Abbreviated Title: Avelumab plus XRT for RRMM
CC Protocol #: 19-C-0078 D
Version Date: 09/01/2020
NCT Number: NCT03910439

A Phase II Pilot Study of Avelumab in Combination with Hypofractionated Radiotherapy in Patients with Relapsed Refractory Multiple Myeloma

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Investigational Agents:

Drug Name:	Avelumab (MSB0010718C)
IND Number:	142027
Sponsor:	Center for Cancer Research
Manufacturer:	EMD Serono, Inc. (Company Study/Tracking #: MS100070-0037)

Commercial Agents: None

PRÉCIS

Background:

- Multiple Myeloma (MM) is a hematologic neoplasm of the plasma cells defined by an M-protein ≥ 3.0 g/dL or bone marrow plasma cells ≥ 10% and presence of end-organ disease.
- Although significant advances in treatment have been made in the past decade, MM remains incurable with median survivals of 5-8 years.
- While therapeutic strides have been made with approvals of immunomodulatory drugs (IMiDs), proteasome inhibitors, and monoclonal antibodies, treatment of relapsed refractory MM (RRMM) remains an unmet need for patients who have exhausted available therapies.
- Extramedullary plasmacytomas arising either from focal bone involvement or from hematogenous spread occur in 7-18% of newly diagnosed MM (NDMM) with an additional 6-20% in RRMM.
- Immune checkpoint inhibitors are being evaluated in combination regimens and evidence exists that radiation therapy (XRT) may synergize with immune checkpoint inhibitors.

Objectives:

- To assess the response rate of avelumab in combination with XRT (BavXRT) in RRMM patients with plasmacytomas or lytic lesions

Eligibility:

- Patients must have previously treated RRMM refractory to, ineligible for, or intolerant of available therapeutic regimens known to provide clinical benefit (e.g, immunomodulatory [IMiD], proteasome inhibitor, and anti-CD38 monoclonal antibody-based treatments).
- Presence of ≥ 1 extramedullary plasmacytoma and/or lytic lesion amenable to XRT
- Age ≥ 18 years
- Adequate organ function, and without serious comorbidity or disease (e.g., autoimmune disease), that would preclude concurrent systemic treatment or radiotherapy.

Design:

- Treatment will consist of a 4-week lead-in with avelumab, followed by concurrent XRT 5Gy x 5 days). Monotherapy avelumab will continue indefinitely until progressive disease (PD) or unacceptable toxicity; 28-day cycles.
- Routine safety and MM-specific clinical labs will be assessed. Additional research bloods will be collected for evaluating immune-subsets, endosomes, and peripheral blood T cell repertoire prior to and following treatment (lead-in and prior to XRT, at disease re-evalutions at at time of response [i.e., CR/PD]).
- Bone marrow biopsies will be evaluated for PD-1/L1 expression, and B and T cell subsets using IHC. Flow cytometry will also be used to evaluate stimulatory and inhibitory immune subsets along with endosomes. Standard clinical histopathology and flow cytometry will also be evaluated.
- Single arm, Simon minimax two-stage phase II trial design. The first stage will enroll 13 patients; if futility is not met, second stage will enroll another 14 patients to define the response rate to BavXRT in this population. Early stopping rules for safety will also be applied.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

To determine the response rate (IMWG 2016 criteria[1]) of avelumab in combination with XRT (BavXRT) in RRMM patients with plasmacytomas or lytic lesions

1.1.2 Secondary Objectives

- To determine complete response (CR) and minimal residual disease negative (MRD) negative CR (MRDnegCR) rates
- To determine reductions in bone marrow (BM) and peripheral blood (PD) plasmacytosis
- To determine systemic radiographic response by FDG PET/CT
- To determine local responses in non-irradiated plasmacytomas and/or FDG avid lytic lesions (abscopal effect)
- To determine progression-free survival (PFS)
- To determine overall survival (OS)
- To determine the safety and tolerability of BavXRT combination
- To determine the activity of avelumab monotherapy after 1 cycle of treatment

1.1.3 Exploratory Objectives

Studies may be performed in select patients where adequate samples and resources are available:

To assess:

- Peripheral blood mononuclear cells (PBMCs):
    - Changes in 123 immune subsets including CD4 and CD8 T cells, natural killer (NK) cells, NK-T cells, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and dendritic cells and 114 refined subsets relating to maturation/function using multicolor flow cytometry
    - Changes in the function of select immune cell subsets (e.g. CD4 and CD8 effector T cells, NK cells, Tregs, and MDSCs)
    - Changes in tumor antigen specific T cells (e.g. brachyury, MUC1, and CEA) using intracellular cytokine staining for IFNg, TNF, and IL2 and the degranulation marker CD107a
    - Changes in T cell clonality/repertoire score using Adaptive Biotechnologies, Inc. TCRseq assay
    - Changes in inflammatory gene signature using Nanostring's nCounter Human PanCancer Immune Profiling Panel
- Peripheral Blood/Plasma/Serum:
    - Changes in the neutrophil to lymphocyte ratio (NLR)
    - Changes in circulating tumor DNA (ctDNA) by Adaptive Biotechnologies, Inc. B cell VDJseq assay
    - Changes in sCD27, sCD40L

  - Changes in cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers
- Bone Marrow:
  - Changes in BM aspirate immune subsets by multicolor flow cytometry to evaluate Tregs, MDSC subsets, monocyte subsets, CD8+ T-cells and CD4+Foxp3- T-cells and functional markers including PD-1, Ki-67, Tim3, CTLA-4 and/or CD40
  - Expression patterns in BM biopsy of PD-1 and PD-L1 by immunohistochemistry (IHC)
  - Evaluate known recurrent MM mutations and other genetic alterations including tumor mutational load (TML) by the MSKCC myTYPE next generation sequencing (NGS) assay
  - Evaluate tumor mutational burden (TMB) and Microsatellite Instability (MSI) status
- Imaging, Diffusion Weighted MRI (DW-MRI):
  - To evaluate radiographic changes (signal intensity) in bone marrow heterogeneity and focal lesions by DW-MRI at baseline and over time
- To evaluate the changes in quality of life (QoL) of patients treated on this protocol using the validated patient reported outcome (PRO) tool developed by the NCI, PROMIS® (Patient-Reported Outcomes Measurement Information System)

1.2 BACKGROUND AND RATIONALE

1.2.1 Introduction

Multiple myeloma (MM) is a neoplasm characterized by the proliferation and accumulation of malignant plasma cells in the bone marrow that lead to the overproduction of monoclonal proteins in the serum or urine. There was an estimated 30,280 new cases in the US, accounting for 1.8% of all new cancer cases with 12,590 deaths in 2017.[2] In 2014, there were an estimated 118,539 people living with myeloma in the United States.[2] End-organ damage and hence myeloma defining criteria resulting from this disorder includes hypercalcemia, renal insufficiency, anemia, and lytic bone lesions (CRAB).[3] Myeloma remains incurable, with a median survival of 5-8 years (previously 3-4 years) in the United States, although newer therapies appear to be improving survival.[4-10] Importantly, two recent studies have provided evidence that all cases of MM are preceded by a premalignant state, monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM), although at this time the biological mechanism of this progression is not understood.[11, 12]

MM is diagnosed when BM C138+ plasma cells are ≥ 10% in number and/or monoclonal band (M-spike) on serum electrophoresis (SPEP) ≥ 3 g/dL with with the addition of CRAB-defined end-organ damage and/or MM defining biomarkers of 1) Involved/un-involved serum free light chain ratio ≥ 100, 2) ≥ 2 focal lesions on spinal MRI, or 3) BM plasmacytosis of ≥60%.[3] Two major criteria have been used in the past to risk stratify patients in terms of prognosis, fluorescent in situ hybridization (FISH)/cytogenetics and International Staging System (ISS) which more recently have been combined into the revised ISS (R-ISS).[13] The table below describes the R-ISS:[13]

R-ISS	Criteria	Prognosis
I	ISS I (Serum β2-microglobulin < 3.5 mg/L, serum albumin ≥ 3.5 g/dL) & non-high risk FISH & normal LDH	mOS not reached HR: 1
II	Not R-ISS Stage I or III	mOS 83 months HR: 3.68 (95%CI: 2.75,4.92)
III	ISS III (Serum β2-microglobulin > 5.5 mg/L) & High Risk FISH or LDH	mOS 43 months HR: 9.95 (95%CI: 6.45,15.36)

1.2.2 Current standard of care and FDA approved therapies for RRMM

The last decade has revolutionized the treatment and management of MM. Key milestones include the development of autologous stem cell transplant (ASCT), immunomodulatory drugs (IMiD), proteasome inhibitors (PI), monoclonal antibodies (MAb) directed against SLAMF7 and CD38 and more recently with promising results of chimeric antigen receptor T cell therapy (CART), bispecific T cell engager therapy (BiTE), and antibody drug conjugates (ADC). In the context of newly diagnosed multiple myeloma (NDMM) and upfront RRMM treatment, 3 drug combinations are superior to 2 drug combinations. However, ultimately patients exhaust available combinations and succumb to disease.[7] Current available therapies are listed below:

Drug class	Drug	FDA: approved indication	Line of Therapy
PI	**Bortezomib**	NDMM and RRMM	≥1st
	Carfilzomib	Monotherapy or in combo with d or Rd for RRMM	≥2nd
	Ixazomib	In combo with Rd for RRMM	≥2nd
IMiD	**Thalidomide**	In combo with d for NDMM	1st
	Lenalidomide	In combo with d for NDMM, RRMM, maintenance	≥1st
	Pomalidomide	In combo with d for RRMM	≥3rd
DNA intercalator	**Liposomal doxorubicin**	In combo with V for RRMM	≥2nd (no prior V)
HDACi	**Panobinostat**	In combo with Vd for RRMM	≥3rd (PI, IMiD)
Anti-CD38	**Daratumumab**	Monotherapy, or with Rev, Velcade, or Pom for RRMM	≥2nd (PI, IMiD)
Anti-SLAMF7	**Elotuzumab**	In combo with Rd for RRMM	2-4th

1.2.3 Extramedullary plasmacytomas and lytic bone lesions in MM and treatment

Extramedullary plasmacytomas (EMPs) in MM patients is a relatively uncommon clinical manifestation and tends to occur in later stages of disease. Soft-tissue extramedullary plasmacytomas (EMPs) can develop either by direct invasion from the medullary compartment disrupting the cortical bone or less commonly by hematogenous metastasis.[14] In a series of

1003 patients, overall, 13% of patients had extramedullary disease (EMD), 7% at diagnosis (85% of cases surrounded the axial skeleton and 15% involved hematogenous spread) and 6% later.[15] Up to 45% of patients with EMD at diagnosis developed EMPs at relapse. In another series of 459 NDMM patients, 16.3% had EMD.[16] More than one site was involved in 20% of patients and in a third EMD patients, the presenting symptoms were mainly due to EMD (palpable masses and neurologic symptoms). Regardless of therapy, extramedullary disease was associated with shorter progression-free and overall survival, as well as the presence of anemia, thrombocytopenia, elevated serum lactate dehydrogenase, and cytogenetic abnormalities in a series of 936 patients with EMD.[17] As evidenced by the above studies, EMD is a major contributor to morbidity in MM.

Lytic bone lesions in MM patients is a relatively common clinical manifestation. In fact, bone lesions are one of the MM defining criteria in CRAB. MM bone disease may manifest in the form of a conventional discrete lytic lesion, radiolucent with underlying plasmacytoma, widespread osteopenia, or multiple lytic lesions affecting any part of the skeleton, preferably spine, skull, and long bones and occurs in up to 80% of patients.[18] As a result of bone disease, patients may suffer from bone pain (70–80%), fractures (50–60%), hypercalcemia (15%), spinal cord compression (2–3%), decreased quality of life, and poor mobility.[19]

Management of both EMD and bone lesions requires multiple disciplines which may include conventional systemic MM agents, radiation therapy, bisphosphonates, pain control, and a subgroup of patients who may need surgical interventions. Radiotherapy, in particular, has been an important aspect of treatment. Upfront external beam radiation is used for patients with EMP or cord compression. Indications include pain control, spinal cord compression, pathological fractures, soft tissue plasmacytomas, and control of local neurological symptoms. Approximately a third of patients will require radiation therapy at some point in their treatment and is most commonly used for pain control with a very high success rate.[20-22] Palliation of lytic bone lesions may be accomplished with up to 30 Gy administered in 5 to 10 fractions, however, a single 8- to 10-Gy fraction is generally recommended as single fractions are increasingly preferred to fractionated treatment.[23] Limited evidence-based data exists regarding the ideal dose, however in a series of 101 patients treated at a single site, the total dose ranged from 3.0 to 60 Gy, with a mean of 25 Gy.[20] Symptom relief was obtained in 97% of the symptomatic sites (92% with < 10 Gy and 98% when ≥ 10 Gy used, respectively) but no dose-response could be demonstrated.

1.2.4 Immune checkpoint inhibitors, approved agents and indications

The last 5 years of drug development have brought the advent of highly successful and efficacious immunotherapies in the treatment of cancer. Newer therapeutic modalities have focused on targeting the immune system.[24] Pathways involved in inhibiting antitumor T-cell responses (activation of the inhibitory coreceptors cytotoxic T-lymphocyte-associated protein 4 [CTLA-4] and programmed cell death 1 [PD-1] on T cells) are thought to allow tumors to evade the immune system. PD-1 inhibitors, a new class of immune checkpoint inhibitors, are thought to block T-cell inhibitory signal pathways by preventing engagement of PD-1 to its ligands (PD-L1/2). Program death-1 (PD-1) protein is a co-T -cell regulatory receptor that mediates immunosuppression by binding to the programmed death ligand 1 (PD-L1) normally expressed on stromal cells, T cell, B cells, macrophages and dendritic cells. Some tumors upregulate PD-L 1 expression as a means of immune evasion. Preclinical data demonstrates that inhibition of the

PD-1/PD-L 1 interaction leads to an enhanced T -cell response and increased tumor killing, and clinical data show promising efficacy with this approach.

The PD-1 pathway can also be blocked by PD-L1 inhibitors. Inhibitors to PD-L1 may be more advantageous than PD-1 inhibitors because they block not only PD-1 but also B7.1, another cell surface receptor involved in inhibition of T-cell activity and priming. Moreover, blocking PD-Ll leaves the PD-1/PD-L2 interaction intact which may be important in maintaining immune homeostasis and preventing autoimmunity.

The PD-1 inhibitors pembrolizumab and nivolumab were the first approved in 2014 for the treatment of melanoma followed by the PD-L1 inhibitors atezolizumab and durvalumab for urothelial cancer and avelumab for Merkel Cell Carcinoma. The table below lists indications that various PD-1/L1 inhibitors are currently approved for.

Agent	FDA Approved Disease Indications										
	Melanoma	NSCLC	RCC	SCCHN	UC	MSI-CRC	HCC	GC/CC	MCC	HD	PMBCL
Atezolizumab (1200 mg q3 wk)		XXX			XXX						
Avelumab (10 mg/kg q2 wk)					XXX				XXX		
Durvalumab (10 mg/kg q2 wk)		XXX			XXX						
Nivolumab (240 mg q2 wk; 480 mg q4 wk)	XXX	XXX	XXX	XXX	XXX	XXX	XXX			XXX	
Pembrolizumab (200 mg q3 wk)	XXX	XXX		XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX

Abbreviations: NSCLC, non-small cell lung cancer; RCC: renal cell carcinoma; SCCHN, Squamous Cell Carcinoma of the Head and Neck; UC, Urothelial Carcinoma; MSI-CRC, Microsatellite Instability-High (MSI-H) or Mismatch Repair Deficient (dMMR) Metastatic Colorectal Cancer; HCC, Hepatocellular Carcinoma; GC, gastric cancer; CC, cervical cancer; MCC, Merkel Cell Carcinoma; HD, Classical Hodgkin Lymphoma; PMBCL, Primary Mediastinal Large B-Cell Lymphoma; [1] Anti-PD-L1 antibody; [2] Anti-PD-1 antibody

1.2.5 Immune checkpoint combinations with XRT and abscopal effect

T cells recognize tumor antigens expressed by cancer cells and induce tumor rejection as self. Although many times tumor infiltrating CD8+ T cells are observed, more often than not, tumor regression in advanced malignancy is not observed due to negative immunoregulatory pathways that inhibit anti-tumor responses.[25] Immune checkpoint inhibitors have revolutionized the treatment of many malignancies with unprecedented clinical benefit in terms of duration of response and overall survival. However, not all patients derive clinical benefit from treatment due to a variety of reasons and therefore there is considerable interest in developing synergistic combinations with other therapies to increase efficacy. Radiotherapy is one potential "drug" candidate. Radiation causes tumors to release (neo)antigens and can modulate the immune system without significant immunosuppression. In addition to direct tumoricidal effect,

radiotherapy interacts with the immune system by inducing the immunogenic recruitment of anti-tumor T cells locally, in addition, it can induce MHC I expression and finally can promote T cell repertoire diversity in terms of anti-tumor antigens.[26] For example, radiotherapy induced cell death generates signals that target Toll-like receptor 4 (TLR4) on dendritic cells (DC), leading to antigen processing and cross-presentation.[27] Additional mechanisms include increased PD-L1 expression, interferon gamma production, tumor vasculature normalization, "danger signals" triggering inflammation and destruction of immunosuppressive stromal cells including Tregs and myeloid derived suppressor cells (MDSC).[27]

The abscopal effect is used to define systemic anti-tumor responses at sites distant from a locally irradiated site. Limited non-clinical animal studies evaluating the abscopal effect exist, however, Camphausen et al., an investigator on this study, showed that irradiation of the leg of immunocompetent mice bearing a syngeneic tumor (lung carcinoma or fibrosarcoma) resulted in tumor growth inhibition.[28] The effect was dose-dependent and more effective with hypofractionation (10 Gy x 5) compared to conventional dosing (2 Gy x 12). Clinical reports of an abscopal effect after radiotherapy alone are not common but a recent report identified 46 cases in the literature including lymphoma, melanoma, and carcinomas.[29] Interestingly, a case report of a RRMM patient who failed multiple treatments including ASCT subsequently achieved a sustained CR after palliative XRT to a gastric plasmacytoma and remained in remission for >15 years.[30]

In terms of immunotherapy/radiation combinations, radiation has been shown to have a synergistic effect with various immunomodulating agents.[31] Synergy with CTLA-4 inhibitors led to decreases in tumor growth in murine mesothelioma, breast cancer, melanoma, pancreatic, and glioblastoma models.[32, 33] These responses have been reported not only in animal models, but in the clinic as well.[34] Synergy between radiation and PD-1 pathway inhibitors have been observed in breast, colon, melanoma, and glioma models.[34-38] A variety of doses have been used including 12 Gy x 1, 20 Gy x 1, 2 Gy x 5, 4 Gy x 5, 8 Gy x 3, and 10 Gy x 1.

