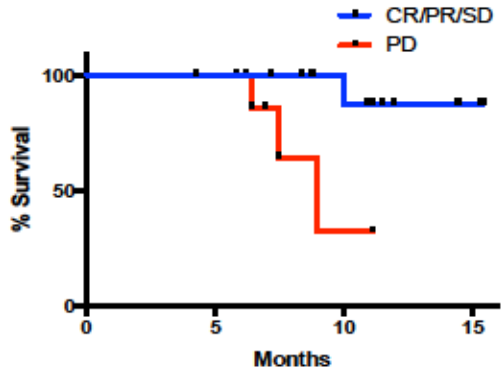


Figure 2

NYU S14-00208, Survival based on OR (median f/u one year)



(Unpublished data, manuscript in preparation)

1.2.9 Immunological checkpoint blockade in NSCLC with anti-PD1

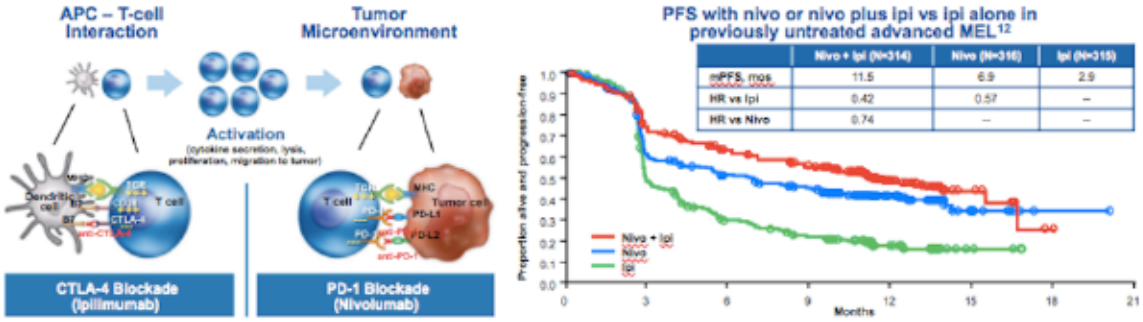
Based on the promising results of immunotherapy using checkpoint inhibitors such as Ipi in the malignant melanoma trials, and the early results seen with NSCLC, interest in other immune checkpoints and their inhibitors have grown rapidly. One such immune checkpoint is regulated by the programmed death 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 “brake” 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 [49]. 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%.

1.2.10 Combination of Anti-CTLA-4 and anti PD-1 in melanoma and NSCLC

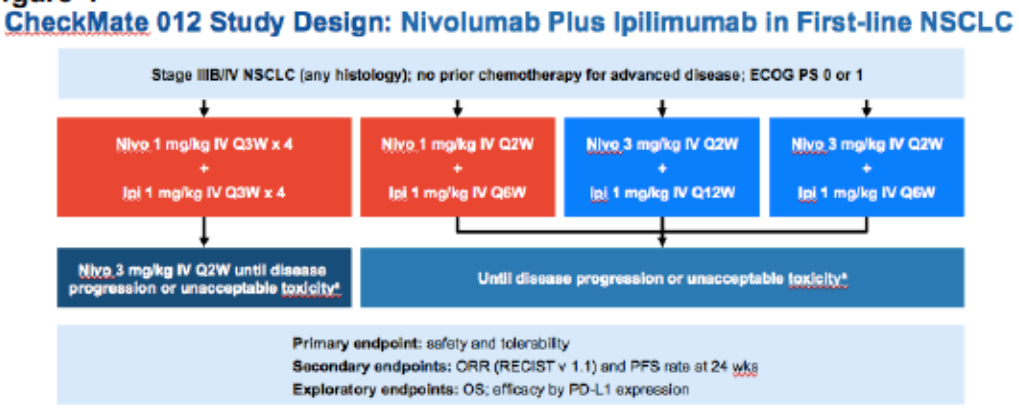
Enhanced therapeutic ratio was demonstrated by the combination of ICB both in melanoma and NSCLC [50, 51]. Figure 3 depicts the rationale for combining the two ICB (left panel and the reported PFS of nivolumab + Ipilimumab in melanoma.

Figure 3



CheckMate 012 tested different combinations of ICB in NSCLC [10], described in Fig.4

Figure 4



First-line therapy with nivolumab plus ipilimumab demonstrated ORR ranging from 39% - 47% and median PFS of 8.1 months. Importantly, nivolumab plus ipilimumab were associated with a favorable safety profile, with no treatment-related deaths and low frequency of treatment-related grade 3–4 AEs leading to discontinuation in only 11%-13% of the treated patients. While clinical activity was observed regardless of tumor PD-L1 expression, enhanced activity in ≥1% PD-L1 expressors was noticed (fig.5)

Figure 5

	≥1% PD-L1 expression				<1% PD-L1 expression			
	Nivo 1 + Ipi 1 Q3W (n = 12)	Nivo 1 Q2W + Ipi 1 Q6W (n = 21)	Nivo 3 Q2W + Ipi 1 Q12W (n = 21)	Nivo 3 Q2W + Ipi 1 Q6W (n = 23)	Nivo 1 + Ipi 1 Q3W (n = 13)	Nivo 1 Q2W + Ipi 1 Q6W (n = 7)	Nivo 3 Q2W + Ipi 1 Q12W (n = 9)	Nivo 3 Q2W + Ipi 1 Q6W (n = 7)
ORR, %	8	24	48	48	15	14	22	0
mPFS, wks (95% CI)	11.5 (7.1,)	21.1 (11.4,)	34.6 (15.9, 35.3)	NR (15.4,)	34.0 (8.9,)	NR (10.1,)	23.1 (4.0,)	10.3 (7.4, 12.7)
PFS rate at 24 wks, % (95% CI)	42 (15, 67)	40 (18, 61)	74 (48, 88)	65 (42, 81)	57 (25, 80)	NC	39 (9, 69)	0

Among patients with <1% PD-1 expression ORR was 0-22%, warranting the investigation of strategies to enhance response. In parallel, a registration phase 3 trial comparing nivolumab or nivolumab plus ipilimumab vs PT-DC in patients with chemotherapy-naïve stage IV or recurrent NSCLC is ongoing (CheckMate 227; NCT02477826).

1.2.11 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.** 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 [40]. An analogous effect was recently reported in a case of melanoma [43] and the first reported case of NSCLC described above [46] and in a trial testing Ipilimumab and RT as second line therapy for metastatic NSCLC [7] (manuscript in preparation). 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 **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 were particularly promising, suggesting that non-ablative localized radiotherapy "repositioned" Ipilimumab in metastatic NSCLC. **These data justify the rationale for testing an induction of ipilimumab and radiation before a nivo/ipi combination**. The trial is powered to demonstrate increase of ORR by 20% with a Simon's optimal two-stage design.

1.3 Summary of Results of Ipilimumab Investigational Program

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.

Nivolumab is a human monoclonal antibody that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes. Binding of PD-1 to its ligand, programmed death ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration.

OPDIVO™ (nivolumab) is approved for use in multiple countries including the United States (US, Dec-2014), the European Union (EU, Jun-2015) and Japan (Jul-2014).

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 stomach or colon.

Complete information on the pre-clinical toxicology studies can be found in the nivolumab Investigator Brochure (IB). Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family. Nivolumab inhibits the interaction of PD-1 with its ligands, PD-L1 and PD-L2 resulting in enhanced T-cell proliferation and interferon-gamma (IFN-γ) release in vitro. Nivolumab binds with high affinity to activated human T-cells expressing cell surface PD-1 and to cynomolgus monkey PD-1. In a mixed lymphocyte reaction (MLR), nivolumab promoted a reproducible concentration-dependent enhancement of IFN-γ release.

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab was well tolerated at doses up to 50 mg/kg, administered weekly for 5 weeks, and at doses up to 50mg/kg, administered twice weekly for 27 doses. While nivolumab alone was well tolerated in cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with other immunostimulatory agents.

Clinical Safety

Ipilimumab immunotherapy was FDA approved for the treatment of metastatic melanoma in March 2011. 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 [45], 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.

Nivolumab monotherapy is approved for the treatment of unresectable or metastatic melanoma in multiple countries including Japan, the US, and the EU. Nivolumab is also approved for the treatment of metastatic NSCLC and renal cell carcinoma in the US.

Qualitative and qualitative information received as of 13-Apr-2016 has been consistent with the established safety profile as observed in clinical trials. No new safety concerns were identified based on the global post marketing reports.

Nivolumab 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 pan hypopituitarism, 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.

Nivolumab in trial 1, randomized, open-label trial in which 370 patients with unresectable or metastatic melanoma received nivolumab 3 mg/kg every 2 weeks (n=268) or investigator's choice of chemotherapy (n=102), either dacarbazine 1000 mg/m2 every 3 weeks or the combination of carboplatin AUC 6 every 3 weeks plus paclitaxel 175 mg/m2 every 3 weeks (see Clinical Studies [47]). The median duration of exposure was 5.3 months (range: 1 day-13.8+ months) with a median of eight doses (range: 1 to 31) in nivolumab-treated patients and was 2 months (range: 1 day-9.6+ months) in chemotherapy treated patients. In this ongoing trial, 24% of patients received nivolumab for greater than 6 months and 3% of patients received nivolumab for greater than 1 year.

Clinically significant adverse reactions were also evaluated in 574 patients with solid tumors enrolled in two clinical trials receiving nivolumab at doses of 0.1 to 10 mg/kg every 2 weeks, supplemented by immune-mediated adverse reaction reports across ongoing clinical trials.

Nivolumab was discontinued for adverse reactions in 9% of patients. Twenty-six percent of patients receiving nivolumab had a drug delay for an adverse reaction. Serious adverse reactions occurred in 41% of patients receiving nivolumab. Grade 3 and 4 adverse reactions occurred in 42% of patients receiving nivolumab. The most frequent Grade 3 and 4 adverse reactions reported in 2% to less than 5% of patients receiving nivolumab were abdominal pain, hyponatremia, increased aspartate aminotransferase, and increased lipase. Other clinically important adverse reactions in less than 10% of patients treated with nivolumab were:

Cardiac Disorders: ventricular arrhythmia

Eye Disorders: iridocyclitis

General Disorders and Administration Site Conditions: infusion-related reactions

Investigations: increased amylase, increased lipase

Nervous System Disorders: dizziness, peripheral and sensory neuropathy

Skin and Subcutaneous Tissue Disorders: exfoliative dermatitis, erythema multiforme, vitiligo, psoriasis.

The most common adverse reaction (>20%) was rash.

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.

Pulmonary Adverse Events:

Pulmonary AEs have been observed following treatment with nivolumab. The recommended management of pulmonary AEs is provided in Appendix 9. The frequency of pulmonary AEs may be greater with nivolumab combination therapies than with nivolumab *monotherapy. The majority of cases reported were Grade 1 or 2, and subjects presented with* either asymptomatic radiographic changes (eg, focal ground glass opacities and patchy infiltrates) or with symptoms of dyspnea, cough, or fever. Subjects with reported Grade 3 or 4 pulmonary AEs were noted to

have more severe symptoms, more extensive radiographic findings, and hypoxia. Pulmonary AEs have been reported in subjects with a variety of tumor types; however, there have been numerically more cases in subjects with NSCLC. It is not clear whether the underlying NSCLC is a distinct risk factor, or if subjects with NSCLC are more likely to develop radiographic changes and symptoms for which it is difficult to distinguish between nivolumab-related and unrelated causes. At this time, no other underlying risk factor, including prior radiotherapy, presence of lung metastases, or underlying pulmonary medical history, has yet to be identified. Asymptomatic subjects were typically managed with dose delay. Subjects with Grade 2 pneumonitis were managed with dose delay, treated with corticosteroids, and had resolution of pneumonitis within days to weeks. In cases where nivolumab treatment was restarted, recurrence of pneumonitis was infrequently reported across the nivolumab program. Subjects with more severe cases of pneumonitis can be difficult to treat. In a few cases, subjects who did not initially respond to corticosteroids were administered anti-tumor necrosis factor therapy (infliximab) and/or cyclophosphamide. In some of these cases, pneumonitis began to resolve following the use of these additional therapies. Guidelines on the recommended management of pneumonitis and other pulmonary AEs are found in. Early recognition and treatment of pneumonitis is critical to its management. Subjects should be advised to seek medical evaluation promptly if they develop new-onset dyspnea, cough, or fever or if they have worsening of these baseline symptoms. As respiratory symptoms are common in subjects with cancer (eg, NSCLC), it is important that an evaluation/work-up distinguishes between non-drug-related causes (eg, infection or progression of disease) and a possible drug-related pulmonary toxicity as the management of these events can be quite different. For symptomatic nivolumab-related pneumonitis, the principal treatment is corticosteroids. All subjects with Grade 3-4 pneumonitis should discontinue nivolumab and initiate treatment with high doses of corticosteroids. Consultation with a BMS medical monitor should be sought for all suspected cases of pneumonitis.

Gastrointestinal Adverse Events

Gastrointestinal AEs have been observed following treatment with nivolumab .Most cases of diarrhea were of low grade (Grade 1-2). Colitis occurred less frequently than diarrhea. High-grade cases of diarrhea and colitis were managed with corticosteroids and, in all cases, the events resolved.

The recommended management of Endocrinopathies management AEs is provided in Appendix 6. The recommended management of GI AEs is provided in Appendix 3. Early recognition and treatment of diarrhea and colitis are critical to their management. Subjects should be advised to seek medical evaluation if they develop new-onset diarrhea, blood in stool, or severe abdominal pain or if they have worsening of baseline diarrhea. As GI symptoms are common in subjects with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (eg, infection or progression of disease) and a possible drug-related AE as the management can be quite different. The principal treatment for high-grade GI AEs is corticosteroids (Appendix 3). Caution should be taken in the use of narcotics in subjects with diarrhea, colitis, or abdominal pain as pain medicines may mask the signs of colonic perforation.

Consultation with a BMS medical monitor should be sought for all moderate- and high-grade cases of GI AEs.

Diverticular Perforation

The prevalence of diverticulosis in the general population is common and increases with age

from 10% under 40 years of age to approximately 50% over 60 years of age. Approximately10% to 25% of subjects with diverticulosis develop diverticulitis. Perforation occurs in 50% to 70% of instances of complicated diverticulitis. Corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), and opioid analgesics are known risk factors for diverticular perforation. Given the high prevalence of diverticulosis and diverticulitis in the general population, it is expected that some nivolumab-treated subjects will have these conditions concurrently with their malignancy. Cases of diverticular perforation while on concomitant corticosteroids (6 cases) or NSAID (1 case) were observed in nivolumab program. While there is insufficient evidence to suggest that diverticulosis or diverticulitis is a predisposing factor for GI perforation following nivolumab administration, clinical caution should be exercised, as appropriate, for subjects on concomitant medications of corticosteroids, NSAID, or opioid analgesics. In addition, be vigilant for signs and symptoms of potential perforation, especially in subjects with known diverticular disease.

Hepatic Adverse Events

Hepatic AEs, including elevated liver function tests (LFTs) and, infrequently, DILI, have been observed following treatment with nivolumab and nivolumab in combination with ipilimumab. Most cases were of low or moderate grade. Higher-grade hepatic AEs, including DILI, were managed with corticosteroids (with or without mycophenolate mofetil) and, in almost all cases, the events resolved. The recommended management of hepatic AEs is provided in Appendix 3. Early recognition and treatment of elevated LFTs and DILI are critical to their management. Subjects should be advised to seek medical evaluation if they notice jaundice (yellow appearance of skin or sclera) or if they develop bruising, bleeding, or right-sided abdominal pain. Physicians should monitor LFTs prior to each nivolumab treatment. As LFT abnormalities are common in subjects with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (eg, infection, progression of disease, concomitant medications, or alcohol) and a possible drug-related AE as the management can be quite different. The principal treatment for high-grade hepatic AEs is corticosteroids (Appendix 4). Consultation with a BMS medical monitor should be sought for all moderate- and high-grade hepatic AEs.

Endocrinopathies

Endocrinopathies have been observed following treatment with nivolumab. Most cases were of low or moderate grade. The events have typically been identified through either routine periodic monitoring of specific laboratories (eg, TSH) or as part of a work-up for associated symptoms (eg, fatigue). Events may occur within weeks of beginning treatment, but also have been noted to occur after many months (while still on treatment). More than 1 endocrine organ may be involved (eg, hypophysitis [pituitary inflammation] may need to be evaluated at the time adrenal insufficiency or thyroid disorder is suspected). Moderate- to high-grade cases were managed with hormone replacement therapy and, in some cases, with the addition of corticosteroids. In some cases, nivolumab treatment was held until adequate hormone replacement was provided.

Guidelines on the recommended management of endocrinopathies are provided in Appendix 6.

Early recognition and treatment of endocrinopathies are critical to its management. Subjects should be advised to seek medical evaluation if they notice new-onset fatigue, lightheadedness, or difficulty with vision or if baseline fatigue worsens. As fatigue is common in subjects with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (eg, progression of disease, anemia, concomitant medications, or depression) and a possible drug-related AE as the management can be quite different. The principal management of endocrinopathies is hormone replacement therapy. For subjects with moderate- or high-grade events, corticosteroids may also be used (Appendix 6). Consultation with a BMS medical monitor

should be sought for all moderate- and high-grade cases of Endocrinopathies.

Hypothyroidism and Hyperthyroidism

OPDIVO can cause autoimmune thyroid disorders. Monitor thyroid function prior to and periodically during OPDIVO treatment. Administer hormone-replacement therapy for hypothyroidism. Initiate medical management for control of hyperthyroidism. There are no recommended dose adjustments of OPDIVO for hypothyroidism or hyperthyroidism.

In patients receiving OPDIVO as a single agent, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 9% (171/1994) of patients; the median time to onset was 2.9 months (range: 1 day to 16.6 months). Approximately 79% of patients with hypothyroidism received levothyroxine and 4% also required corticosteroids. Resolution occurred in 35% of patients.

Hyperthyroidism occurred in 2.7% (54/1994) of patients receiving OPDIVO as a single agent; the median time to onset was 1.5 months (range: 1 day to 14.2 months). Approximately 26% of patients with hyperthyroidism received methimazole, 9% received carbimazole, 4% received propylthiouracil, and 9% received corticosteroids. Resolution occurred in 76% of patients.

