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Version 3.0

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Protocol Amendments

Protocol type	*Version number*	*Version date*
Amendment 2	***3.0***	***06/28/2019***
Amendment 1	2.0	03/12/2019
Initial	1.0	09/06/2018

TITLE: Radiotherapy and Immunotherapy for Systemic Effect in Myeloma (RISE-M) Trial

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Principal Investigators:

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Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCM.

List of Abbreviations

All abbreviations used throughout the protocol must be defined.

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CTSC	Clinical Translational Science Center
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBFA	Human Research Billing Analysis Form
HUD	Humanitarian Use Device
ICF	Informed Consent Form
IDE	Investigational Device Exemption
IMWG	International Myeloma Working Group
IND	Investigational New Drug
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAP	Unanticipated Problem
WCM	Weill Cornell Medicine
MV	Megavolts
Gy	Gray, derived unit of ionizing radiation dose
MeV	Megaelectronvolts

Protocol Amendments

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Amendment 2	***3.0***	***06/28/2019***
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SUMMARY OF CHANGES: version 3.0 version date : 06/28/2019

1. Adding Dr. Usama Gergis as a co-investigator.
2. Removing Pragya Yadav as the data manager and adding Drew Ribadeneyra as the data manager.
3. Removing Sheetal Ramnath as the study coordinator.
4. Adding Ramya Krisha Chunduru as the regulatory coordinator.
5. Removing Sharanya Chandrasekhar as the study contact.

Summary of changes:

1. Changing personnel : Replacing Research Nurse Linda Tegnestam with Kathleen Pogonowski
2. Changing personnel: Data Manager (Myeloma team): Brielle Liotta
3. Section 3.3 - Exclusion criteria #12 - Prior exposure to immune checkpoint inhibitor
4. Correcting Nivolumab infusion from 60 mintues to 30 minutes as per BMS and FDA treatment prescriptions.
5. Clarifying stool collection timepoints : Baseline, end of radiation, C4D1 and C7D1
6. saliva collection added to the calendar for C1D1 (before infusion)
7. Adding investigator : Dr. Cara Rosenbaum
8. Updated Appendix 2 : for stool collection timepoints.
9. Clarifying radiation given as 5 fractions and removing “consecutive days” language.
10. Removing immune monitoring blood samples from section 8.1, 14.2 and 14.5.
11. Revising bone marrow aspiration to 20ml.

Informed consent changes :

1. Clarifying that patients will receive 5 fractions of radiation treatment.

Protocol Summary

Full Title:	Radiotherapy and Immunotherapy for Systemic Effect in Myeloma (RISE-M) Trial
Short Title:	RISE-M Trial
Clinical Phase:	II
Principal Investigator:	Dr. Himanshu Nagar, MD
Sample Size:	N = 30
Accrual Ceiling:	This study will enroll 30 subjects and screen up to 50.
Study Population:	Eligible patients will have multiple myeloma.
Accrual Period:	2 years
Study Design:	Eligible patients have multiple myeloma with measurable disease in the blood and a targetable soft tissue or bony lesion with radiotherapy. All eligible patients will receive immunotherapy (Nivolumab) plus radiotherapy, 6 Gy x 5 fractions, to a targetable lesion. Immunotherapy treatment starts with the first radiotherapy fraction. Nivolumab will be given every 2 weeks. Patients will have specified laboratory values measured bi-monthly and evaluated for response at 12 weeks as defined by International Myeloma Working Group Criteria. Patients will continue to receive their respective immunotherapy until disease progression or dose limiting toxicity is reached.
Study Duration:	Patients will have approximately 25-30 visits and a follow up for up to 3 years.
Study Agent/Intervention:	Patients with multiple myeloma will receive Nivolumab intravenously at 240 mg every two weeks. Infusions will be given over 30 minutes (not bolus or IV push). Patients will continue to receive infusions every two weeks until disease progression or dose limiting toxicity is reached. Patients will receive 5 fractions of radiation. A dose of 6 Gy x 5 days will be administered.
Primary Objective:	The primary aim is to estimate the overall response at 12 weeks using IMWG criteria in patients with multiple myeloma when treated with immunotherapy and radiotherapy.

Secondary Objectives: The secondary aims are: 1) to estimate the median progression free survival and the median overall survival for patients treated with immunotherapy and radiotherapy, and 2) to assess the toxicity (per CTCAE version 4.0) of patients treated with immunotherapy and radiotherapy.

Exploratory Objectives: Exploratory analyses will include: 1) investigate the expression of PD-L1 and PD-L2 on myeloma plasma cells prior to and at various time points after immune checkpoint blockade and radiotherapy, 2) investigate the population of T cells (cytotoxic T cells, helper T cells and regulatory T cells) and their expression of immune checkpoint receptors (PD-1, CTLA-4, LAG3, 2B4, BTLA, TIM3, A2aR) prior to and at various time points after checkpoint blockade and radiotherapy, 3) investigate mutational burden of pre-treatment myeloma samples in the bone marrow and target site utilizing FoundationOne Heme
4) investigate the T cell receptor (TCR) repertoire in the bone marrow aspirateprior to and at various time points after immune checkpoint blockade and radiotherapy and 5) microbiome analysis prior to and after immunoradiotherapy.

Endpoints: The primary endpoint will be the overall response rate using IMWG criteria. Other end points include progression free survival and overall survival.

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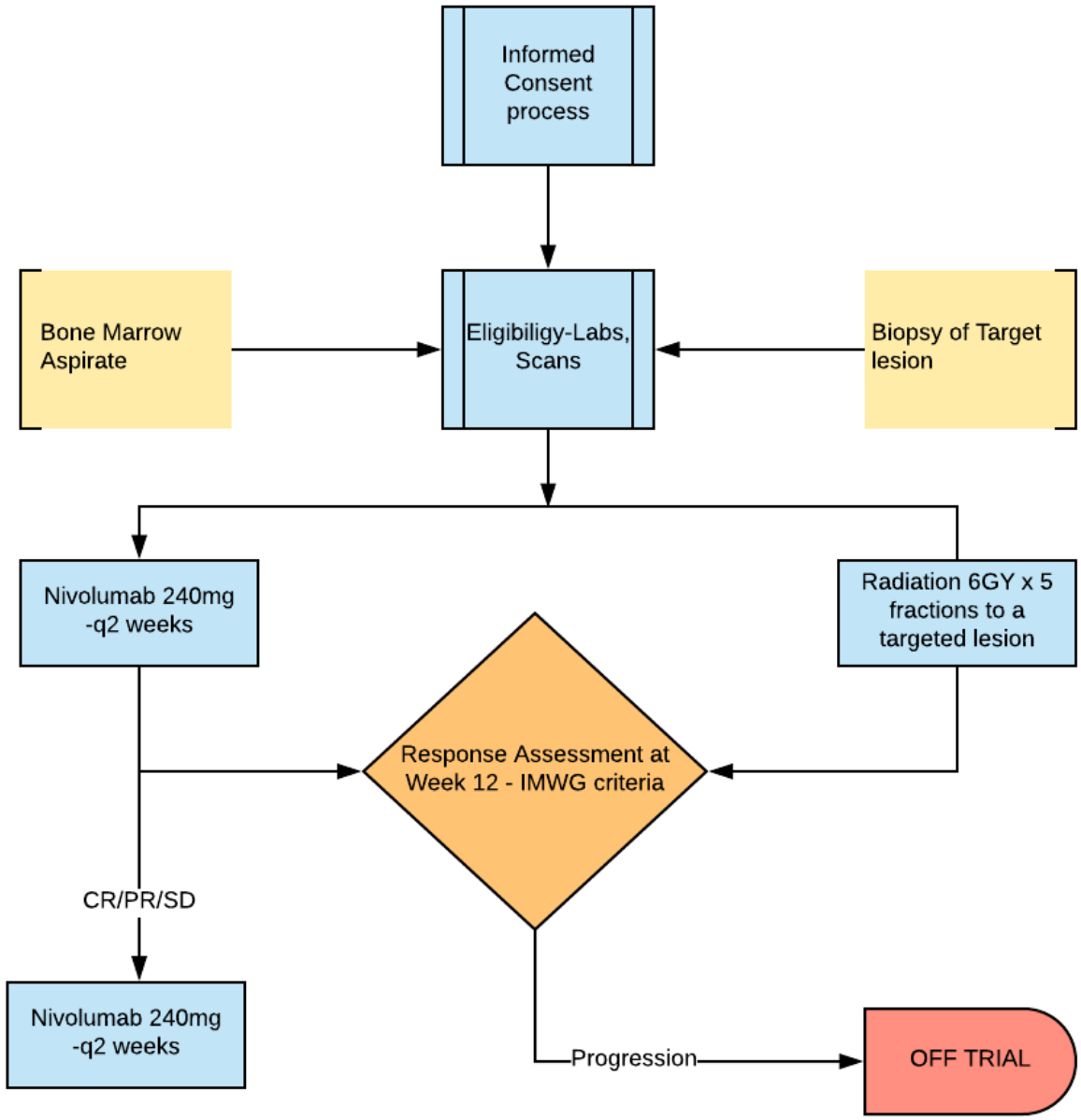
SCHEMA



Bone marrow aspirate pre-Nivolumab infusion on C2D1, C4D1, C7D1, C10D1 and Q 12 weeks thereafter and as needed to confirm CR.