1.2.6 PD-1/L1 inhibitors in MM

Despite the overwhelming success of monotherapy PD-1/L1 inhibitors in the treatment of solid tumors, their monotherapy activity is marginal and disappointing in MM. For example, in the phase 1b study of nivolumab treatment in various hematologic malignancies, the response rate in the MM cohort was a modest 4% compared to B and T cell lymphomas.[39] Interestingly, in that study, the one response which was observed occurred in a patient who had received palliative radiation. Combination regimens blocking the PD-1 pathway however, have generated initial excitement. In MM, tumor cells, pDCs, and MDSCs express PD-L1,while the microenvironment of the bone marrow including resident cytotoxic T, NK, and NK-T cells express PD-1.[40, 41] Preclinical studies have shown that inhibition of the PD-1/PD-L1 interaction inhibits accessory cell induced MM proliferation and survival while activating host T- and NK-cell anti-MM tumor responses which can be augmented by the addition of lenalidomide.[42] These preclinical data led to a series of single arm clinical studies with reported response rates of 33 to 76%.[41, 43, 44] Two important phase 3 randomized studies evaluated lenalidomide or pomalidomide, dex, and +/- pembrolizumab. Unfortunately, at an interim analysis, the PD-1 inhibitor containing arms were associated with a detriment in overall survival leading to placing the study on hold and release of an FDA statement including Kaplan-Meier graphs.[45] The reasons and mechanisms of action leading to this detriment with the combination is currently unknown, however, it is unlikely that combinations with IMiDs will move forward.

1.2.7 Avelumab drug product, preclinical and clinical pharmacology

Avelumab (company code: MSB0010718C) binds PD-L1 and blocks the interaction between PD-L1 and PD-1. This removes the suppressive effects of PD-L1 on anti-tumor CD8+ T cells, resulting in the restoration of cytotoxic T cell response. The active pharmaceutical ingredient in avelumab drug product is a fully human antibody (calculated molecular weight of 143,832 Dalton) of the immunoglobulin G (IgG) 1 isotype that targets PD-L1, the ligand for PD-1.[46]

The nonclinical pharmacology studies have shown that avelumab functionally enhances T cell activation in vitro and significantly inhibits the growth of PD-L1 expressing tumors in vivo. Avelumab binds to human PD-L1 with a high affinity of 0.7 nM and not to any other B7 family proteins, and competitively blocks the interaction of PD-L1 with PD-1.[46] The in vitro study results have shown that by binding to PD-L1, avelumab effectively enhances T cell activation as measured by interleukin (IL)-2 or interferon-gamma production. In addition, as a fully human IgG1 antibody, avelumab has the potential to trigger the antibody-dependent cell-mediated cytotoxicity (ADCC) against target cells expressing PD-L1. As a monotherapy, avelumab has demonstrated anti-tumor activity against murine MC38 colon carcinoma tumors that are characterized by a high level of PD-L1 expression. A dose-dependent trend was observed, and 400 µg per dose (20 mg/kg) was identified as the optimally effective dose when given every third day for 3 total doses.[46]

Full pharmacokinetic (PK) profiles were evaluated in mice and cynomolgus monkeys, since these species have similar binding affinity to PD-L1 to humans, and therefore these species are likely to have similar target-mediated clearance. The anti-PD-L1 antibodies from research batches and precursor molecules tested in single-dose PK studies demonstrated pronounced nonlinear PK characteristics in mice and cynomolgus monkeys in single-dose studies at doses below 20 mg/kg, suggesting a combination of first order catabolic clearance and saturable target-mediated clearance. Similar terminal half-lives (t1/2) ranging from 58 to 70 hours at doses between 20 and 140 mg/kg were observed in toxicity studies in mice and monkeys. Since avelumab represents a foreign protein to the immune system of animals, anti-avelumab antibodies in rodents and nonhuman primates were observed and have been considered in interpreting the nonclinical data, with higher doses generally resulting in lower immunogenicity incidence. This is potentially due to the interference of avelumab trough concentrations with the measurement of antidrug antibody (ADA), and did not affect exposure or impact the conclusions of the toxicity studies. The immunogenicity incidence against the human antibody avelumab in animals is not deemed predictive for human subjects.

Unlike mouse models, neither in the pilot 4-week IV repeat-dose toxicity study nor in the pivotal 13-week study were clinical signs of hypersensitivity or avelumab-related infusion reactions seen in cynomolgus monkeys after repeated treatment with avelumab at dose levels of 20, 60, and 140 mg/kg, respectively. For the pilot 4-week study as well as for the pivotal 13-week IV repeat-dose toxicity study, a no observed adverse effect level (NOAEL) of 140 mg/kg for systemic toxicity was established.

Clinical Pharmacology: Human PK of avelumab have been characterized using both on compartmental analyses (NCA) and population PK (Pop PK) analysis. The PK profile of avelumab is typical for a human antibody, i.e. with a low clearance and volume of distribution. Based on the NCA after the first dose, maximum plasma concentration observed post-dose (Cmax) and area under the serum concentration-time curve (AUC) increased dose-proportionally at 3 mg/kg and higher, while concentration at the end of the dose interval (Ctrough) increased

dose-proportionally at 10 mg/kg and higher. Based on Pop PK analysis, serum clearance (CL) of avelumab was estimated to be 0.0246 L/hr [95% confidence interval (CI): 0.0239, 0.0252] for a typical subject. The volume of distribution at steady state (Vss) was estimated to be 4.72 L (95% CI: 4.63, 4.82). The half-life (t1/2) was estimated to be 6.1 days [146 hour (95% CI: 140, 152)]. Avelumab accumulation on Cmax and AUC was estimated to be 1.25-fold when given once every 2 weeks. Pop PK analysis and simulations demonstrated that age, race, baseline tumor PD-L1 status, urothelial carcinoma (UC) tumor type, or hepatic impairment had no influence on the avelumab CL or the central and peripheral volumes of distribution, and no influence of renal impairment on CL. Body weight was found to positively correlate with PK exposure. Baseline albumin, sex, the 3 mg/kg dose level, baseline tumor burden, and metastatic Merkel cell carcinoma (mMCC) tumor type in the final Pop PK model reduced the unexplained variability in CL by 5.1%, but none of those parameters is expected to have a clinically meaningful effect on PK.

Avelumab PK are not expected to be affected by concomitant administration of other drugs. Avelumab did not induce cytokines in vivo for human subjects to concentrations needed to affect transporters involved in the distribution or CYP450 metabolism for small molecule drugs. The 10 mg/kg dose once every 2 weeks achieved the high target occupancy (mean TO > 90%) of PD-L1 in PBMC during the whole dose interval as determined from ex vivo studies. Based on the in vitro TO data and the observed trough serum avelumab levels, TO was predicted to reach or exceed 95% throughout the entire dose interval for more subjects in 10 mg/kg dose group than those in 3 mg/kg dose group from the dose escalation cohorts of Study EMR100070-001. Immunogenicity assessment included all subjects from Studies EMR100070-001 and EMR100070-003 treated with 10 mg/kg of avelumab once every 2 weeks and who had at least one valid ADA result. Treatment-emergent ADA incidence was 64 of 1558 subjects (4.1%) across the integrated safety analysis population. No apparent clinically meaningful impact of immunogenicity on PK, efficacy or safety of avelumab was observed.

1.2.8 Avelumab clinical safety and efficacy

Avelumab is currently in clinical development across multiple Phase 1-3 clinical studies, however, as of the date of Investigator Brochure v7 (10/2016), there are no completed clinical trials to report. [46] The safety data summarized in the Investigator's Brochure include data from all subjects treated with 10 mg/kg every 2 weeks from studies JAVELIN Solid Tumor (1650 patients) and JAVELIN Merkel 200 Part A (88 subjects) as pooled safety dataset with a data cutoff of 09 June 2016. Most of the observed adverse events (AEs) were either in line with those expected in patients with advanced solid tumors or with class effects of monoclonal antibody (mAb) blocking the PD-1/PD-L1 axis. Infusion-related reactions including drug hypersensitivity reactions and immune-mediated adverse reactions (immune-related pneumonitis, immune-related colitis, immune-related hepatitis, immune-related endocrinopathies (thyroid disorders, adrenal insufficiency, new onset type I diabetes mellitus, pituitary disorders), immune-related nephritis and renal dysfunction and other immune-related AEs (myositis, myocarditis, Guillain-Barré syndrome, uveitis) have been identified as important risks for avelumab. The tables below list the appropriate treatment emergent AEs (TEAE).

Most Frequently Reported (≥ 5%) Treatment-Related TEAEs in the Pooled Safety Dataset

Body System or Organ Class Preferred term	(N=1738) N (%)
Number of Subjects With At Least One Event	1164 (67.0)
Endocrine disorders Hypothyroidism 87 (5.0)	111 (6.4) 87 (5.0)
Gastrointestinal disorders Nausea Diarrhea	356 (20.5) 150 (8.6) 123 (7.1)
General disorders and administration site conditions Fatigue Chills Pyrexia	554 (31.9) 307 (17.7) 116 (6.7) 106 (6.1)
Injury, poisoning and procedural complications Infusion related reaction	302 (17.4) 295 (17.0)
Investigations	200 (11.5)
Metabolism and nutrition disorders Decreased appetite	149 (8.6) 90 (5.2)
Musculoskeletal and connective tissue disorders	170 (9.8)
Nervous system disorders	132 (7.6)
Respiratory, thoracic and mediastinal disorders	104 (6.0)
Skin and subcutaneous tissue disorders	253 (14.6)

Grade ≥ 3 treatment-related TEAEs were observed in 177 subjects (10.2%) in the pooled safety dataset. The most frequently reported (at least 3 subjects, 0.2%) Grade ≥ 3 treatment-related TEAEs were fatigue, lipase increased (17 subjects each; 1.0%), GGT increased, infusion related reaction (10 subjects; 0.6%), AST increased (8 subjects; 0.5%), pneumonitis (7 subjects; 0.4%), anemia, blood CPK increased (6 subjects each; 0.3%), diarrhea, asthenia (5 subjects each; 0.3%), autoimmune hepatitis, ALT increased, amylase increased, hyponatremia, hypophosphatemia (4 subjects each; 0.2%). Other Grade ≥ 3 treatment-related TEAEs that were observed in 3 subjects (0.2%) included lymphopenia, adrenal insufficiency, hypothyroidism, colitis, vomiting, autoimmune disorders, lymphocyte count decreased, transaminase increased, decreased appetite and hypokalemia. In the pooled safety dataset, overall, a total of 4 deaths (0.2%) were due to TEAEs related to study treatment. The causes of death included pneumonitis, hepatic failure, and respiratory distress.

In the pooled safety dataset (N=1738), a total of 247 patients (14.2%) experienced immune related adverse events (irAEs). The median time to first onset of an irAE was 11.7 weeks. The most frequent irAEs were thyroid disorders including hypothyroidism (5.2%), hyperthyroidism (0.4%) and thyroiditis (0.2%), immune-related rash (5.2%), immune-related colitis (1.5%),

immune-related pneumonitis (1.2%), immune-related hepatitis (0.9%), adrenal insufficiency (0.5%), and immune-related myositis (0.5%). In addition, irAEs reported in 0.1% of patients in the pooled safety dataset included: Type 1 diabetes mellitus, immune-related nephritis and renal dysfunction, pituitary disorder, uveitis, and Guillain-Barré syndrome. The majority of irAEs were Grade 1 or Grade 2 in severity, with 39 (2.2%) being of Grade ≥ 3 severity. Fatal outcome was reported in 1 (0.1%) patient with immune-related pneumonitis, and 2 (0.1%) patients with immune-related hepatitis.

Clinical efficacy as provided in the USPI for approved indications:

JAVELIN Merkel 200 trial

Efficacy Endpoints	Results (N=88)
Overall Response Rate (ORR)	
Overall response rate, (95% CI)	33.0% (23.3%, 43.8%)
Complete response (CR) rate, (95% CI)	11.4% (6.6%, 19.9%)
Partial response (PR) rate, (95% CI)	21.6% (13.5%, 31.7%)
Duration of Response (DOR)	N=29
Range in months	2.8 to 23.3+
Patients with DOR ≥ 6 months, n (%)	25 (86%)
Patients with DOR ≥ 12 months, n (%)	13 (45%)

JAVELIN Solid Tumor Trial: urothelial carcinoma cohorts

Efficacy Endpoints	≥ 13 Weeks Follow-Up (N=226)	≥ 6 Months Follow-Up (N=161)
Confirmed Overall Response Rate (ORR)		
Overall Response Rate n (%)	30 (13.3%)	26 (16.1%)
(95% CI)	(9.1, 18.4)	(10.8, 22.8)
Complete Response (CR) n (%)	9 (4.0%)	9 (5.6%)
Partial Response (PR) n (%)	21 (9.3%)	17 (10.6%)
Duration of Response (DOR)		
Median, months (range)	NE (1.4+ to 17.4+)	NE (1.4+ to 17.4+)

1.2.9 Rationale for combining PD-L1 inhibitor with XRT in RRMM

Pre-clinical models suggest that methods involving the restoration of robust anti-tumor innate immune activation may overcome resistance to checkpoint inhibitors. Therefore, we hypothesize that targeted radiation can change the MM microenvironment niche to sensitize myeloma cells to PD-1 inhibitors and activate systemic anti-tumor T cell and immune responses. The rationale for using radiation as the synergistic partner to induce an abscopal effect is that radiation:

1. Induces tumor cell death through DNA damage and creates neoantigens
2. Increases PD-L1 expression on MM cells and immune cells
3. Increases secretion of IFN gamma
4. Enhances MHC class I surface expression and induces neo-antigen presentation on MM cells
5. Activates dendritic cells and cross presentation of antigens
6. Increases density of tumor infiltrating lymphocytes and modulates Treg's to create the "inflamed phenotype" which plays major role in XRT efficacy
7. Induces cell adhesion molecules and modifies the tumor vasculature to enhance immune cell extravasation

Furthermore, patients will receive radiation at sites of disease (plasmacytomas and bone lesions) and will therefore also derive direct benefit from the local therapy.

A second potential mechanism of action for avelumab is the induction of ADCC. Myeloma cells have been well characterized as having increased PD-L1 expression.[47, 48] As a fully human IgG1 antibody, avelumab has the potential to trigger the antibody-dependent cell-mediated cytotoxicity (ADCC) against target cells expressing PD-L1 which has been shown in preclinical models.

In summary, preclinical models of PD-1 pathway blockade in MM have shown promising results as this pathway appears to play an important role as the tumor cells express relatively high levels of PD-L1 and T cells which show increased PD-1 expression not observed in normal plasma cells.[49-51] We hypothesize that the addition of local radiotherapy will synergize with systemic PD-L1 inhibition to lead to clinical benefit by priming the immune system. In support of this, using a 5T33 murine mouse multiple myeloma model, it has been shown that radiation therapy in combination with blocking the PD-L1 pathway with an antibody resulted in longer survival compared to a control IgG antibody.[52] In another preclinical J558L myeloma model, the authors showed that PD-L1 blockade with an antibody elicited rejection of murine myeloma when combined with radiation.[50] The term abscopal effect was coined to describe an immune-mediated anti-tumor response to radiation by malignant cells located distant from the irradiated site and has been demonstrated in various solid tumors including melanoma, renal cell carcinoma, breast cancer, and hepatocellular carcinoma amongst others.[53] Recently, more and more studies are indicating that combining radiation with immunotherapy may boost abscopal response rates. The table below describes the preclinical studies supporting the combination of immunotherapy.