In patients receiving OPDIVO with ipilimumab, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 22% (89/407) of patients; the median time to onset was 2.1 months

(range: 1 day to 10.1 months). Approximately 73% of patients with hypothyroidism or thyroiditis received levothyroxine. Resolution occurred in 45% of patients.

Hyperthyroidism occurred in 8% (34/407) of patients receiving OPDIVO with ipilimumab: the median time to onset was 23 days (range: 3 days to 3.7 months). Approximately 29% of patients with hyperthyroidism received methimazole and 24% received carbimazole. Resolution occurred in 94% of patients. Please refer to Appendix 6 for Endocrinopathy management algorithm.

Immune-mediated endocrinopathies, including life-threatening cases, can occur with YERVOY.

Monitor patients for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper-or hypothyroidism. Patients may present with fatigue, headache, mental status changes, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated.

Monitor clinical chemistries, adrenocorticotropic hormone (ACTH) level, and thyroid function tests at the start of treatment, before each dose, and as clinically indicated based on symptoms. In a limited number of patients, hypophysitis was diagnosed by imaging studies through enlargement of the pituitary gland.

Withhold YERVOY dosing in symptomatic patients and consider referral to an endocrinologist. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent, and initiate appropriate hormone replacement therapy

Skin Adverse Events

Rash and pruritus were the most common skin AEs observed following treatment with nivolumab. The rash was typically focal with a maculopapular appearance occurring on the trunk, back, or extremities. Most cases have been of low or moderate grade. In some cases, rash and pruritus resolved without intervention. Topical corticosteroids have been used for some cases of rash. Anti-histamines have been used for some cases of pruritus. More severe cases responded to systemic corticosteroids.

Subjects should be advised to seek medical evaluation if they notice new-onset rash. Early consultation with a dermatology specialist and a biopsy should be considered if there is uncertainty as to the cause of the rash, or if there is any unusual appearance or clinical feature associated with it. Other drugs that may cause rash should be considered in the differential and, if possible, discontinued. In addition, careful evaluation of potential benefit-risk is necessary when considering the use of nivolumab or ipilimumab in a patient who has previously experienced a severe or life-threatening skin adverse reaction on a prior immune-stimulating therapy.

Guidelines on the recommended management of skin AEs are provided. The principal treatment for skin AEs, such as rash and pruritus, consists of symptomatic management. Topical corticosteroids can be used for low- to moderate-grade focal rash. Systemic corticosteroids should be used for diffuse and high-grade rash. Consultation with a BMS medical monitor should be sought for all moderate- and high-grade cases of skin AEs.

Rare cases of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), some with fatal outcome, have been observed. If symptoms or signs of SJS or TEN appear, nivolumab or nivolumab in combination with ipilimumab should be withheld and the patient referred for specialized care for assessment and treatment. If the patient has confirmed SJS or TEN, permanent discontinuation of nivolumab or nivolumab in combination with ipilimumab is recommended.

Renal Adverse Events

Elevated creatinine and biopsy-confirmed tubulointerstitial nephritis and allergic nephritis have been infrequently observed following treatment with nivolumab. The frequency of renal AEs may be greater with nivolumab combination therapies than with nivolumab monotherapy. Most cases were Grade 2 or 3 and based on creatinine elevation. Subjects with a history of RCC or prior nephrectomy did not appear to be at higher risk. Events were managed with corticosteroids and, in all cases, renal function partially or fully improved.

The recommended management of renal AEs is provided in Appendix 8. Physicians should monitor creatinine regularly. As creatinine abnormalities are common in subjects with cancer and other comorbidities, it is important that an evaluation/work-up distinguishes between non- drug-related causes (e.g., dehydration, concomitant medications, hypotension, or progression of disease) and a possible drug-related AE as the management can be quite different. The principal treatment for renal AEs is corticosteroids (Appendix 8). Consultation with a BMS medical monitor should be sought for all moderate- and high-grade cases of renal AEs.

Neurologic Adverse Events

Neurologic AEs have been uncommonly observed following treatment with nivolumab. The frequency of neurologic AEs may be greater with nivolumab + ipilimumab combination therapies than with nivolumab monotherapy or other nivolumab combinations. Neurologic AEs can manifest as central abnormalities (e.g., aseptic meningitis, encephalopathy, or encephalitis) or peripheral sensory/motor neuropathies (e.g., Guillain-Barre Syndrome, myasthenia gravis complicated with sepsis and fatality). The onset has been observed as early as after a single treatment with the nivolumab + ipilimumab combination. The recommended management of neurologic AEs is provided in Appendix 7. Early recognition and treatment of neurologic AEs is critical to its management. Subjects should be advised to seek medical evaluation if they notice impairment in motor function (e.g., weakness), changes in sensation (e.g., numbness), or

symptoms suggestive of possible central nervous system abnormalities such as new headache or mental status changes. As neurologic symptoms can be common in subjects with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (eg, progression of disease, concomitant medications, or infection) and a possible drug-related AE as the management can be quite different. The principal treatments for neurologic toxicity are dose delay, corticosteroids, and IV immunoglobulin as outlined in the safety algorithm (Appendix 7). For high-grade related neurological AEs, nivolumab should be discontinued. Consultation with a BMS medical monitor should be sought for all moderate- and high-grade cases of neurologic AEs.

Infusion Reactions

Infusion reactions, including high-grade hypersensitivity reactions, following administration of nivolumab are uncommon. Investigators are advised to monitor for fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty in breathing during and immediately after administration of nivolumab. Study protocols provide explicit guidance on the management of infusion-related reactions.

Lipase/Amylase Elevations

Asymptomatic elevations in lipase and amylase have been reported. In monotherapy studies, lipase and amylase levels were not systematically monitored, so an estimate of the frequency of asymptomatic lipase/amylase elevations is unknown. In studies evaluating the safety of the nivolumab + ipilimumab combination in multiple tumor types, lipase and amylase levels were systematically monitored, and elevations in any grade of lipase/amylase were consistently noted in approximately 10% to 30% of subjects. Very few subjects reported associated symptoms (e.g., abdominal pain) or radiographic findings (e.g., stranding) consistent with pancreatitis. Thus, there does not seem to be clinical significance to the elevated laboratory values.

As lipase/amylase abnormalities are not uncommon in subjects with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (e.g., progression of disease, concomitant medications, or alcohol) and a possible drug-related cause as the management can be quite different. The recommended management of nivolumab-related elevated lipase/amylase values centers around close observation. Physicians should ensure that subjects have no associated symptoms consistent with pancreatitis, such as abdominal pain.

Corticosteroids do not seem to alter the natural history of lipase/amylase elevations. Laboratory values tend to fluctuate on a day-to-day basis and eventually return to baseline or low grade over the course of weeks, whether or not subjects receive corticosteroids. Asymptomatic elevations should be monitored approximately on a weekly basis, and nivolumab should be held per protocol instructions. For sustained asymptomatic Grade 4 elevations, nivolumab should be discontinued per protocol instructions. For subjects with elevated lipase/amylase and symptoms consistent with possible pancreatitis, nivolumab should be discontinued, and consultation with a gastroenterologist should be considered. Consultation with a BMS medical monitor should be sought for all high-grade cases of elevated lipase/amylase.

Uveitis and Visual Complaints

Immune therapies have been uncommonly associated with visual complaints. Inflammation of components within the eye (e.g., uveitis) is an uncommon, but clinically important, event. Uveitis may occur more frequently with nivolumab + ipilimumab combination therapy than with nivolumab monotherapy or nivolumab in combination with other therapies. An ophthalmologist should evaluate visual complaints with examination of the conjunctiva, anterior and posterior

chambers, and retina. Topical corticosteroids may be used to manage low-grade events. Low-grade events that do not resolve and high-grade events should be managed with systemic corticosteroids. Consultation with a BMS medical monitor should be sought for all cases of ocular inflammatory events. Complaints of double vision should also prompt medical evaluation. In addition to ocular inflammatory events, a work-up should also consider pituitary inflammation as a cause.

Other Immune-mediated Adverse Events

For suspected immune-related adverse reactions, adequate evaluation should be performed to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, nivolumab or nivolumab in combination with ipilimumab should be withheld and corticosteroids administered. Upon improvement, nivolumab or nivolumab in combination with ipilimumab may be resumed after corticosteroid taper. Nivolumab or nivolumab in combination with ipilimumab must be permanently discontinued for any severe immune-related adverse reaction that recurs and for any life-threatening immune-related adverse reaction.

Overdose, Warnings, and Precautions

There is no available information concerning overdose with nivolumab. Depending on the symptoms and/or signs leading to the suspicion of overdose, supportive medical management should be provided. There is no specific antidote.

Precautions for Women of Childbearing Potential

However, 2 cases of human in-utero exposure to nivolumab (involving the fetuses of female partners of male subjects receiving nivolumab) were reported; one woman had a live birth noted as preterm (37 weeks) and the pregnancy outcome in the other woman has not been reported. Additionally there was 1 case of an ectopic pregnancy in a female patient. Given the potential risk suggested by preliminary data from nonclinical and clinical data, dosing during pregnancy will continue to be prohibited. In addition, women of childbearing potential (WOCBP) receiving nivolumab will be instructed to adhere to contraception for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 7 months after the last dose of nivolumab.

These durations have been calculated using the upper limit of the half-life for nivolumab (~26 days) and are based on the recommendation that WOCBP use contraception for 5 half-lives plus 30 days, and men who are sexually active with WOCBP use contraception for 5 half-lives plus 90 days after the last dose of nivolumab. Females should not breastfeed while receiving nivolumab and for any subsequent protocol-specified period. For ONO studies, refer to individual study protocols for the duration of WOCBP contraception use.

Embryo-Fetal Toxicity

Based on its mechanism of action and data from animal studies, nivolumab can cause fetal harm when administered to a pregnant woman. In animal reproduction studies, administration of nivolumab to cynomolgus monkeys from the onset of organogenesis through delivery resulted in increased abortion and premature infant death. Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to use effective contraception during treatment with a nivolumab-containing regimen and for at least 5 months after the last dose of nivolumab.

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 multi-organ 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.

Drug related deaths are rare with nivolumab treatment (~0.4%). For details on all drug-related deaths please refer to the most recent version of the IB.

Clinical Efficacy

Treatment with ipilimumab has demonstrated clinically important and durable tumor responses in several malignancies including melanoma, prostate cancer, and renal cell carcinoma. Our preliminary data suggest that when combined with radiotherapy, ipilimumab is "re-positioned" in this role in metastatic lung cancer [7, 46] with 33% OR in patients completing four cycles of ipilimumab and radiotherapy.

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 = 0.022). 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 nivolumab, and radiotherapy in patients with metastatic NSCLC. Ipilimumab, although it is safe and FDA approved for the treatment of metastatic melanoma, it is associated with AEs that can cause morbidity and (rarely) even death.

Specifically, at the dose used in this protocol (3 mg/kg, the FDA approved dose) will be used

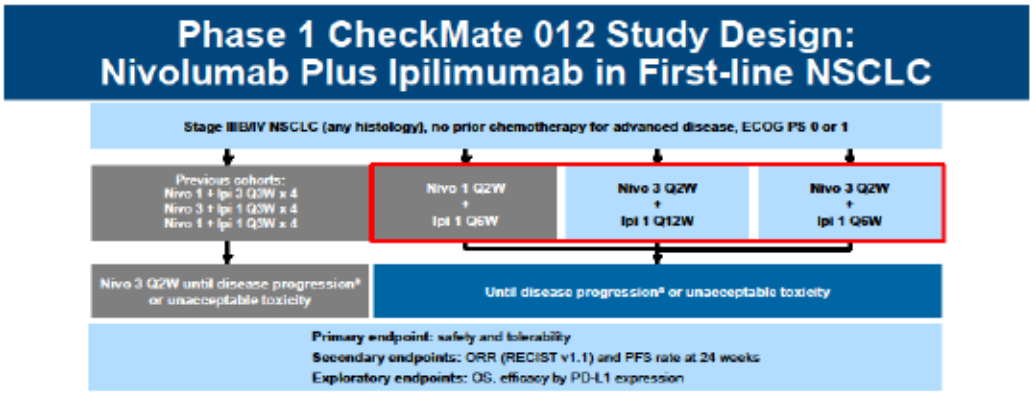
only once, in combination with initial radiotherapy. The risk of irAEs is approximately 60% based on the clinical trials in metastatic melanoma. Of these AEs, only 17% of patients had Grade 3-5 irAEs, with about 1% risk of fatal AEs, although the risk is probably lower now that an effective approach 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%). 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.

The encouraging ORR of 33% among the patients in NYU S14-00208 support efficacy of this approach. 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, a single dose of ipilimumab at 3 mg/kg 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 and III 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.

Hellmann et al reported at ASCO 2016 the results on different dose and combinations of Ipi and Nivo. Based on the success of the combination in melanoma (reference) three schedules were studied in Stage IIIB/IV NSCLC as first line therapy with ECOG PS 0-1 (Fig. 6). When compared to Nivo alone both schedules of Nivo/Ipi demonstrated increased grade III/IV toxicity, but comparable incidence of treatment discontinuation based on AE (Fig. 7).

Noticeably either combination demonstrated increased ORR compared to Nivo alone (39-47%, compared to 23%) as shown in figure c. Best PFS was achieved by the Nivo 3mg/kg q2 weeks and Ipi 1mg/kg q12 weeks.

Figure 6



- The safety and tolerability of the nivolumab–ipilimumab combination was improved with less frequent ipilimumab dosing[5]
- Schedules with nivolumab 3 mg/kg also showed increased clinical efficacy in a previous analysis[5]
- Here, we report longer follow-up on nivolumab 3 mg/kg plus ipilimumab schedules[5]

[a]Patients tolerating study treatment permitted to continue treatment beyond RECIST v1.1–defined progression if considered to be deriving clinical benefit
[5]February 2016 database lock
Ipilimumab and nivolumab dosing are shown in mg/kg IV (eg, nivo 1 = nivolumab 1 mg/kg IV)

4

Figure 7

Nivolumab Plus Ipilimumab in First-line NSCLC: Safety Summary

	Nivo 3 Q2W + Ipi 1 Q12W (n = 38)		Nivo 3 Q2W + Ipi 1 Q6W (n = 39)		Nivo 3 Q2W (n = 52)	
	Any grade	Grade 3–4	Any grade	Grade 3–4	Any grade	Grade 3–4
Treatment-related AEs, %	82	37	72	33	71	19
Treatment-related AEs leading to discontinuation, %	11	5	13	8	10	10

- There were no treatment-related deaths
- Treatment-related grade 3–4 AEs led to discontinuation at a third of the rate seen with older combination arms using higher or more frequent doses of ipilimumab[8]

Combination data based on a February 2016 database lock; monotherapy data based on a March 2015 database lock

6

Figure 8.

Nivolumab Plus Ipilimumab in First-line NSCLC: Summary of Efficacy

	Nivo 3 Q2W + Ipi 1 Q12W (n = 38)	Nivo 3 Q2W + Ipi 1 Q6W (n = 39)	Nivo 3 Q2W (n = 52)
Confirmed ORR, % (95% CI)	47 (31, 64)	39 (23, 55)	23 (13, 37)
Median duration of response, mo (95% CI)	NR (11.3, NR)	NR (8.4, NR)	NR (5.7, NR)
Median length of follow-up, mo (range)	12.9 (0.9–18.0)	11.8 (1.1–18.2)	14.3 (0.2–30.1)
Best overall response, %			
Complete response	0	0	8
Partial response	47	39	15
Stable disease	32	18	27
Progressive disease	13	28	38
Unable to determine	8	15	12
Median PFS, mo (95% CI)	8.1 (5.6, 13.6)	3.9 (2.6, 13.2)	3.6 (2.3, 6.6)
1-year OS rate, % (95% CI)	NC	69 (52, 81)	73 (59, 83)

NC = not calculated (when ~25% of patients are censored); NR = not reached
Combination data based on a February 2016 database lock; monotherapy data based on a March 2015 database lock except for OS data, which are based on an August 2015 database lock

8

2.0 Study Rationale

Combining RT and CTLA-4 blockade, before PD-1/ CTLA-4 blockade

The encouraging preliminary results from our trial of ipilimumab and localized radiotherapy converge with the promising data reported by Rizvi et al in CHECKMATE 012, in a similar population of patients with metastatic NSCLC [10](51).

3.0 STUDY OBJECTIVES

Primary objectives

2.1 To demonstrate safety of combining initial concurrent radiotherapy and ipilimumab as an induction regimen to the combination of Ipi/Nivo in NSCLC

2.2 To enhance the ORR to the combination of Ipi/Nivo in NSCLC by preceding it with a combination of Ipi/RT to convert the irradiated tumor into an in situ vaccine

Secondary objectives:

2.3 To explore whether early changes in TCR repertoire in peripheral blood are associated with response to treatment

2.4 To explore if serum markers levels and their changes are associated with response

2.5 To explore associations of ORR with changes in the microbiome

2.6 To measure progression free survival (PFS) and overall survival (OS).

4.0 STUDY DESIGN

A Simon's two-stage optimal design is chosen for this trial, thus 15 patients are required for stage 1. If 5 or more responses are seen then the trial will proceed to stage 2 and an additional 29 patients will be enrolled and treated. If a total of ≥ 18 objective responses are demonstrated the trial will achieve its main endpoint.

We expect to accrue up to a total of 48 patients, independently from their PD-L1 status.

Objective 2.1: To demonstrate safety of combining initial concurrent radiotherapy and Ipilimumab as an induction regimen to the combination of Ipi/Nivo in NSCLC

A Simon's two stage design study of combined RT and Ipi (RT-Ipi), followed by maintenance of ipi/nivo is proposed for patients with metastatic NSCLC. Bristol-Myers Squibb (BMS) will provide drug and study support (ipilimumab and nivolumab) to patients on study (see letter of commitment from BMS).

Eligibility criteria: Patients >18 years of age with histologically proven metastatic NSCLC, at least two measurable sites of disease, adequate organ and bone marrow function, ECOG PS 0-1 or Karnofsky ≥ 70% 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 or CT (chest, abdomen, and pelvis) 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 X 5 daily fractions will be delivered to the biopsied lesion. Patients will receive ipi 3 mg/kg i.v. over 30 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. At day 22 patients are started on the ICB combination of nivolumab 360 mg q 3 weeks, ipi 1mg/kg q 6 weeks, administered until evidence of progression. Patients will be re-imaged by CT between days 70 ± 7 days (Week 9) and evaluated for response.