One course of radiation (6 Gy x 5 fractions), with first radiation AFTER IV Nivolumab.

All Infusions will have a ± 3 day window. Nivolumab infusion 240mg q 2 week.

1 Study Objectives

1.1 Primary Objectives

The primary objective of the study is to estimate the proportion of patients with systemic response at 12 weeks using IMWG criteria in patients with multiple myeloma when treated with immunotherapy and radiotherapy. Systemic response is defined as partial or complete best response within this time

1.2 Secondary Objectives

The secondary objectives of the study are: 1) to estimate the median progression free survival and the median overall survival for patients treated with immunotherapy and radiotherapy, and 2) to assess the toxicity (per CTCAE version 4.0) of patients treated with immunotherapy and radiotherapy.

1.3 Exploratory Objectives

Exploratory analyses will include: 1) investigate the expression of PD-L1 and PD-L2 on myeloma plasma cells prior to and at various time points after immune checkpoint blockade and radiotherapy, 2) investigate the population of T cells (cytotoxic T cells, helper T cells and regulatory T cells) and their expression of immune checkpoint receptors (PD-1, CTLA-4, LAG3, 2B4, BTLA, TIM3, A2aR) prior to and at various time points after checkpoint blockade and radiotherapy, 3) investigate mutational burden of pre-treatment myeloma samples in the bone marrow and target site utilizing FoundationOne Heme and 4) investigate the T cell receptor (TCR) repertoire in the bone marrow aspirate prior to and at various time points after immune checkpoint blockade and radiotherapy.

2 Background

2.1 Disease

Multiple myeloma remains a disease that requires innovative treatment with an increasing incidence of cases in the United States with a 50% mortality rate at 5 years [1]. Interest in the role of the immune system in attacking malignancies across multiple tumor types has grown tremendously over the past few years with the advent of immune checkpoint inhibitors, including multiple myeloma [2].

The immunomodulatory molecule PD-L1 has higher expression levels on malignant plasma cells and myeloma-propagating pre-plasma cell in the bone marrow of patients with multiple myeloma compared to healthy donors and patients with MGUS and smoldering multiple myeloma [3].

Preliminary results from the ongoing KEYNOTE-023 phase I study that combined an anti-PD1 antibody with Lenalidomide and Dexamethasone in heavily pretreated patients showed a response rate of 76% [4]. Preliminary results from an ongoing phase II study that combined an anti-PD1 antibody with Pomalidomide and Dexamethasone in heavily pretreated patients showed a response rate of 60% [5]. Adverse events were consistent with previous studies of the drug in other cancers.

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

The promising data on the effect of PD1 blockade in multiple myeloma render this an optimal clinical setting to test this regimen with radiotherapy to assess the role of radiotherapy in inducing abscopal responses. Thus, we are proposing a pilot trial to assess the efficacy of combining PD1 blockade with radiotherapy in patients with relapsed/refractory multiple myeloma.

2.2 Investigational Agent

Nivolumab (Opdivo®) is a fully human IgG4 PD-1 immune checkpoint inhibiting antibody that selectively blocks the interaction between PD-1, which is expressed on activated T cells, and PD-1 ligand 1 (PD-L1) or 2 (PD-L2), which are expressed on both immune and tumor cells. The interaction between PD-1 and PD-L1 or PD-L2 normally results in inhibition of cellular immune responses.

2.3 Rationale

2.3.1 Overview

An attractive area of research supports the use of immune manipulations to recover patients' initial antitumor immunity. It is a strategy that has the advantages of being both natural and potentially long-lasting [6]. We propose combining immunotherapy with radiotherapy directed to a target site to establish a "hub" for in situ immunization against an irradiated tumor enhancing "tumor rejection" in additional areas. The use of radiotherapy has been explored as a viable approach to establish an individualized vaccine. In a "proof of principle" clinical trial, radiotherapy was combined with GM- CSF, a dendritic cell recruiter and activator, in patients with metastatic solid tumors and was shown to be safe and effective at establishing abscopal responses in 27% of treated patients. Those who achieved an abscopal response not only possessed a healthier immune system (as demonstrated by a lower neutrophil to lymphocyte ratio) at baseline but also demonstrated a longer median survival[7]. Additionally, our group recently completed the accrual of a phase II clinical trial for patients with metastatic non-small cell lung cancer treated with ipilimumab, a CTLA-4 inhibitor and T-cell activator, plus radiotherapy to a metastatic site. Partial and complete responses were achieved (per RECIST version 1.1) in 18% of treated patients (approximately 30% of treated patients had an abscopal response), whereby CTLA-4 blockade alone historically achieved response rates of only approximately 5 percent [7,8]. An example of a patient with a complete response to treatment is shown (Figure 1). The patient was treated to one liver metastasis, 6 Gy x 5, while receiving CTLA-4 blockade. The post-treatment PET/CT (completed in January 2013) demonstrated a complete response to therapy approximately 4 months after the initiation of treatment. The patient remains alive >3 years post treatment [9].

2.3.2 PD1/PDL1 and T-Cell Activation

The mechanistic rationale for the use of immunotherapy with anti-programmed death 1 (PD-1) receptor and anti-programmed death ligand 1 (PD-L1) antibodies are displayed in Figure 1 [10]. Antigen-presenting cells uptake tumor antigens released from cancer cells and present them to naïve T cells. This interaction results in activation or tolerization of the T cells, depending on the balance of stimulatory and inhibitory signals. Once activated, T cells can recognize and kill cancer cells that express and present the antigens in association with major histocompatibility (MHC) molecules, leading to immune-mediated tumor elimination. To avoid improper/excessive activation the immune system has developed several "checkpoints" that hinder T cell activation and/or effector function. Among them, the PD-1 receptor is expressed on T cells upon activation and inhibits immune responses through engagement of PD-L1 and PD-L2 on antigen presenting and cancer cells. While this pathway plays an important role in protection of normal tissue from collateral damage during an inflammatory

response, it has been shown to be often exploited by tumor cells to evade T cell mediated tumor rejection. In fact, monoclonal antibody-mediated blockade of PD-1 or its ligand PD-L1 has been successful in the clinic in multiple tumor types [11]. Identification of patients who can benefit from blockade of PD-1/PDL-1 pathway and of combination treatments that can increase the number of responding patients is an area of active investigation, which appears to be related to, but not fully explained by PD-1/PD-L1 expression levels.