Selected preclinical studies demonstrating the abscopal effect with the combination of radiotherapy and immunotherapy (adapted from Ngwa et al.)[53]

Tumor model	Radiation dose	Immunotherapy type, dose, timing, route	Boosting the abscopal effect: conclusion
Subcutaneous MC38 colon adenocarcinoma[54]	3 × 8 Gy	Anti-PD1, anti-CD137 or both; 300 µg; after each radiotherapy fraction; intraperitoneal	Brachytherapy with immunotherapy can potentiate the abscopal effect
Subcutaneous CT26 colorectal carcinoma[55]	20 Gy	Anti-CTLA4; 250 µg; before radiotherapy; intraperitoneal Anti-TNFRSF4; 250 µg; after radiotherapy; intraperitoneal	In combining radiotherapy and immunotherapy, ideal timing of radiotherapy is dependent on the mechanism of action of

Tumor model	Radiation dose	Immunotherapy type, dose, timing, route	Boosting the abscopal effect: conclusion
			the respective immunotherapy utilized
Subcutaneous Lewis lung carcinoma[56]	6 Gy	Anti-CD40; 20 μg; after radiotherapy; intratumoural	Intratumoural administration of anti-CD40 boosts the abscopal effect, and further research on the use of SRBs to boost this effect is justified
Subcutaneous 67NR mammary carcinoma[57]	3 × 8 Gy	FLT3L; 10 μg × 10; after radiotherapy; intratumoural	Fractionated radiotherapy with FLT3L induces abscopal effects
Subcutaneous B16-F10 melanoma & Subcutaneous TSA mammary adenocarcinoma [58]	20 Gy; 3 × 8 Gy	Anti-CTLA4, anti-PD1, anti-PDL1; 200 μg per mouse; before, concurrent with and after radiotherapy; intraperitoneal	The combination of radiotherapy, anti-CTLA4 and anti-PDL1 promotes response and immunity through distinct mechanisms
Subcutaneous TUBO mammary carcinoma & MCA38 colon carcinoma[35]	12 Gy	Anti-PDL1; 200 μg × 4; before, concurrent with and after radiotherapy; intraperitoneally	There is close interaction between radiotherapy, T cells and PDL1 in boosting the abscopal effect
Subcutaneous colon26 adenocarcinoma[59]	2 Gy × 5 consecutive days per cycle × 2 cycles	IL-2; 20,000 IU in 0.1 mL of PBS; after radiotherapy; intratumoural	Intratumoural injection of IL-2 boosts both the local and abscopal effects of local radiotherapy
Subcutaneous TSA mammary adenocarcinoma & MCA38 colon carcinoma[60]	20 Gy × 1, 8 Gy × 3 or 6 Gy × 5	Anti-CTLA4 antibody (9H10); 200 μg × 3; concurrent with or after radiotherapy; intraperitoneal	Fractionated but not single-dose radiotherapy in combination with anti-CTLA4 boosts the abscopal effect
Subcutaneous SCCVII[61]	4–10 Gy	DC; 1×10^6 cells; after radiotherapy; intratumoural	A combination of intratumoural DCs and radiotherapy can induce strong local and abscopal responses
Subcutaneous 4T1 mammary carcinoma[62]	12 × 2 Gy	Anti-CTLA4; 200 μg × 3; after radiotherapy; intraperitoneal	Combining local radiotherapy with anti-CTLA4 is a promising new strategy against poorly immunogenic metastatic cancers
Subcutaneous 67NR mammary carcinoma[63]	2–6 Gy	FLT3L; 0.5 mg per kg body weight × 10; after radiotherapy; intraperitoneal	T cells mediate the abscopal effect
Subcutaneous C3 sarcoma and MethA fibrosarcoma[64]	10 Gy × 3–5 cycles	DC; $2–4 \times 10^6$ cells per mouse; after radiotherapy; intravenous	The combination of radiotherapy and DC administration may be an attractive new approach to treat advanced cancer

Tumor model	Radiation dose	Immunotherapy type, dose, timing, route	Boosting the abscopal effect: conclusion
B16 melanoma[65]	2, 5, 10, 20 Gy	Anti-PD-1 antibody, pre and post radiation	Combination of PD-1 inhibitor and radiation produces persistent abscopal effect

In conclusion, both animal models and clinical anectodal cases support the current study of combining radiation with checkpoint blockade.

1.2.10 Avelumab and XRT dose and schedule rationale

Avelumab is currently approved for 2 indications at a dose of 10 mg/kg and schedule of every 2 weeks. We propose to use a fixed or flat dose of 800 mg IV given every 2 weeks. Per personal communication with the manufacturing sponsor (EMD Serono) this is the equivalent appropriate flat dose per their data. As most PD-1/L1 drug sponsors are developing or have developed flat doses, we have proposed to do the same with avelumab for this study.

In terms of radiation, an ideal dose and schedule should activate the immune system and decrease the level of immunosuppression. Thus far there is some discrepancy based on preclinical studies of the optimal dose and regimen of XRT to be used in conjunction with immunotherapy.[66, 67] However, it appears that hypofractionated doses of XRT improve synergy with immune-oncology drugs compared to conventional doses or one time high doses. For example, in preclinical models, hypofractionated XRT in daily doses of 6 to 8 Gy times 5 or 3 days, over a single high dose treatment of 20 Gy, were more effective in generating an anti-tumor immune response with anti-CTLA-4 inhibitors.[60] Since human tumor cells are generally more sensitive to radiation than mouse cells, and since hematologic tumors are generally more sensitive to radiation than epithelial tumors, daily doses of 5 Gy over 5 days to a multiple myeloma lesion are expected to produce similar immunological sequelae to the aforementioned 6 to 8 Gy over 3 to 5 days. In addition, delayed anti-CTLA-4 inhibition was inferior to concurrent administration. Moreover, the most impressive clinical results with solid tumors have been observed when preclinical XRT hypofractionated doses (i.e. 6 Gy x 5) with ipilimumab were used.[68, 69] Based on successful preclinical results combining XRT with PD-1/L1 inhibition, many early phase and a couple of late phase clinical trials have been initiated with results pending. A review by Kang et al, identified 19 phase 1, 29 phase 1/2, and 2 phase 3 registered clinical trials encompassing a variety of both hematologic and solid cancers.[66] The results of these trials are much anticipated as they will give clinical insight regarding the various XRT schema used including 6 Gy × 5, 8 Gy × 1, 2, or 3 and 20 Gy × 1, 2, or 3.

In terms of our proposed study in RRMM, the chosen XRT regimen of 5 Gy for 5 days is generally aligned with both preclinical work and ongoing clinical studies to activate an anti-myeloma immune response. Furthermore, a 5 Gy x 5 fraction regimen can be considered an ablative palliative dose that is likely to provide local control of the lesion (without recurrence in the treatment field) and restoration of function in as many cases as radiation is expected to restore. Thus, this regimen is sufficient to provide direct local palliation to plasmacytoma and lytic lesions; it is expected to be well-tolerated with minimal risk of toxicity; and it is a dose and fractionation scheme similar to dose/fractionation approaches which can be radiobiologically synergistic with concurrent immunotherapy.

The study design incorporates a lead-in period of one cycle (two doses) of avelumab prior to radiotherapy. The rationale for this study design is to ensure that maximal PD-L1 blockade and T cell priming occurs prior to radiation to induce tumor cell immunogenicity. Preclinical studies have shown that a delay in PD-L1 inhibition does not lead to synergy. Therefore, concurrent administration of the combination is essential for synergy by converting the irradiated tumor into an in situ vaccine for systemic disease control.**[66]** For example, in a mouse model, delay of PD-L1 pathway inhibition was ineffective in enhancing long term survival of the mice.**[36]** Furthermore, the majority of clinical trials are evaluating the concomitant administration of radiation and checkpoint blockade.**[66]** To ensure maximal PD-1 pathway inhibition, the lead-in period will ensure a study state concentration of avelumab. In human repeated dosing studies, avelumab reached the steady state between the second and third dose.**[46]** Furthermore, Pop PK modeling confirmed that the steady state is expected to be reached by the third dose.**[46]** Therefore, it is expected that at 1 cycle of avelumab treatment, drug levels will reach study state and with the addition of radiation then, maximal synergy will occur.

1.2.11 Rationale for performing exploratory studies

Immunotherapies including PD-1/L1 inhibitors have revolutionized treatment paradigms for many malignancies. However, not all patients respond to therapy and the most appropriate biomarker has not yet been identified. Although, PD-L1 expression has been associated with improved clinical responses, in itself it may not be adequate.[70] Newer biomarkers have been discovered and are currently being developed.[71] For example, tumor mutational burden (TMB) and micro-satelite instability (MSI) have been found to be important in predicting response to checkpoint blockade.[72, 73] Along these lines, T cell repertoire, clonality, and diversity have been shown to be important in predicting response to checkpoint blockade.[74, 75] It has been suggested that immune gene signatures may play an important role in predicting response to immunotherapy. For example, two signatures, the “Interferon-gamma 10-gene” and the “Expanded-immune 28-gene,” were found to be associated with ORR and PFS in a validation cohort of PD-1 inhibitor treated patients.[76] The Nanostring Human Pan Cancer Gene Expression Panel is a gene expression panel which combines components involved in the interaction between the tumor, microenvironment and immune response in cancer for characterization of disease biology. Furthermore, immune subset populations in the micro environment and tumor infiltrating lymphocytes are important mediators of final clinical activity of checkpoint inhibitors.[71, 74] This entails the balance in the micro environment and periphery of activating (CD4 and CD8 T cells, natural killer (NK) cells, NK-T cells) and inhibitory subsets (regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs)). Other studies have shown that the neutrophil to lymphocyte ratio (NLR) may be an important predictor of checkpoint inhibitor response.[77] Tumor antigen specific T cells play an important role in anti-myeloma responses. For example, brachyury expression has been shown to be increased in MM.[78] MUC1 and CEA also appear to be important T cell anti-myeloma tumor antigens.[79, 80]

It is now well known that mismatch repair defects (MMRd) and MSI-High tumors forecast a response to checkpoint blockade not only in colon cancer but in multiple other solid tumors as well. For example, in a study of 68 patients with 12 different solid tumor types with evidence of MMRd were given a PD-1 inhibitor which was associated with an ORR of 53%.[81] Pembrolizumab is now approved for second line treatment of any MMRd and MSI-H solid tumor. This approval was based on data from five single-arm multi-cohort trials (KEYNOTE-

016, KEYNOTE-164, KEYNOTE-012, KEYNOTE-028, and KEYNOTE-158) including 15 cancer types. Among all patients, ORR was 39.6% with 78% of responders having a duration of 6 months or greater. Studies have investigated the frequency of microsatellite instability in MM, for example, Velangi et al. reported MSI phenotype in 54% of 26 newly diagnosed MM patients.[82] Timuragaoglu et al. examined 98 patients with plasma cell dyscrasias and found that MMRd became more frequent as the disease progressed from MGUS/SMM (7.7%) to 20.7% in MM/plasma cell leukemia.[83] More recently, Miyashita et al. used high-resolution florescent MSI assay to demonstrate MSI in 2 of 20 patients with myeloma.[84] The Illumina TruSight Oncology 500 (STO 500) NGS assay is able to target multiple variant types, including TMB and MSI. Evaluating for MMRd/MSI-High, along with TMB in this study is of upmost clinical significance.

The purpose of our proposed exploratory studies is to evaluate weather the above stated markers at baseline will correlate with a clinical response in RRMM. Secondly, we would like to evaluate these markers as patients go into a clinical response and/or have progressive disease to evaluate whether clinical changes are associated with biologic changes presumably due to activation of the immune system and/or failure of overcoming myeloma-mediated immunosupression.

1.2.12 Imaging – DW-MRI

DW-MRI has recently been found as a potential complementary imaging modality that focusses on the actual myeloma plasmacytoma disease rather than subsequent bone destruction. The MY-RADS group state that because skeletal survey and CT predominantly help to detect the destructive effects of myeloma on trabecular and cortical bone rather than disease within the bone marrow space, sensitivity and capability as a restaging tool are inherently limited. [81] Myeloma infiltrates within bone marrow can be observed on CT if they lie within the marrow spaces adjacent to fatty bone marrow. However, in trabecular bone spaces (i.e., vertebral bodies), myeloma infiltrates are difficult to evaluate given factors including the trabeculae, degenerative changes, benign lesions, and osteoporosis. In contrast, MRI allows direct imaging of the bone marrow given its superb sensitivity, soft-tissue contrast, and early detection of focal myeloma lesions. Although PET/CT can also detect myeloma lesions, MRI is more sensitive especially with newer techniques; in particular, DW-MRI have shown a sensitivity of 77% compared to 47% for PET/CT. [82] Current whole-body MRI protocols can incorporate DW- MRI sequences that are sensitive to cellular density and viability, and are important for disease detection and monitoring. Another benefit of DW-MRI sequences are that they are quick to perform (approximately 30-45 minutes) and interpret. Finally, the relationship of apparent diffusion coefficient values with cell density has the potential to assess response to treatment and response heterogeneity prior to changes in lesion size. [83, 84] The recent MY-RADS recommendations were published in an attempt to promote standardization and decrease variations in the acquisition, interpretation, and reporting of whole-body MRI, and to allow better response assessments across cancer centers. MY-RADS recommendations do require validation within clinical trials, including assessments of reproducibility and, therefore, we will be guided by the group's recommendations especially in regard to MRI data acquisition and analysis protocols. [81]

1.2.13 Patient Reported Outcome/Quality of Life: PROMIS

Patient-Reported Outcomes Measurement Information System (PROMIS) is a health-related patient reported outcome (PRO) quality of life measurement instrument that was developed by NIH/NCI to standardize patient-reported outcomes for national use by research clinicians. Two

versions of PROMIS are available to researchers: computer-adaptive tests (CATs) and short forms.[85-90] The short forms are brief, static instruments that have demonstrated similar reliability to the longer, dynamic CATs, which provide precise measures for studying populations with widely varied responses and longitudinal self-report data. Each short form includes 4 to 8 items, measures reported health outcomes in the past 7 days on a Likert type scale and takes less than 5 minutes each to complete; collectively we anticipate the questionnaires chosen for this study to take approximately 20 minutes in total.

PROMIS instruments, measuring a broad range of health domains, have been validated for adults (≥18) with a variety of health conditions. Results of PRO for patients with RRMM treated with the carfilzomib, lenalidomide, dexamethasone regimen have been published before as, which is a significantly different drug combination than the this study.[91] In addition, recently, QoL and other EORTC PROs were published in patients post salvage HDM-ASCT.[92] In the current study, we hope to gain insight into PROs when a monoclonal antibody drug is combined with radiation therapy.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have a documented diagnosis of multiple myeloma defined by the International Myeloma Working Group Criteria (IMWG)[3]. Patients at initial diagnosis must have had a serum M-protein ≥3 g/dL and/or bone marrow plasma cells ≥10%, and at least one of the following:

- Anemia: Hemoglobin ≤10 g/dL, or
- Renal failure: serum creatinine ≥ 2.0 mg/dL, or
- Hypercalcemia: Ca ≥10.5 mg/dL, or
- Lytic bone lesions on X-ray, CT, or PET/CT, or
- ≥2 focal lesions on spinal MRI, or
- ≥60% bone marrow plasma cells, or
- Involved/un-involved serum free light chain ratio ≥ 100

2.1.1.2 Have at least one extramedullary plasmacytoma or lytic lesion which at the discretion of the investigators is amenable to and clinically indicated for localized radiation therapy

2.1.1.3 Must have *Relapsed* or *Relapsed and Refractory* Multiple Myeloma. Patients must have documented evidence of progressive disease (PD) as defined by the IMWG criteria on or after their last regimen and must have achieved a minimal response (MR) or better to at least one prior regimen. Definitions by the IMWG:[93]

- Relapsed and refractory: disease that is nonresponsive while on salvage therapy or progresses within 60 days of last therapy in patients who have achieved minor response (MR) or better
- Relapsed: disease that progresses and requires the initiation of salvage therapy but does not meet criteria for either *primary refractory* or *relapsed and refractory* MM categories

2.1.1.4 Patients must have been previously treated for MM and be refractory to, not a candidate for (ineligible), or intolerant of available therapeutic regimens known to provide clinical benefit including immunomodulatory (IMiD), proteasome inhibitor, and anti-CD38 monoclonal antibody-based treatments.