Assessment of response: Response is defined as an objective radiographic response of the measurable metastatic site(s) outside the radiation field evaluated by CT at 3 months after the start of treatment. Lesions will be measured using RECIST 1.1 [52] 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.

Objective 2.2: To enhance the ORR to the combination of Ipi/Nivo in NSCLC by preceding it with a combination of Ipi/RT to convert the irradiated tumor into an in situ vaccine.

The trial is designed to enhance ORR to the combination of Ipi/Nivo, by an initial induction of Ipi/RT to one of the metastatic lesions. Since a) approximately 2/3 of chemo-refractory metastatic NSCLC cancers are expected to express <1% PDL1; b) Checkmate 012 demonstrated a 19%

objective response rate (ORR) in these patients and c) our recent study of radiation and ipi demonstrated 18% ORR based on intent to treat, the current trial is powered to test in patients with metastatic NSCLC whether the initial addition of local radiotherapy during ipilimumab can increase the ORR to immune checkpoints independently from PD-L1 expression. In order to test this hypothesis a Simon's two-stage design is chosen for this trial, thus 15 patients will be enrolled in the first stage with an upper limit of 4 responses for 1st stage rejection of the combined regimen. If 5 or more objective responses are seen then the trial will proceed to the second stage and an additional 29 patients with PD-L1 negative tumors will be enrolled and treated, for a total target accrual of 44 evaluable patients. A patient is deemed evaluable if they have measurable disease at baseline and had a 9 week imaging evaluation. To ensure 44 evaluable patients, up to 4 additional patients will be enrolled if necessary to replace patients who withdrew or did not have a 9 week evaluation. If there are more than 44 evaluable patients, the decision rule will be made based on the first 44 evaluable patients. Patients who were replaced because they were not evaluable, will not be part of the denominator.

Objective 2.3: To explore whether early changes in TCR repertoire in peripheral blood are associated with response to treatment

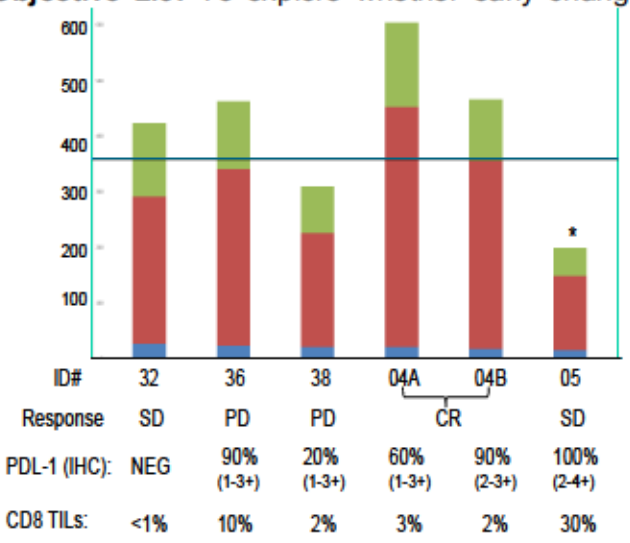


Figure 9. Total exonic mutation burden in NSCLC patients. Frameshiftsindels (blue) missense (red) and synonymous (green) mutations were established by whole-exome NGS in tumor tissue removed pre-treatment in 4 pts (#32, 36, 38, 04) and post-treatment in 1 pts (#05). For pts #4 2 separate metastases (04A and 04B), resected from frontal lobe and cerebellum approximately 2 months before enrollment in the trial were available. Tumor tissue was also stained for and CD8 and PDL-1 expression. Expression of PDL-1 is indicated as percentage positive tumor cells and intensity. Average CD8+ T cells infiltrating the tumor is indicated.

Rationale and preliminary data

Our underlying hypothesis is that RT+Ipi induces expansion of pre-existing tumor-specific T cells and/or priming of T cells against novel tumor antigens (antigenic spread), detectable as expansion of novel T cell clones in peripheral blood and tumor after completion of RT and the first cycle of ipi (day 22). This hypothesis is supported by preliminary data from NYU S14-00208/NCT02221739. TCRB repertoire analysis was performed using genomic DNA (gDNA) from peripheral blood mononuclear cells (PBMC) and ImmunoSEQ hsTCRB (Deep) assay in the 7 responders (CR+PR) and 9 non-responders (PD). Samples were analyzed at baseline and at day 22, after completing the first cycle of ipilimumab and RT. A significant increase in T cell clones with different abundance pre- and post-treatment in responders (CR+PR) versus non-responders (PD) was seen (Figure 10), suggesting that the magnitude of TCR repertoire changes correlates with clinical

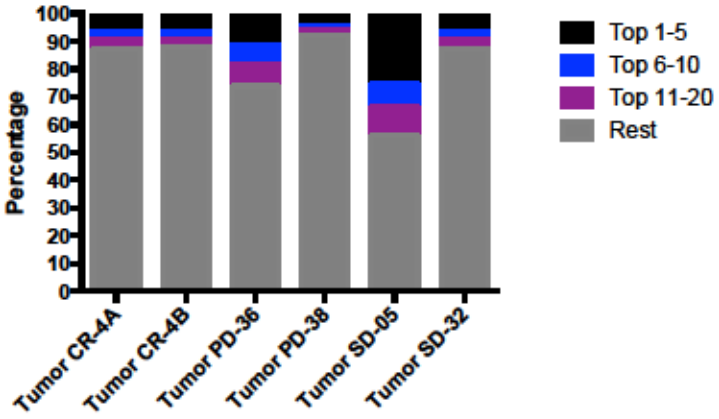


Figure 10. TCR clonal abundance in the tumor of NSCLC patients. TCRB repertoire analysis was performed using genomic DNA (gDNA) isolated from FFPE tissue and ImmunoSEQ hsTCRB technology. Representation of the most frequent clonotypes in each tumor.

activity of the combination. Further analysis of the mutational burden and TCR repertoire was performed in the pre-treatment tumor tissue available from 4 patients, including 1 CR, 1 SD, and 2 PD. In addition, 1 patient with SD had post-treatment tissue available for evaluation. Overall, mutational burden in these 5 patients was in the low to medium range (Figure 9) compared to the NSCLC cases analyzed for response to anti-PD-1 by Rizvi et al. [10](53). Interestingly, expression of PDL-1 was seen in both responding and non-responding patients (Figure 9). Among patients with tissue available for evaluation, 4/4 responders (CR+PR), 3/4 non-responders (PD), 0/1 SD, and 2/8 NE had PDL-1+ tumors, using expression in >1% tumor cells as cutoff for positivity (not shown). One patient with SD (#05) did not have pre-treatment tissue available but a biopsy taken post-treatment revealed high PDL-1 expression and brisk CD8 T cell infiltration (Figure 9). Consistent with the development of tumor-specific T cell responses, TCR repertoire analysis showed the presence of markedly expanded clones in the tumor of pts #5 (Figure 10). In contrast, tumors from pts #4, 32, and 38, which did not have a prominent CD8 infiltrate showed minimal clonal expansion (Figures 9 and 10). Importantly, further analysis revealed that the two metastases from pts #4 shared >60% of missense mutations, and 8.16% TCR CDR3 sequences. Of the 323 total clones shared between the 2 tumor sites, 22 of the top 30 clones were present at baseline in the blood, and 28 persisted overtime (Figure 11 and data non shown). In addition, analysis of the top 100 clones present in the blood at day 22, showed the emergence of two new clones not present at baseline. One clone was not present in the tumors and showed marked reduction in frequency at day 64, but persisted at lower levels until F/U. The other clone remained elevated and was found to be present in only one of the two tumors, where it was the 132nd clone in frequency (Figure 11). We are currently investigating whether these clones are found within the CD8 or CD4 compartment in the peripheral blood with the aim of investigating if they recognize a neoantigen presented by MHC class I or class II in collaboration with Dr. Robert Schreiber (? Robert Schneider). Overall, our data from NYU S14-00208/NCT02221739 testing the

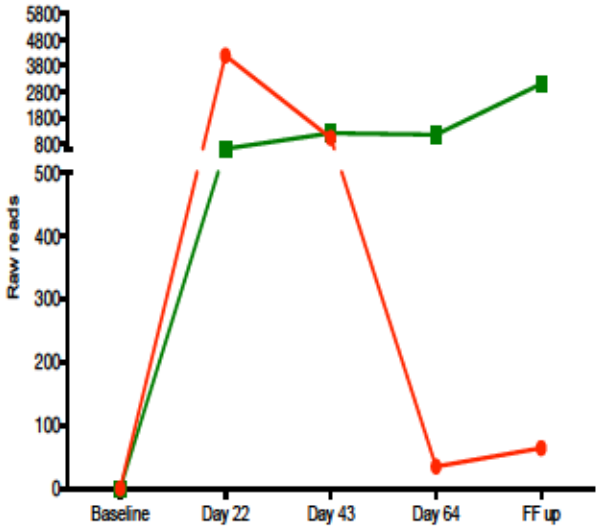


Figure 11. Emergence of new T cell clones in the blood at day 22 that persist overtime in patient #4. Two clones not present at baseline were identified among the top 100 clones at day 22. One clone was not shared with the two tumors (red line). The other was found at low level in one but not the other tumor (green line) and maintained at high levels even at the 6 month F/U.

combination of RT and ipi support the hypothesis that priming and/or expansion of tumor-specific T cells occurs within the first 3 weeks from start of treatment and can be detected in the blood by TCR repertoire analysis.

The results presented also highlight the critical importance of obtaining data from tumor and blood in order to separate between clonal expansions of T cells that are likely to be tumor specific versus those associated with immune-related toxicity. In fact, a large number of clones were found to be expanded in the blood between day 43 and 88, with brisk decrease after termination of Ipi administration (not shown), consistent with prior reports of a similar effect of anti-CTLA4 antibodies related to toxicity but not response [53].

Experimental methods
Serial blood for immune monitoring studies will be collected at baseline, end of radiation therapy, at the time of the first three infusions of ipilimumab, at the time of the first three infusions of nivolumab, at response evaluation (day 70), and at 6 month follow up. Small aliquots of PBMC 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. Biopsies of the lesion to be irradiated will be obtained at baseline in all patients. Optional post-treatment biopsy will be performed at Day 22 in patients with an accessible, non-irradiated metastasis. We expect approximately 50% of the patients to comply with the second tissue acquisition. The choice of a non-irradiated lesion is motivated by safety concerns, after RT.

Sub-sites:

Samples collected at sub sites will be shipped directly to the WCM via overnight shipping. We request that the sites notify the WCMC research team regarding number of patient samples being shipped and the tracking number of the specimens. Please refer to Appendix 1 for more information.

a- Evaluation of systemic T cell populations and MDSC.

In NCT02221739 we have observed a significant increase in Treg and in PD-1+ CD8 T cells in pts with PD but not in responders at day 22. In contrast a significant decline in MDSC was seen in responders (not shown). Additional trends were detected in other cell subsets including CD56bright and CD56dim NK cell subsets and in their expression of NKG2D. While hypothesis-generating these changes were not statistically significant, in part due to the small number of patients evaluated. To further evaluate the mechanistic and predictive value of the changes in immune cell subsets in peripheral blood during treatment with RT+Ipi and Ipi+Nivo, multi-color flow cytometry will be used to characterize T cells, NK cells and myeloid cells for the expression multiple markers, including 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 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[54]. Additionally, T cells will be stained for the proliferation marker Ki67, and transcription factor EOMES, since changes in these markers have been reported to be associated with outcome and immune-related adverse events (irAE). CD8 T and NK cells will also be stained for NKG2D expression, a receptor implicated in their function and in response to anti-CTLA-4 (see below). Treg cells will be characterized for expression of surface markers (CD4, CD25, CD127, CD45RA) and transcription factors (FoxP3, Helios). Finally, MDSC (Lin^- $CD14^+HLA\text{-}DR^{neg/low}$) will be evaluated to validate the significance of their decrease at day 22 as a potential predictor of response to treatment. Functional assays to test the suppressive activity or effector function of MDSC, NK, and T cell subsets will be performed if warranted.

b- Evaluation of TCR repertoire changes in blood and tumor following treatment.

TCRB repertoire analysis will be performed using genomic DNA (gDNA) from peripheral blood mononuclear cells (PBMC) and ImmunoSEQ hsTCRB (Deep) assay as described above. Genomic DNA (2 ug) will be prepared from paraffin tumor tissue and snap frozen PBMC. If <2ug are obtained, all available extracted DNA will be used. For each tumor sample, percentage of T cell infiltration in the tumor will be estimated using a droplet digital PCR technique. TCRB overlap between tumor and blood, and between each site before and after treatment, will be calculated for each patient as described [55]

c- Evaluation of tumor mutational burden by whole-exome NGS and identification of potential neoantigens recognized by T cells.

To investigate the mutational burden in NSCLC patients and identify tumor-specific mutations, whole exome NGS of tumor from pre-treatment (and post-treatment, if availabe) biopsy and normal tissue (blood) will be performed at the WCMC genomic core and data analyzed with the support of the bioinformatics core. To this end, DNA from each sample will be used to prepare barcoded libraries using Kapa Low-Throughput Library Preparation Kit Standard (Kapa Biosystems). The pooled capture library will be sequenced in on an Illumina HiSeq 2500 sequencer using the 2x100 paired-end cycle protocol. Sequencing results will be demultiplexed and converted to FASTQ format using Illumina Bcl2FastQ software. The reads adapter and quality trimmed with Trimmomatic [http://www.ncbi. nlm.nih.gov/pubmed/24695404] will be aligned to the human genome using the Burrows-Wheeler Aligner [http://www.ncbi.nlm.nih.gov/pubmed/19451168]. PCR duplicates are marked using Picard toolkit [http://www. ncbi.nlm.nih.gov/pubmed/23104886]. Further local indel realignment and base-quality score recalibration will be performed using the Genome Analysis Toolkit (GATK) [http://www.ncbi.nlm.nih.gov/pubmed/21478889]. SNGs will be generated with MuTect [http://www. ncbi.nlm.nih.gov/pubmed/23396013] and indels with SomaticIndelDetector. ANNOVAR [http://www.ncbi.nlm. nih.gov/pubmed/20601685] will be used to annotate variants with functional consequence on genes as well as identifying presence in dbSNP. For each tumor we will obtain data on the number and type of single nucleotide variations (SNVs) and short insertions and deletions (Indels), and non-synonymous mutations. We will explore whether the number of total and non-synonymous mutations correlates with response to treatment.

In addition, in collaboration with Dr. Robert Schreiber at NYU, we will explore the tumor neoantigen landscape. Non-synonymous mutation data will be filtered for gene expression using data obtained from RNA-Seq performed on the same tumor, and potential T cell epitopes analyzed using predictions for proteosomal processing and HLA class I and class II binding using NetChop Cterm3.0 and NetMHC3.2 algorithms.

Objective 2.4: To explore whether serum markers levels and their changes are associated with response.

Rationale and preliminary data

Our preclinical data show that RT-induced cancer cell intrinsic IFNβ production is critical for the priming of T cell responses capable of mediating an abscopal effect in combination with anti-CTLA-4 (Vanpouille-Box et al., manuscript in preparation). Importantly, a similar mechanism may be active in patients, since we detected a significant increase in IFNb at day 22 in serum of responders to RT+ipi (not shown). In addition, recent data have demonstrated the predictive nature of baseline levels of sMICA and CXCL11 for pts treated with ipi[56]. Interestingly, while high levels of sMICA were associated with poor survival in ipi-treated melanoma patients, in NYU NCT02221739 trial we have found a statistically significant increase in antibodies to sMICA

(p=0.01) and sMICB (p=0.0004) at day 22 compared to baseline only in responding pts, suggesting that antibodies that block the soluble MICA molecules may restore recognition of the MICA/B+ tumor cells by NK and CD8 T cells[57]

Experimental methods

Serial serum samples will be used to measure IFN□ and CXCL11 by ELISA. sMICA, sMICB and antibodies anti-sMICA and anti-sMICB will be measured in serum by ELISA using recombinant MICA/B protein and anti-human MICA mAb, in collaboration with Dr. Kai Wucherpfennig (Dana Farber) with whom we have an ongoing collaboration. MICA expression in tumors will be tested by IHC. sMICA/B and anti-sMICA/B antibody levels, and expression levels of NKG2D in circulating CD8 and NK cells will be plotted over time to identify the nature of the changes and summarized using longitudinal mixed effects regression models that incorporate the repeated observations over time (fixed effects) within the same patient (random effect) and missing observations. Baseline levels and changes from baseline at the time of 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.

Objective 2.5: To explore associations of ORR with changes in the microbiome

While immune checkpoint blockade results in remarkably prolonged disease control in a subset of patients [58, 59] modulators of this phenotype are not well understood. Accumulating evidence suggests the gut microbiota play a critical role in response to both anti-PD-L1 efficacy and CTLA-4 blockade: two recently published studies by Vetizou *et al.* and Sivan *et al.* demonstrated in mouse models that the gut microbiome critically impacts response to immunotherapy [58, 59]. Remarkably, by altering the composition of commensal microbiota, the response to CTLA-4 blockade and anti-PD-L1 therapy could be manipulated and therapeutic response markedly enhanced. These pre-clinical studies provide strong rationale for our hypothesis that the human gut microbiome composition will correlate with response to ICB and RT. Identification of commensal organisms harbored in patients with heightened antitumor immune response can ultimately be manipulated to achieve desired outcomes in a greater proportion of patients. Fecal transplant is an established clinical technique whereby microbiota can be transferred into humans to induce select therapeutic outcomes, providing direct clinical applicability of any findings [30].

The study by Vetizou *et al.* underscores the dependence of CTLA-4 blockade on commensal microbiota [59]. First, the authors demonstrate that anti-CTLA-4 therapy is ineffective in mice that are housed in germ-free cages or treated with antibiotics.

By transferring specific gut microbiota back into antibiotic-treated or germ-free mice, the anticancer efficacy of CTLA-4 blockade could be recovered. The transfer of T cells specific to *Bacteroides fragilis*, or immunization with dendritic cells exposed to *B. fragilis* polysaccharides, also restored the antitumor effect of CTLA-4 blockade. Additionally, Vetizou *et al.* explored human gut microbiome changes in 25 malignant melanoma patients treated with CTLA-4 blockade, who at baseline fell into three microbial cluster patterns (Clusters A-C). During treatment, the microbial composition altered, becoming more similar to cluster pattern "C" and distant from cluster pattern "B". Subsequent fecal transplantation from cluster C patients into mice resulted in a significant response to CTLA-4 blockade, whereas transplantation from cluster B patients had no response, providing evidence that host gut microbial composition has a significant impact on likelihood of ICB success.