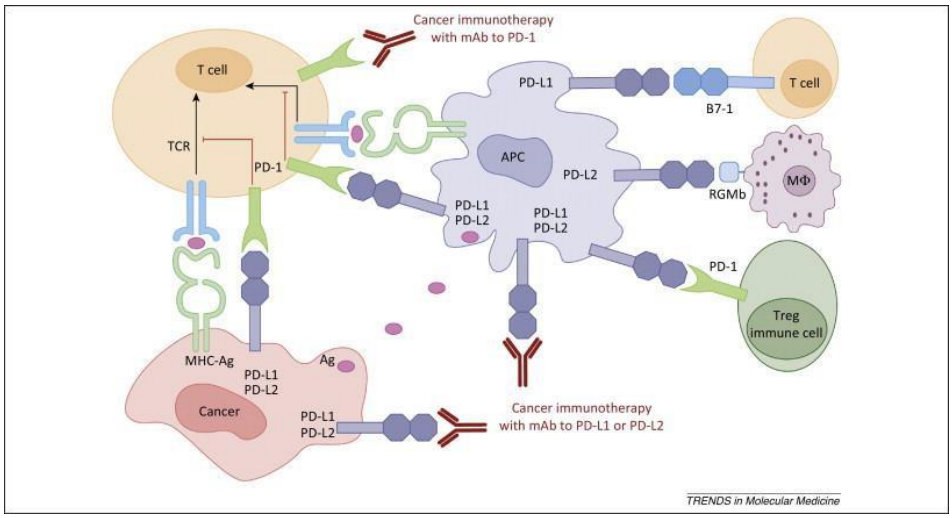


Figure 1. The mechanistic rationale for the use of immunotherapy with anti-programmed death 1 (PD-1) receptor and anti-programmed death ligand 1 (PD-L1) antibodies are displayed. Antigen-presenting cells uptake tumor antigens released from cancer cells and present them to naïve T cells. This interaction results in activation or tolerization of the T cells, depending on the balance of stimulatory and inhibitory signals.

2.3.3 Multiple Myeloma and PD1/PDL1

Multiple myeloma is associated with progressive immune dysregulation characterized by decreased antigen presenting and effector cell function, loss of myeloma reactive effector T cell populations and a bone marrow microenvironment that promotes immune escape [12-14]. The role that the PD-1/PD-L1 pathway plays in mediating immune escape and the corresponding therapeutic efficacy of PD-1/PD-L1 blockade in multiple myeloma has emerged as an area of great interest [2]. PD-L1 is highly expressed on plasma cells isolated from patients with multiple myeloma but not on normal plasma cells [3]. Notably, PD-L1 expression is up-regulated in the setting of relapsed and refractory disease suggesting a role in the development of clonal resistance [15]. PD-L1 expression is associated with increased proliferation and increased resistance to anti-myeloma therapy [16]. PD-1 is expressed on circulating T cells isolated from patients with advanced multiple myeloma, while expression of PD-1 on T cells is reduced in patients who achieve minimal disease state [17]. Additionally, increased PD-1 expression is observed on NK cells derived from myeloma patients associated with loss of effector cell function restored via PD-1 blockade [18]. These findings support the role of the PD-1/PD-L1 pathway in contributing to immune escape in multiple myeloma and suggest that blockade may be an effective therapeutic strategy.

The clinical efficacy of PD-1 blockade is most pronounced in malignancies such as melanoma and

Hodgkin lymphoma characterized by the presence of infiltrating effector cells in the tumor bed. Additionally, therapeutic efficacy has been correlated with mutational burden and thought to be associated with the presence of neoantigens derived from mutational events producing non-self-epitopes targeted by high affinity T cells [19]. In contrast, myeloma is characterized by low levels of infiltrating effector cells and a modest mutational burden as compared to solid tumors suggesting a more restricted neoantigen profile. As such, it is likely that immune checkpoint blockade will be more potent when coupled with therapies that stimulate neoantigen presentation. Such approaches, including combining immune checkpoint blockade with radiotherapy, immunomodulatory drugs and cellular therapies such as tumor vaccines, are currently being studied in clinical trials.

2.3.4 Abscopal Effect of Radiotherapy

Originally described in 1953, the abscopal effect of radiotherapy is a remote effect of ionizing radiotherapy on tumors outside of the radiotherapy field [20]. The phenomenon was termed the abscopal effect, from the Latin *ab* (position away from) and *scopus* (mark or target). The abscopal effect of radiotherapy is rare and its mechanism remains unexplained, although a variety of biologic events have been proposed including the involvement of the immune system [21-23]. Investigators have reported findings consistent with the abscopal effect, which was the result of recovered anti- tumor immunity after radiotherapy [24-26]. Notably, Saba et al. reported a case of a patient who originally presented with advanced multiple myeloma at the age of 50 who failed multiple therapeutic regimens and stem cell transplant. Subsequently, the patient achieved a sustained complete remission after receiving palliative radiotherapy to a gastric plasmacytoma and has remained in remission for over 15 years [27].

2.3.5 Radiotherapy and Immunity

Tumor-targeted radiotherapy can generate immune-stimulating effects and not immune suppression as was previously thought [28,29]. Moreover, it has become clear that radiotherapy can induce profound effects on tumor cells and on the tumor microenvironment that can enhance or trigger an anticancer immune response.

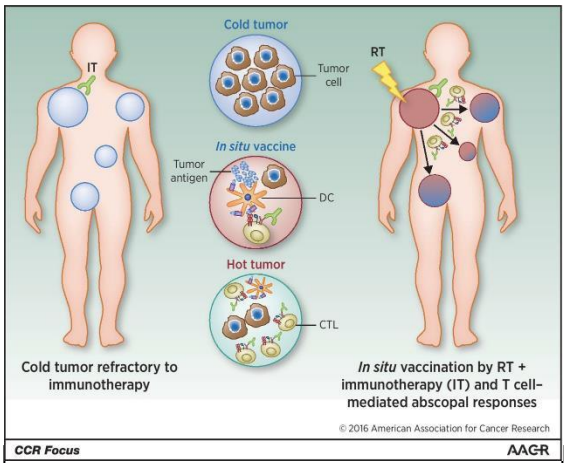


Figure 2. Local radiotherapy in combination with immune checkpoint blockade to break immune tolerance of established primary tumors has been shown to promote effector T cell mediated anti-tumor responses.

First, radiotherapy can induce an immunogenic cell death (ICD) of cancer cells [30,31]. ICD is a way of cell dying that releases tumor antigens and evokes an immune response. ICD defines cell death associated with the release of immunostimulatory molecular signals, collectively termed “danger signals” that activate the antigen presenting cells, dendritic cells (DCs), to uptake the released tumor antigens and express costimulatory molecules and cytokines that together promote the activation of T cells and their differentiation into anti-tumor effectors. Secondly, radiotherapy has been shown to alter the tumor milieu by enhancing trafficking of immune cells, inducing inflammatory cytokines, adhesion molecules and NKG2D ligands promoting the recognition of cancer cells by cytotoxic T cells (CTL) [9]. Thirdly, radiotherapy induces upregulation of MHC class I molecules, which are crucial for T cell activation [32]. This effect is time and dose dependent at least *in vitro* with a plateau between 48 hours and 72 hours after exposure

[32]. Fourthly, radiotherapy upregulates tumor-associated antigens, which are expressed at the surface of cells in association with MHC class I antigens [33,34]. The upregulation of both MHC-I antigens and tumor-associated antigens may evoke the recognition of cancer cells by the immune system. Finally, there is evidence that radiotherapy alters the MHC class I associated peptide profile [32]. Some of these peptides were specifically induced by radiotherapy and may therefore serve as radiotherapy-specific antigens, which makes the recognition of these cells by the immune system more likely.

Local radiotherapy in combination with immune checkpoint blockade to break immune tolerance of established primary tumors has been shown to promote effector T cell mediated anti-tumor responses [35,36]. The elicited immune response was effective against spontaneous metastases as well as the primary tumor. Regressing primary tumors demonstrated an increased infiltration of effector T cells and an expanded pool of circulating tumor- specific memory effector T cells was observed. These results demonstrate that tumor-directed radiotherapy may induce a therapeutically effective anti-tumor response in combination with immune checkpoint blockade, suggesting that the irradiated tumor can act as a vaccine and generate anti-tumor T cells that mediate local and systemic tumor rejection.

2.3.6 Harnessing the Pro-Immunogenic Effects of Radiation in Cancer Treatment: A New Paradigm

Experimental work done in two syngeneic mouse models (Lewis lung tumors and mammary carcinomas) 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 [37,38]. The findings inspired a clinical trial testing the combination of subcutaneously injected GM-CSF (a growth factor and activator of dendritic cells) with radiotherapy to a metastatic site in patients with solid tumors. GM-CSF increased 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 x 10 fractions, abscopal responses were detected in 27% of the patients accrued to the trial [7]. Those who achieved an abscopal response from therapy presented with a better pretreatment immune system (as demonstrated by a baseline neutrophil to lymphocyte ratio < 4) and subsequently demonstrated an improved overall survival. 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, express on the surface of dendritic cells. These compounds can activate both lymphoma B-cells as well as nearby antigen-presenting cells, particularly plasmacytoid dendritic cells, as previously demonstrated in a murine lymphoma model [39].