2.1.1.5 Documented measurable disease within the 4 weeks prior to registration defined by any one of the following:

- Monoclonal Bone marrow plasma cells ≥5%
- Serum monoclonal protein ≥0.2 g/dl
- Urine monoclonal protein >200 mg/24 hour
- Serum immunoglobulin free light chain >10 mg/dL AND abnormal kappa/lambda ratio
- A measurable lesion on PET/CT or MRI

2.1.1.6 Be ≥ 18 years of age on day of signing informed consent

Note: The estimated 2017 US incidence of MM of patients under the age of 20 is 0.0%; therefore, children are excluded from enrollment in this study.[2]

2.1.1.7 ECOG performance status ≤2 (**Appendix A**)

2.1.1.8 Adequate organ function as evidenced by the following laboratory parameters:

• Absolute neutrophil count (ANC)	≥ 1000 /mcL
• Platelets	≥ 75,000 / mcL
• Hemoglobin	≥ 8 g/dL (transfusions permitted)
• Serum creatinine **OR** Measured CrCl or eGFR by CKD-EPI formula may be used to estimate CrCl/eGFR	≤ 1.5 X ULN (except if due to myeloma) **OR** ≥ 30 mL/min/1.73 m^2 for subject with creatinine levels > 1.5 X ULN
• Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels > 1.5 ULN (except if due to myeloma)
• AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN (except if due to myeloma)

2.1.1.9 The effects of avelumab on the developing human fetus are unknown, however, given the known role of PD-1/PD-L1 in maintaining the maternal/fetal tolerance, avelumab can be expected to have an adverse effect on pregnancy, including embryo-lethality. Women of child-bearing potential (WOCBP) and men must agree to use highly effective contraception (such as implants, injectables, combined oral contraceptives, IUDs, sexual abstinence or vasectomised partner) prior to study entry and for the duration of study treatment, and for at least 30 days after the last dose of avelumab. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

NOTE: WOCBP is defined as any female who has experienced menarche and who has not undergone successful surgical sterilization or who is not postmenopausal.

2.1.1.10 Negative serum or urine pregnancy test at screening for WOCBP.

2.1.1.11 Ability of patient to understand and the willingness to sign a written informed consent document

2.1.2 Exclusion Criteria

2.1.2.1 Patients with clinically unstable lesions (e.g., impending cord compression) where a delay in receiving XRT would be detrimental are not eligible

2.1.2.2 Current or prior anti-cancer treatment prior to the first dose of avelumab as defined below:

- Chemotherapy, targeted small molecule therapy, or other anti-cancer treatment not otherwise specified below within 2 weeks
- Radiation therapy within 2 weeks
- Anti-cancer monoclonal antibody (mAb) treatment within 4 weeks
- Use of an investigational agent (e.g., biologic, drug, or other) within 4 weeks
- Allogeneic stem cell transplant

2.1.2.3 No autoimmune disease, as follows:

- Active (acute or chronic) autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent. Patients with type I diabetes, vitiligo, psoriasis, or hypo- or hyperthyroid diseases not requiring immunosuppressive treatment may be eligible.
- History of serious autoimmune-related disorders including immune colitis, inflammatory bowel disease, pneumonitis, or pulmonary fibrosis whether drug-mediated or not.

2.1.2.4 Current use of immunosuppressive medication, EXCEPT for the following:

- Intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection)
- Systemic corticosteroids at physiologic doses
- Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)

2.1.2.5 Uncontrolled intercurrent illness including, but not limited to the following that may limit interpretation of results or that could increase risk to the patient in the judgment of the investigator:

- Patients with a positive hepatitis B core antibody [HBcAb] and negative surface antigen (HBsAg) may be included if HBV DNA is undetectable
- Patients who are positive for HCV antibody must be negative for HCV by polymerase chain reaction (PCR) to be eligible for study participation
- Known acquired immunodeficiency syndrome (AIDS). Controlled and stable HIV positivity is allowed
- Prior organ transplantation including allogenic stem-cell transplantation
- Clinically significant cardiovascular disease: cerebral vascular accident/stroke (<

6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class III), or serious cardiac arrhythmia requiring medication. Mild arrhythmias, e.g. stable atrial fibrillation, may be allowed at the discretion of the investigator

- Active infection requiring systemic therapy (minor infections may be allowed at the discretion of the investigator)
- Known mental or physical illness that would interfere with cooperation with the requirements of the trial or confound the results or interpretation of the results of the trial and, in the opinion of the treating investigator, would make the patient inappropriate for entry into the study.

2.1.2.6 Persisting toxicity related to prior therapy (Grade > 1); however, alopecia, sensory neuropathy Grade ≤ 2, or other Grade ≤ 2 not constituting a safety risk based on investigator's judgment are acceptable.

2.1.2.7 Vaccination with live vaccines within 4 weeks of the first dose of avelumab and while on study is prohibited (inactivated vaccines may be administered).

2.1.2.8 Pregnant or lactating females. Because there is an unknown but potential risk for adverse events in nursing infants, on-study breastfeeding is not allowed.

2.1.2.9 History of allergic reactions or hypersensitivity to avelumab or any component in its formulations, including known severe hypersensitivity reactions to monoclonal antibodies (Grade ≥ 3) unless felt to be in the best interests of the patient at the discretion of the investigator.

2.1.2.10 Known additional malignancy that is symptomatic or requires active systemic treatment (at the discretion of the PI, exceptions may be made if in the best interest of the patient).

2.1.2.11 Other severe acute or chronic medical conditions including immune colitis, inflammatory bowel disease, immune pneumonitis, pulmonary fibrosis or psychiatric conditions including recent (within the past year) or active suicidal ideation or behavior; or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

2.1.3 Recruitment Strategies

Study participants will be recruited from the population of patients screened in the lymphoid malignancies clinic of the National Institutes of Health. These will include both referrals from outside physicians as well as patient self-referrals. Of note, our outside physician referral network has a high representation of minorities.

This study will be posted on NIH websites and on NIH social media forums. Study-specific public service announcements and informational fliers will be used for recruitment activities. All information to be posted or distributed publicly will be submitted to the IRB for review and approval in advance of use.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent **or** the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which screening activities will be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

NOTE: Assessments and procedures to confirm study eligibility should be completed within 28 days prior to registration (unless otherwise noted).

2.2.3 Clinical Evaluations

- Confirmation of disease diagnosis (i.e., review of pathology and/or hematopathology, as required)
- Disease history, including: diagnosis, treatment (e.g., systemic treatments, radiation and surgeries), status, and significant prior/ongoing side effects and symptoms
- Complete medical history, including: all active conditions considered to be clinically significant by the treating investigator
- Physical examination, including: height (screening only), weight, vital signs (i.e., temperature, pulse, respiratory rate, and blood pressure); review of concomitant medications and symptoms/side effects; and, assessment of performance status

2.2.4 Laboratory Evaluations

NOTE: Results from outside NIH are accepted.

- CBC with differential
- Chemistry panels: Acute Care (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine), Mineral (serum calcium, phosphate, magnesium and albumin) and Hepatic (alkaline phosphatase, ALT, AST, total and direct bilirubin) and 24-hour urine creatinine clearance (if needed to measure CrCl in cases where serum creatinine > 1.5 X ULN)
- Thyroid function tests, including: thyroid stimulating hormone (TSH), total triiodothyronine (T3), free thyroxine (T4)

- Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody, Hepatitis C antibody (HCV) [qualitative]) (within 3 months). If Anti HCV is positive, will follow with HCV RNA PCR
- Anti-HIV-1/2 Antibody (within 3 months)
- Urine and/or serum HCG in WOCBP (within 3 days prior to enrollment and initiation of study therapy)
- Uric acid, LDH, and Beta-2 Microglobulin
- Serum protein electrophoresis (SPEP) and immunofixation to assess for presence and quantity of monoclonal protein (M-protein)
- Random urine sample for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria). Collect a 24 hour urine sample if patient's serum monoclonal protein is ≤ 0.2 g/dL.
- Serum free light-chain studies
- Quantitative immunoglobulins

2.2.5 Imaging Studies

NOTE: For the purposes of screening, outside radiology may be used, however, a baseline performed at NIH will be needed.

- ^{18}F-FDG PET/CT to confirm extramedullary disease and/or lytic lesions (in certain scenarios, at the discretion of the investigator, a chest, abdomen and pelvis CT or MRI without contrast may substitute).

2.2.6 Other Procedures

- 12-lead EKG
- Optional: Bone marrow biopsy for screening may be done if patient does not have measurable disease otherwise (see eligibility). If done for screening/eligibility purposes, it does not have to repeated as a baseline measure.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here..

2.3.1 Treatment Assignment Procedures

2.3.1.1 Cohorts

Number	Name	Description
1	RRMM	Patients with relapsed/refractory multiple myeloma (RRMM), including at least one extramedullary plasmacytoma or lytic lesion amenable to and clinically indicated for localized radiation therapy

2.3.1.2 Arms

Number	Name	Description
1	Experimental therapy	Avelumab 800 mg IV every two weeks in combination with radiation therapy

2.3.1.3 Arm Assignment

Single arm/group assignment; open-label and non-randomized (i.e., subjects in Cohort 1 directly assigned to Arm 1).

2.4 BASELINE EVALUATION

The following should be performed within 28 days prior to the first dose of avelumab unless otherwise noted; tests performed as part of screening do not need to be repeated if they were performed within the specified window prior to the first dose of avelumab.

NOTE: Results from an outside NIH institution are accepted at the discretion of the investigator.

2.4.1 Clinical Evaluations

- Medical history (interim)
- Physical examination, including weight, and vital signs (i.e., temperature, pulse, respiratory rate, and blood pressure); review of concomitant medications and symptoms/side effects; and, assessment of performance status

2.4.2 Peripheral Blood/Urine Laboratory Evaluations

NOTE: Results from outside NIH are accepted.

- Required within 3 days:
  - Urine and/or serum HCG in WOCBP
- Required within 14 days:
  - CBC with differential; reticulocyte count
  - Chemistry panels, including: Acute Care Panel (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine), Mineral Panel (serum calcium, phosphate, magnesium and albumin), and Hepatic Panel (alkaline phosphatase, ALT, AST, total and direct bilirubin)
  - Others: LDH, Uric acid, Total protein, Beta-2 Microglobulin
  - Serum protein electrophoresis (SPEP) and immunofixation to assess for presence and quantity of monoclonal protein (M-protein). Random urine sample for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria). Collect a 24 hour urine sample if patient's serum monoclonal protein is < 1.0 g/dl.
  - Serum free light-chain studies
  - Quantitative serum immunoglobulins
  - Coagulation panel, including: PT/INR and aPTT
  - Thyroid function tests, including: thyroid stimulating hormone (TSH), total triiodothyronine (T3), free thyroxine (T4)
- Required within 28 days:
  - Lymphocyte Phenotype: T, B and NK cell subsets

2.4.3 Bone Marrow Biopsy and Aspirate Evaluations

- Histopathological evaluation on bone marrow aspirate and biopsy (DLM)

- Immunophenotyping of aberrant clonal plasma cells by multiparametric flow cytometry (LP: Stetler-Stevenson Lab)
- Interphase FISH (Mayo Laboratories)

2.4.4 Imaging Studies

NOTE: The following required to be done at NIH.

- ^{18}F-FDG PET/CT
- Diffusion Weighted Whole Body MRI (DW-MRI) (exception: patients with a contra-indication or inability to perform due to administrative or logistical reasons) (MIP)
- Skeletal Survey (CC)

  NOTE: Results from NIH only. In certain scenarios, at the discretion of the investigator, a CT chest, abdomen and pelvis may substitute or be in addition to the above diagnostic imaging modalities.
- Radiation therapy imaging for planning and treatment position verification purposes, per institutional standards and treating radiation oncologist (must be done locally by ROB).

2.4.5 Research Correlates

NOTE: See Section **5** for additional information.

2.4.6 Questionnaires/Patient-Reported Outcomes (PROs)

NOTE: See Section **5.3.6** and **Appendix G**

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single-arm, open-label, single institution phase 2 study of avelumab in combination with hypofractionated radiation therapy (BavXRT) in up to 27 patients with relapsed/refractory multiple myeloma (RRMM) with extramedullary disease who have progressed on 2 or more prior lines of therapy. Avelumab monotherapy will continue after cycle 2 (concurrent avelumab and radiotherapy) until disease progression, unacceptable toxicity, or other criteria meeting stopping criteria (see Section **3.8**).

The primary endpoint of ORR per IMWG criteria will be augmented with secondary endpoints including safety and tolerability of BavXRT and exploratory correlative studies.

All ongoing testing and procedures will take place per the Study Calendar (Section **3.7**).

NOTE: Treatment is planned to be administered on an outpatient basis. At the investigator's discretion (e.g., for additional monitoring, patient social reasons, scheduling logistics, etc.), patients may be treated on an inpatient basis. Any cases of planned hospitalization are not considered reportable serious adverse events per Section **6.1.2**.

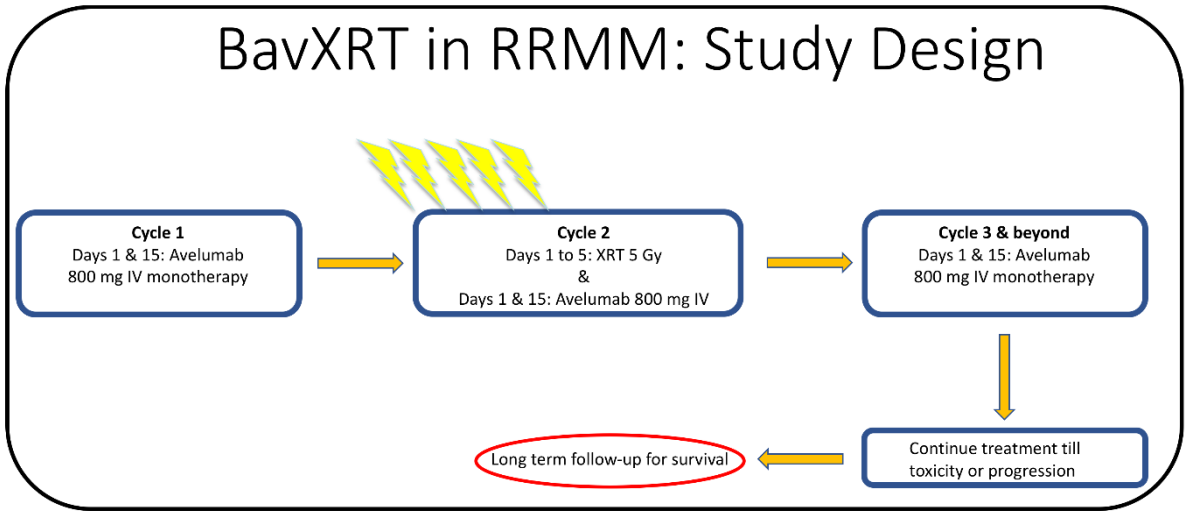
3.1.1 Cycle 1 and 3 and beyond

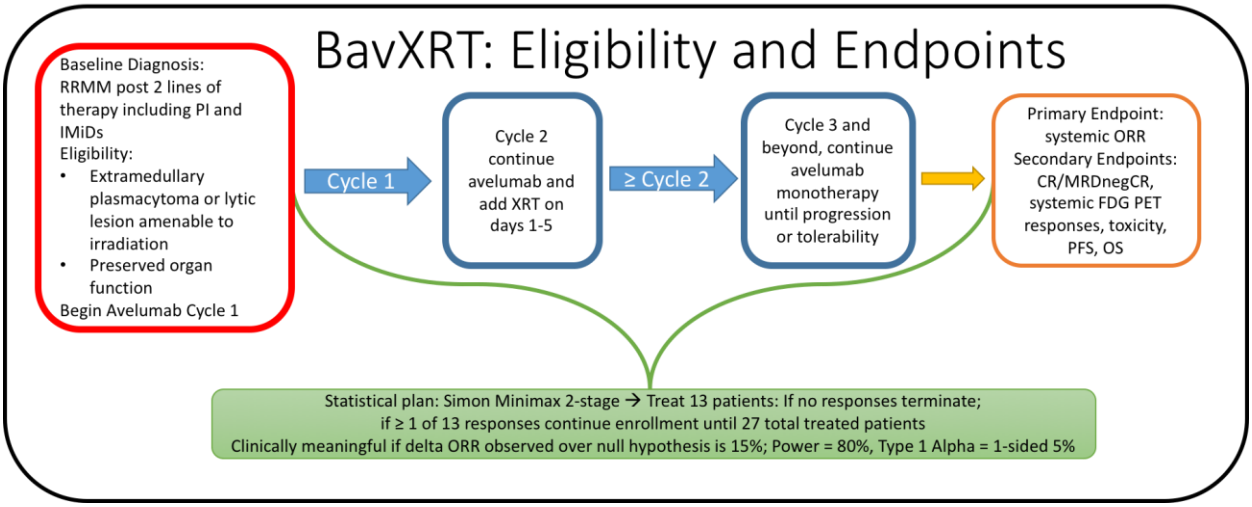
- Patients will receive avelumab 800 mg IV every two weeks

3.1.2 Cycle 2 only

- Patients will receive XRT to at least one extramedullary plasmacytoma or lytic lesion daily for 5 days starting day 1.

- Patients will receive avelumab 800 mg IV every two weeks





3.2 DRUG ADMINISTRATION

3.2.1 Avelumab (MSB0010718C) Dosage

Patients will receive avelumab 800 mg IV over 60 minutes (+/- 20 minutes) on days 1 and 15 of each 28-day or 4-week cycle. If radiation therapy on Day 1 of Cycle 2 is delayed (+/- 5 days), subsequently, Day 15 of Cycle 2 will occur 14 days after. Any avelumab administration day may be adjusted +/- 5 days as needed due to scheduling or other administrative reasons. The maximum window between cycles is 14 days.

3.2.2 Avelumab Preparation

Visually inspect vial for particulate matter and discoloration. Avelumab is a clear, colorless to slightly yellow solution. Discard vial if the solution is cloudy, discolored, or contains particulate matter.

Withdraw the required volume of avelumab from the vial(s) and inject it into a 250 mL infusion bag containing either 0.9% Sodium Chloride Injection or 0.45% Sodium Chloride Injection.