Sivan *et al.* likewise provides compelling data supporting the importance of gut microbiota in

determining treatment efficacy to ICB [58]. The authors compared growth of melanoma cell lines in two sets of mice harboring distinct gut microbiota, with differential tumor growth and response to PD-L1 therapy, and identified a specific bacterial species, *Bifidobacterium,* to be associated with antitumor immunity. By transferring *Bifidobacterium* from JAX mice to poorly-responding TAC mice, the authors were able to improve baseline anti-tumor immunity, and significantly enhance anti-PD-L1 therapy response. The therapeutic effect of *Bifidobacterium* was established to arise from altered dendritic cell function and increased priming of tumor-specific CD8+ T cells. Only live bacteria could achieve this effect, implying that augmentation of dendritic cell function requires direct interaction of host cells with gut microbiota [30]. These novel findings signify that the gut microbiome composition impacts response to anti-PD-L1 therapy and can be manipulated to enhance treatment response, providing impetus for similar analyses in human subjects.

Taken together, the above preclinical studies lend strong support towards collection of gut microbiome samples from patients enrolling on all future immunotherapy studies. We propose to collect stool samples from study patients before treatment and at day 21, 3 months and 6 months for analysis of microbiome composition and changes after ICB and RT. Both pre-clinical and clinical data demonstrate that the addition of RT to ICB creates a robust immune response, eliciting anti-tumor immune responses in otherwise non-responding patients. Given that we expect a similar heightened response in our proposed study, our patient cohort with its expected dichotomous outcomes will be particularly suitable for microbiome analysis. The results will be an important step towards understanding, and ultimately harnessing, the interplay between the human gut microbiome and anti-tumor immune response.

Methods Stool collection will occur before treatment, at day 22, day 70 and at 6 months follow up to explore changes of the microbiome during ICB. Bacterial diversity and taxonomy will be estimated by high throughput sequencing (HTS) of 16S rRNA amplicons, using the MiSeq platform in the Genomic Technology Core Lab. Methods have been established in the Blaser Lab and proven to yield accurate and reproducible results.[60, 61] Analysis will be based on the QIIME (Quantitative Insights into Microbial Ecology) software package developed by the Knight lab, [62] and machine learning analyses developed by NYU Center for Health informatics and Bioinformatics (CHIBI) investigators working on NYU Human Microbiome Program studies [63, 64](64,65). Appendix 10 describes the protocol for stool collection.

Sub-sites:

Samples collected at sub sites will be shipped directly to the WCM via overnight shipping. We request that the sites notify the WCMC research team regarding number of patient samples being shipped and the tracking number of the specimens. Please refer to Appendix 10 for more information.

5.0 SUBJECT SELECTION CRITERIA

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

5.1 Inclusion Criteria

1) Ability to understand and the willingness to sign a written informed consent document;
2) Histologic diagnosis of NSCLC;
3) Patients with an EGFR sensitizing mutation must have received an EGFR tyrosine kinase inhibitor (osimertinib, erlotinib, gefitinib or afatinib) and patients with ALK translocation must have received anti-ALK therapy.

4) Patients must have at least two distinct lesions, 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) If the patient has received prior systemic therapy, an interval of 2 weeks from last therapy is required;
6) Patients must have recovered from the toxic effect(s) of the most recent anti-cancer treatment to NCI CTCAE Grade 1 or less (except alopecia).
7) Patients must have adequate organ and marrow function as defined by initial laboratory tests:
   - WBC ≥2000/uL
   - ANC ≥1.5/uL
   - Platelets ≥ 100 x 10^3/uL
   - Hemoglobin ≥ 9 g/dL
   - Creatinine ≤ 1.5 x ULN
   - AST/ALT ≤ 2.5 x ULN, or ≤ 5 x ULN if liver metastases are present.
   - Bilirubin ≤ 1.5 x ULN, (except patients with Gilbert's Syndrome, who must have a total bilirubin ≤ 3.0 mg/dL;
8) Performance status ECOG 0-1 or Karnofsky ≥ 70%;
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 2 weeks (including radiosurgery treated lesions) and patients are not symptomatic or steroid dependent; Baseline MRI will be required." Note these lesions are not considered primary targets for the purposes of this protocol.
12) Patients in this study may not use vaccines for the treatment of cancer for up to one month before the first dose of ipilimumab. Concomitant systemic or local anti-cancer medications or treatments are prohibited while receiving study treatments.
13) Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 5 months 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 ≥ 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.
14) Men should use avoid impregnating women during study and for 7 months after the study.

5.2 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 auto immune disease (e.g., rheumatoid arthritis, progressive systemic sclerosis [scleroderma]), systemic lupus erythematosus, autoimmune vasculitis [e.g., Wegener's granulomatosis];

3. Patients with a history of symptomatic interstitial lung disease OR a history of (non-infectious) pneumonitis that required oral or IV steroids or current pneumonitis. Patients with resolved RT pneumonitis are eligible.
4. Patients with uncontrolled HIV infection. Note: participants with a plasma HIV viral load less than 200 copies/mL are eligible.
5. Patients with active Hepatitis B and Hepatitis C infection. Patients undergoing active therapy will be excluded from the trial.
6. 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;
7. 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; Tyrosine Kinase inhibitors such as Erlotinib;
8. Women and men who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 5mos (women) or 7mos(men) weeks after cessation of study drug, or have a positive pregnancy test at baseline, or are pregnant or breastfeeding;
9. Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness.
10. Patients with mixed NSCLC/SCLC histology are excluded from this trial.

6.0 STUDY THERAPY

Each patient will receive ipilimumab intravenously at 3 mg/kg on C1D1. Infusions will be given over 30minutes (not bolus or i.v. push).

Ipilimumab 3mg/kg will be administered on Day 1. On Day 22, ipilimumab (1mg/kg) will be given along with nivolumab (360mg). Subsequently, ipilimumab (1mg/kg) will be given every 6 weeks and nivolumab (360mg) will be given every 3 weeks.

Radiation will be delivered daily, Monday-Friday, for five consecutive days, at the Stich Radiation or DHK Radiation Oncology at Weill Cornell Medicine-New York Presbyterian. A dose of 6 Gy x 5 days will be tested. All patients will be re-imaged by CT on Day 70 (±7 days).

PRODUCT INFORMATION TABLE:

Product Description and Dosage Form	Potency	Primary Packaging	Appearance	Storage Conditions (per label)

		(Volume)/ Label Type		
Nivolumab BMS-936558-01 Solution for Injection[a]	100 mg (10 mg/mL)	10 mL vial	Clear to opalescent colorless to pale yellow liquid. May contain particles	2 to 8□C. Protect form light and freezing
Ipilimumab Solution for Injection	200 mg (5 mg/mL)	40 mL vial	Clear, colorless to pale yellow liquid. May contain particles	2 to 8°C. Protect from light and freezing.

*Nivolumab may be labeled as BMS-936558-01 Solution for Injection

If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab and ipilimumab include laboratory coats and gloves.

For additional details on prepared drug storage and use time of nivolumab or ipilimumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) and ipilimumab Investigator Brochure section for "Recommended Storage and Use Conditions"

Handling and Dispensing

The investigator should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as per product information and the Investigator Brochure and per local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact BMS immediately.

Please refer to the current version of the Investigator Brochure and/or shipment reference sheets for additional information on storage, handling, dispensing, and infusion information for nivolumab.

Destruction

Sponsor/Investigator drug destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the Sponsor SOPs and a copy provided to BMS upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, i.e, incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for BMS to review throughout the clinical trial period as per the study agreement.

If conditions for destruction cannot be met, please contact BMS.

It is the Sponsor Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

Dose calculations and administration

Describe timing of first dose from registration/randomization. For preparation and administration details to be included in your protocol [per institutional policies], please refer to the current Investigator Brochure, (Section 3.2) and/or pharmacy reference Appendix.

Day 1 ipilimumab will be given at a dosing of 3mg/kg. On day 22, nivolumab (360mg) and ipilimumab (1mg/kg) will be given together. Subsequently, ipilimumab (1mg/kg) will be given every 6 weeks and nivolumab (360mg) every 3 weeks. Both nivolumab and ipilimumab can be administered over 30 minutes.

When study drugs (ipilimumab or nivolumab) are to be administered on the same day, separate infusion bags and filters must be used for each infusion. It is recommended that nivolumab be administered first. The second infusion will always be ipilimumab, and will start approximately 30 minutes after completion of the nivolumab infusion.

Ipilimumab and nivolumab may be diluted in 0.9% Sodium Chloride Solution or 5% Dextrose solution.

The dosing calculations should be based on the body weight. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the dose, the dose must be recalculated. All doses should be rounded up or to the nearest milligram per institutional standard.

Following the first doses of the combination of nivolumab and ipilimumab on day 22, nivolumab will be given every three weeks at a dose of 360 mg. Patients may be dosed no less than 12 days from the previous dose of drug; and dosed up to 3 days after the scheduled date if necessary.

Dose Modifications

There will be no dose modifications permitted. Dose reductions or dose escalations are not permitted.

Management Algorithms for Immuno-Oncology Agents

Immuno-oncology (I-O) agents are associated with adverse events that can differ in severity and duration than adverse events caused by other therapeutic classes. Nivolumab and ipilimumab are considered immuno-oncology agents in this protocol. Management algorithms have been developed to assist investigators in assessing and managing the following groups of adverse events: Gastrointestinal, Renal, Pulmonary, Hepatic, Endocrinopathies, Skin, and Neurological.

Early recognition and intervention are recommended according to the management algorithms; and in addition include ophthalmologic evaluations for any visual symptoms in order to evaluate for nivolumab or ipilimumab related uveitis.

The recommendations are to follow the algorithms in the nivolumab investigator brochure for immune related events; while the ipilimumab investigator brochure contains similar algorithms, the algorithms in the nivolumab brochure have been aligned to accommodate combinations as

well as nivolumab monotherapy.

Therefore, the algorithms recommended for utilization are attached for reference.

For subjects expected who require more than 4 weeks of corticosteroids or other immunosuppressants to manage an adverse event, consider the following recommendations

Antimicrobial/antifungal prophylaxis per institutional guidelines to prevent opportunistic infections such as Pneumocystis jiroveci and fungal infections.

Early consultation with an infectious disease specialist should be considered. Depending on the presentation, consultation with a pulmonologist for bronchoscopy or a gastroenterologist for endoscopy may also be appropriate.

In patients who develop recurrent adverse events in the setting of ongoing or prior immunosuppressant use, an opportunistic infection should be considered in the differential diagnosis.

Additional details on the safety of nivolumab and ipilimumab, including results from clinical studies, are available in the IB.

Dose Delay Criteria

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories.

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab, ipilimumab or both). All study drugs must be delayed until treatment can resume.

Nivolumab and ipilimumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for asymptomatic amylase or lipase, AST, ALT, or total bilirubin:
- Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay. It is recommended to consult with the principle investigator for Grade 3 amylase or lipase abnormalities.
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Criteria to Resume Treatment

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes. If the design of the study defines that doses may not be omitted [skipped], strongly recommend adding "Doses may not be skipped"

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

Discontinuation Criteria

Treatment should be permanently discontinued for the following:

Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment

Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions

Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation

Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below

Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation

Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:

- AST or ALT > 8 x ULN
- Total bilirubin > 5 x ULN
- Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN

Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:

Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.

- Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks with the following exceptions:
- Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab or ipilimumab dosing

Treatment of Nivolumab or Ipilimumab Related Infusion Reactions

Since nivolumab and ipilimumab contain only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (version 5.0) guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated

for 24 hours).

Stop the nivolumab or ipilimumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab or ipilimumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab or ipilimumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilator support indicated).

Immediately discontinue infusion of nivolumab or ipilimumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab or ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

The section below is Optional and should be considered if part of assessment criteria. All decisions to continue treatment beyond initial progression documented in the study record and part of an agreement with BMS. Subjects should be re-consented with an ICF describing any reasonably foreseeable risks or discomforts.

Treatment Beyond Progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD.

Subjects will be permitted to continue treatment beyond initial RECIST 1.1 defined PD as long as they meet the following criteria:

- Investigator-assessed clinical benefit and subject is tolerating study drug.

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment.

Subjects should discontinue study therapy upon further evidence of further progression, defined

as an additional 10% or greater increase in tumor burden volume from time of initial progression (including all target lesions and new measurable lesions).

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase in short axis to at least 15 mm).

For statistical analyses, this will need to be described in patient population information. E.g. Subjects who continue treatment beyond initial investigator-assessed, RECIST 1.1-defined progression will be considered to have investigator-assessed progressive disease at the time of the initial progression event.

Subjects will have the opportunity to receive all 4 doses of Induction Phase ipilimumab.

Other Immune-mediated Adverse Events

For suspected immune-related adverse reactions, adequate evaluation should be performed to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, nivolumab or nivolumab in combination with ipilimumab should be withheld and corticosteroids administered. Upon improvement, nivolumab or nivolumab in combination with ipilimumab may be resumed after corticosteroid taper. Nivolumab or nivolumab in combination with ipilimumab must be permanently discontinued for any severe immune-related adverse reaction that recurs and for any life-threatening immune-related adverse reaction.

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 9 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.

7.0 OTHER GUIDANCE

7.1 Infusion Reactions Associated with Ipilimumab and Nivolumab

Infusion reactions should be graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 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.

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.

8.0 RADIATION THERAPY GUIDELINES

8.1 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 Stich Radiation Center, WCM, 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.

8.2 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, to an isodose surface encompassing ≥90% of the PTV.

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) as a reference image set. Both kV and MV images may be used to verify setup.

IGRT Target Localization: In addition to the portal imaging, 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. If shifts are made based upon the CBCT images, the portal images will be repeated.

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 (66) and the H. Lee Moffitt Cancer Center experience using this fractionation schedule (67).

After completion of the first course of radiation + ipilimumab at a 3mg/kg dose on Day 1 (or within 24 hours of RT), the patient will start on day 22 the regimen of Nivolumab 360 mg q3 weeks and Ipilumumab 1mg/kg q6 weeks until evidence of progression.

Response assessment will be performed during Day 70 (± 7 days) by imaging with PET/CT or CT (CAP).

8.3 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.

8.4 On Study Evaluations

As summarized in the Study Calendar, patients are evaluated pre-treatment for definition of metastatic sites with PET/CT or CT (CAP). 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.

8.5 Off Study Criteria

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable toxicity (defined in Section 15),
- 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.

9.0 PROHIBITED AND RESTRICTED THERAPIES DURING THE STUDY

9.1 Prohibited Therapies

Patients in this study may not use vaccines for the treatment of cancer for up to one month before the first dose of ipilimumab. Concomitant systemic or local anti-cancer medications or treatments are prohibited while receiving study 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 CTLA-4 or anti PD-1 or PD-L1 inhibitors or agonists;
- Immunosuppressive agents;
- Chronic systemic corticosteroids.

10.0 STUDY CALENDAR

Procedure	Screening (Day0-28)	Day 1[h]	Day 2	Day 3	Day 4	Day 5	Day 22[h]	Nivo q3 wks[h]	Ipi q6 wks[h]	Resp. Eval Day 70 (±7days)	Nivo q3 wks[h]	Ipi q6 Wks[h]	6 month Follow up	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]		X[a]	X[a]		
Physical Examination	X				X		X	X	X		X	X	X	X
ECG (12 Lead)	X													
Vital Signs	X				X		X	X	X		X	X	X	X
Adverse Events Assessment	X				X		X	X	X		X	X	X	X
Serum Chemistry[b]	X	X			X		X	X	X		X	X	X	X
Hematology[b]	X	X			X		X	X	X		X	X	X	X
Urinalysis	X													
Hep B and Hep C panel	X													
Endocrine Panels[c]	X									X			X	X
Radiation Therapy[d]		X	X	X	X	X								
Ipilimumab Treatment [f]		X[f]					X[f]		X[f]			X[f]		X[f]
Nivolumab Treatment [g]							X[g]	X[g]			X[g]			X[g]
Bloods/T-cell Response[e]	X					X	X	X[e]	X[e]	X			X	
Stool Sample collection[i]	X						X			X			X	
Tumor biopsy[j]	X						X							
PET/CT Imaging or CT (chest abdomen and Pelvis)	X									X			X	Q 12 W
MRI Brain	X													
Efficacy assessment										X				X

a. Only for pre-menopausal female patients
b. SOC labs (COMP, CBC w/diff,).
c. TSH, T3, T4 required at screening, every 9-12 weeks thereafter while on therapy with ipilimumab and Nivolumab and once a year unless warranted by symptoms or signs detected at follow up visits. May be requested by treating physician if clinically necessary.
d. 6 Gy X5, daily
e. Blood for immune monitoring studies will be collected at baseline, end of radiation therapy, at the time of the first three infusions of ipilimumab, at the time of the first three infusions of nivolumab, at response evaluation (day 70), and at 6 month follow up. See Appendix 2 for details.
f. Ipilimumab infusion 1mg/kg q6 weeks. After Day 70 (+/- 7 days response assessment, if patients have disease progression, patients will come off study. Patients who demonstrate partial, stable or complete response will continue to receive ipilimumab q6 weeks.
g. Nivolumab infusion 360mg q 3 weeks. After Day 70 (+/- 7 days) response assessment, if patients have disease progression, patients will come off study. Patients who demonstrate partial, stable or complete response will continue to receive nivolumab q3 weeks.
h. All Infusions will have a ± 3 day window
i. Stool samples will be collected on Day 1, Day 22, and Day 70 and at 6 month follow up. See appendix 11 for details.
j. Biopsies will be optional and will be obtained at baseline and at day 22.

11.0 PROCEDURES BY VISIT

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. There are two levels of withdrawal and it should be documented whether one or both levels are met. The first level is withdrawal from protocol treatment and testing schedule. The second level is withdrawal from all subsequent follow-up (e.g. disease progression and overall survival). A patient may choose to withdraw from further treatment and protocol assessments but is willing to be followed for disease progression and survival.