Another combination strategy to overcome immune-tolerance consists of the blockade of the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), a negative regulator of T cell activation. Prolonged survival and some cures occurred in a syngeneic model of poorly immunogenic mammary carcinoma, a process requiring CD8+T cells [35]. Postow et al. recently reported a clinical case report with the same combination [40]. 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 41.

Saba et al. describe a case of a patient who originally presented with multiple myeloma in 1996 at the age of 50. She failed multiple chemotherapeutic regimens including high-dose melphalan with autologous stem cell transplantation. Subsequently, the patient achieved a sustained complete remission, after receiving palliative radiotherapy to a symptomatic gastric plasmacytoma. She has remained in remission for >15 years 27. These results, although anecdotal, support the concept that local radiotherapy and immunotherapy can synergize to produce a therapeutically effective anti-tumor immune-response.

Combining radiotherapy with immunotherapy is promising and presents advantages. Because of its localized nature, radiotherapy is devoid of most systemic effects commonly encountered with chemotherapy, thereby limiting interference with a 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 a personalized in situ vaccine. Our group recently presented results of a phase II clinical trial of radiotherapy with CTLA-4 blockade in lung cancer patients, whereby 18% of treated patients achieved a partial or complete response RECIST version 1.1 (30% of treated patients achieved an abscopal response). Similar to the trial of radiotherapy in combination with GM-CSF for metastatic solid tumors, patients who achieved stable disease or a complete/partial response subsequently demonstrated an improved median survival compared to those who had disease progression upon completion of their treatment 7. Thus, we are proposing a pilot trial that combines PD1 axis blockade and radiotherapy in patients with relapsed/refractory multiple myeloma.

2.4 Risk/Benefit Assessment

Patients enrolled on this study have a diagnosis of multiple myeloma. Early results from immune checkpoint blockade therapy in multiple myeloma have shown to have a tolerable safety profile [4,5]. Based on data from two recently halted clinical trials (KEYNOTE-183 and KEYNOTE-185) , the U.S. Food and Drug Administration issued a statement to inform the public, health care professionals, and oncology clinical investigators about the risks associated with the use of pembrolizumab (a drug in the same class of nivolumab) in combination with dexamethasone and an immunomodulatory agent (lenalidomide or pomalidomide) for the treatment of patients with multiple myeloma. The FDA required that all patients in these trials be discontinued from further investigation with this drug, because interim results from both trials demonstrated an increased risk of death for patients receiving pembrolizumab when it was combined with an immunomodulatory agent as compared to the control group. Pembrolizumab is not approved for treatment of multiple myeloma.

The FDA has lifted partial clinical holds placed on the phase I CheckMate-039 and phase II CA204142 trials exploring nivolumab-based regimens in patients with relapsed/refractory multiple myeloma. The agency placed the partial holds on the studies in October 2017, along with a third study, the phase III CheckMate-602 trial, also examining a nivolumab combination in myeloma. In this current trial, nivolumab will be administed as a single agent and not combined with dexamethasone or immunomodulatory agents. A Phase Ib study that included 26 patients with multiple myeloma testing the safety and efficacy of nivolumab showed acceptable drug-related adverse events which occurred in 63% of patients where most were grade 1 or 2.

2.5 Correlative Studies Background

Aim 1: *Investigate the expression of PD-L1 and PD-L2 on myeloma plasma cells prior to and at various*

time points after checkpoint blockade and radiotherapy.

Rationale: PD-L1 is highly expressed on plasma cells isolated from patients with multiple myeloma but not on normal plasma cells and PD-L1 is not expressed on plasma cells isolated from patients with monoclonal gammopathy of unknown significance (MGUS) [3,43-45]. It will be important to confirm and quantify this finding in our study. Additionally, little is known about the expression of PD-L2 on the plasma cells from patients with multiple myeloma, which may affect the immunogenicity of patients with multiple myeloma.

Experimental Design: Expression of these ligands on myeloma plasma cells will be assessed from bone marrow biopsies by immunohistochemistry and flow cytometry staining with anti CD45, CD19, CD38 and CD138 to identify plasma cells, as well as PD-L1 (CD274) and or PD-L2 (CD273). As pre- treatment and serial post-treatment biopsies will be taken, we will compare responses rates to expression levels of PD-L1 and PD-L2 on myeloma cells at various time points.

20 ml bone marrow aspirate and core aspirate will be collected and sent to immunopathology.

Aim 2*: Investigate the population of T cells (cytotoxic T cells, helper T cells and regulatory T cells) and their expression of immune checkpoint receptors (PD-1, CTLA-4, LAG3, 2B4, BTLA, TIM3, A2aR) prior to and at various time points after immune checkpoint blockade and radiotherapy.*

Rationale: The clinical efficacy of PD-1 blockade is most pronounced in malignancies characterized by the presence of infiltrating effector cells in the tumor bed [19]. In contrast, myeloma is characterized by low levels of infiltrating effector cells, so the role of this biomarker in multiple myeloma is unclear. It is also unknown whether bone marrow T cell profiles reflect the T cell profile of the tumor itself in multiple myeloma. Therefore, we will quantify and characterize the T cell population in the site to be irradiated, bone marrow before immune checkpoint blockade and radiotherapy. Additionally, the T cell population will be similarly assayed on serial bone marrow biopsies and compared.

Experimental Design: T cells are broadly categorized into three major groups based on function: cytotoxic T cells, helper T cells (Th), and regulatory T cells (Tregs). Differential expression of markers on the cell surface, as well as their distinct cytokine secretion profiles, allow different subsets to be quantified. For example, CD8+ cytotoxic T cells destroy infected target cells through the release of perforin, granzymes, and granulysin, whereas CD4+ T helper cells have little cytotoxic activity and secrete cytokines that act on other leukocytes such as B cells, macrophages, eosinophils, or neutrophils. Tregs suppress T cell function by several mechanisms including binding to effector T cell subsets and preventing secretion of their cytokines. T cell diversity will be examined by flow cytometry using the following surface marker antibodies/transcription factors along with the expression of immune checkpoint receptors on these cells.

T Cell Subset	Surface Markers/Transcription Factors
Cytotoxic	CD8

Th1	CD4+CXCR3+
Th2	CD4+CCR4+CCR6–
Th9	CD4+CCR4– CCR6+
Th17	CD4+CCR4+CCR6+
Th22	CD4+CCR4+CCR6+CCR10+
NK/T	CD3+CD56+
Tregs	CD4+CD25+FoxP3+

Aim 3: *Investigate mutational burden of pre-treatment myeloma samples in the bone marrow and target site utilizing FoundationOne Heme.*

Rationale: The efficacy of PD-1 blockade has been correlated with mutational burden and thought to associate with the presence of neoantigens derived from mutational events producing non-self epitopes [19]. In contrast, myeloma is characterized by a modest mutational burden as compared to solid tumors suggesting a more restricted neoantigen profile.

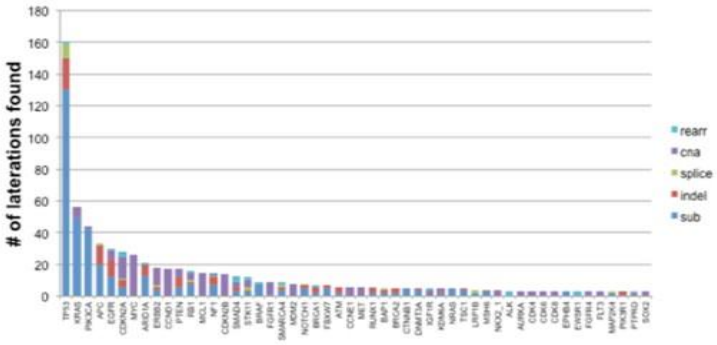


Figure:FoundationOne Heme genomic

Experimental Design: Genomic profiles of biopsied tumors will be performed using FoundationOne Heme, a comprehensive platform that applies next-generation sequencing to identify all 4 types of genomic alterations (base substitutions, insertions and deletions, copy number alterations and rearrangements) across all genes known to be drivers of hematologic malignancy. The test sequences the entire coding region of 405 cancer-related genes and employs RNA sequencing across 265 genes to capture a broad range of gene fusions and provides an interpretive report (Figure), including “tumor mutational burden” (TMB), a reproducible estimate of mutation rates across all coding sequences in the genome. Spearman rank correlation will be used to explore the significance of the correlation between TMB and response.