Gently invert the bag to mix the diluted solution and avoid foaming or excessive shearing.

Inspect the solution to ensure it is clear, colorless, and free of visible particles.

Discard any partially used or empty vials.

3.2.3 Avelumab Administration

- Infusion: Administer the diluted solution over 60 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micron). Do not co-administer other drugs through the same intravenous line.
- Premedication: To mitigate infusion related reactions, premedication with an antihistamine and paracetamol (acetaminophen) 30 to 60 minutes prior to the first 4 infusions of avelumab is mandatory (e.g., 25-50 mg diphenhydramine and 650 mg acetaminophen). At the discretion of the investigator, ranitidine 150 mg PO or IV 30-60 minutes prior to avelumab may also be administered. Premedication should be administered for subsequent avelumab infusions based upon clinical judgment and presence/severity of prior infusion reactions. This may be modified based on local treatment standards and guidelines, as appropriate.
- Setting: Avelumab should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.
- Observation period: Following avelumab infusions, patients must be observed for 30 minutes post infusion for potential infusion related reactions. Infusions may be done peripherally or via central venous access device (not required by study), etc.

3.3 RADIATION THERAPY ADMINISTRATION

3.3.1 Personnel

Radiation therapy to extramedullary and/or lytic lesions will be administered by Radiation Oncology investigators from the Radiation Oncology Branch of the CCR/NCI/NIH.

3.3.2 Radiation Dosage and Schedule

At any given lesion, selected as a target for this study, 5 Gy per fraction will be delivered on 5 consecutive treatment days (although consecutive days are ideal, a treatment may be delayed up to 3 days for administrative purposes) for a total dose 25 Gy. Treatment will be administered on Cycle 2 Day 1-5 (+/- 7 days). Radiation Oncology Branch Associate Investigators will be the points of contact (1st: Dr. Jennifer Jones; 2nd: Dr. Kevin Camphausen). Depending on the radiation field for a given lesion, alternate dosing and schedules will be allowed.

3.3.3 Radiation Sites

Only lesions which are safely amenable to radiation will be considered for radiation. Sites may include lytic or extramedullary lesions of the long bones, axial skeleton, and soft tissue. At the

discretion of the investigator, more than a single site may be irradiated if clinically indicated. Doses to critical structures, including spinal cord, bowel, kidneys, lungs, heart, and brain, will be limited to standard dose constraints that are known to be associated with lower than 2-5% average risk of long term radiation-related side effects. Lesions that normally would not have a clinical indication for radiation will not be irradiated. In addition, we will treat all lesions that clinically require it.

3.3.4 Additional Irradiation

At the discretion of the investigator, a patient may receive palliative radiation to additional sites of disease.

3.4 DOSE MODIFICATIONS

3.4.1 Avelumab

- Dose reductions are not allowed.
- Doses should be delayed in general for Grade ≥ 3 toxicity until toxicity resolves to Grade ≤ 1 unless otherwise stated in the protocol. Management should follow accepted medical and institutional standards.
- Dose delays should only be instituted for adverse reactions that are at least possibly due to (suspected) avelumab.
- Laboratory abnormalities which are clinically insignificant and/or unrelated to avelumab in the judgement of the investigator will not lead to dose delays.
- Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to Grade ≤ 1 with medical management will not lead to dose delays.
- Management guidelines for infusion-related adverse reactions are found in **Appendix B: Treatment Modification for Symptoms of Infusion-Related Reactions** and should be followed along with institutional standards.
- Immune-related adverse reactions (irAE) should lead to treatment modifications and management per **Appendix C**.
- Patients may temporarily suspend study treatment for up to 28 days from the last dose if they experience toxicity that is considered related to the study drug and requires a dose to be held.
- Local tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected myelomatous lesions will not be considered adverse events or lead to dose interruptions.

3.4.2 Radiation Therapy

- Very limited toxicity is expected with the planned dose and schedule of XRT (Section **3.3**) and delays in XRT are not expected. However, both dose delays and dose adjustments (increase or decrease) are allowed at the discretion of the treating radiation oncologist in conjunction with the Principal Investigator and ROB Associate Investigators.
- Any local toxicity (i.e. rash, pain) will be managed per institutional standards.
- Local tumor flare phenomena defined as local pain, irritation, or rash localized at sites of known or suspected tumor, , may occur within days of the radiation treatment.

These responses are typically self-limited and are able to be managed by standard supportive measures, including topical Aquaphor and/or other institutional standards.

3.5 ON STUDY EVALUATIONS

Upon confirmation of eligibility and successful registration, and following completion of the Screening/Baseline visits, patients will begin treatment with BavXRT.

After Cycle 1, pre-dose assessments may be performed up to 3 days prior to Day 1 of a cycle, except where otherwise noted. The results from all applicable procedures/tests must be reviewed prior to initiation of each cycle of treatment for consideration of dose modifications. Additional clinical and other appropriate evaluations may be performed as clinically indicated.

Treatment will continue indefinitely until disease progression, unacceptable treatment-related toxicity, or other reasons outlined in Section **3.8.1**.

Refer to the Study Calendar (Section **3.7**) for all tests and procedures to be conducted during screening/baseline, on study/during treatment, and upon discontinuation of treatment and during follow-up.

3.5.1 Cycle 2 Day 1 Evaluation

We hypothesize that avelumab monotherapy will not be sufficient and that concomitant administration of XRT will be needed for synergy and clinical responses. Therefore, at the Cycle 2 Day 1 evaluation, if the patient meets criteria for biochemical progression per IMWG, the patient will remain on treatment if clinically stable and without overt clinical progression in the judgement of the investigator. In terms of evaluating best response, PD on Cycle 2 Day 1 and treatment continuation will not be included.

3.5.2 Treatment Past Progression ("Pseudo-progression")

Patients will not be allowed to remain on treatment past IMWG defined PD unless per the investigator they are receiving objective clinical benefit.

3.6 POST-TREATMENT EVALUATIONS

The following evaluations are required after the decision to discontinue protocol treatment. Any other evaluations and tests should be performed as clinically indicated. Any adverse events which are present at the time of study discontinuation should be followed in accordance with the safety requirements.

3.6.1 Safety Follow-Up Visit

The following will be performed at the end of treatment (+2 weeks), about 30 days following the last dose of treatment (+7 days), and post-treatment prior to (or at) disease progression. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day safety follow-up visit must occur before the first dose of the new therapy.

3.6.2 Extended Safety Follow-Up

Given the potential risk for delayed immune-related toxicities, in addition to above, safety follow-up must be performed 90 (+14) days after the last dose of avelumab administration. This may be performed either via a clinic visit or via a telephone call/email with subsequent clinic visit requested in case any concerns are noted during the telephone call/email.

3.6.3 Follow-Up – Prior to Disease Progression

If a patient discontinues treatment for a reason other than disease progression, or unless otherwise noted, follow-up post treatment will occur at the following time points: every 3 months (+/- 2 weeks) for first year after completion of therapy, every 6 months for years 2-5 (+/- 4 weeks), and then annually thereafter (+/- 6 weeks) at the discretion of the investigator. All effort will be made to follow up patients in clinic to include relevant blood work and response assessment. However, these follow ups may be performed by phone/email if patients are unable to attend clinic at the discretion of the investigator.

3.6.4 Follow-Up – Survival/Post-Disease Progession

Upon disease progression, contact will be for survival evaluation about every 3 months only while the patient is on study unless otherwise indicated at the judgement of the investigator. See Study Calendar (Section **3.7**) for additional information.

3.7 STUDY CALENDAR

Procedure	Screening[1]	Baseline[2]	Treatment Cycles[3]							Disease Evaluations[4]	End of Treatment[5]	Post-Treatment Follow-Up	
			Cycle 1				Cycle 2		Cycle 3+				
Study Cycle Day: *(1 cycle = 28 days or 4 weeks)*			1	8	15	22	1	15[8]	1			Safety[6]	Follow-Up (Prior to PD)[7]
Physical Exam (including, history, vitals, weight, and height [screening only]); ECOG PS	X	X*		X	X	X	X	X	X		X	X	X
Confirmation of Diagnosis	X												
CBC with Differential	X	X*		X	X	X	X	X	X		X	X	X
Reticulocyte Count		X*		X	X	X	X	X	X		X	X	X
Chemistry Panels (i.e., Acute care, Mineral. Hepatic)	X	X*		X	X	X	X	X	X		X	X	X
LDH, Uric Acid, Total Protein		X*		X	X	X	X	X	X		X	X	X
PT/INR and aPTT		X											
Thyroid Function (i.e., TSH, T3, T4)	X	X*					X	X	X		X	X	X
Urinalysis			X	X	X	X	X	X	X		X	X	X
Pregnancy Test (urine/serum HCG; WOCBP)[9]	X	X					X		X				
Hepatitis B and C Testing (within 3 months)	X												
HIV Antibody Testing (within 3 months)	X												
Myeloma Tests[10]	X	X*					X	X	X		X	X	X
12-lead EKG	X												
T/B/NK cell subsets		X	X				X	X	X		X	X	X
Skeletal Survey		X						X[#]		X	(X)	X	X
^{18}F-FDG-PET/CT Scan[11] & DW MRI	X	X						X[#]	X[11]	X	(X)	X	X
Radiation Therapy Imaging		X											
Bone Marrow Aspiration/Biopsy[12]		X						X[#]		X	(X)	(X)	
Symptoms/Toxicity Assessment, Concomitant Medication Review	X	X	X	X	X	X	X	X	X		X	X	X
Research Samples[#13]		X	See Table in Section 5.1										
PROs/Questionnaires[#]		X					X				X		X
Survival													X (Post-PD)

(X) = Optional. **NOTE:** Other tests/assessments may be performed at the discretion of the investigator as clinically indicated.

[#]See Section **5**, Correlative Studies for Research, for additional information on the research blood, urine, bone marrow sampling, and questionnaires..

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[1] Screening evaluations should be performed within 28 days, with the following exceptions: Confirmation of diagnosis (no time limit); HIV antibody, Hepatitis B surface antigen and Hepatitis C antibody (within 3 months). **NOTE:** Screening tests performed within the time frame for baseline do not need to be repeated.
[2] Baseline evaluations should be performed within 28 days prior to first dose of avelumab unless otherwise noted, tests performed as part of screening do not need to be repeated if performed within the specified window. **NOTE:** Items marked with an asterick (*) are required within 14 days prior to first dose.
[3] 14 day maximum window between cycles due to scheduling or other administrative reasons. Cycle 1 (monotherapy): Avelumab Day 1, 1 5; Cycle 2 (dual therapy): Avelumab Day, 15, Radiation therapy Day 1,2,3,4,5; Cycle3+ (monotherapy): Avelumab Day 1, 15. Treat indefinitely until disease progression, or unacceptable toxicity. After Cycle 1 pre-dose assessment may be performed up to 3 days prior to Day 1 of a cycle except when otherwise noted. If radiation therapy on Day 1 of Cycle 2 is delayed (±5 days), subsequently, Day 15 of Cycle 2 will occur 14 days after. Any avelumab administration day may be adjusted ±5 days as need for administrative or logistical reasons.
[4] During treatment, if and when patient reaches partial response (PR), complete response (CR), and/or progressive disease (PD), and at the end of treatment which is optional.
[5] Within 2 weeks after last treatment
[6] 30 days (+7) following last dose (via clinic/in person, preferred), and 90 days (+14) after last dose of avelumab (via clinic or phone/email). If initiating new anti-cancer therapy within 30 days after last dose of avelumab, 30-day safety follow-up visit must occur before first dose of new therapy.
[7] Post-treatment follow-up to be done every 3 months (±2 weeks) for first year after therapy completion, every 6 months for years 2-5 (±4 weeks), and then annually thereafter (±6 weeks) at the discretion of the investigator. Follow-ups may be done by phone/email or by clinic visit at the discretion of the investigator. In cases of remote follow-up (i.e., phone/email), routine outside blood, urine, bone marrow, imaging results may be collected and used for analysis at the discretion of the PI; otherwise, follow-up will involve verbal reporting and survival follow-up only. Any other evaluations and tests should be performed as clinically indicated. Upon progression or start of new therapy, survival follow-up may take place about every 3 months (±28 days) (i.e., phone/email preferred).
[8] At Cycle 2 Day 15, disease evaluations/re-assessment should be performed ±5 days; if radiation therapy is delayed, perform 14 days (±5 days) after start of radiation therapy. **NOTE:** This applies to all items marked with "#;" the timing of other C2D15 assessment may also be adjusted at the discretion of the PI in the case of radiation delay(s).
[9] Urine and/or serum HCG in women of childbearing potential (at screening/baseline and within 3 days prior to initiation of study therapy).
[10] Myeloma tests include serum protein electrophoresis (SPEP) and serum immunofixation to assess M-protein quantity and presence, random urine sample for urine protein electrophoresis (UPEP) and urine immunofixation (Bence-Jones proteinuria- M- protein in urine), serum free light chains, quantitative immunoglobulins and beta-2 microglobulin
[11] In certain scenarios, at the discretion of the investigator, a chest, abdomen and pelvis CT or MRI may substitute for PET. At Cycle 2 Day 15, re-assessment should be performed +/- 5 days; if radiation therapy is delayed, perform 14 days (±5 days) after start of radiation therapy. Patients will also receive PET/CT imaging at cycle 4 day 1 (±5 days) and at any point per the disrection of the investigator for non-reasearch clinical reasons. Diffusion weighted MRI (DW-MRI) will also be performed at the discretion of the PI. DW-MRI will be performed by standard techniques in the NCI Molecular Imaging Program. In follow-up, PET/CTs and DW-MRIs will be done approximately annually, if feasible.
[12] Histopathological evaluation, multiparametric flow cytometry- immunotyping of aberrant clonal plasma cells, Interphase FISH (only at baseline)
[13] Samples for correlative research, including optional biopsy based on investigator discretion and safety (see Section **5**).

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all patients complete a safety visit approximately 30 and 90 days following the last dose of study therapy. Additional safety visits and follow-up will continue as per Section **3.6**.

3.8.1 Criteria for removal from protocol therapy

- Confirmed disease progression not including exceptions outlined in Section **3.5**
- Unacceptable toxicity
- Intercurrent illness that prevents further administration of treatment
- Requirement for use of prohibited therapies as listed in Section **4.2**
- Pregnancy
- Patient withdrawal from protocol therapy
- Noncompliance with trial treatment or procedure requirements
- Investigator's decision to withdraw patient
- Study is cancelled for any reason

3.8.2 Off-Study Criteria

- Patient requests to be withdrawn from study
- Patient is lost to follow-up
- Death
- Study is cancelled for any reason
- Screen failure

4 CONCOMITANT MEDICATIONS/MEASURES

Medications or vaccinations specifically prohibited in the exclusion criteria (see Section **2.1.2)** are not allowed during the ongoing trial. If there is a clinical indication for a prohibited medication/measure during the trial, discontinuation from trial therapy may be required.

4.1 ACCEPTABLE MEDICATIONS

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF. Bisphosphonates (e.g. zolidronic acid) or other bone modifying agents may be given to treat myeloma bone disease at the discretion of the investigator per institutional standards.

All concomitant medications received starting from the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded.

4.2 PROHIBITED MEDICATIONS

Patients are prohibited from receiving the following therapies during treatment on this trial:

- Other therapy for the disease under study not specified in this protocol, unless specifically noted as permitted
- Investigational agents outside of this clinical study protocol
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

Patients who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the protocol treatment but may remain on study for follow up. Patients may receive other medications that the investigator deems to be medically necessary.