12.0 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 treatment, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

13.0 DETAILS OF PROCEDURES

13.1 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.

13.2 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 5.0 (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.

13.3 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 drugs (ipilimumab and nivolumab) 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.

13.4 Criteria for Evaluation

Safety evaluation

All patients who receive at least one dose of ipilimumab or nivolumab will be considered evaluable for safety parameters. Adverse events will be collected and documented at every visit. Serious Adverse events will be documented and immediately reported to the all concerned authorities as per institutional and sponsor guidelines. This study will be conducted in accordance with the guidelines of the 2001 NCI approved data Safety and Monitoring plan for the WCM Cancer Institute. Reports to the Data Safety and Monitoring Committee will include the following information: accruals, targets, responses, adverse events and evidence of reporting to appropriate review committees.

At the time of protocol initiation the WCM Data and Safety Monitoring Board (DSMB) will review the IRB approved protocol, the data and safety monitoring plan and any stopping guidelines (15.0, page 56). A WCM DSMB data and safety analysis will be performed every six months following the accrual of the first study subject. The WCM DSMB may also convene as needed if stopping criteria are met or other safety issues arise from communications of the Principal Investigator and/or IRB.

Efficacy Evaluation

For the purposes of this study, patients should be evaluated for response at day 70(+/-7 days), then re-evaluated every 3 months, until evidence of disease progression. Confirmatory scans should also be obtained 4 weeks following initial documentation of objective response or progressive disease.

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1; Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Selection of Lesions -

- Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Refer to Appendix 13 for evaluation of radiated target lesions.

- Malignant lymph nodes: To be considered pathologically enlarged and measurable,a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.
- Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1

to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

- Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.
- Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

- Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

Methods for Evaluation of Measurable Disease:

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

- Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

- Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situation.
- PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.
- Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in
- Their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.
- Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.
- Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
- Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease
- FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the

positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

Response Criteria

Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).
- Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm).
- (Note: the appearance of one or more new lesions is also considered progressions).
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
- Evaluation of Non-Target Lesions
- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).
- Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.
- Although a clear progression of "non-target" lesions only is exceptional, the opinion of the

treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

Evaluation of Overall Response Criteria

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. Revised Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 and immune-related response criteria (irRC; Nishino 2013) are summarized in tables within this section.

13.5 Response According to Revised Response Evaluation Criteria in Solid Tumors (Version 1.1)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when confirmation is Required
CR	CR	No	CR	≥ 4 weeks confirmation
CR	Non-CR/Non-PD	No	PR	≥ 4 weeks confirmation
CR	Not Evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥ 4 weeks confirmation from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR.
Any	PD	Yes or No	PD	
Any	Any	Yes	PD	

CR = Complete response; PD = Progressive Disease; PR=Partial response; SD =Stable disease.

This protocol will be monitored by the WCM Data Safety Monitoring Committee which oversees the safety of all WCM 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.

14.0 RESPONSE ENDPOINTS

Ipilimumab and Nivolumab are 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 increase 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 Nivolumab 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 Nivolumab 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.

15.0 STUDY SUSPENSION CRITERIA

Stopping Rules: The study will suspended if there are 4 grade 4+ AEs in first ten patients or > 30% of grade 4+ AEs thereafter that are deemed at least possibly related to study treatment.

16.0 ADVERSE EVENT REPORTING

Collection of Safety Information

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). Potential drug induced liver injury (DILI) is also considered an important medical event.

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, and must be immediately reported.

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.

16.1 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 CTCAEv5.

This is a Weill Cornell Medicine 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 WCM IRB through the institution's web-based portal, Research Navigator.

The causal relationship to study drug is determined by a physician and should be used to assess all AEs. The causal relationship can be one of the following:

Related: 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.

.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

- Adverse events classified as "serious" require expeditious handling and reporting to WCM to comply with regulatory requirements.
- All SAEs whether related or unrelated to the ipilimumab and nivolumab, must be immediately reported to WCM 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 WCM 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 WCM. 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, WCM 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 and nivolumab, 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 100 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 WCM of any SAE that may occur after this time period which they believe to be certainly, probably, or possibly related to ipilimumab and nivolumab.

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 and nivolumab. 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 nivolumab 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 and nivolumab exposure, including during at least 6 half-lives after product administration, the ipilimumab and nivolumab will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). Exceptions to ipilimumab and nivolumab 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.

Adverse events that are considered non-serious events, including lab abnormalities will be documented in REDCap database and will be reported to BMS on a regular basis. In addition to that, WCMC Data safety monitoring committee will be reviewing the study on an annual basis and will review the adverse events collected on the study.

17.0 STATISTICAL METHODOLOGY

17.1 Overview

The primary endpoint of this study is ORR. A response is defined as a PR or CR as a best response (or that has been confirmed). The trial will be considered to be successful if the ORR, originally achieved in similar patient populations of metastatic NSCLC treated in a protocol of Ipilimumab and local radiotherapy to a metastatic lesion, is increased by 20% or more. It is assumed that the ORR in the first-line is 40% and the ORR in the second-line is 18% and that there will be approximately 60% first-line patients and 40% second-line (and beyond) patients. This yields an historical ORR of 0.31 (= (0.60x0.40) + (0.40x0.18)). The trial design is a single arm, multi-center, Simon optimal two-stage design. Tumor responses will be assessed at baseline, at Day 70 (± 7 days), and at specified intervals as stated in section 10.0. Patients will be evaluated for response at day 70 day ± 7 days. Patients will be enrolled in two stages with 15 patients in the first stage, and potentially an additional 29 patients in the second stage. The decision to enroll patients into the second stage will depend on the number of responses observed in the first stage.

17.2 Sample size justification

As mentioned, a Simon's two-stage optimal design [65] will be used. The null hypothesis is that the true response rate is 31% will be tested against a one-sided alternative. If there are 4 or fewer responses in the first 15 patients, the study will be stopped. Otherwise 29 additional patients will be accrued for a total of 44 patients. The null hypothesis will be rejected if 17 or more responses are observed in 44 patients. This design yields a type I error of 0.10 and power of 90% when the true response rate is 51%. The target sample size is 44 evaluable patients and up to 48 patients (4 extra patients) will be accrued to account for non-evaluable patients or patients who withdraw prior to the 9-week evaluation.

17.3 Primary analysis

The primary analysis will be an evaluation of the ORR. The trial decision rules are to conclude the treatment strategy is not worth further evaluation if in the first evaluable 15 patients, there are 4 or fewer patients with a response (PR or CR). If in the first 23 evaluable patients, there are 5 or more patients with responses, an additional 29 evaluable patients will be enrolled. If in the 44 evaluable patients, there are 18 or more responses, it will be concluded that this treatment strategy is worth further evaluation (the null hypothesis will be rejected). If in the 44 evaluable patients, 17 or fewer responses are observed, the treatment will not be recommended for further evaluation in this patient population (non-selected, metastatic NSCLC patients). The ORR will be estimated with a one-sided 90% exact binomial procedure (66).

The primary safety analysis will be descriptive summaries with frequencies and relative frequencies of all observed AEs. Specifically, the number (proportion) of patients who experience a grade 3+ AE and a grade 4+ AE will be determined with an exact binomial estimate and corresponding 95% confidence intervals. The regimen will be considered to be safe if there are fewer than 4 grade 4+ AEs in the first ten patients during the ipi+RT phase and fewer than 30% of grade 4+ AEs in the total sample during the ipi+RT phase. Assessment of adverse events will be conducted within 3 weeks from accrual of patient #10. In addition, all observed AEs and corresponding grades will be summarized as counts and proportions over the entire portion of the treatment period. In addition, the number and proportion of patients who experience a grade 3+ AE and grade 4+ at during the entire treatment period will be summarized as counts and

proportions.

17.3 Secondary analyses

Progression free survival (PFS) is defined as time from study enrollment until documented disease progression or death, whichever occurs earlier. Patients who are alive at time of the data cutoff and do not have documented disease progression will be censored. Overall survival (OS) will be measured from the time of study enrollment until death from any cause. Patients alive at the time of data cutoff will be censored. The Kaplan-Meier estimator and curve will be used to summarize the PFS and OS data. Duration of response (DOR) will be measured from the time of documented response until disease progression. If a patient has not progressed at the time of data cutoff, they will be censored. Duration of response will be summarized with Kaplan-Meier incidence curves and will only include patients with a documented response (the clock starts at the time of response and the incident event is disease progression). The median DOR and corresponding 95% CI will be generated. The time to disease progression (TTP) also be estimated with K-M incidence curves. The start will be the time of study enrollment. The event will be documented disease progression. Patients who died prior to documented progression or are alive and progression free at time of data cutoff will be censored. The median TTP and corresponding 95% CI will be generated.

Changes in the gut microbiome will be assessed globally and at each individual timepoint. In the global analysis of measure of diversity, the data will be summarized by a normalized area under the curve (AUC). The AUC of responders will be compared to the AUC of non-responders using a two-group t-test (or non-parametric test if more appropriate). In addition, logistic regression will assess the relationship of the AUC for the diversity index and responder status. This model may adjust for baseline levels of diversity and other relative clinical variables. Changes in the diversity index will also be evaluated at each timepoint and compared between responders and none responders using a two-group t-test (or non-parametric test if needed). A similar analysis will be done with the changes in serum marker levels over time. A global analysis will be done using a normalized AUC and making a comparison between responders and non-responders. There will also be analyses done for changes at each timepoint between responders and non-responders. For these analysis, we will also generate graphs of the data that show the changes over time in the measurements for each individual patient (i.e. a spaghetti plot). We will superimpose a loess curves on the plot: one for the entire cohort, one for responders and one for non-responders). Given the relatively small sample size, these analyses are exploratory and no adjustment will be made for multiple comparisons.

17.4 Study monitoring

There will be monthly reports generated for study accrual, observed AEs, and data timeliness. The study team will review these reports. In addition, the study will undergo periodic review by the Weill Cornell data safety and monitoring board (DSMB) semi-annually.

17.5 DSMB Safety Review

The protocol will be reviewed by the Data Safety Monitoring Board (DSMB) on a semi-annual basis. Safety reports will be submitted to the DSMB every six months.

ADMINISTRATIVE SECTION

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 WCM protocol manager.

All revisions (protocol amendments, administrative letters, and changes to the informed consent) will be submitted to the WCM 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.

Subsite:

All sub sites will follow local institutional guidelines to manage the study locally. All regulatory requirements will be governed by the local institutional guidelines.

18.0 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.

18.1 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.

18.2 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 WCM.

18.3 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.

Sub-sites:

Samples collected at sub sites will be shipped directly to the WCM via overnight shipping. We request that the sites notify the WCMC research team regarding number of patient samples being shipped and the tracking number of the specimens. Please refer to Appendix 1 for more information.

18.4 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 WCM, Radiation Biology Division 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, Redcaps. 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 Clinical trials office of the Department of Radiation Oncology (located in W2). 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 WCM 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 de-identified 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 de-identified database using only the unique identifier (study ID number).

Serial blood for immune monitoring studies will be collected at baseline, at the end of radiation therapy, at the time of the first three infusions of ipilimumab, at the time of the first three infusions of nivolumab, at response evaluation (day 70), and at 6 month 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) 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 Silvia C. Formenti, M.D. at WCM, 525 East 68th Street, BOX 169, NY-10065.

18.5 Genetic Testing

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

18.6 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 Weill Cornell Medicine. Patient records will be made available for inspection to auditing agencies to satisfy regulatory requirements.

18.7 Research Conflict of Interest

Dr. Ronald Scheff, who is Co-Principal Investigator on this study, has been paid as a member of the speakers' bureau and advisory board for Bristol Myers Squibb (BMS), and maker of one of the study drugs, Nivolumab.

APPENDIX 1: Laboratory Correlate Manual

Please refer to the study lab manual for more information on shipping and handling research specimens.

At Baseline BIOPSIES: >3 core biopsies from the lesion selected to be irradiated is obtained and delivered to Dr. Demaria's lab.
One core will be assigned for histology, one for for RNA/DNA, and one for PDX generation.

Blood: Six 10 mL lavender top (EDTA) tubes—to WCM Core Lab

One 10 mL red top (serum) tube—serum to be processed and frozen by WCM Core Lab (Total research blood draw this day 70 mL)

First 3 ipi infusions: Blood: Six 10 mL lavender top (EDTA) tubes—to WCM Core Lab

One 10 mL red top (serum) tube—serum to be processed and frozen by WCM Core Lab (Total research blood draw this day 70 mL)

Day 22: Blood: Six 10 mL lavender top (EDTA) tubes—to WCM Core Lab

One 10 mL red top (serum) tube—serum to be processed and frozen by WCM Core Lab (Total research blood draw this day 70 mL)

Optional tissue biopsy will be obtained at Day 22.

First 3 Nivo infusions: Blood: Six 10 mL lavender top (EDTA) tubes—to WCM Core Lab

One 10 mL red top (serum) tube—serum to be processed and frozen by WCM Core Lab (Total research blood draw this day 70 mL)

Day 70: Blood: Three 10 mL lavender top (EDTA) tubes—to WCM core lab

(Total research blood draw this day 30 mL)

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

Blood: Six 10 mL lavender top (EDTA) tubes—to WCM Core Lab

One 10 mL red top (serum) tube—serum to be processed and frozen by WCM Core Lab (Total research blood draw this day 70 mL)

Biospy collection:

Prior to sample collection

1. Please email Sharanya Chandrasekhar – shc2043@med.cornell.edu and Pragya Yadav – pry2003@med.cornell.edu once a patient consents with the biopsy schedule.

2. The sub site will alert about a patient enrolment as soon as possible, and at least a week in advance, and communicate the estimated time of arrival (ETA) of patient biopsy to Lab. [Note: The physician consenting the patient should make sure that the radiologist performing the biopsy is aware that this is a research biopsy and that if possible >3 cores **at least 18G are needed**).

Patient sample collection

1. Please prepare a 50 ml Falcon Tube filled with 20 ml sterile RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2mM Glutamine, Penicillin 100U/ml, Streptomycin 100 ug/ml (complete medium). Keep tube on ice until biopsies are collected.

2. The core sample needs to be immediately placed into the complete medium and placed on ice and shipped to WCM using frozen ice packs on the same day of collection. ***Please do not freeze the samples.***

WCM research team:

Sharanya Chandrasekhar – shc2043@med.cornell.edu
Pragya Yadav – pry2003@med.cornell.edu

Shipping information:

1. Please ship samples ambient on the day of collection.
2. Notify via email to the WCM research team

Please ship samples ambient to the following address:

Dr. Sandra Demaria
413 East 69th Street – Belfer Research Building
Demaria Lab, Rm # E209
New York, NY 10021
Tel # 646 962 2260

APPENDIX 3 GI Toxicity Management Algorithm

GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade 2 or 3/4
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	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • 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): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

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

Updated 05-Jul-2016

APPENDIX 4 Hepatotoxicity Management Algorithm

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or T. bili > ULN to 1.5 x ULN	• Continue I-O therapy per protocol	• Continue LFT monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: •Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 g BID • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

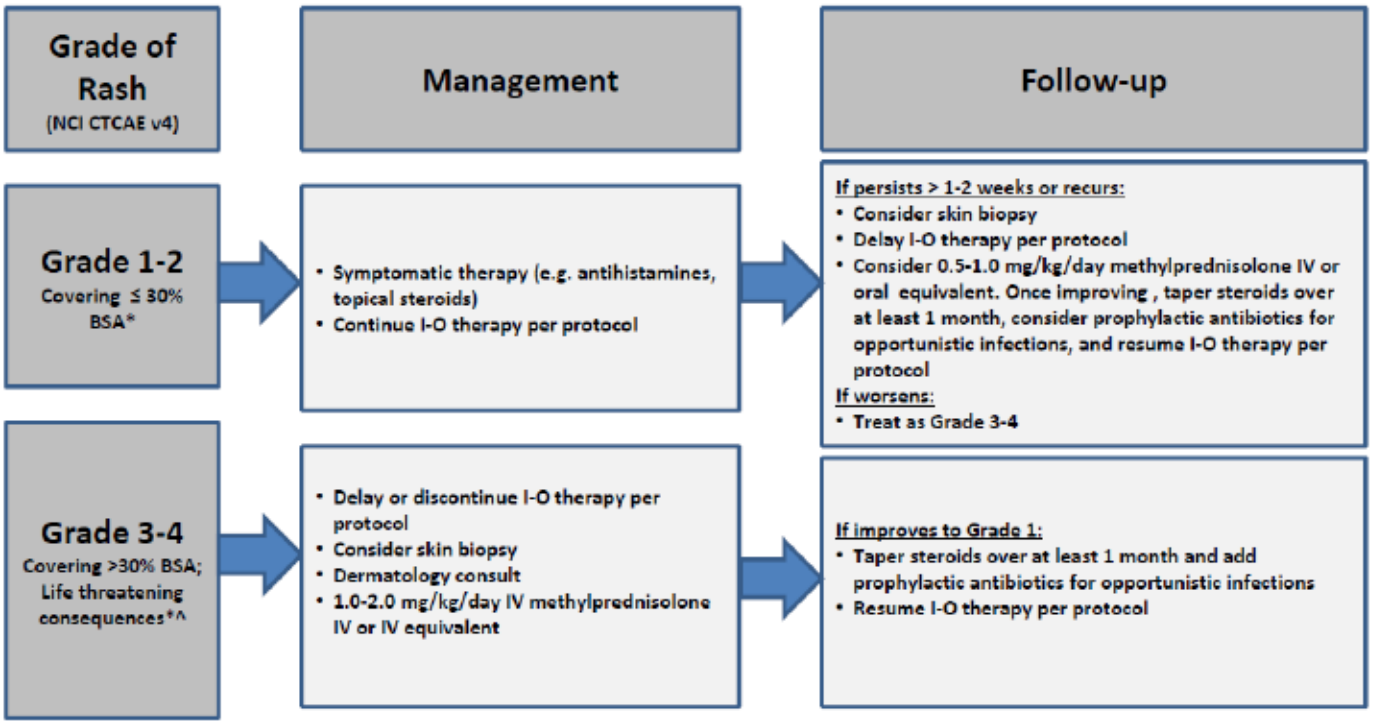
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Updated 05-Jul-2016