Aim 4: *Investigate the T cell receptor (TCR) repertoire in the bone marrow aspirate prior to and at various time points after immune checkpoint blockade and radiotherapy.*

Rationale: At the center of the process of the immune system distinguishing self from nonself is

recognition and binding of a TCR to an antigen displayed in the major histocompatibility complex (MHC) on the surface of an antigen-presenting cell (APC). The naïve TCR repertoire is formed by somatic rearrangements of non-contiguous genes belonging to the variable (*V*), diversity (*D*) and joining (*J*) families (combinatorial diversity). These are adjacent to the constant (*C*) gene in the heterodimeric α–β or γ–δ TCR. Further random insertion and deletion of nucleotides at the rearrangement positions create junctional diversity of the highly variable complementarity-determining region 3 (CDR3 region). The CDR3 region is unique for every T cell clone and encodes the receptor portion that makes the majority of TCR contacts with antigenic peptides bound by the MHC. The frequency of a specific CDR3 sequence indicates the abundance of its T cell clone. Thus, comprehensive TCR sequencing could provide a representative and quantitative repertoire analysis of specific tumor immunity. [46] In a neighboring research project (PI: Demaria), TCR repertoire analysis was used to investigate effects of anti-CTLA-4 and/or radiation therapy treatment on the TCRβ repertoire of tumor infiltrating lymphocytes (TILs) in a breast carcinoma mouse model. The 4T1 tumor model is not sensitive to single treatment using anti-CTLA-4 or radiotherapy, but respond well to the combination of both treatments. With TCR repertoire analysis, treatment-specific clusters of TILs were identified and to show that highly abundant clones rarely were unique to only one animal. Additionally, the data suggest the major role of radiotherapy is to increase the frequency of mid-ranked clones whereas anti-CTLA-4 primarily expands the top-ranked clones. However, when used together, radiotherapy and anti-CTLA-4 increased both top- and mid-ranked clones (Rudqvist et al., manuscript in preparation).

Experimental Design: TCR sequencing from bone marrow aspirate will be performed using the immunoSEQ assay provided by Adaptive Biotechnologies (AB). DNA from samples will be sent for sequencing to AB after which one part of the analysis will be performed utilizing AB's analytical platform the ImmunoAnalyzer 3.0. Furthermore, data will be downloaded and processed using various in-house developed tools for TCR repertoire analysis. Since the recombination process of the TCR is a random process with large variation between individuals, Dr. Rudqvist (postdoc in Radiation Oncology at Weill Cornell Medicine) has developed a method for calculating a similarity metric between TCR repertoires (preliminary data not shown). With this approach, questions such as when and how a favorable T cell repertoire develops can more easily be assessed.

2.6 Microbiome Correlative studies

2.6.1 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 [47,48] 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 [47,48]. 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 [49].

The study by Vetizou *et al.* underscores the dependence of CTLA-4 blockade on commensal microbiota [48]. 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 [47]. 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 [49]. 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 21, at 3 months and 6 months 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.[50,51] Analysis will be based on the QIIME (Quantitative Insights into Microbial Ecology) software package developed by the Knight lab, [52] and machine learning analyses developed by NYU Center for Health informatics and Bioinformatics (CHIBI) investigators working on NYU Human Microbiome Program studies [53,54]. Appendix 2 describes the protocol for stool collection.

3 Subject Selection

3.1 Study Population

Patients with a diagnosis of multiple myeloma who meet the inclusion and exclusion criteria will be eligible for participation in this study.

3.2 Inclusion Criteria

1. Subject is, in the investigator's opinion, willing and able to comply with the protocol requirements.
2. Subject has given voluntary written informed consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to their future medical care.
3. Must have received 2 consecutive cycles of systemic myeloma therapy.
4. Documented refractory or relapsed and refractory (R/R) multiple myeloma
    a. patients had less than minimal response, or had achieved at least a minimal response to previous treatment, but progressed within 6 months
    b. patients who have failed treatment with, are intolerant to, or ane not candidates for all available therapies known to be active for treatment of relapsed or refractory myeloma
    c. patients must have failed, be intolerant or are ineligible to treatment with at least 3 lines of therapy, including an IMiD, proteasome inhibitor and anti-CD38 agent
5. Targetable plasmacytoma, either intra-or extramedullary that is visualized on imaging (PET/CT or MRI) and is causing symptoms (eg. pain, neurological compromise) or potential to cause symptom as per clinician's judgement; and measurable disease at screening, defined as one or more of the following:
    a. Serum IgG, IgA, or IgM M-protein ≥ 0.5 g/dL
    b. Urine M-Protein ≥ 200 mg excreted in a 24-hour collection sample
    c. Involved serum free light chain (sFLC) > 100 mg/L provided the FLC ratio is abnormal
6. Males and Females at least 18 years or legal age of consent per local regulations.
7. Women of childbearing potential (WOCBP) must have two negative serum or urine pregnancy tests (minimum sensitivity 25 mIU/mL or equivalent units of HCG). One 10-14 days prior to start of the study drug and one within 24 hours prior to the start of study drug.
8. Investigators shall counsel WOCBP and male subjects who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy Investigators shall advise WOCBP and male subjects who are sexually active with WOCBP on the use of highly effective methods of contraception. Highly effective methods of contraception have a failure rate of < 1% when used consistently and correctly.
9. No condition which would cause unacceptable risk.

3.3 Exclusion Criteria

1. Subjects with solitary bone or extramedullary plasmacytoma as the only evidence of plasm cell dyscrasia.
2. Subjects with monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), Waldenstrom's macroglobulinemia, or POEMS syndrome (plasma cell dyscrasia with poly neuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes).
3. Subjects with active plasma cell leukemia (defined as either 20% of peripheral blood white blood cell count comprised of plasma/CD138+ cells or an absolute plasma cell count of 2 x 109/L).
4. Subjects within 100 days of stem cell transplantation.
5. Subjects within 4 weeks of surgery, unless cleared by surgeon.
6. Women who are of childbearing potential not complying to the above described contraceptive measures or are breastfeeding, and sexually active fertile men whose partners are WOCBP if they are not complying to the above described contraceptive measures.
7. Any uncontrolled or severe cardiovascular or pulmonary disease determined by the investigator, including:
    a. NYHA functional classification III or IV, congestive heart failure, unstable or poorly controlled angina, uncontrolled hypertension, arrhythmia, or myocardial infarction in the past 12 months
    b. Subjects with interstitial lung disease that is symptomatic or may interfere with the detection or management of suspected drug-related pulmonary toxicity
8. Active infection or know HBV/HCV/HIV.
9. Subjects with an active, known or suspected autoimmune disease. Subjects with type I diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis, or alopecia) not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.
10. Subjects with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of initiation of study drug. Inhaled or topical steroids, and adrenal replacement steroid doses > 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.
11. Previous radiotherapy to the area of the target area.
12. Prior exposure to immune checkpoint inhibitor.
13. Persistent toxicities of ≥ Grade 1 from prior treatment (including chemotherapy, targeted therapy, experimental agents, radiation, or surgery).

4 Registration Procedures

4.1 Patient Registration

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

- Legible copy of the HRBAF
- First and last page of signed informed consent form

Registration must be completed within 24 hours of the signing of informed consent.