5 CORRELATIVE STUDIES

5.1 SUMMARY

Research correlatives listed below are not all inclusive and may or may not be performed based on investigator discretion and available resources. Of note, the table below lists the optimal collection methods/tubes but may be modified at the discretion of the investigator. The following sample types will be collected for correlative research studies:

Research Samples and Questionnaire Calendar:

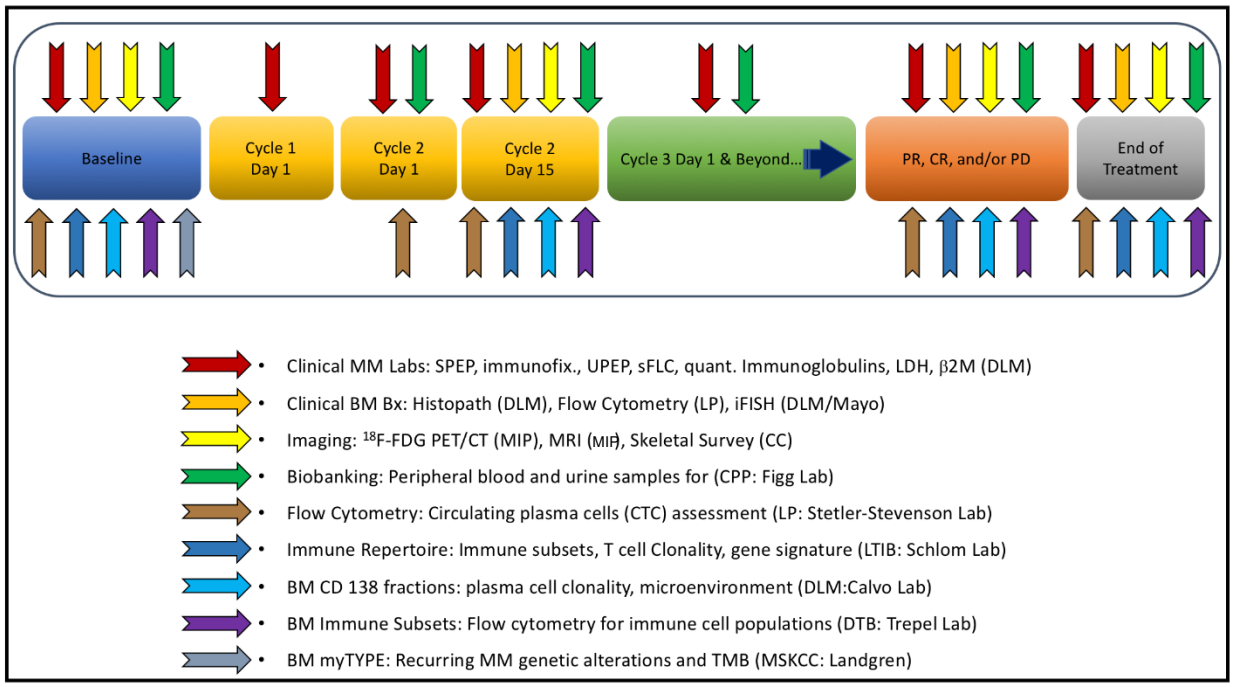
Sample	Collection Details*	Time Points									Supervising Laboratory/ Investigator
		Baseline	C2 D1	C2 D15	D1,C3 & beyond	≥ PR	≥ CR	PD	End of Treatment	Safety/ Follow-Up	
Blood Samples											
Biobank Storage (ctDNA, gDNA, exosomes)	2 x 10 mL Streck tubes 2 x 7-10 mL K_2EDTA tubes	X	X	X	X	X	X	X	(X)	X	Figg Lab
Circulating Tumor Cells (CTCs)	2 x 10 mL in NaHep tubes	X	X	X		X	X	X	(X)	X	Stetler-Stevenson Lab
PBMCs, Immune Markers, Functional Studies Immunoseq T cell Repertoire Inflammatory Gene Signature	4-6 x 10 mL NaHep tubes and 1-2 x 8 mL SST tubes	X		X		X	X	X	(X)	X	Schlom Lab
Bone Marrow Biopsy/Aspirate											
Histology/IHC	FFPE	X		X		X	X	X	(X)		CC/DLM
Multiparametric Flow Cytometry	1 x 3 mL in heparinized syringe	X		X		X	X	X	(X)	(X)	Stetler-Stevenson Lab
CD138+ sorting/VDJ seq	1 x 2-5 mL K_2EDTA tube	X		X		X	X	X	(X)	(X)	Calvo Lab
BMMCs/Immune Subsets	1 x 1 mL CPB (or EDTA) tube	X		X		X	X	X	(X)	(X)	Trepel Lab
FISH	1 x 1-2 mL in NaHep tube	X								(X)	Mayo send out
myTYPE NGS	Aspirate sections (standard media/ handling)	X									CC/DLM; MSKCC Landgren
Urine											
Biobank Storage	Sterile Container	X	X	X	X	X	X	X	(X)	X	Figg Lab
Other Procedures											
Plasmacytoma Biopsy	FFPE	Optional: up to 3 anytime on study at discretion of PI based on safety									Laboratory of Pathology
PROMIS Questionnaires+	Electronic (tablet)	X	X	X	X				(X)		Study Team

(X) = Optional

*Tubes/media may be adjusted at the time of collection based upon materials available or to ensure the best samples are collected for planned analyses. Any of the above collections may also be performed at the discretion of the investigator at monthly time points for patients who may be off-treatment but not off-study.

[+]The electronic PROMIS questionnaire can be accessed by designated study team members at https://scribe.cancer.gov/

TIME POINTS FOR CORRELATIVE RESEARCH/SELECT CLINICAL MYELOMA SAMPLES:



5.2 SAMPLE COLLECTION AND PROCESSING

5.2.1 Summary

The planned analyses described below may be done on leftover and/or shared sample portions from the respective laboratories, as needed. In addition to the prospectively collected samples below, leftover portions of samples sent for routine laboratory testing (e.g., plasma from CBC/hematologies) may also be retrieved for research tests prior to being discarded. The planned prospective analyses are identified below; laboratories may share resources or collaborate on analyses, if appropriate (e.g., isolation/analysis of DNA is not prospectively planned by the Trepel lab, yet may be incorporated if needed during the planned analyses).

Portions of all samples may be banked for future research analyses; prospective consent will be obtained during the informed consent process.

For adult subjects: The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. Correlative studies associated with blood/bone marrow/urine specimens will be performed and related to clinical outcome if the results of the study indicate a clinical or translational rationale for analyzing the samples. Such studies may include but are not limited to the following:

5.2.2 Peripheral Blood and Urine Samples

- Biobanking and Storage: CPP/Figg Lab
  For questions, please contact Dr. Figg's Clinical Pharmacology Program (CPP) at 240-760-6180; additionally, for pre-notification of planned samples (at least 24 hours in advance, the Friday before is preferred) email NCIBloodcore@mail.nih.gov. After sample collection, please page 102-11964 for immediate pick-up. For any questions

regarding sample processing, you may also contact NCIBloodcore@mail.nih.gov by e-mail or at 240-760-6180. For processing see **Appendix D**.

- Circulating Plasma Cells (CTCs): LP/ Stetler-Stevenson Lab
  For questions about processing, please contact the Flow Cytometery Section of the Laboratory of Pathology (LP). Collect two 10 ml of blood in Sodium heparin tubes. Mix by gentle inversion 5-6 times. Label tube with patient name, unique identifier number and date. Deliver immediately to the Flow Cytometry Laboratory 3S240 (specimens containing hematopoietic neoplasms have a tendency to clot and must be processed immediately). Call for STAT Escort pickup and delivery if you cannot deliver the specimen yourself (301-496-9295). Flow Cytometry will be performed per LP's standard processing.

- Immune Subsets/Function, T cell repertoire, Inflammatory Signature: LTIB:/Schlom Lab
  Ideally, 6 Na heparin (green top) 10 ml and 2 serum separator (8 ml SST) tubes should be collected, however, 4 Na heparin 10 ml and 1 SST 8ml red top tubes is the very minimum acceptable. Please notify the Frederick laboratory when specimens are being shipped and email Frederick prior to shipping to notify the lab. Emails should be sent to Theresa Burks, burkst@mail.nih.gov and Caroline Jochems, jochemscm@mail.nih.gov. Once samples are drawn in phlebotomy, send to OP12 clinic via escort for courier pickup.

  On days samples are drawn, Jen Bangh at the Clinical Support Laboratory should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens to be delivered to Frederick National Laboratory. All samples will be labeled with the following identifier system:

  - Patient's enrollment #
  - Trial number
  - Patient's initials Example: 01-ABC

  These labels are used only to send the samples from the NIH Clinical Center to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research. The Clinical Support Laboratory will process all samples, appropriately discard the label on the blood tube, except for one label that is kept on the original paperwork, and then store the samples with unique identifiers, to which only NCI study personnel will have the code to link to patient specific clinical information.

5.2.3 Bone Marrow Biopsy and Aspirate Samples

- Immunohistochemistry of FFPE Bone Marrow Core Biopsy: CC/DLMP
  Per standard protocol of the Hematopathology Section of CC/DLM, Dr. Maric will lead the effort of characterizing expression of markers including PD-1 and PD-L1 by IHC of FFPE Bone Marrow core sections. No special collection is required for this.

- Immunophenotyping by Flow Cytometry Aspirate: LP/Stetler-Stevenson Lab
  For questions about processing, please contact the Flow Cytometery Section of the Laboratory of Pathology (LP). Collect 3 mL of aspirate in one heparinized (0.5 ml) syringe. Mix by gentle inversion 5-6 times. Label tube with patient name, unique identifier number and date. Deliver immediately to the Flow Cytometry Laboratory 3S240 (specimens containing hematopoietic neoplasms have a tendency to clot and must be processed immediately). Call for STAT Escort pickup and delivery if you cannot

deliver the specimen yourself (301-496-9295). Flow Cytometry will be performed per LP's standard processing.

- Aspirate CD138 +/- Sorting, VDJ and Microenvironment: DLM/Calvo Lab
  For aspirate designated for potential CD138 sorting, Illumina TMB/MSI sequencing (see **5.3.2.1**), VDJ sequencing, exome sequencing, and microenvironment studies, send 2-5 mL aspirate sample in K_2EDTA tube to the Calvo Laboratory in the Department of Laboratory Medicine Clinical Center (Attn: Dr. Weixin Wang (Building 10 Room 2C418A). Place aspirate sample in EDTA syringe immediately on ice. Transfer within 30 minutes of sampling to the lab for processing. For baseline samples, cells will be sorted in CD138+ and – fractions. Cells will be viably frozen at -20ºC and batched. See **Appendix E** for detailed laboratory protocol regarding CD138 sorting.

- BMMCs and Immune Subsets by Flow Cytometry: DTB/Trepel Lab
  Upon subject scheduling and immediately after sample collection, contact the Trepel Lab, Developmental Therapeutics Branch, NCI for pick-up: Jane Trepel: trepelj@mail.nih.gov; Min-Jung Lee: min-jung.lee@nih.gov; Akira Yuno: akira.yuno@nih.gov; and, Sunmin Lee: leesun@mail.nih.gov; and/or 240-760-6330.

  Bone Marrow Mononuclear Cells (BMMCs) will be isolated from fresh Bone Marrow aspirate samples. Collect aspirate in sodium citrate tubes (e.g., CPB, blue/black speckled top tubes); gently invert tubes 8-10 times immediately after collection. BMMCs will be isolated per routine laboratory techniques.

- Standard Fish Cytogenetics: Mayo Clinical Labs
  For baseline bone marrow aspirates, samples will be sent for standard clinical interphase Fluorescent In Situ Hybridization (FISH) through the CC/DLM routine processing to Mayo Clinical Labs. Optionally, at the discretion of the investigator, outside documented FISH/Cytogenetics results may be used to substitute. The name of the specific assay is Plasma Cell Proliferative Disorder, FISH (Test ID: PLASF)

- myTYPE Targeted NGS Assays: Landgren Lab (MSKCC)
  Clot sections and/or aspirate based DNA will be sent to Dr. Landgren under an MTA to conduct a specific myeloma targeted NGS panel. Only coded, linked samples and data will be shared for these assays.

5.2.4 Other Tissue Samples/Extramedullary plasmacytoma

- Optionally and the discretion of the investigator, a core needle biopsy (or similar) procedure may be performed on extramedullary soft tissue or bone plasmacytomas. The procedure will only be performed on amenable lesions where the procedure will only be minimal risk for the patient (i.e., non-significant risk). The biopsy will be performed per routine standard of care, by Surgery Consultants or Interventional Radiology, as appropriate; with imaging guidance, if appropriate (e.g., CT-guided). A procedure-specific consent form will be signed by the patient prior to the procedure. If a biopsy procedure is performed, more than one lymph node and at more than one anatomic site may be collected, provided the additional procedures are acceptable risk to the patient. In the event of core needle biopsy, these are obtained typically by using a 16-18G needle at the discretion of the provider performing the procedure. Conscious sedation may be used, if warranted, and the use and risks are acceptable to the patient.

Potential site(s) of biopsy include, but are not limited to: bone lesions, extramedullary disease/masses, and lymph nodes. The type of procedure to be done and manner in which it will proceed (e.g., excision/core, single vs. multiple sites of biopsy) will be discussed with the patient prior to the biopsy procedure. The patient will be reminded that all sampling for research is voluntary.

No more than three (3) optional biopsies will take place in any patient during the study.

- Sample handling/processing

  When performed, biopsies will be placed in sterile collection/core cylinder tubes (e.g., formalin); gently invert/inspect tubes with media 8-10 times immediately after collection to ensure the core(s) is completed immersed in the media.

  Tissue samples will be handled/processed as below prior to planned analyses, as appropriate:

  Any required routine review for histopathologic confirmation of diagnosis and/or grade will occur per standard of care (e.g., H&E, immunohistochemistry), if required. Formalin samples will be fixed and paraffin-embedded per routine techniques.

5.3 BIOMARKER AND RESEARCH METHODS

The technology platforms that are able to interrogate genomic structure and function are constantly in flux; therefore, the exact nature of the methodologies that will be employed will be assessed at the time that the samples are collected and ready for analysis. All of the below biomarker and research methods will be considered exploratory.

5.3.1 Immune Subset Analysis

IHC and flow cytometry will be used to evaluate bone marrow immune subsets. Bone marrow aspirates will be processed, stored, and assessed by the Trepel Lab using multiparameter flow cytometry for immune subsets including but not necessarily limited to Tregs, MDSC subsets, monocyte subsets, CD8+ T-cells and CD4+Foxp3- T-cells. Assessment will include functional markers, i.e. PD-1, Ki-67, Tim3, CTLA-4 and/or CD40 (Trepel Lab). Cell analysis and histological (e.g., H&E), immunohistochemical review and analysis will be performed per standard and established research techniques (e.g., PD-1/PD-L1, -L2 expression [Dako], CD20, Bcl-2, and other IHC analyses)(CC/DLM).

The following immune assays may be performed at the Laboratory of Tumor Immunology and Biology at the NCI's Center for Cancer Research (CCR) in select patients where adequate samples are available:

PBMC:

1. PBMCs may be analyzed for changes in standard immune cell types (CD4 and CD8 T cells, natural killer [NK] cells, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], and dendritic cells) as well as 123 immune cell subsets, as per established methods.

2. PBMCs from selected subjects may be analyzed for function of specific immune cell subsets, including CD4 and CD8 T cells, NK cells, Tregs, and MDSCs.
3. PBMCs may be analyzed for tumor antigen-specific immune responses to CEA, MUC-1 and Brachyury using an intracellular cytokine staining assay. PBMCs will be stimulated in vitro with overlapping 15-mer peptide pools encoding the tumor-associated antigens listed above; control peptide pools will involve the use of human leukocyte antigen peptide as a negative control and CEFT peptide mix as a positive control. CEFT is a mixture of peptides of CMV, Epstein-Barr virus, influenza, and tetanus toxin. Post-stimulation analyses of CD4 and CD8 T cells will involve the production of IFN-γ, IL-2, TNF, and the degranulation marker CD107a. If sufficient PBMCs are available, assays may also be performed for the development of T cells to other tumor-associated antigens.
4. To determine T-cell receptor clonality, DNA may be extracted from cryopreserved PBMC or tumor tissue (if available) using the Qiagen DNeasy Blood and Tissue Kit. TCR Vb CDR3 sequencing will be performed at the NCI genomic core facility using the Immunoseq kit from Adaptive Biotechnologies at the survey (tumor) or deep (PBMC) resolution. In this assay, a multiplex PCR system amplifies and quantifies the rearranged CDR3b sequences from sample DNA. Analysis of the sequences will be performed using the ImmunoSeq ANALYZER from Adaptive Biotechnologies.
5. To determine changes in inflammatory gene signatures, RNA may be extracted from cryopreserved PBMC or tumor tissue (if available) using the Qiagen RNeasy Plus Kit. Nanostring will be performed at the NCI genomic core facility using Nanostring's nCounter Human PanCancer Immune Profiling panel that quantifies expression of 770 genes.

Analyses of soluble factors:

1. Sera may be analyzed pre- and post-therapy for the following soluble factors: sCD27, sCD40 ligand using commercial ELISA kits.

   Sera may be analyzed for changes in cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers using ELISA or multiplexed assays (e.g. Mesoscale, Luminex, cytokine bead array).

5.3.2 Circulating Myeloma Cells (CTCs) and Bone Marrow Myeloma Cells

CTCs and bone marrow plasma cell will be isolated and evaluated per established flow cytometry techniques. Immunophenotyping of aberrant plasma cells by flow cytometry currently involves, but is not limited to, the use of the following reagents: CD138, CD19, CD45, CD38, and CD56. Characteristic changes in immunophenotypically abnormal plasma cells (CD138 positive) include but are not limited to absent CD19 and CD45, decreased CD38, and increased CD56. These studies will be performed under the direction of Maryalice Stetler-Stevenson of the flow cytometry unit in the NCI Laboratory of Pathology

5.3.2.1 Bone Marrow, CD138+/- Fractions

Bone Marrow CD138 positive (plasma cells) and negative (microenvironment cells) will be fractioned per routine methods in the Calvo Lab. DNA from CD138+ fractions may be sent to Adaptive Biotechnologies for amplification and sequencing of the VDJ segment of the immunoglobulin receptor to determine clonality using well established NGS techniques. In

addition, CD138+ fractions may be sent to Dr. Raffeld's Lab (NCI/LP) to undergo targeted sequencing using the Illumina TSO500 assay which LP is currently clinically validating to detect TMB and MSI status of tumor tissues. Negative fractions will be evaluated for microenvironmental changes in response to therapy in the Calvo Lab.