APPENDIX 5: Skin Toxicity Management Algorithm

Skin Adverse Event Management Algorithm

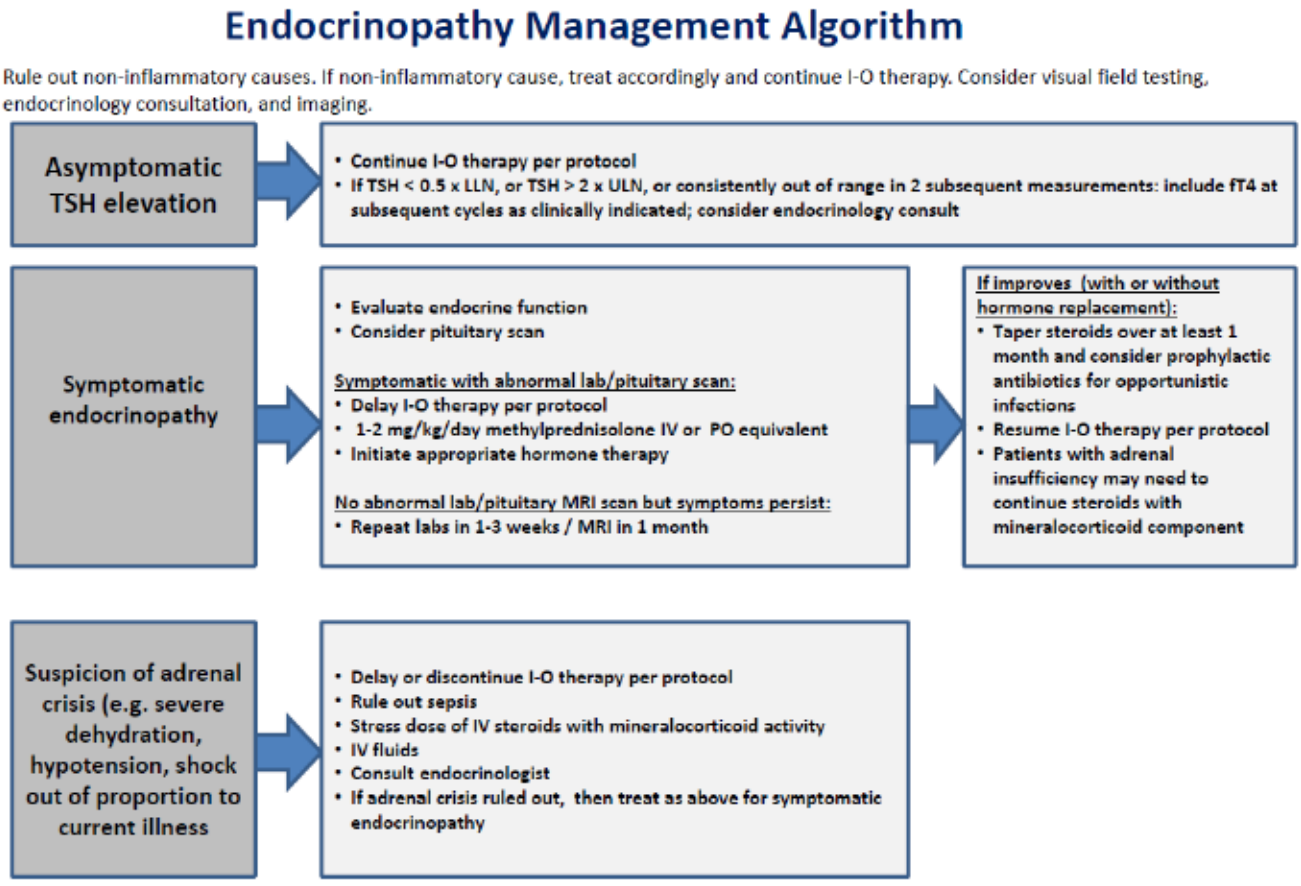
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.
^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Updated 05-Jul-2016

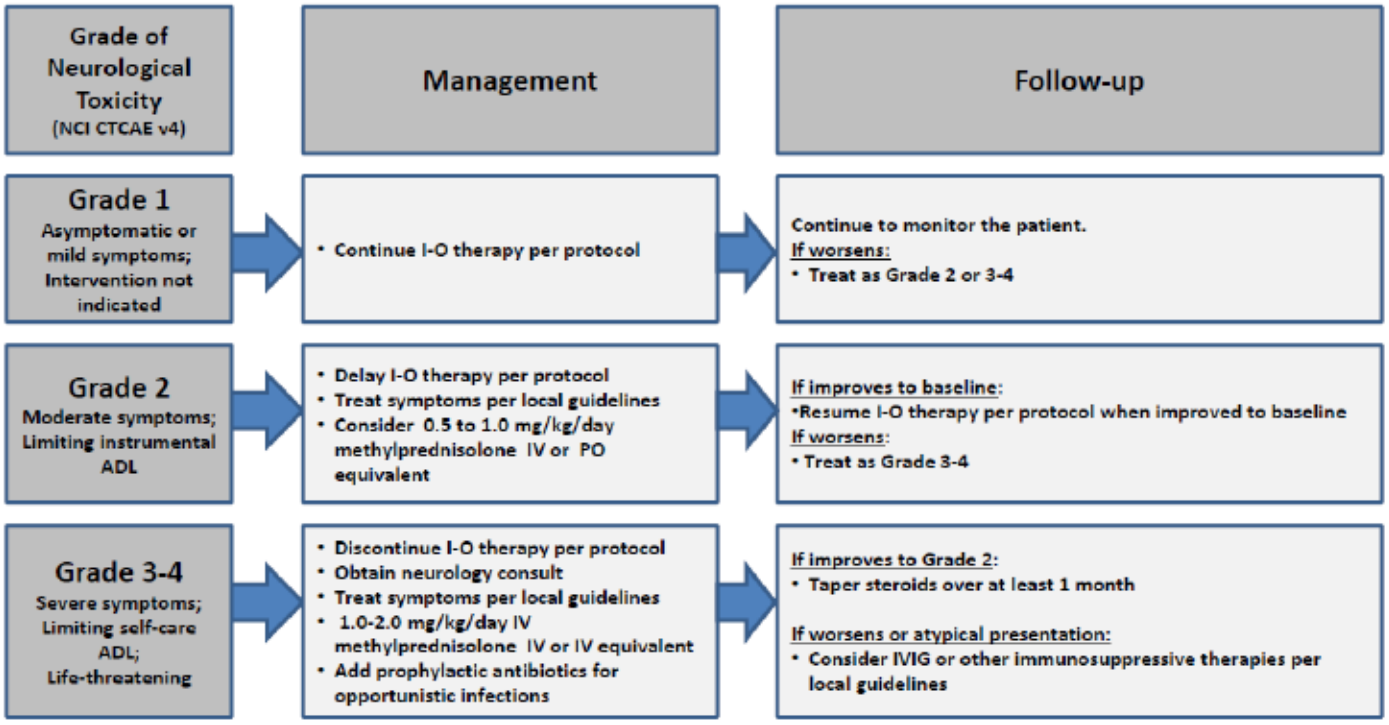
APPENDIX 6: Endocrinopathy Management Algorithm



APPENDIX 7: Neurological Toxicity Management Algorithm

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



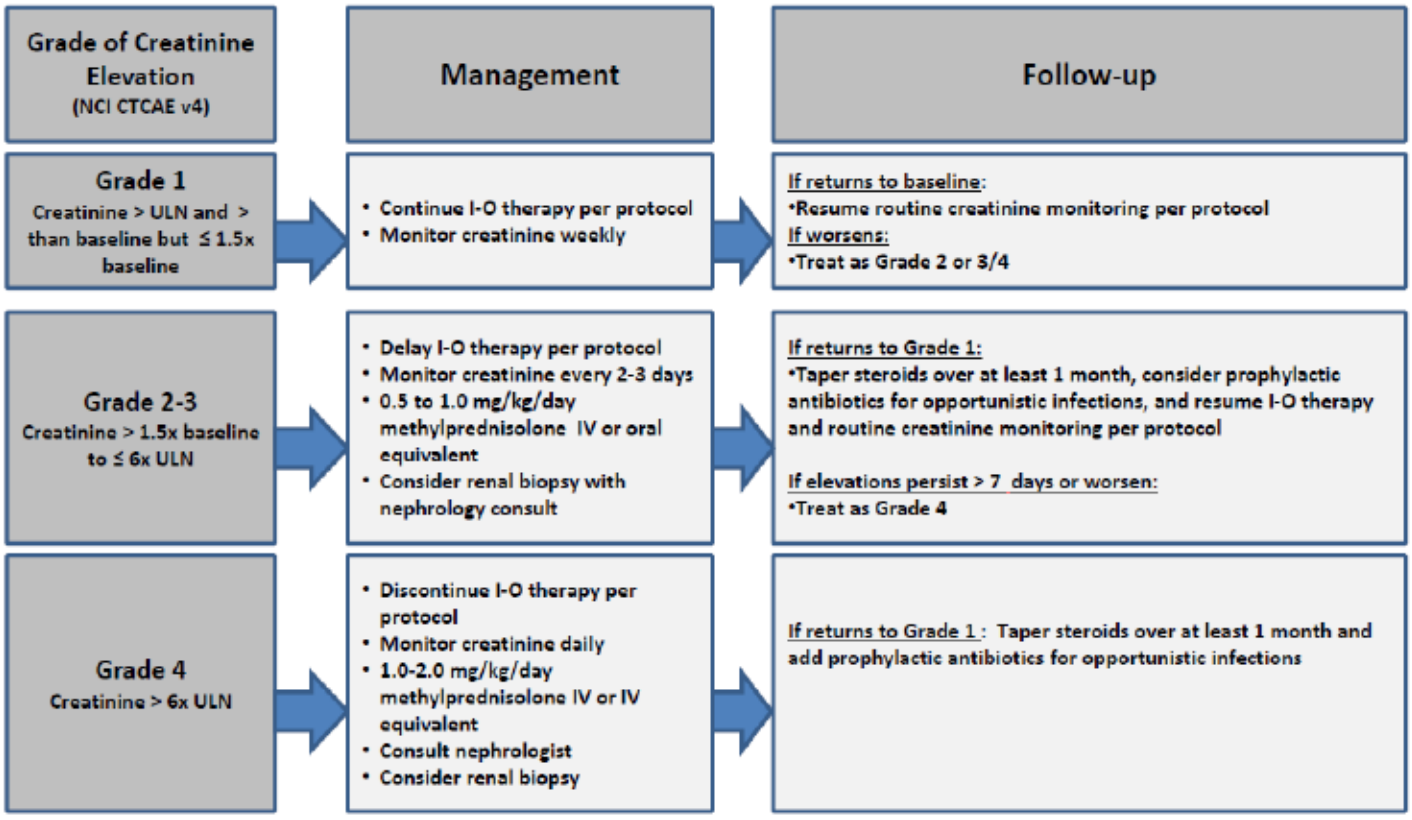
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Updated 05-Jul-2016

APPENDIX 8: Renal Toxicity Management Algorithm

Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



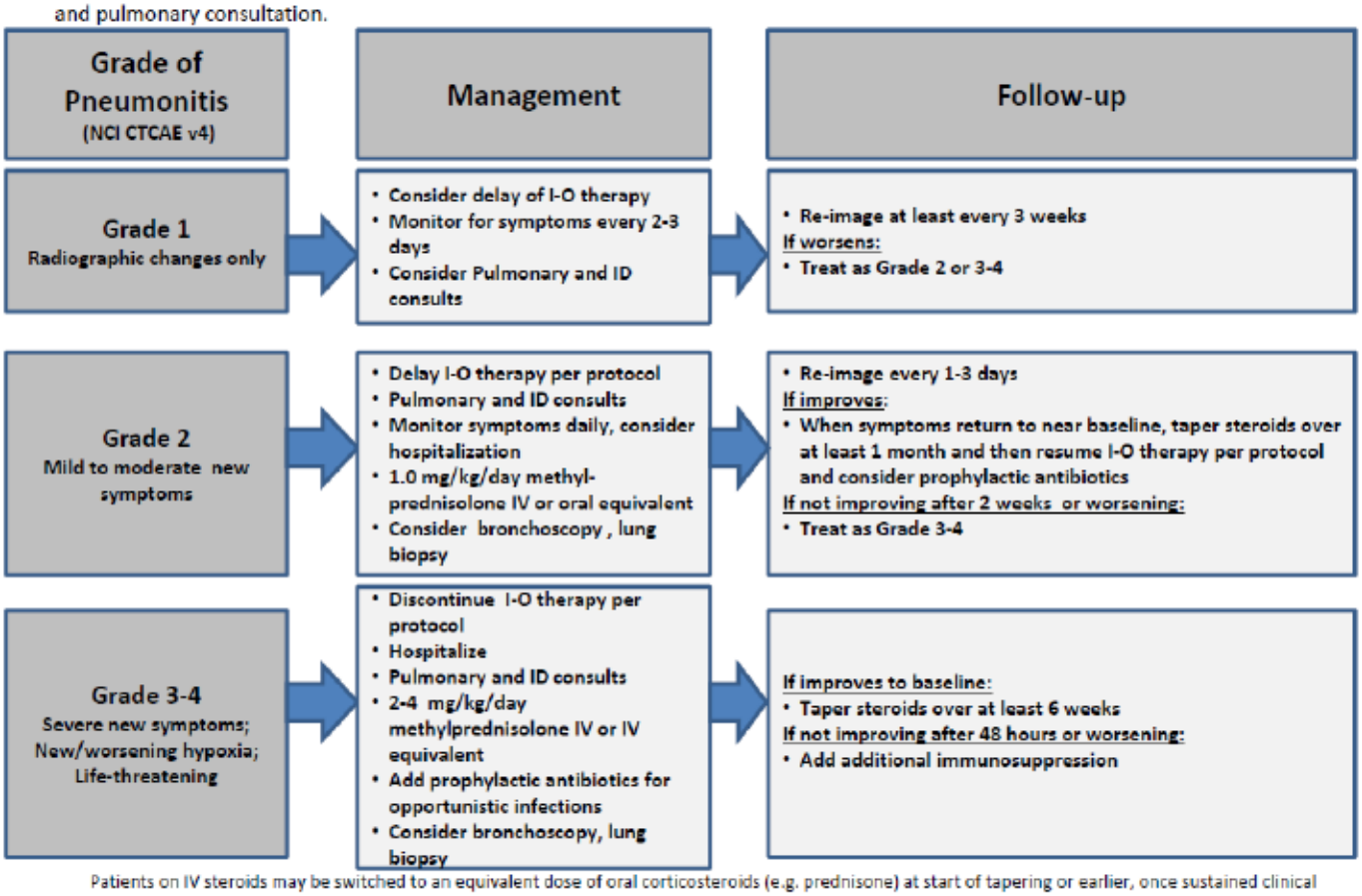
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Updated 05-Jul-2016

APPENDIX 9: Pulmonary Toxicity Management Algorithm

Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Updated 05-Jul-2016

APPENDIX 10 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
irPFS	Immune-Related Progression Free Survival
irPR	Immune-Related Partial Response
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

Abbreviation	Term
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
PTV	Planning Target Volume
RCOIC	Research Conflict of Interest Committee
RECIST	Response Evaluation Criteria In Solid Tumors
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

APPENDIX 11 INSTRUCTIONS FOR COLLECTION OF STOOL SAMPLES AT HOME:

Stool samples will be collected on Day 1, Day 22, Day 70 and at 6 month follow up.

Stool Collection

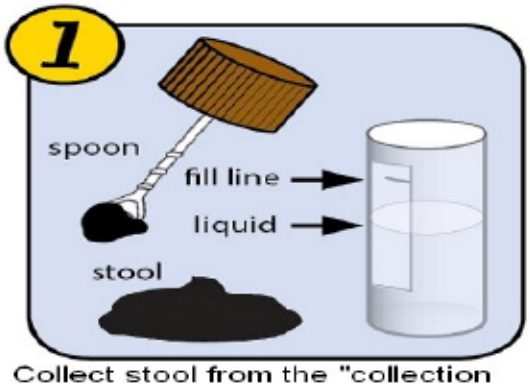
Step 1: Place the collection hat under the toilet seat

Step 2: Your stool should "land" in the collection hat.

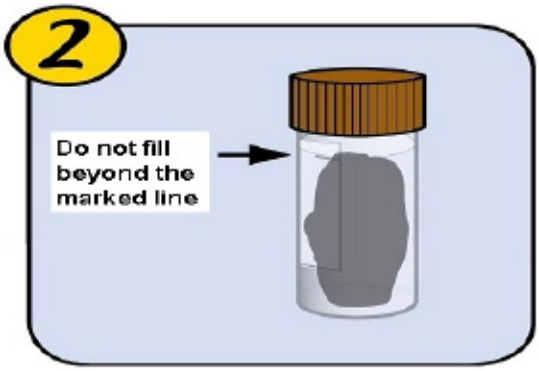
- Do not place toilet tissue in the hat
- Do not urinate in the hat – this is very important – if urine comes in contact with the stool sample discard the sample and start again. You should rinse the collection hat and dry it with a paper towel.

Step 3: "Scoop" one sample into each of the 2 collection tubes.

- After your sample has "landed" in the collection hat
  - Unscrew the blue cap from the tube
  - Using the "scoop", collect a pea-sized sample from the middle of the stool. Do not touch the stool or the scoop or inside of collection tube.
  - Place the scoop with the sample replace it on the tube and tighten the blue cap.



Collect stool from the "collection hat" or diaper and transfer to the vial, making sure that the sample with liquid does not go beyond the fill line. The liquid does not have to reach as high as the fill line, a pea-sized scoop is sufficient.



Replace the cap on the vial tightly and shake vial with liquid until the stool has dissolved into the liquid.

Step 4: Flush the remaining stool

Step 5: Pack the sample for return

Label the collection bag with your:

- Name
- Date
- Time of collection

Place the specimens into the collection bag labeled urine and stool. Store the samples in the refrigerator until you leave for your appointment (drop off site).

STEP 6: When you are ready to transport the sample to the drop off site, remove the frozen gel packs from your freezer. Place one pack in the bottom of the provided lunch

19.0 NIVOLUMAB (BMS-936558) PHARMACY REFERENCE MATERIAL

As this is provided for guidance only, please see investigator brochure for additional information regarding preparation and administration

Nivolumab has a concentration of 10mg/mL and is provided in a 10mL vial. Ten or five vials are provided in a carton.