5 Study Calendar

5.1 Schedule of Assessment

Procedure	Screening (Day0	C1D1 (±3 days)	C1D15 (±3 days)	Cycles 2-4		Cycles 5+	Cycles 5+	End of Study
				D1	D15	D1	D15	
Informed Consent	X							
Medical History	X	X		X		X	X	X
Physical Exam	X	X		X		X	X	X
Vital Signs + weight	X	X	X	X	X	X	X	X
Performance Status (ECOG)	X	X		X		X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X
Second Primary Malignancy	X	X	X	X	X	X	X	X
Adverse Events Assessment	X	X	X	X	X	X	X	X
Bone Marrow Aspiration/Biopsy[a]	X[a]			X[a]		X[a]		X[a]
Biopsy of Targeted Lesion	X							
Radiologic bone imaging:								
PET/CT, MRI	X				X			X
CBC w/ plts & diff	X	X	X	X	X	X	X	X
CMP, Mg, Uric Acid, LDH	X	X	X	X	X	X	X	X
Serum or urine pregnancy test	X	X	X	X	X	X	X	X
TSH	X			X		X		
Beta 2 microglobulin	X			X				
Response Assessment								
Serum quantitative immunoglobulins	X	X		X		X		X
free light chains, SPEP & SIFE	X	X		X		X		X
24-Hour urine for urine total protein	X	X		X		X		X
UPEP & UIFE	X	X		X		X		X
TREATMENT								
Nivolumab Infusion[c]		X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	
Radiation to Targeted Lesion[b]		X[b]						
RESEARCH PROCEDURES								
Stool Samples (before nivo infusion)[d]		X[d]			X[d]	X[d]		
Saliva Sample collection (before nivo)		X						

Foot notes:

a. Bone marrow aspirate pre-nivolumab infusion on C2D1, C4D1, C7D1, C10D1 and Q 12 weeks thereafter and as needed to confirm CR

b. One course of radiation (6GY x 5 fractions), with first radiation on D1 AFTER IV nivolumab

c. All Infusions will have a ± 3 day window.

d. Stool samples will be collected on Day1, end of Radiation, C4D1 and C7D1.

5.1.1 Screening Visit – (Day 0 - day28)

After proper documentation of the informed consent process, patients will undergo screening procedures which will include standard of care labs, specified laboratory values as defined by International Myeloma Working Group (IMWG) Criteria, PET/CT –MRI, baseline imaging, bone marrow aspiration biopsy, and target lesion biopsy as outlined in section 5.1 schedule of assessments.

5.1.2 Treatment Phase

Once eligible, patients will receive immunotherapy, nivolumab, intravenously at 240 mg every two weeks. Infusions will be given over 30 minutes (not bolus or IV push). Patients will also receive radiation of 6 Gy x 5 fractions the first week of starting immunotherapy. Immunotherapy treatment starts with the first radiotherapy fraction. Nivolumab will be given every 2 weeks. Patients will have specified laboratory values measured monthly and evaluated for response as defined by International Myeloma Working Group Criteria. Patients will continue to receive their respective immunotherapy for up to three years or until disease progression or dose limiting toxicity is reached. Patients will remain on therapy for up to 6 cycles in the setting of stable disease and for up to a year in the setting of a PR or better by 6 cycles.

5.2 Response Assessment at Week 12

Patients will have specified laboratory values measured monthly and evaluated for response as defined by International Myeloma Working Group Criteria. Patients without progression of disease as defined by IMWG criteria including assessment of plasmacytoma(s) by PET/CT will continue to receive nivolumab every 2 weeks.

5.3 Duration of Therapy and Criteria for Removal from Study:

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

1. Progression of disease as defined by IMWG critera,
2. Intercurrent illness that prevents further administration of treatment,
3. Unacceptable adverse event(s),
4. Patient decides to withdraw from the study, or
5. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.4 Duration of Follow Up

Patients will be followed for 3 years after removal from study or until death, whichever occurs first. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event

6 Radiation Therapy Guidelines

6.1 Planning

After informed consent is obtained, a targetable plasmacytoma 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 region. These images will be used in 3D treatment planning in accordance with the dose specification constraints.

The CTV is defined as the plasmacytoma 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.

6.2 Treatment

Radiotherapy is delivered by external beam using linear accelerators capable of delivering ≥ 4megavoltage x- rays. The PTV will encompass all of the biopsied plasmacytoma 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 plasmacytoma in "real-time", the operator may automatically align the plasmacytoma 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.

After completion of the first course of radiotherapy + nivolumab on Day 1 (or within 24 hours or RT), the patient will start on day 15 the regimen of nivolumab 240 mg q2 weeks.

Response assessment will be performed every 2 weeks as standard of care.

6.3 Treatment modifications for Radiation Adverse Events:

Dosing delay: The patient should have resolution or return to pre-treatment baseline of all grade 3-4 toxicities prior to start of the next immunotherapy treatment.

6.4 On Study Evaluations:

As summarized in the Study Calendar, patients are evaluated pre-treatment for measurable disease in the blood and a targetable soft tissue or bony lesion with radiotherapy. Patients will have specified laboratory values measured bi-monthly and evaluated for response as defined by International Myeloma Working Group Criteria. Response assessments will be performed every 2 weeks.

7 Pharmaceutical Information

7.1 Investigational Agent

Nivolumab is a human monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Nivolumab is an IgG4 kappa immunoglobulin that has a calculated molecular mass of 146 kDa. Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

7.2 Availability

Nivolumab is an investigational agent supplied to investigators by Bristol-Myers Squib

7.3 Preparation and Administration of Nivolumab

Dose Calculations

For Multiple myeloma: Nivolumab is given every 2 weeks. The recommended dose of nivolumab is a flat dose of 240mg administered as an intravenous infusion over 30 minutes every 2 weeks until disease progression or unacceptable toxicity.

Visually inspect drug product solution for particulate matter and discoloration prior to administration. Nivolumab is a clear to opalescent, colorless to pale-yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

7.4 Dose Modifications, Delays or Discontinuation of Nivolumab

Recommendations for OPDIVO modifications are provided in Table 1. There are no recommended dose modifications for hypothyroidism or hyperthyroidism. Interrupt or slow the rate of infusion in patients with mild or moderate infusion reactions. Discontinue OPDIVO in patients with severe or life-threatening infusion reactions.

Table 1: Recommended Dose Modifications for OPDIVO

Adverse Reaction	Severity*	Dose Modification
Colitis	Grade 2 diarrhea or colitis	Withhold dose[a]
	Grade 3 diarrhea or colitis	Withhold dose[a] when administered as a single agent
		Permanently discontinue when administered with ipilimumab
	Grade 4 diarrhea or colitis	Permanently discontinue
Pneumonitis	Grade 2 pneumonitis	Withhold dose[a]
	Grade 3 or 4 pneumonitis	Permanently discontinue
Hepatitis/non-HCC[b]	Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) more than 3 and up to 5 times the upper limit of normal (ULN) or total bilirubin more than 1.5 and up to 3 times the ULN	Withhold dose[a]
	AST or ALT more than 5 times the ULN or total bilirubin more than 3 times the ULN	Permanently discontinue
Hepatitis/ HCC[b]	• If AST/ALT is within normal limits at baseline and increases to more than 3 and up to 5 times the ULN • If AST/ALT is more than 1 and up to 3 times ULN at baseline and increases to more than 5 and up to 10 times the ULN • If AST/ALT is more than 3 and up to 5 times ULN at baseline and increases to more than 8 and up to 10 times the ULN	Withhold dose[c]

Table 1: Recommended Dose Modifications for OPDIVO

Adverse Reaction	Severity*	Dose Modification
	If AST or ALT increases to more than 10 times the ULN or total bilirubin increases to more than 3 times the ULN	Permanently discontinue
Hypophysitis	Grade 2 or 3 hypophysitis	Withhold dose[a]
	Grade 4 hypophysitis	Permanently discontinue
Adrenal Insufficiency	Grade 2 adrenal insufficiency	Withhold dose[a]
	Grade 3 or 4 adrenal insufficiency	Permanently discontinue
Type 1 Diabetes Mellitus	Grade 3 hyperglycemia	Withhold dose[a]
	Grade 4 hyperglycemia	Permanently discontinue
Nephritis and Renal Dysfunction	Serum creatinine more than 1.5 and up to 6 times the ULN	Withhold dose[a]
	Serum creatinine more than 6 times the ULN	Permanently discontinue
Skin	Grade 3 rash or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Withhold dose[a]
	Grade 4 rash or confirmed SJS or TEN	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose[a]
	Immune-mediated encephalitis	Permanently discontinue
Other	Other Grade 3 adverse reaction First occurrence Recurrence of same Grade 3 adverse reactions	 Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue
	Grade 3 myocarditis	Permanently discontinue
	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse reactions lasting 12 weeks or longer	Permanently discontinue

* Toxicity was graded per National Cancer Institute Common Terminology Criteria for Adverse Events. Version 4.0 (NCI CTCAE v4).
[a] Resume treatment when adverse reaction improves to Grade 0 or 1.
[b] HCC: hepatocellular carcinoma.
[c] Resume treatment when AST/ALT returns to baseline.

8 Correlative Studies

8.1 Bone Marrow tissue aspirate

In addition to this, 20 ml sample of bone marrow tissue will be collected at the same intervals.