5.3.2.2 Myeloma Targeted NGS Panel: myTYPE

As resources allow, baseline and/or other bone marrow clot section slides may be sent to Memorial Sloan Kettering Cancer Center (MSKCC) for NGS targeted sequencing of myeloma cells using the myTYPE assay under the direction of Ola Landgren, MD, PhD. myTYPE captures 120 genes including driver mutations, signaling pathways, therapeutic targets, IGH translocations, and copy number variations. Baits were designed to capture the entire IgH locus where majority of the chromosome 14 breakpoints occur, genome wide single nucleotide polymorphisms (SNPs) for hyperdiploidy and other CNAs, as well as exons of 120 frequently mutated somatic genes in multiple myeloma. The target regions from bone marrow samples are amplified and then sequenced using 126 bp paired end reads using Illumina HiSeq with a mean target depth of ~600x. Data is analyzed using validated bioinformatic algorithms including CNVkit, Brass and Delly. This will not include sequencing matched controls.

5.3.3 Figg Lab Biobanked Samples (ctDNA, gDNA, and exosomes)

Blood research samples will also be collected for biobanking purposes and future experiments. The purpose of these assays will be to further characterize underlying biology and correlate with clinical outcomes in an exploratory manner. Future experiments and research work not discussed in this protocol will first be incorporated into the protocol prior to initiation. Appropriate Material Transfer Agreements (MTA) will be executed prior to sample shipment. These exploratory studies are not limited to but may include the following:

Peripheral blood nanoscale particle analysis (exosome profiling, microparticles, and other extracellular vesicles):

Dr. Jennifer Jones will lead studies based on profiling exosomes and changes with treatment. Samples will be isolated, batched, and stored in Dr. Figg's lab. At the time of analysis, samples will be pulled and delivered to Dr. Jones' lab to perform studies per routine lab procedures.

Next Generation Sequencing (NGS) collaboration with Office of Hematology and Oncology Products (OHOP) FDA :

This study may be incorporated in to the multi-protocol CCR collaborations with OHOP/FDA in terms of the translational and correlative aspects utilizing OHOP's NGS laboratory to perform NGS assays on various human samples enrolled on a variety of CCR clinical trials. The transfer of samples will be performed under a CCR "umbrella" MTA. The samples will be locally biobanked in the Figg Lab and batched at the NIH until time for shipment, at which time, the samples will be sent to:

ATTN: Elliot Rosen, Ph.D.
DBRR III/OBP/OPQ/CDER
Food and Drug Administration
10903 New Hampshire Ave,
Bldg. 52/72, Room 2248,
Silver Spring, MD 20993
Tel: 240-402-7353

Email: elliot.rosen@fda.hhs.gov

The correlative NGS assays to be performed will be dependent on final agreement in investigating mutually important questions of interest with our collaborators. These include but are not limited to all or some of the following but are all optional:

1. Germline single nucleotide polymorphisms (SNP) and copy number variations (CNV) DNAseq: Whole exome or genome sequencing will be performed on peripheral blood to determine baseline germline SNPs and CNVs. Whole blood samples will be collected at baseline for germline DNA extraction. This DNA will be used to analyze and compare germline vs. somatic/tumor genetic alterations based on sequence data. Furthermore, germline normal polymorphic variation will be analyzed as genome-wide association study (GWAS) to determine whether certain normal variations predispose patients with treated SMM to progress to biochemical or overt symptomatic disease. Additionally, these germline variations may be analyzed for association with treatment related adverse events.
2. ctDNA: Targeted DNAseq will be performed on ctDNA for assessment of changes in myeloma clone copy numbers. This may be performed by Adaptive Biotechnologies by their well established assay. Alternatively, a novel assay may be developed in collaboration with the FDA. This assay, in addition to the VDJ targeted commercial assay to determine presence of the malignant clone, the library will also include other known recurrent genetic aberrations. Approximately 45% of myeloma patients have hyperdiploidy of one of the odd numbered chromosomes, the other 45% have specific translocations/deletions, well characterized, including translocations of 6;14, 11;14, 4;14, 14;16, 16;18, 17p del, and cMYC. Therefore, the assay will focus on both VDJ as with other hematologic malignancies and recurrent genetic alterations similar to the approach used in solid malignancies. Blood collection will involve two 10 mL EDTA tubes, alternatively, Streck tubes may be used if processing of the sample will be delayed by more than 2 hours.
3. Gene expression profiling: RNAseq will be performed on CD138+ myeloma cells and correlated with DNAseq results. If performed, samples will be processed in collaboration with the Calvo Lab.

5.3.4 Future Use

Any blood, tissue, or other human tissues or portions leftover from other analyses will be stored for future research.

5.3.5 Imaging – DW-MRI

5.3.5.1 Schedule

Whole body DW-MRI scans will be performed on patients at baseline, on day 15 of cycle 2, cycle 4, if and when patient reaches a PR, CR, and/or PD, and at the end of treatment. During follow-up period, scans may be done at any time point at the discretion of the investigator.

5.3.5.2 Procedures

DW-MRI exploits differences in the diffusion of water in various tissues to internal physiology. The image contrast in reflects the difference in rate of diffusion between tissues. All attempts will be made to perform the DW-MRI scans on the same day as PET/CTs, but is not mandatory.

For DW-MRI, no external contrast will be used and fasting is not required. Standard clinical operating procedures will be used for image collection.

5.3.5.3 Results

Patients will be given the results of the DW-MRI scans. But given the exploratory and research nature of the scans, these results will not be used for clinical decision-making purposes.

5.3.6 Patient Reported Outcomes

The PROMIS QoL instrument will be administered to all consenting patients who speak English or Spanish. These instruments will be administered at baseline; at cycle 2 day 1 and 15; and start of every cycle beginning with cycle 3. Since the measures have not been validated in other languages, participation in this portion of the study will be limited to subjects who speak either English or Spanish.

PROMIS, the Patient Reported Outcome Measurement Information System (PROMIS®), is funded by the National Institutes of Health and provides clinicians and researchers access to efficient, precise, valid, and responsive measures of health and well–being in clinical trial patients. The short forms that will be implemented in this study involve questions in the domains of physical function, anxiety, depression, fatigue, sleep disturbance, ability to participate in social roles and activities, pain interference, pain intensity, and cognitive function (see **Appendix G**). A computer tablet-based application also exists which can utilize computer adaptive tests (CAT) which can lower patient burden; that is, adapting the template 37 questions presented on the paper short forms based on patient response. The questionnaires are not expected to take more than 20 minutes to complete.

As feasible, the PROMIS data may be captured based on this CAT PROMIS tablet application; alternatively, the paper short forms presented in the appendix will be used. Data will be secured on the tablet based on 2 passwords. When results are ready for analysis, they will be exported in excel format and submitted for storage in C3D or Labmatrix, as appropriate. Patients already enrolled onto the study may begin completion of PROs at the next scheduled visit after amendment activation at the discretion of the investigator.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.4.1 General

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting patients will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

If the patient withdraws consent his/her data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.4.2 Laboratory of Tumor Immunology and Biology (Schlom Lab)

All data associated with the patient samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80˚C freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

The NCI Frederick Central Repositories is managed under subcontract to Leidos Biomedical, Inc., Frederick, Inc. NCI Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited-access facilities with sufficient security, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance will applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontactors Federal-wide Assurance. The subcontractor's role limited to clinical research databases and repositories containing patient specimens. The subcontractor does not conduct or have any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects. The subcontractor's IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

It is the intent and purpose of the subcontractor to accept only coded (linked) samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens, as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdraw request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input, and withdraw authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

Samples will be used for research analysis, including immunologic monitoring as outlined previously. All specimens for analysis will be requested from Leidos Biomedical, Inc. and will be delivered by Leidos Biomedical, Inc. couriers to the Laboratory of Tumor Immunology and Biology.

The samples will be processed/stored through:

Leidos Biomedical Research
Attn: Ms. Theresa Burks
1050 Boyles Street
Bldg. 469/Room 121
Frederick, MD 21702
Phone 301-846-5125

On days samples are drawn, Jen Bangh at the Clinical Support laboratory should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens.

5.4.3 Developmental Therapeutics Branch (Trepel Lab)

Under the direction of Dr. Trepel, all samples processed by the Developmental Therapeutics Branch Laboratory will be uniquely barcoded, with data entered using a secure computerized database and backup hardcopy process per standard laboratory practice. All lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

Samples are stored in labeled boxes in secured freezers (i.e., -20ºC to -80ºC, or other, as appropriate) according to stability requirements; these freezers are located onsite. Access to stored clinical samples is restricted and limited to research personnel for approved analyses only (as per the IRB approved protocol).

Upon completion of planned analyses by the Trepel lab, leftover samples may be stored for future analyses at the Clinical Support Laboratory, Leidos Biomedical Research, Inc.in Frederick, MD (see below).

5.4.4 Clinical Pharmacology Program (Figg Lab)

5.4.4.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) of the Clinical Pharmacology Program under the direction of Dr. Figg will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.4.4.2 Sample Storage

Barcoded samples are stored in barcoded boxes in a locked freezer at appropriate temperatures (e.g., -20ºC to -80ºC) according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.4.5 Hematopathology Section of Laboratory of Pathology (other tissue samples) and Department of Laboratory Medicine (Bone Marrow)

Archival and/or freshly collected and processed tumor tissue may be stored in the Hematopathology Section of Laboratory of Pathology until ready for planned and/or future research assays if the patient has agreed to allowing specimens to be used in future research studies. IRB approval will be obtained before using any samples to conduct studies that are not described within this protocol. Samples will be stored under conditions appropriate to the type of sample and processing (e.g., ambient or frozen).

Tissue that is given to the technician will be assigned an accession number (HP#) in the HP Case Log book; sample tracking also takes place with a FileMaker Pro database called HP Patient Information and Specimen Inventory. A Patient background sheet may be filled out and filed with any accompanying paperwork, with final reports and any supplemental reports that follow added as completed.

5.4.6 Adaptive Biotechnologies Corp.

Only coded (linked) samples will be shared with Adaptive Biotechnologies Corporation for the studies as described (see Sections **5.3.1**, **5.3.2.1** and **5.3.3**. The samples and data will sent in batches at the address listed below:

Adaptive Biotechnologies Corp.
1551 Eastlake Ave E
Suite 200
Seattle WA 98102

5.5 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.5.1 Scope of genetic/genomic analysis

The research correlates for this study are expected to include DNA/RNA sequencing of tumors, including circulating tumor (ct) DNA. In addition, whole exome sequencing may include evaluation for known lymphoma mutations. For any genetic studies performed, the results will

be deposited in a database such as dbGaP per NIH requirements. Although there is controlled access to such a database, such a submission carries theoretical risks of revealing the identity of the subject. This is discussed in the consent.

5.5.2 Privacy and confidentiality of medical information/biological specimens

Confidentiality for genetic samples will be maintained as described (Section **5.4**). In addition, a Certificate of Confidentiality has been obtained for this study.

5.5.3 Management of Results

These NGS exploratory investigations will be performed solely for research purposes, as these assays are not as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing. There is no plan for looking for incidental findings that are relevant to other diseases. Therefore, results will not be shared with patients or referring physicians. Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists).

5.5.4 Genetic Counseling

Subjects will be contacted with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH to have genetic education and counseling to explain this result; at the time of any such event(s), these activities will be funded by the NCI/CCR in consideration of the specific circumstances. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.6 EXPLORATORY/RESEARCH RADIATION EXPOSURE

An exploratory objective of this study is to describe the effects of the study treatment in tumor tissue with optional biopsies. To satisfy this exploratory objective, subjects will receive radiation from up to three (3) CT-guided biopsies for research purposes on this protocol.

CT-guidance may be used for up to three (3) optional tumor biopsies during the study. The procedures for performing each biopsy will follow clinical policies, no special procedures apply to the additional procedures for research purposes (see Section **5.2.4**).

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

6.1.1 Summary

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be

kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1 through 90 days after last treatment. Beyond the 90 day period after the last drug was administered, only adverse evnts which are serious and related to the study intervention need to be recorded.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.2 Data Collection/Recording Exceptions

6.1.2.1 Abnormal Laboratory Values

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

6.1.2.2 Hospitalizations

Any cases of planned or prolonged hospitalization are not considered reportable serious adverse events if for the following reasons:

- Closer monitoring and/or prophylaxis of TLS at any cycle

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.

_X_ Coded, linked data in another public repository

_X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_X_ Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?
Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov, dbGaP.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements.

_X_ Publication and/or public presentations.

When will the data be shared?
_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

Patient response and overall response rates (ORR) will be assessed according to the 2016 International Myeloma Working Group (IMWG) response criteria.[1] (See **Appendix F**.)

6.3.1 Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence per IMWG criteria.

6.3.2 Duration of Response

The duration of response (DoR) is measured from the time measurement criteria are met for a response of PR or better until the first date that recurrent or progressive disease is objectively documented. Patients without a progression event will be censored at the date response assessment was last evaluated.

6.3.3 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from the date of study enrollment until time of disease progression, or death, whichever occurs first. Patients without a progression or death event will be censored at the date response assessment was last evaluated. Note that, a progression or death event occurring up to cycle 2 day 1 will not be included in the definition as patients have not had received combination therapy until that time.

6.3.4 Overall Survival

Overall survival (OS) is defined as the duration of time from the date of study enrollment until time of death. Patients without a death event will be censored at the date survival assessment was last evaluated (e.g., clinic visit, phone call).

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version

5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS / DATA SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet approximately weekly when patients are being actively treated on the trial to discuss each patient. Decisions about trial continuation will be made based on the efficacy data from prior patients at appropriate time points per the statistical plan.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a

pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.5**.

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.5**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at:
https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

There are no execeptions to expedited reporting requirements.

8.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

All events listed below must be reported in the defined timelines to OSROsafety@mail.nih.gov. The CCR Office of Regulatory Affairs will send all reports to the manufacturer(s) as described below, unless otherwise noted.

The investigational agent, avelumab, is being supplied by EMD Serono and the following are their requirements for safety reporting.

The following reportable events must be submitted to EMD Serono within 2 business days or 3 calendar days (whichever comes first) using the applicable safety report form provided. The Principal Investigator/study team will submit reportable events to the Sponsor as well as ensure that any other local reporting requirements are completed, if required (e.g., IRB). The Sponsor will assume responsibility for submitting the reportable event(s) to EMD Serono.

The reportable events to EMD Serono include:

- Serious Adverse Events
- Exposure during Pregnancy or Breastfeeding (even if not associated with an adverse event)
- Occupational exposure (even if not associated with an adverse event)
- Potential drug-induced liver injury (Hy's Law cases): These events are considered important medical events and should be reported as SAEs.

Contact information for submission of reportable events to EMD Serono:

Fax: +49 6151 72 6914

OR

E-mail: GlobalDrugSafety@merckgroup.com, specifying:

- PROTOCOL Number and/or Title
- EMD Serono assigned Study Number
- SUBJECT Number
- SITE Number/PI Name

8.6 REPORTING PREGNANCY

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 30 days after study drug.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 30 days after the last dose should, if possible, be followed up and documented.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 STUDY ENDPOINTS

- Primary Endpoint:
    - The primary endpoint of this study is to determine the overall response rate (ORR) in patients with multiple myeloma treated with avelumab and irradiation.
- Secondary Endpoints:
    - The secondary endpoints are to determine:
        - Complete Response (CR) rate and Minimal Residual Disease (MRD) negative CR rate,
        - Reductions in bone marrow (BM) and peripheral blood (PD) plasmacytosis,
        - Radiographic reduction in size, and/or FDG avidity (PET/CT) of extramedullary lesions,

        - Radiographic reduction in size, and/or FDG avidity (PET/CT) of non-irradiated extramedullary lesions (abscopal effect)
        - Progression-free survival (PFS)
        - Overall survival (OS)
        - All Grade, Grade 3-4, and serious adverse events, and
        - ORR post cycle 1 (prior to XRT)
- Exploratory Endpoints
    - Correlative Studies may be performed in select patients where adequate samples are available:
        - Peripheral blood mononuclear cells (PBMCs):
            - Changes in 123 immune subsets including CD4 and CD8 T cells, natural killer [NK] cells, NK-T cells, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], and dendritic cells and 114 refined subsets relating to maturation/function using multicolor flow cytometry
            - Changes in the function of select immune cell subsets (e.g. CD4 and CD8 effector T cells, NK cells, Tregs, and MDSCs)
            - Changes in tumor antigen specific T cells (e.g. brachyury, MUC1, and CEA) using intracellular cytokine staining for IFNg, TNF, and IL2 and the degranulation marker CD107a
            - Changes in T-cell clonality score using adaptive biotechnologies TCRseq assay
            - Changes in inflammatory gene signature using Nanostring's nCounter Human PanCancer Immune Profiling Panel
        - Plasma/Serum:
            - Changes in sCD27, sCD40L
            - Changes in cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers
            - Changes in VDJ copy number of MM clones
        - Bone Marrow:
            - Changes in immune subsets
            - Changes in VDJ copy number of MM clones
            - DNA genetic alterations and TMB of MM cells

10.2 STATISTICAL HYPOTHESIS AND SAMPLE SIZE DETERMINATION

Historically, anti-PD-1 monotherapy in MM has an overall response rate (PR+VGPR+CR+sCR) of approximately 5%. It would be desirable to demonstrate that the combination of avelumab and irradiation has a substantially higher response rate, of at least 10-15%. As such, this study will be conducted using a Simon minimax two-stage phase II trial design to rule out an unacceptably low ORR of 5% (p0=0.05) in favor of an improved response rate of 20% (p1=0.20). With alpha=0.05 (probability of accepting a poor treatment=0.05) and beta = 0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll 13 evaluable patients, and if 0 of the

13 have a clinical response, then no further patients will be accrued in this trial. If 1 or more of the first 13 patients have a response, then accrual would continue until a total of 27 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 3 patients with a response out of 27 patients, this would be an uninterestingly low response rate. If there were 4 or more of 27 (14.8%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 51.3%.