Storage Conditions & Handling:

- Store at 2-8°C (36-46°F), protect from light, freezing, and shaking.
- If any temperature excursions are encountered during storage, please report these to BMS for assessment via the Temperature Excursion Response Form.

- As with all injectable drugs, care should be taken when handling and preparing nivolumab. Whenever possible, nivolumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique.
- Partially used vials should be disposed at the site following procedures for the disposal of anticancer drugs.

After final drug reconciliation, unused nivolumab vials should be disposed at the site following procedures for the disposal of anticancer drugs. For further information, please either discuss with your BMS CSR&O protocol manager or refer to your site IP Destruction policies and procedures

Use Time/Stability: Please refer to the appropriate section of the current Investigator Brochure or Addendum. Due to parameters surrounding the use time of nivolumab and ipilimumab, the time of preparation should be noted in the Pharmacy Source documents [accountability logs] or in study files as required for investigator sponsored research [FDA and GCP]

The administration of BMS-936558-01 injection prepared for dosing nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 20 hours in a refrigerator at under refrigeration conditions (2°-°-8°C (, 36°-46°F) and used within 24 hours, and a maximum of 4 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and under room light. The maximum 4-hour period under room temperature and room light conditions for undiluted and diluted solutions of BMS-936558-01 injection in the IV bag includes the product administration period.

Preparation and Administration:

1. Visually inspect the drug product solution for particulate matter and discoloration prior to administration. Discard if solution is cloudy, if there is pronounced discoloration (solution may have a pale-yellow color), or if there is foreign particulate matter other than a few translucent-to-white, amorphous particles. *Note: Mix by gently inverting several times. Do not shake.*
2. Aseptically withdraw the required volume of nivolumab solution into a syringe, and dispense into an IV. bag. 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. **Do not** enter into each vial more than once. **Do not** administer study drug as an IV push or bolus injection
3. Add the appropriate volume of 0.9% Sodium Chloride Injection solution or 5% Dextrose Injection solution. *It is acceptable to add nivolumab solution from the vials into an appropriate pre-filled bag of diluent.*
4. ***Note: Nivolumab infusion concentration must be at or above the minimum allowable concentration of 0.35 mg/mL [IBV13 Addendum Section 3.2.2]***
5. *Note: It is not recommended that so-called "channel" or tube systems are used to transport prepared infusions of nivolumab.*
6. Attach the IV bag containing the nivolumab solution to the infusion set and filter.
7. At the end of the infusion period, flush the line with a sufficient quantity of approved diluents.

20.0 IPILIMUMAB PHARMACY REFERENCE MATERIAL

Ipilimumab vials (40 mL) are shipped in quantities of four..

Ipilimumab (BMS-736016) Injection (5 mg/ml) must be stored refrigerated (2-8°C, 36-46°F) with protection from light and from freezing. Ipilimumab may be stored in IV infusion bags (PVC, non-PVC/non-DEHP) or glass infusion containers for up to 24 hours at room temperature (20-25°C, 68-77°F) or refrigerated (2-8°C, 36-46°F). This would include any time in transit and the total time for infusion. Drug must be completely delivered within 24 hours of preparation.

Storage Conditions & Handling:

Ipilimumab injection may be stored undiluted, 200 mg/vial (5 mg/mL), or following dilution to concentrations between 1 mg/mL and 4 mg/mL in 0.9% Sodium Chloride Injection (USP), or 5% Dextrose Injection (USP) in PVC, non-PVC/ or glass containers for up to 24 hours in the refrigerator (2°C to 8°C) or at room temperature/room light. For longer storage, ipilimumab should be kept refrigerated (2°C to 8°C) with protection from light.

Ipilimumab injection must not be frozen.

Partially used vials or empty vials of Ipilimumab Injection should be discarded at the site according to appropriate drug disposal procedures.

Preparation and Administration

As this is provided for guidance only, please see investigator brochure for additional information regarding preparation and administration.

1. As ipilimumab is stored long term at refrigerated temperatures (2-8°C) and protected from light, allow the appropriate number of vials of ipilimumab to stand at room temperature for approximately five minutes.
2. Ensure that the ipilimumab solution is clear colorless, 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.
3. Aseptically transfer the required volume of ipilimumab solution into a syringe. [Note: A sufficient excess of ipilimumab is incorporated into each vial to account for withdrawal losses].
4. Do not draw into each vial more than once. Discard partially used vials or empty vials.
5. Ipilimumab solution should be added to an appropriate size infusion container to accommodate the calculated final volume.

   Total dose should be calculated using the most recent subject weight; if weight on dosing day differs by 10% from prior weight used to calculate dosing, the dose should be recalculated and study drug adjusted accordingly.

   Mix by GENTLY inverting several times. DO NOT shake.

   Ipilimumab injection may be diluted in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP.

6. Visually inspect the final solution. If the initial diluted solution or final solution for infusion is not clear or contents appear to contain precipitate, the solution should be discarded.
7. Immediately after the infusion is complete, flush with an adequate amount of 0.9% Sodium Chloride injection (USP) or 5% Dextrose injection (USP) to completely flush the residual fluid (dead space) in your administration set (approximately 30-50mL); this will ensure that all active drug is delivered to the study participant
8. Safely discard any unused portion of the infusion solution. Do not store for reuse.

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

It is possible that sites may have more than one ipilimumab clinical study ongoing at the same time. <u>It is imperative that only product designated for this protocol be used for this study.</u>

21.0 REFERENCES

1. Bishop, M.R., et al., *Allogeneic lymphocytes induce tumor regression of advanced metastatic breast cancer.* J Clin Oncol, 2004. **22**(19): p. 3886-92.
2. Galon, J., et al., *The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures.* Immunity, 2013. **39**(1): p. 11-26.
3. Formenti, S.C. and S. Demaria, *Radiation therapy to convert the tumor into an in situ vaccine*. Int J Radiat Oncol Biol Phys, 2012. **84**(4): p. 879-80.
4. Formenti, S.C. and S. Demaria, *Combining radiotherapy and cancer immunotherapy: a paradigm shift.* J Natl Cancer Inst, 2013. **105**(4): p. 256-65.
5. Matsumura, S., et al., *Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells.* J Immunol, 2008. **181**(5): p. 3099-107.
6. Demaria, S., et al., *Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated.* Int J Radiat Oncol Biol Phys, 2004. **58**(3): p. 862-70.
7. E.B. Golden , A.C., M.B. Fenton-Kerimian, S. Demaria, S.C. Formenti, *Abscopal Responses in Metastatic Non-Small Cell Lung Cancer (NSCLC) Patients Treated on a Phase 2 Study of Combined Radiation Therapy and Ipilimumab: Evidence for the In Situ Vaccination Hypothesis of Radiation.* International journal of Radiation Oncology*Biology*Physics, 2015. **93**(3): p. Supplement, Pages S66-S67.
8. Borghaei, H., et al., *Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(17): p. 1627-39.
9. Brahmer, J., et al., *Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(2): p. 123-35.
10. Rizvi NA, G.S., Goldman J et al. , *Safety and Efficacy of First-line Nivolumab (Antiprogrammed Death-1 [PD-1]) and Ipilimumab in Non-small Cell Lung Cancer (NSCLC)*. Presented at WCLC 2015 Annual Meeting in Denver, CO, 2015.
11. Lenschow, D.J., T.L. Walunas, and J.A. Bluestone, *CD28/B7 system of T cell costimulation*. Annu Rev Immunol, 1996. **14**: p. 233-58.

12. Schwartz, R.H., *Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy.* Cell, 1992. **71**(7): p. 1065-8.

13. Chen, L., et al., *Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4.* Cell, 1992. **71**(7): p. 1093-102.
14. Townsend, S.E. and J.P. Allison, *Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells.* Science, 1993. **259**(5093): p. 368-70.
15. Allison, J.P., A.A. Hurwitz, and D.R. Leach, *Manipulation of costimulatory signals to enhance antitumor T-cell responses.* Curr Opin Immunol, 1995. **7**(5): p. 682-6.
16. Townsend, S.E., et al., *Specificity and longevity of antitumor immune responses induced by B7-transfected tumors.* Cancer Res, 1994. **54**(24): p. 6477-83.
17. Linsley, P.S., et al., *CTLA-4 is a second receptor for the B cell activation antigen B7.* J Exp Med, 1991. **174**(3): p. 561-9.
18. Thompson, C.B. and J.P. Allison, *The emerging role of CTLA-4 as an immune attenuator.* Immunity, 1997. **7**(4): p. 445-50.
19. Kearney, E.R., 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): p. 1032-6.
20. Krummel, M.F. and J.P. Allison, *CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation.* J Exp Med, 1995. **182**(2): p. 459-65.
21. Krummel, M.F., T.J. Sullivan, and J.P. Allison, *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): p. 519-23.
22. Walunas, T.L., et al., *CTLA-4 can function as a negative regulator of T cell activation.* Immunity, 1994. **1**(5): p. 405-13.
23. Melief, C.J., *Tumor eradication by adoptive transfer of cytotoxic T lymphocytes.* Adv Cancer Res, 1992. **58**: p. 143-75.
24. Antonia, S., J.J. Mule, and J.S. Weber, *Current developments of immunotherapy in the clinic.* Curr Opin Immunol, 2004. **16**(2): p. 130-6.
25. Rosenberg, S.A., *Progress in human tumour immunology and immunotherapy.* Nature, 2001. **411**(6835): p. 380-4.

26. Egen, J.G., M.S. Kuhns, and J.P. Allison, *CTLA-4: new insights into its biological function and use in tumor immunotherapy.* Nat Immunol, 2002. **3**(7): p. 611-8.

27. Leach, D.R., M.F. Krummel, and J.P. Allison, *Enhancement of antitumor immunity by CTLA-4 blockade.* Science, 1996. **271**(5256): p. 1734-6.

28. Yang, Y.F., 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): p. 4036-41.

29. Hurwitz, A.A., et al., *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): p. 10067-71.

30. van Nood, E., et al., *Duodenal infusion of donor feces for recurrent Clostridium difficile.* N Engl J Med, 2013. **368**(5): p. 407-15.

31. Dranoff, G., *CTLA-4 blockade: unveiling immune regulation.* J Clin Oncol, 2005. **23**(4): p. 662-4.

32. Mole, R.H., *Whole body irradiation; radiobiology or medicine?* Br J Radiol, 1953. **26**(305): p. 234-41.

33. Ehlers, G. and M. Fridman, *Abscopal effect of radiation in papillary adenocarcinoma.* Br J Radiol, 1973. **46**(543): p. 220-2.

34. Ganss, R., et al., *Combination of T-cell therapy and trigger of inflammation induces remodeling of the vasculature and tumor eradication.* Cancer Res, 2002. **62**(5): p. 1462-70.

35. Nikitina, E.Y. and D.I. Gabrilovich, *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): p. 825-33.

36. Ohba, K., et al., *Abscopal regression of hepatocellular carcinoma after radiotherapy for bone metastasis.* Gut, 1998. **43**(4): p. 575-7.

37. Chakravarty, P.K., et al., *Flt3-ligand administration after radiation therapy prolongs survival in a murine model of metastatic lung cancer.* Cancer Res, 1999. **59**(24): p. 6028-32.

38. Formenti, S.C. and S. Demaria, *Systemic effects of local radiotherapy.* Lancet Oncol, 2009. **10**(7): p. 718-26.

39. Kingsley, D.P., *An interesting case of possible abscopal effect in malignant melanoma.* Br J Radiol, 1975. **48**(574): p. 863-6.
40. Demaria, S., 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): p. 728-34.
41. Golden, E.B., et al., *Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: a proof-of-principle trial.* Lancet Oncol, 2015. **16**(7): p. 795-803.
42. Brody, J.D., et al., *In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study.* J Clin Oncol, 2010. **28**(28): p. 4324-32.
43. Postow, M.A., et al., *Immunologic correlates of the abscopal effect in a patient with melanoma.* N Engl J Med, 2012. **366**(10): p. 925-31.
44. Gulley, J.L., et al., *Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer.* Clin Cancer Res, 2005. **11**(9): p. 3353-62.
45. Hodi, F.S., et al., *Improved survival with ipilimumab in patients with metastatic melanoma.* N Engl J Med, 2010. **363**(8): p. 711-23.
46. Golden, E.B., et al., *An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer.* Cancer Immunol Res, 2013. **1**(6): p. 365-72.
47. Lynch, T.J., 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**(17): p. 2046-54.
48. Zatloukal P, H.D., 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 2009. **27**(15).

49. Topalian, S.L., et al., *Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.* N Engl J Med, 2012. **366**(26): p. 2443-54.

50. Antonia, S., et al., *Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study.* Lancet Oncol, 2016. **17**(3): p. 299-308.
51. Motzer, R.J., *Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma.* The New England Journal of Medicine, 2015: p. 373:1803-1813.
52. Benedict, S.H., et al., *Stereotactic body radiation therapy: the report of AAPM Task Group 101.* Med Phys, 2010. **37**(8): p. 4078-101.
53. Robert, L., et al., *CTLA4 blockade broadens the peripheral T-cell receptor repertoire.* Clin Cancer Res, 2014. **20**(9): p. 2424-32.
54. Maecker, H.T., *Multiparameter flow cytometry monitoring of T cell responses.* Methods Mol Biol, 2009. **485**: p. 375-91.
55. Emerson, R.O., et al., *High-throughput sequencing of T-cell receptors reveals a homogeneous repertoire of tumour-infiltrating lymphocytes in ovarian cancer.* J Pathol, 2013. **231**(4): p. 433-40.
56. Koguchi, Y., et al., *Serum Immunoregulatory Proteins as Predictors of Overall Survival of Metastatic Melanoma Patients Treated with Ipilimumab.* Cancer Res, 2015. **75**(23): p. 5084-92.
57. Jinushi, M., F.S. Hodi, and G. Dranoff, *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, 2006. **103**(24): p. 9190-5.
58. Sivan, A., et al., *Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy.* Science, 2015. **350**(6264): p. 1084-9.
59. Vetizou, M., et al., *Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota.* Science, 2015. **350**(6264): p. 1079-84.
60. Cho, I., et al., *Antibiotics in early life alter the murine colonic microbiome and adiposity.* Nature, 2012. **488**(7413): p. 621-6.

61. Cox, L.M., et al., *The nonfermentable dietary fiber hydroxypropyl methylcellulose modulates intestinal microbiota.* FASEB J, 2013. **27**(2): p. 692-702.
62. Caporaso, J.G., et al., *QIIME allows analysis of high-throughput community sequencing data*. Nat Methods, 2010. **7**(5): p. 335-6.

63. Segal, L.N., et al., *Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation.* Microbiome, 2013. **1**(1): p. 19.
64. Statnikov, A., et al., *A comprehensive evaluation of multicategory classification methods for microbiomic data.* Microbiome, 2013. **1**(1): p. 11.

65. Simon R (1989). *Controlled Clinical Trials* 10: 1-10.

66. Clopper, C. and Pearson, E.S. (1934). *The use of confidence or fiducial limits illustrated in the case of the binomial*. Biometrika, 26, pp. 404-413.

22.0 SUB-STUDY OF 1607017434 (BMS-CA209-632)

Innovative immuno monitoring in a clinical trial of radiotherapy and immune checkpoint blockade.

1. INVESTIGATORS:

P.I. Name: Silvia Formenti, M.D.
Title and Department: Chair, Radiation Oncology
Telephone: 212-746-3608
Email Address: formenti@med.cornell.edu

Co-P.I. Name: Sandra Demaria, M.D.
Title and Department: Professor, Radiation Oncology and Pathology
Telephone: 212-746-6068
Email Address: szd3005@med.cornell.edu

RESEARCH PROPOSAL

ABSTRACT. Radiotherapy (RT) is under investigation in the clinic for its ability to induce anti-tumor immunity and enhance responses to immune checkpoint blockade (ICB). An improved understanding of the mechanisms underlying the immune-stimulatory activity of RT is much needed. The influence of dose, per fraction, type of fractionation and the interplay with tumor oncogenic alterations need elucidation to move from an empirical to a precision use of RT, to enhance the therapeutic benefits of radiation in the context of immunotherapy. We have recently identified the DNA exonuclease Trex1 as a regulator of radiation-induced anti-tumor immunity and shown that Trex1 induction is dependent on the radiation dose per each fraction of exposure (2). RT induces the accumulation of dsDNA in the cancer cell cytoplasm where it is sensed by cyclic GMP-AMP (cGAMP) synthase (cGAS, *MB21D1*), which activates downstream adaptor Stimulator of Interferon Genes (STING, *TMEM173*) to induce IFN□ and downstream IFN-stimulated genes (ISGs). Trex1 acts by clearing cytoplasmic dsDNA, thus removing the substrate for cGAS-STING pathway induced IFN-type I-production and the consequent anti-tumor CD8 T cell activation.
Our findings have opened the way to identify candidate biomarkers that predict for optimal RT and ICB use. For instance, cancer cells expression of cGAS and STING is required for the immunogenicity of radiation. Thus, cGAS and STING, which are epigenetically inactivated in a significant percentage of cancers, are candidate biomarkers to predict which tumor may benefit from the addition of radiation to ICB. Furthermore, downstream of cGAS and STING other factors such as IRF3 have been shown to be defective in some cancers, precluding IFN-I activation. Therefore, an assay that measures the ability of radiation to elicit IFN-I production by a specific tumor and establishes the optimal radiation dose/fractionation to maximize its response will advance the field of RT and ICB combinations by guiding patient selection and the choice of precision RT. Finally, the assay will enable investigations on the effect of distinct oncogenic transformation on the threshold for TREX1 induction.
We propose to generate Patient Derived Tumor Xenograft (PDTX) from pre-treatment tumor biopsies of non-small cell lung cancer (NSCLC) patients enrolled in a prospective trial investigator-initiated at WCM/MCC (P.I. Dr. Formenti, NCT03168464) and supported by BMS (BMS # CA209-632). In this trial RT is delivered to a metastatic site in NSCLC to determine its ability to enhance responses of CTLA-4 blockade by ipilimumab (ipi), in combination with PD-1 blockade by nivolumab (nivo). PDTX from cancer biopsies of accrued patients will be grown to an average of 5 mm diameter, and irradiated with different

dose per fraction, followed by measurements of expression of Trex1, IFN□ and other ISGs. We have established feasibility of this approach, in a primary patient-derived NSCLC (2). Circulating T cells collected pre and post-treatment from patients will be adoptively transferred to PDTX mice to test if they home to the tumor and can mediate tumor rejection. The goal is to develop a companion murine protocol to the clinical trial to: (1) explore the induction of IFN-I and Trex 1 as biomarkers to tailor the radiation regimen in a personalized way; (2) study the interaction between radiation and the molecular signature of a given tumor; and (3) study the T cell responses that mediate tumor rejection and identify their antigenic targets. This proposal leverages a multidisciplinary team of investigators with complementary expertise in clinical radiation oncology and immunotherapy (Dr. Formenti), immunotherapy pre-clinical and translational studies (Dr. Demaria), PDTX and molecular characterization of tumors (Dr. Inghirami). SPECIFIC AIMS. NCT03168464 is a phase I-II study of patients with metastatic NSCLC testing the hypothesis that initial local RT during anti-CTLA-4 blockade with Ipilimumab is safe and increases the objective response rate to the combination of ipilimumab and nivolumab by 20%. The accrual goal is 44 evaluable patients. A mandatory biopsy is performed at baseline and blood is collected longitudinally. We propose to use part of the tissue from this individual patient biopsy to generate PDTX mice in order to assess functional biomarkers of response to RT, and the interaction of T cells isolated from patients' blood during the course of treatment with the tumor. The aims are:

1) To determine the rate of successful generation of PDTX using biopsy tissue from the patients enrolled in the trial (expected accrual rate 20-25 patients/year)
2) To determine the expression of cGAS and STING and the induction of *ifnb1*, ISGs, and Trex1 after in vivo tumor irradiation of PDTX with various doses per fraction.
3) To determine feasibility of using autologous PBMC collected during the course of treatment to study the TCR repertoire and antigen specificity of T that mediate tumor rejection in patients.