8.2 Tissue biopsies

4 core pre treatment tissue biopsies will be taken for diagnostic confirmation and correlative studies.

8.3 Other studies :

A baseline sputum (saliva) sample will be obtained from all patients for gene expression profiles.

8.4 Microbiome samples

Stool collection for microbiome analysis will be collected at Baseline, end of radiation, C4D1 and C7D1. to explore changes of the microbiome during ICB. Appendix 2 describes the protocol for stool collection.

9 Measurement of Effect

9.1 Standard IMWG Response Criteria

Response to multiple myeloma will be assessed based on the International Myeloma Working Group Criteria (IMWG) as described in the table below.

Standard IMWG response criteria*	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)++
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level < 100mg per 24 hours.
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24h; If the serum and urine M-protein are unmeasurable, a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥ 50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥ 30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required.
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required.

Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease‡‡,Ω	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0·5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD§§ of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease
Clinical relapse	Clinical relapse requires one or more of the following criteria: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD§§ of the measurable lesion; Hypercalcaemia (>11 mg/dL); Decrease in haemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma; Hyperviscosity related to serum paraprotein

Relapse from complete response (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia)

*****Derived from international uniform response criteria for multiple myeloma. Minor response definition and clarifications derived from Rajkumar and colleagues. When the only method to measure disease is by serum FLC levels: complete response can be defined as a normal FLC ratio of 0·26 to 1·65 in addition to the complete response criteria listed previously. Very good partial response in such patients requires a ≥90% decrease in the difference between involved and uninvolved FLC levels. All response categories require two consecutive assessments made at any time before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions or extramedullary plasmacytomas if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments do not need to be confirmed. Each category, except for stable disease, will be considered unconfirmed until the confirmatory test is performed. The date of the initial test is considered as the date of response for evaluation of time dependent outcomes such as duration of response.

******All recommendations regarding clinical uses relating to serum FLC levels or FLC ratio are based on results obtained with the validated Freelite test (Binding Site, Birmingham, UK).

++Presence/absence of clonal cells on immunohistochemistry is based upon the κ/λ/L ratio. An abnormal κ/λ ratio by immunohistochemistry requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is κ/λ of >4:1 or <1:2. ‡‡Special attention should be given to the emergence of a different monoclonal protein following treatment, especially in the setting of patients having achieved a conventional complete response, often related to oligoclonal reconstitution of the immune system. These bands typically disappear over time and in some studies have been associated with a better outcome. Also, appearance of monoclonal IgG κ in patients receiving monoclonal antibodies should be differentiated from the therapeutic antibody.

§§Plasmacytoma measurements should be taken from the CT portion of the PET/CT, or MRI scans, or dedicated CT scans where applicable. For patients with only skin involvement, skin lesions should be measured with a ruler. Measurement of tumor size will be determined by the SPD.

‡‡Positive immunofixation alone in a patient previously classified as achieving a complete response will not be considered progression. For purposes of calculating time to progression and progression-free survival, patients who have achieved a complete response and are MRD-negative should be evaluated using criteria listed for progressive disease. Criteria for relapse from a complete response or relapse from MRD should be used only when calculating disease-free survival.

Ω In the case where a value is felt to be a spurious result per physician discretion (eg, a possible laboratory error), that value will not be considered when determining the lowest value.

10 Data Reporting / Regulatory Considerations

10.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

10.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

10.2 Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Principal Investigator. *Bristol-Myers Squibb* will have the opportunity to review and approve the changes prior to submission of these changes to the local IRB.

11 Statistical Considerations

The primary endpoint of this study is response rate. The trial design is a single arm, single-center, Simon optimal two-stage design. Patients will be evaluated for response at 12 weeks (84 days ± 7 days). Patients will be enrolled in two stages with 13 patients in the first stage, and potentially an additional 13 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, as described below.

11.1 Sample Size/Accrual Rate

As mentioned, a Simon's two-stage optimal design [Simon, 1989] will be used. The null hypothesis that the true response rate is 20% will be tested against a one-sided alternative. If there are 3 or fewer responses in these 13 patients, the study will be stopped. Otherwise 13 additional patients will be accrued for a total of 26 patients. The null hypothesis will be rejected if 10 or more responses are observed in 26 patients. This design yields a one-sided type I error of 0.025 and power of 60% when the true response rate is 40%. The target sample size is 26 evaluable patients and up to 30 patients (4 extra patients) will be accrued to account for non-evaluable patients or patients who withdraw prior to evaluation.

11.2 Stratification Factors

None

11.3 Analysis of Endpoints

11.3.1 Analysis of Primary Endpoints

The primary analysis will be an evaluation of the response rate. The trial decision rules are to conclude the treatment strategy is not worth further evaluation if in the first evaluable 13 patients, there are 3 or fewer patients with a response. If in the first 13 evaluable patients, there is at least 4 patients with a response, an additional 13 evaluable patients will be enrolled. If in the first 26 evaluable patients, there are 10 or more responses are seen, it will be concluded that this treatment strategy is worth further evaluation (the null hypothesis will be rejected). If in the first 26 evaluable patients, 9 or fewer responses are observed, the treatment will not be recommended for further evaluation in this patient population. The response rate will be estimated with a one-sided 95% exact binomial procedure (66). Analyses will be performed in R (R Core Team, 2017).

11.3.2 Analysis of Secondary Endpoints

With adequate follow-up time, secondary endpoints of progression-free survival (PFS) and overall survival (OS) will be assessed by Kaplan-Meier survival analysis and 95% confidence intervals for median PFS/OS will be calculated using Greenwood's formula. PFS will be defined as the time from first treatment day until objective or symptomatic progression. OS will be defined as the time from first treatment day until death. The frequency of subjects experiencing toxicities will be tabulated. Toxicities will be assessed and graded according to CTCAE v. 4.0 terminology. Exact 95% confidence intervals around the toxicity proportions will be calculated to assess the precision of the obtained estimates.

11.3.3 Interim Analysis

None Planned.

11.3.4 Reporting and Exclusions

11.3.5 Evaluation of toxicity

All patients will be evaluable for toxicity from the time of their first treatment with Investigational agent. A DLT will be defined as any grade 4 non-hematological toxicity or any toxicity that interrupts treatment for more than 6 weeks, treatment-related deaths, or grade 3 treatment-related non-hematological toxicities. Once 8 patients have been enrolled, we will use a continuous Bayesian toxicity stopping rule that will pause the study for review if the posterior probability that the DLT event rate is greater than 20% is > 80%, using an uninformative prior of Beta(1,1). The trial will reviewed if 3 or more of the first 8 patients experience DLTs, or 4 or more of the first 9-12, or 5 or more of the first 13-16, 6 or more of the first 17-21, 7 or more of the first 22-25.

11.3.6 Evaluation of response.

All patients included in the study will be assessed for response to treatment if they have received at least *1* nivolumab infusion.

11.4 Analysis of Correlative Endpoints

11.4.1 Microbiome

11.5 Statistical analysis of microbiome and metagenome composition

Statistical analysis of microbiome and metagenome composition will be performed in the R statistical programming environment [56] using package *phyloseq* [57], which incorporates and builds upon community ecology packages such as ade4 and vegan and employs the flexible graphic system ggplot2, to easily visualize complex data relationships. For 16S data, we will evaluate the adequacy of sequencing efforts using rarefaction plots. Alpha diversity index for each will be characterized through dominance, equitability, richness, evenness. The diversity metrics will be calculated at Operational Taxonomic Unit (OTU) and higher taxonomical levels to best characterize the community structure. We will test for associations of each of these alpha diversity metrics with the time relative to radiation exposure, using one-way ANOVA after even-sampling the observations to a depth cut-off maximizing the number of samples and depth. In addition, rank-abundance plots will be used to visualize differences in abundance of dominant taxa in the clinical and phenotypic groups. We will utilize skyline plots to visualize the patterns of community structure in terms of relative abundances in the collected samples between before and after the radiation treatment or between case and control samples. Similarly, for metagenomic data, skyline plots will be used to reveal functional compositions of the samples. Heat-maps will be plotted to visualize clustering patterns in the data.