SIGNIFICANCE. Preclinical data on the critical role of IFN-I in the development of therapeutically relevant tumor-specific immune responses (3) are supported by clinical findings documenting the endogenous activation of IFN-I signaling in spontaneously regressing tumors (4), and in metastases from cancers highly infiltrated by T cells (5, 6). Thus, several strategies are being pursued to activate IFN-I signaling within the microenvironment of tumors devoid of T cells to convert them into T cell-rich tumors that would respond to ICBs (6-8). We have recently shown that RT by itself has the potential to effectively induce the secretion of IFN-I within the tumor microenvironment, but it must be administered at optimal doses and schedules to generate robust anti-tumor immunity (2). Poorly immunogenic tumors avoid generation of anti-tumor T cells by excluding DCs (9). In these tumors, RT activation of IFN-I by cancer cells can be an efficient and clinically viable means to recruit and activate BATF3-dependent DCs, and hence prime a T-cell response that overcomes resistance to ICBs and mediates abscopal effects (2). Cancer cell-intrinsic IFN-I activation in response to radiation is dependent on the expression of cGAS and STING and on a cellular microenvironment that allows for the productive activation of cGAS/STING signaling (2). Recent data indicate that cGAS and/or STING expression is down-regulated, often by epigenetic mechanisms, in over a third of colorectal cancers and melanomas, and in some melanoma cells the ability of STING to activate the transcription factors NF-kB or IRF3 is compromised (10, 11). While the frequency of expression of cGAS and STING in lung cancer has not been reported, we have found loss of STING expression in some patients from a previous trial (NCT02221739, S.Formenti, P.I., (1)) (Figure 1). Thus, it is important to determine if cGAS and/or STING expression in malignant cells can serve as a biomarker to identify the patients who may benefit from the addition of RT to ICBs. A routine immunohistochemical (IHC) staining can be employed for this purpose (Figure 1). Along similar lines, evaluating the methylation status of the *MB21D1* and/or

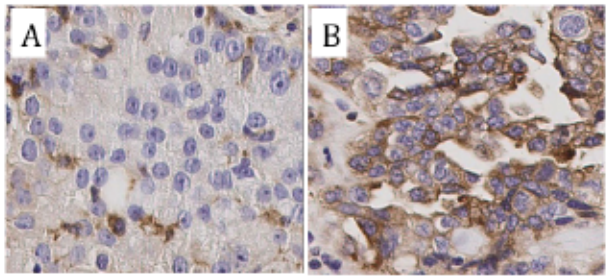


Figure 1. Example of (A) STING- non-responder, (B) STING+ responder NSCLC from RT+Ipi trial (1).

TMEM173 promoters may inform on the possibility of using de-methylating agents to recover IFN-I secretion in response to RT.

The global functionality of the IFN-I-secreting machinery in cancer cells and the identification of the most immunostimulatory RT dose and schedule for each specific tumor also need to be defined in each individual case. In a panel of mouse and human carcinoma cells, the RT dose threshold for TREX1 induction at levels that are sufficient to degrade cytosolic DNA ranged from 12 to 18Gy (2). Specific mutations that affect the DNA damage response in a given cancer are likely to also influence the threshold. While we are currently investigating the molecular mechanisms that regulated Trex1 induction by RT under different funding mechanism (R01CA201246), testing tumor samples from the NCT03168464 trial will provide a unique opportunity to explore the range of RT doses that are most immunogenic in NSCLC patients, and generate preliminary information on cGAS, STING, TREX1, IFN-I and ISGs as biomarkers of response to combinations of RT and ICBs in the clinic. Moreover, patients PBMC applied to the PTDX model to test for their ability to recognize the tumor could lead to development of an assay for early response prediction, and identification of the tumor (neo) antigens recognized in irradiated and non-irradiated tumors. Collectively, the proposed exploratory studies have the potential to generate preliminary data to move the field forward by both providing biomarkers for improved patient selection and enabling precision radiotherapy, through personalized dose per fraction choices to boost the efficacy of ICB.

INNOVATION. We were the first to implicate the anti-tumor immune response in mediating the abscopal effect of RT (12), and to demonstrate that local RT converted a tumor unresponsive to ICB into a responsive one (13). We have also shown that hypo-fractionated (8GyX3 or 6GyX5) but no single high dose (20Gy) RT was effective at inducing abscopal effects in combination with anti-CTLA-4 in pre-clinical models (14). Furthermore, we proved that RT regimens, similar to the ones effective in mice, could induce abscopal effects in lung cancer patients treated with RT and ipilimumab (1, 15). We have recently discovered a completely novel mechanism, the RT dose-dependent regulation of cancer cell-intrinsic IFN-I activation by the DNA exonuclease Trex1. Our recently published data show that in poorly immunogenic tumors, refractory to treatment with ICBs, RT induces cancer cells to produce IFN□ that recruits DCs to prime CD8 T cells that mediate abscopal responses (2). The validity of this mechanism has never been demonstrated in patients. Moreover, while PTDX have been used to perform co-clinical trials to test targeted therapies in lung cancer (16), this research paradigm has not been previously used to study the response to RT and immunotherapy (17). Thus, results of this feasibility project have the potential to address a crucial clinical question and lead to a cutting edge original approach for immunomonitoring during trials testing RT and immunotherapy combinations.

APPROACH. In NCT03168464, patients with metastatic NSCLC will be treated with non-ablative RT (6GyX5) directed to one lesion with Ipi 3 mg/kg (± 24hrs from first RT dose). On day 22 the combined treatment of ipi plus nivo will start (nivo 360mg q 3 weeks, ipi 1mg/kg q 6 weeks), and administered until evidence of progression. Patients will be evaluated for response (defined as an objective response by RECIST of the measurable metastatic sites outside the radiation field) at week 9. Blood will be collected at baseline, day 22 and day 70 (at time of response evaluation), and at 6 months follow up. A mandatory biopsy will be performed prior to treatment start. Tissue from this biopsy will be used to generate PTDX mice. Lymphocytes and serum will be separated from blood and cryopreserved.

Aim 1: *To determine the rate of successful generation of PTDX using biopsy tissue from the patients enrolled in the trial (expected accrual rate 20-25 patients/year).* As per IRB Protocol #1607017434, 3 tissue cores will be collected by interventional radiology, from the lesion to be irradiated. Cores will be triaged by the PDTX Shared Resource core and tissue allocated for RNASeq (in RNA later), DNA and protein separation (snap frozen), histology and IHC (FFPE), and s.c. implantation into NSG mice to generate PDTX. Sequencing will be performed before implantation and after growth in the mice to confirm that PDTX maintain the original tumor genetics.

Aim 2: *To determine the expression of cGAS and STING and the induction of of ifnb, ISGs, and Trex1 after in vivo tumor irradiation of PTDX with various doses and fractionations*. Expression of cGAS and

STING will be assessed by RNASeq and the positivity in the cancer cells confirmed by IHC ((10) and Fig 1). Methylation status of cGAS and STING promoter will be tested by ATACseq in tumors that are negative. Once PTDX tumors have reached an average diameter of ~5 mm (approximately 2-4 weeks post-implantation) one of the two tumors will be irradiated while the contralateral tumor will serve as untreated control. For each RT dose level the N=3 is chosen based on our experience that statistically significant differences can be detected in the expression of IFN-I and ISGs as well as Trex1 using this group size (2). RT will be delivered at 6GyX1, 6GyX5, 8GyX1, 8GyX3, 15GyX1, 20GyX1. Mice will be sacrificed at 24 hours post-completion of RT and tumors divided into two portions, one for RNA and protein extraction and the other for IHC. Expression of cGas, STING, Trex1, *Ifnb1*, cxcl10, Mx1, Ifnar1 and other ISGs (2) will be measured by qRT-PCR. Protein levels of IFN□ and CXCL10 will be measured using the human procarta kits (Affymetrix-eBioscience). To detect the presence of cytosolic dsDNA tumor sections will be stained as described (2). We will also determine if tumor fragments can be used for testing the induction of *Ifnb1* and *Trex1* by single dose radiation in short-term ex-vivo cultures. Preliminary data suggest that we can obtain results comparable to in vivo irradiation (not shown). This will allow us to save time and money by selecting the most effective single dose in ex vivo culture for further testing in fractionation experiment in vivo, since we found that both single dose size and fractionation are important for optimal RT-induced IFN-I pathway activation (2).

Aim 3: *To determine feasibility of using autologous PBMC collected during the course of treatment to study the TCR repertoire and antigen specificity of T that mediate tumor rejection in patients*. PDTX mice will be injected i.p. with 2-5x10^6 autologous PBMC collected before and after treatment initiation (day 22 and 70), as described (18). Recruitment of the adoptively transferred patient T cells to untreated and irradiated tumors will be tested in the presence and absence of ipi and nivo, given at doses comparable to the doses used in the trial. Phenotype and TCR repertoire of the T cells infiltrating the PDTX will be compared to the T cells within the original tumor biopsy and in the peripheral blood of patients. Tumors will be harvested a week after treatment and tumors divided into portions, for DNA, RNA and protein extraction (to be used for TCR repertoire, mutation/neoantigen analysis, and immune signatures analysis) and histology/IHC. Immune pathway associated with tumor rejection will be analyzed using Nanostring platform. Density and distribution of CD8 and CD4 T cells, expression of proliferation (Ki67), and effector function (granzyme), and regulatory activity (FoxP3) markers, as well as PDL-1 and Caspase 3 (cell death marker) will be analyzed by immunostaining. If warranted single cell analysis will be performed on the T cells infiltrating the tumors in additional PTDX tumors.

Expected results, pitfalls and alternative approaches. We expect to have sufficient biopsy tissue from ≥70% of the patients, and successfully establish PDTX in ≥70% of the latter. With an accrual rate of 20-25 patients/year we can generate 5-7 PDTX in 9-12 months. Presuming a response rate of ≥30% based on prior experience (1), at least 2 patients should develop robust anti-tumor T cell responses during treatment. By comparing phenotype and TCR repertoire of T cells isolated at baseline and at time of response evaluation (day 70) in the patients' blood and PDTX tumors we will obtain evidence about the immuno-PDTX suitability to study anti-tumor T cells and identify their antigenic targets. To overcome the problem of early xGvHD development after PBMC engraftment in NSG mice, we will use b2m-deficient NSG mice, which we and others have proven have delayed and limited xGvHD for 12 months or more (19). While lymphopenia in the patients may limit baseline PBMC yield for adoptive transfer experiments, ipi is likely to significantly increase lymphocyte counts and the chance that sufficient PBMC will be available from post-treatment time points.

REFERENCES

1. Golden, E. B., A. Chachoua, M. B. Fenton-Kerimian, S. Demaria, and S. C. Formenti. 2015. Abscopal responses in metastatic non-small cell lung cancer (NSCLC) patients treatded on a phase 2 study of combined radiation therapy and ipilimumab: evidence for the in situ vaccination hypothesis of radiation. *Int J Radiat Oncol Biol Phys* 93: S66-67.

2. Vanpouille-Box, C., A. Alard, M. J. Aryankalayil, Y. Sarfraz, J. M. Diamond, R. J. Schneider, G. Inghirami, C. N. Coleman, S. C. Formenti, and S. Demaria. 2017. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun* 8: 15618.

3. Dunn, G. P., A. T. Bruce, K. C. Sheehan, V. Shankaran, R. Uppaluri, J. D. Bui, M. S. Diamond, C. M. Koebel, C. Arthur, J. M. White, and R. D. Schreiber. 2005. A critical function for type I interferons in cancer immunoediting. *Nat Immunol* 6: 722-729.

4. Wenzel, J., B. Bekisch, M. Uerlich, O. Haller, T. Bieber, and T. Tuting. 2005. Type I interferon-associated recruitment of cytotoxic lymphocytes: a common mechanism in regressive melanocytic lesions. *Am J Clin Pathol* 124: 37-48.

5. Harlin, H., Y. Meng, A. C. Peterson, Y. Zha, M. Tretiakova, C. Slingluff, M. McKee, and T. F. Gajewski. 2009. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res* 69: 3077-3085.

6. Woo, S. R., L. Corrales, and T. F. Gajewski. 2015. The STING pathway and the T cell-inflamed tumor microenvironment. *Trends Immunol* 36: 250-256.

7. Bald, T., J. Landsberg, D. Lopez-Ramos, M. Renn, N. Glodde, P. Jansen, E. Gaffal, J. Steitz, R. Tolba, U. Kalinke, A. Limmer, G. Jonsson, M. Holzel, and T. Tuting. 2014. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. *Cancer Discov* 4: 674-687.

8. Zitvogel, L., L. Galluzzi, O. Kepp, M. J. Smyth, and G. Kroemer. 2015. Type I interferons in anticancer immunity. *Nat Rev Immunol* 15: 405-414.

9. Spranger, S., R. Bao, and T. F. Gajewski. 2015. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* 523: 231-235.

10. Xia, T., H. Konno, J. Ahn, and G. N. Barber. 2016. Deregulation of STING Signaling in Colorectal Carcinoma Constrains DNA Damage Responses and Correlates With Tumorigenesis. *Cell Rep* 14: 282-297.

11. Xia, T., H. Konno, and G. N. Barber. 2016. Recurrent Loss of STING Signaling in Melanoma Correlates with Susceptibility to Viral Oncolysis. *Cancer Res* 76: 6747-6759.

12. Demaria, S., B. Ng, M. L. Devitt, J. S. Babb, N. Kawashima, L. Liebes, and S. C. Formenti. 2004. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys* 58: 862-870.

13. Demaria, S., N. Kawashima, A. M. Yang, M. L. Devitt, J. S. Babb, J. P. Allison, and S. C. Formenti. 2005. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin Cancer Res* 11: 728-734.

14. Dewan, M. Z., A. E. Galloway, N. Kawashima, J. K. Dewyngaert, J. S. Babb, S. C. Formenti, and S. Demaria. 2009. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res* 15: 5379-5388.

15. Golden, E. B., S. Demaria, P. B. Schiff, A. Chachoua, and S. C. Formenti. 2013. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. *Cancer Immunol Res* 1: 365-372.

16. Kim, H. R., H. N. Kang, H. S. Shim, E. Y. Kim, J. Kim, D. J. Kim, J. G. Lee, C. Y. Lee, M. H. Hong, S. M. Kim, H. Kim, K. H. Pyo, M. R. Yun, H. J. Park, J. Y. Han, H. A. Youn, M. J. Ahn, S. Paik, T. M. Kim, and B. C. Cho. 2017. Co-clinical trials demonstrate predictive biomarkers for dovitinib, an FGFR inhibitor, in lung squamous cell carcinoma. *Ann Oncol* 28: 1250-1259.

17. Byrne, A. T., D. G. Alferez, F. Amant, D. Annibali, J. Arribas, A. V. Biankin, A. Bruna, E. Budinska, C. Caldas, D. K. Chang, R. B. Clarke, H. Clevers, G. Coukos, V. Dangles-Marie, S. G. Eckhardt, E. Gonzalez-Suarez, E. Hermans, M. Hidalgo, M. A. Jarzabek, S. de Jong, J. Jonkers, K. Kemper, L. Lanfrancone, G. M. Maelandsmo, E. Marangoni, J. C. Marine, E. Medico, J. H. Norum, H. G. Palmer, D. S. Peeper, P. G. Pelicci, A. Piris-Gimenez, S. Roman-Roman, O. M. Rueda, J. Seoane, V. Serra, L. Soucek, D. Vanhecke, A. Villanueva, E. Vinolo, A. Bertotti, and L. Trusolino. 2017. Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nat Rev Cancer* 17: 254-268.

18. Sanmamed, M. F., I. Rodriguez, K. A. Schalper, C. Oñate, A. Azpilikueta, M. E. Rodriguez-Ruiz, A. Morales-Kastresana, S. Labiano, J. L. Pérez-Gracia, S. Martín-Algarra, C. Alfaro, G. Mazzolini, F. Sarno, M. Hidalgo, A. J. Korman, M. Jure-Kunkel, and I. Melero. 2015. Nivolumab and Urelumab Enhance Antitumor Activity of Human T Lymphocytes Engrafted in Rag2-/-IL2Rγnull Immunodeficient Mice. *Cancer Res* 75: 3466-3478.

19. King, M. A., L. Covassin, M. A. Brehm, W. Racki, T. Pearson, J. Leif, J. Laning, W. Fodor, O. Foreman, L. Burzenski, T. H. Chase, B. Gott, A. A. Rossini, R. Bortell, L. D. Shultz, and D. L. Greiner. 2009. Human peripheral blood leucocyte non-obese diabetic-severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse model of xenogeneic graft-versus-host-like disease and the role of host major histocompatibility complex. *Clin Exp Immunol* 157: 104-118.