Univariate analyses. To circumvent instabilities associated with rare species, which are difficult to detect uniformly in all samples, we will focus our primary univariate analyses only on highly abundant taxa, i.e. those present at 1% or more relative abundance across all specimens. This approach will also help to reduce multiple comparison-related Type I-error inflation, which will be formally controlled by false discovery rate (FDR) [58]. The differences in presence or absence of specific taxa and functional categories will be assessed by χ^2-test at all taxonomical levels. *Paired*-Sample Wilcoxon Signed Rank *Test* will be used to establish the differences in relative abundance of taxa between before and after the radiation treatment or between paired samples. Depending on the dynamics observed, subsequent analyses may focus on less abundant taxa, and at higher depth, as indicated above.

Multivariate analyses. Multivariate association of the entire microbiome/metagenome with clinical and phenotypic factors will be examined with Principal Coordinate Analysis (PCoA) on Jensen-Shannon, weighted and unweighted Unifrac, and χ^2 (correspondence analysis) distances. Likewise, these distances will be used to build non-parametric multivariate Analysis of variance (ANOVA) models (ADONIS) [59] to allow for simultaneous measurement of univariate and interaction effects of the clinico-phenotypic variables on the microbiome. Starting with the microbiota and metagenomic features significantly different in univariate analyses, we will use the dimension-reduction method, canonical correlation analysis (CCA) to identify the bacteria taxa which are related radiation treatment, according to benchmarked methodologies of our prior studies [54,60]. We will utilize a phylogenetic structure-constrained penalty function to impose phylogenetic relationships among bacteria on the model selection [61].

Longitudinal analysis: We will study the evolution of microbiome over time and how that evolution is associated with the radiation treatment. The relative abundances at each taxonomical level will

be first normalized by log-ratio transformation [62]. Then the transformed relative abundance of each individual taxa at multiple time points will be fitted by the linear mixed model along with the time effect and all subject-specific characteristics as the independent covariates. For the nonlinear trend, we will combine the nature splines with linear mixed model in the data analysis. The same model will be applied on the indices calculated in the ecology microbial analysis. For the joint analysis of more than one taxon. we will use MCMCglmm R package to implement the multivariate generalized linear mixed model. This package gives a large flexibility in analyzing correlated multiple longitudinal response by allowing different types of covariance structure [63].

12 Adverse Event Reporting Requirements

CTCAE Version 4.0 will be used for tracking the Adverse Events in this study.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation.

Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

12.1 Adverse Event Definition

12.1.1 Collection of Safety Information

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

12.1.2 Attribution of the AE:

Definite – The AE *is clearly related* to the study treatment.

Probable – The AE *is likely related* to the study treatment.

Possible – The AE *may be related* to the study treatment.

Unlikely – The AE *is doubtfully related* to the study treatment.

Unrelated – The AE *is clearly NOT related* to the study treatment.

12.1.3 Recording of adverse events

At every clinical visit, the patients will be assessed for adverse events. Adverse events will be documented in patient's charts (EPIC). All adverse events will be recorded on a patient specific AE log. The AE log will be maintained by the research staff and kept in the patient's research chart. AEs will also be updated in REDCAP.

A ***serious AE*** **or reaction** is any untoward medical occurrence that at any dose:

- 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.

12.2 Reporting of Serious Adverse Events (SAEs)

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

This is 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, Data Safety Monitoring Board (DSMB) 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 and to DSMB.

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 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 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 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 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 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 nivolumab exposure, including during at least 6 half-lives after product administration, nivolumab will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). Exceptions to 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 Datasafety monitoring committee will be reviewing the study on an annual basis and will review the adverse events collected on the study.

12.3 Off Study Criteria

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

3. Patient decides to withdraw from the study, or
4. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
5. Authorized Physicians must notify the data manager and Principle Investigator when a patient is taken off study.

13 PROHIBITED AND RESTRICTED THERAPIES DURING THE STUDY

13.1 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 nivolumab. 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.

13.2 Data and Safety Monitoring Plan (DSMP)

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).

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.

13.3 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 IRB approved physicians 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. The study samples obtained will be for the purpose of immune-monitoring study when funds become available.

14 APPENDIX 1: Laboratory Correlate Manual

14.1 At Baseline:

1. Tissue Biopsies (4 core biopsies)

4 core biopsies will be collected by interventional radiology form the same lesion and placed in one container as described below.

- Immediately after tumor exeresis, put the specimen into a appropriately labeled container (i.e. 50ml Falcon, specialized OR containers etc.) into sterile saline (ideally including Penicillin and Streptomycin)
- Keep the container 4^{O}C on wet ice (if possible)
- Bring as soon as possible the tissue sample from the operating theatre to the pathology triage area: THIS needs to be coordinated with Dr. Inghirami and pathology.
- Identify the sample using only the trial ID# of the patient and date for all subsequent steps
- On arrival, place the tissue sample in a Petri dish, maintaining it in sterile conditions (use sterile tweezers etc.)

Place the tissue in a sterile Petri dish containing 1-2 ml of tissue culture medium supplemented with 10% FCS and antibiotics

TRIAGE:

Priority list, depending on amount of viable tissue:

1. Place one portion in RNA later (for RNA Seq), which will be stored frozen [Needs to be in emergency power-line freezer for clinical samples in Rad Onc]

2. PDX: Cut viable tissue into in strip of 2-4 x2mm long with a maximum width of 1 mm (alternative prepare cubes of 1 mm3)

- Fresh sample can be used immediate for animals implants
- Please use 2-4 1mm3 cube tissue cubes for each s.c. implantation site

3. Histology: One core (or a portion of a core) and be processed for paraffin embedding. The blocks will be used for 1 H&E, and stored for multiplexing IHC

Freeze dry samples (for protein and DNA extraction etc.), ~3-5x1mm portion

5. Biobank (unlikely that sufficient tissue will be available, but should be considered): Put 4 tissue fragments into 1 ml freezing medium (RPMI 1640, 20% FCS, 10% DMSO

Place printed bar coded identification vials and place them to cryovials. Prepare at least 3-5 bar coded vials for multiple experiments/implants (biobank). Place the tissue vials into an appropriate container with isopropyl alcohol at – 80°C for least 12 hours.
Transfer and store the cryovials into a liquid nitrogen tank. Record into the data base sample, vials (RNA, DNA, protein and viable) and location.

14.2 Baseline Bone Marrow Aspirate and Core

20ml bone marrow aspirate will be obtained at baseline from all patients. The samples will be sent to immunopathology

14.3 Baseline Saliva sample

Approximately 5ml saliva (sputum) will be obtained from patients for genetic profiling.

15 APPENDIX 2. INSTRUCTIONS FOR COLLECTION OF STOOL SAMPLES AT HOME:

Stool samples will be collected on Day1, end of Radiation, C4D1 and C7D1.

Stool Collection

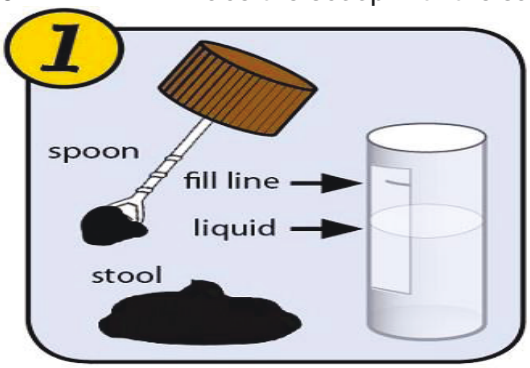
Step 1: Place the collection hat under the toilet seat

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

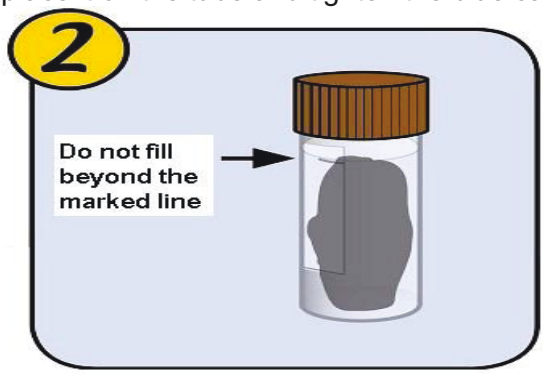
- Do not place toilet tissue in the hat
- Do not urinate in the hat – this is very important – if urine comes in contact with the stool sample discard the sample and start again. You should rinse the collection hat and dry it with a paper towel.

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 bag. Place the stool specimen container.

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