It is expected that approximately 8-10 patients per year may enroll onto this trial. Thus, it is expected that 3 years may be required to enroll up to 27 evaluable patients. To allow for a small number of inevaluable patients and screen failures, the accrual ceiling will be set at 30 patients.

10.3 POPULATIONS FOR ANALYSES

Modified intention to treat: all patients who receive at least one dose of avelumab will be included in the statistical analyses performed.

10.3.1 Evaluable for toxicity:

All patients will be evaluable for toxicity from the time of their first treatment with avelumab.

10.3.2 Evaluable for objective response:

Only those patients who have measurable disease present at baseline and have received at least one dose of therapy will be considered evaluable for response.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

Response fractions, and time to event endpoints will be reported along with appropriate confidence intervals.

10.4.2 Analysis of the Primary Endpoints

The fraction of patients who experience a PR, VGPR, CR, or sCR using the study treatment will be determined by dividing the number of responders by the total evaluable patients. The fraction will be reported along with 90% and 95% two-sided confidence intervals

10.4.3 Analysis of the Secondary Endpoints

- CR rate: the fraction of patients who experience a CR or sCR using the study treatment will be determined by dividing the number of these responders by the total evaluable patients. The fraction will be reported along with 90% and 95% two-sided confidence intervals.
- MRDnegCR rate: the fraction of patients who experience an MRDnegCR using the study treatment will be determined by dividing the number of these responders by the total evaluable patients. The fraction will be reported along with 90% and 95% two-sided confidence intervals.
- Reduction of BM and PD plasmacytosis: Percent change in plasma cells in the PB and BM from baseline will be reported descriptively.

- Reduction of size, and/or FDG avidity of extramedullary lesions: Percent reduction of size, and/or FDG avidity, radiographicaly, of extramedullary lesions compared to baseline will be reported descriptively.
- PFS: will be determined using the Kaplan-Meier method, considering those who progress or die without progression as failures, and censoring those who do not.
- OS: will be determined using the Kaplan-Meier method.
- Safety and tolerability: overall tolerability in terms of adverse events will be evaluated with descriptive statistics to determine the safety of receiving avelumab in the context of irradiation in RRMM patients. NCI CTCAE v.5 will be used to grade adverse events.
- ORR post cycle 1: a milestone ORR at start of cycle 2 will be calculated as above to determine ORR of avelumab monotherapy after 1 cycle of therapy.

Each of these will be calculated starting from the date the patient enrolled onto the trial which corresponds to the date the patient signed the informed consent document. Appropriate confidence intervals will be reported for each of these measures. (Only PFS events occurring during the initiation and of the combination of irradiation and avelumab and after will be counted as this proof of principal study hypothesis is that the combination of BavXRT will be active.)

10.4.4 Safety Analyses

Early stopping rules for toxicities will be put in place. Specifically, if within the first 6 patients, there are two Grade ≥ 3 toxicities, or if at any time after 6 patients have been treated, the cumulative fraction with Grade ≥ 3 toxicity is 33% or greater, then the trial will suspend accrual and will only reopen following review of the safety data and amendment to modify the treatment appropriately. Adverse events which are clinically insignificant or can be resolved with conservative measures (e.g. laxatives for constipation) or which are not at least possibly due to the experimental treatment will not be included in this early stopping strategy.

10.4.5 Baseline Descriptive Statistics

Demographic and baseline clinical characteristics of all patients will be reported.

10.4.6 Planned Interim Analyses

As indicated above futility will be determined using the Simon two-stage design, after the required number of evaluable patients have been enrolled in the first stage, an analysis of the response rate will be undertaken to determine if there are sufficient responses to proceed to the second stage.

10.4.7 Sub-Group Analyses

None will be performed

10.4.8 Tabulation of Individual Participant Data

None will be performed

10.4.9 Exploratory Analyses

All exploratory objectives listed above will be analyzed using descriptive statistics. Various percentile confidence intervals and nominal P-values may be determined.

Changes in QoL/PROs based on the PROMIS instrument will be evaluated relative to any degree of response and summary statistics will be described over time. As these evaluations may be performed in a post hoc manner based on the response results obtained, the findings will need to

be carefully presented in the context of the exploratory nature of the analyses undertaken; results will not be evaluated in "real-time."

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

This study will be incorporated into an existing CRADA between the National Cancer Institute and EMD Serono (#02666/iMTA #44957-18).

11.2 MATERIAL TRANSFER AGREEMENT (MTA)

11.2.1 Memorial Sloan Kettering Cancer Center (MSKCC)

An MTA will be established between the National Cancer Institute (NCI) and Dr. C. Ola Landgren at Memorial Sloan Kettering Cancer Center (MSKCC) prior to the sharing of any data/samples (see Section **5.2.3**). (MTA # [PENDING])

11.2.2 Food and Drug Administration (FDA)

12 AN MTA WILL BE EXECUTED/REVISED TO ALLOW THE SAMPLES DESCRIBED IN SECTION 5 TO BE SHIPPED TO THE OFFICE OF BIOTECHNOLOGY AT THE FDA AS PART OF THE COLLABORATIVE EFFORT. THE MTA WILL BE A GLOBAL CCR MTA TO INCLUDE MULTIPLE PROTOCOLS, HOWEVER, DR. KAZANDJIAN WILL BE THE RESPONSIBLE PI REGARDING THE MULTIPLE MYELOMA PROTOCOLS BOTH AT THE NIH AND FDA SITE. (MTA #43656)HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

MM is an almost always incurable plasma cell neoplasm that comprises approximately 10% of all hematologic malignancies, affecting 30,000 patients annually. Recent studies have shown that MM is preceded by MGUS and SMM. Rate of progression at 5 years from high risk SMM to MM is 72-76% at 5 years with a median TTP of < 2 years. MM affects all genders and races. Incidence rates of myeloma is higher among African Americans (AA) compared to Caucasian Americans (CA), affecting 14.3 AA males per 100, 000 males and 10.0 AA females per 100,000 females compared to 6.7 CA males per 100, 000 males and 4.1 CA females per 100, 000 women. The median age at death for myeloma is 75 years of age. As such, we expect that the majority of patients enrolled in this trial will be older adults of either gender or race. MM patients enrolled on this study will consist of patients referred to and screened at the NIH Clinical Center. There will be no subject selection bias with regard to gender, ethnicity, or race. This protocol excludes lactating and pregnant women from receiving this investigational drug to avoid any possible risks to the fetus or newborn.

12.2 PARTICIPATION OF CHILDREN

Pediatric patients with RRMM are extremely rare. Patients under the age of 18 are excluded from this study because inclusion of a rare younger patient will not provide adequate generalizable information to justify their inclusion in this study. Based on the most recent SEERS data, the incidence of MM in people less than the age of 20 was 0.0%.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent

may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.4**), all subjects deemed capable of doing so by the PI will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

NOTE: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that are incapacitated or become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

In cases where the subject's LAR is unable to be present in person, obtaining informedconsent via technology and/or electronic processes is permissible.

12.4 Evaluation of Benefits and Risks/Discomforts

Currently, MM is an incurable malignancy with frequent complications of skeletal fractures, anemia, renal failure and hypercalcemia. Conventional radiographs reveal that 79% of patients will have observed skeletal abnormalities at time of diagnosis. Treatment of patients with RRMM and extramedullary disease with avelumab and XRT may not only treat/reduce skeletal/soft tissue related events and prevent morbidity from irreversible bone damage seen with MM but also by synergizing together, the combination may cause a systemic abscopal effect. Therefore, not only will patients benefit from local radiation but may also benefit in terms of the other symptoms caused by systemic and diffuse myeloma including the CRAB criteria. Although effective treatments exist for MM, once a patient progresses through IMiD and PI based therapies, available drug options become limited. Risks of the study include exposing patients to immune-related adverse drug reactions seen with anti-PD-L1 inhibitors without potential benefit of a systemic response. However, patients will still benefit from the well-established modality of XRT locally. Of importance, the relative adverse drug reaction profile of avelumab is favorable compared to other newer cell-based therapies or older cytotoxic chemotherapy regimens. Procedures required for obtaining samples/data for experimental purposes (venipuncture, urine collection, PET/CT scan, DW-MRI, bone marrow biopsy, and potential radiation from CT-guided biopsy procedures) are of limited risk to the patient. Although patients will suffer some additional pain or discomfort from the PET/CT scans and annual bone marrow biopsies, clinical experience has shown that the medical risk is limited.

12.4.1 Risks Related to Imaging

[^{18}F]-FDG PET/CT (alternatively, CT scans), MRIs, CT-guided biopsies, and skeletal surveys may be used to monitor a patient's disease on this study. Some of these scans expose a patient to radiation as discussed below.

In addition, [^{18}F]-FDG PET/CT involve use of contrast (oral and/or IV). The [^{18}F]-FDG PET/CT scans will entail the injection of 10mCi dose for all ages and body sizes. An IV line may need to be inserted for administration of the contrast agent and can cause pain at the site where the IV is placed. There is also a small risk of bruising or infection. If a contrast agent is given with the scan there is a small risk of having a reaction to the contrast. In the small group of patients who have a reaction, the most common symptoms are nausea, pain in the vein where the contrast was given, headache, a metallic or bitter taste in the mouth, and a warm or flushing feeling that lasts from 1-3 minutes. Rarely, these symptoms may require treatment. In very rare cases, people have had more severe allergic reactions that result in skin rashes, shortness of breath, wheezing, or lowering of the blood pressure.

12.4.2 Radiation Exposure

The primary source radiation exposure on the study is from 5x5Gy of radiation therapy to bone lesions. In addition, a much smaller amount of radiation will be derived from up to 9 FDG/PET CT scans, 5 skeletal surveys and 3 CT guided biopsies. The risks of radiation are as described in the consent form.

12.5 Consent Process and Documentation

The informed consent document will be provided to the participant or consent designee(s) (e.g., legally authorized representative [LAR] if participant is an adult unable to consent) for review prior to consenting. A designated study investigator (see **NOTE** at the bottom of this section) will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

NOTE: Please note that consent for treatment (consent labeled Affected Patient) must be obtained by a designated appropriately licensed study investigator (e.g., MD, NP, PA, DO). However, study investigators not falling into this category (e.g. RNs) who are designated as able to obtain consent, may do so for non-treatment procedures such as screening.

Consent for the optional biopsies performed on this study will be obtained at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.6 Inclusion of Women and Minorities

Both men and women and members of all races are eligible for this trial.

13 PHARMACEUTICAL INFORMATION

This investigation of avelumab requires an IND as investigational, not commercial, drug supply lots are being provided for this clinical trial by the pharmaceutical collaborator (EMD Serono).

13.1 AVELUMAB (IND #142027)

13.1.1 Source

Investigational supplies of Avelumab will be provided by EMD Serono for use by subjects in this clinical trial.

13.1.2 Toxicity

The following immune-related adverse events have occurred in patients receiving Avelumab as a single agent (1,738 patients):

- Immune-related hypothyroidism/hyperthyroidism (6%)
- Immune-related colotis (1.5%)
- Immune-related pneumonitis (1.2%)
- Immune-related hepatitis (0.9%)
- Immune-related adrenal insufficiency (0.5%)
- Immune-related Type 1 Diabetes Mellitus (0.1%)
- Immune-related nephritis and renal dysfunction (0.1%)

Other following clinically significant, immune-related adverse reactions have been reported. The following occurred at an incidence of less than 1%: immune-related myocarditis including fatal events, immune-related pancreatitis including fatal events, immune-related myositis, hypopituitarism, uveitis, myasthenia gravis/myasthenic syndrome, and Guillain-Barré syndrome.

Please refer to the IB for more detailed toxicity information.

13.1.3 Formulation and Preparation

Avelumab drug product is a sterile, clear, and colorless concentrate for solution presented at concentration of 20 mg/mL in European Pharmacopeia (Ph. Eur.) and United States Pharmacopeia (USP) type I glass vials closed with a rubber stopper and sealed with an aluminum Flip Off® crimp seal closure.

Each single-use vial contains 200 mg of avelumab as a preservative-free acetate-buffered solution (pH 5.2) containing Mannitol, and Polysorbate 20 (Tween 20).

For avelumab drug product, only excipients that conform to the current Ph. Eur. and/or the current USP are used.

13.1.4 Stability and Storage

Supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial supplies must be recorded by an authorized person at the trial site. Supplies may not be used for any purpose other than that stated in the protocol.

Avelumab drug product must be stored at 2°C to 8°C until use. Store diluted solution at room temperature up to 77°F (25°C) for no more than 8 hours from the time of dilution *OR* under refrigeration at 36°F to 46°F (2°C to 8°C) for no more than 24 hours from the time of dilution. If

refrigerated, allow the diluted solution to come to room temperature prior to administration. The storage condition is based on data from ongoing long term stability studies with avelumab. Avelumab drug product stored at room (23°C to 27°C) or higher temperatures for extended periods of time might be subject to degradation. Avelumab drug product must not be frozen. Rough shaking of the solution must be avoided.

13.1.5 Administration procedures

For administration in clinical trials, avelumab drug product may be diluted with 0.9% saline solution (sodium chloride injection) supplied in an infusion bag; alternatively, a 0.45% saline solution can be used if needed. The chemical and physical in-use stability for the infusion solution of avelumab in 0.45% or 0.9% saline solution has been demonstrated for a total of 24 hours at room temperature. However, from a microbiological point of view, the diluted solution should be used immediately. If not used immediately, it can be considered that the diluted product is sufficiently stable from a microbiological perspective for up to 8 hours when stored at ambient room temperature or up to 24 hours at 2°C to 8°C. The in-use storage times and conditions prior to administration are the responsibility of the user.

Prior to the preparation of the dilution for final infusion, allow each vial to equilibrate to room temperature. Use a disposable syringe equipped with a needle of suitable size to remove a volume of sodium chloride solution to be replaced by avelumab from the infusion bag and discard the removed solution. Use a new disposable syringe equipped with a needle of suitable size to inject a volume of avelumab drug product identical to the discarded volume of sodium chloride solution into the infusion bag. Gently invert the mixture 10 times. Infusion bags must not be shaken, in order to avoid foaming or excessive shearing of the protein solution. The preparation must be carefully inspected as it should result in a homogeneous looking clear solution, free of visible particles.

13.1.6 Returns and Reconciliation

Unused investigational products will be destroyed per routine pharmacy procedure.

14 REFERENCES

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15 APPENDICES

15.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

15.2 APPENDIX B: TREATMENT MODIFICATION FOR SYMPTOMS OF INFUSION-RELATED REACTIONS

NCI-CTCAE Grade	Treatment Modification for Avelumab
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the avelumab infusion rate by 50% and monitor closely for any worsening.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	Temporarily discontinue avelumab infusion. Resume infusion at 50% of previous rate once infusion-related reaction has resolved or decreased to at least Grade 1 in severity and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life-threatening • Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. • Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop avelumab infusion immediately and disconnect infusion tubing from the subject. Subjects have to be withdrawn immediately from study avelumab and must not receive any further avelumab treatment.
- If avelumab infusion rate has been decreased by 50% or interrupted due to an infusion reaction, it must remain decreased for the next scheduled infusion. If no infusion reaction is observed in the next scheduled infusion, the infusion rate may be returned to baseline at the subsequent infusions based on investigator's medical judgment.- If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice.	

IV = intravenous; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Event; NSAIDs = nonsteroidal anti-inflammatory drugs.

15.3 APPENDIX C: MANAGEMENT OF IMMUNE-MEDIATED ADVERSE REACTIONS

15.3.1 Gastrointestinal irAEs

Severity of Diarrhea/Colitis (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 1 Diarrhea: increase < 4 stools/day over Baseline; mild increase in ostomy output compared to baseline Colitis: asymptomatic	Continue avelumab therapy Symptomatic treatment (e.g. loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2, 3 or 4.
Grade 2 Diarrhea: 4 to 6 stools per day over Baseline; moderate increase in ostomy output compared to baseline; limiting instrumental ADL IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; mucus or blood in stool	Withhold avelumab therapy Symptomatic treatment	If improves to Grade ≤ 1: Resume avelumab therapy If persists > 5-7 days or recurs: Treat as Grade 3 or 4.
Grade 3 to 4 Diarrhea (Grade 3): increase of ≥ 7 stools per day over Baseline; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL incontinence; IV fluids ≥ 24 h; interfering with ADL Grade 4: Life-threatening consequences; urgent intervention indicated Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, urgent intervention indiacted perforation	Withhold avelumab for Grade 3. Permanently discontinue avelumab for Grade 4 or recurrent Grade 3. 1.0 to 2.0 mg/kg/day prednisone IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy	If improves: Continue steroids until Grade ≤ 1, then taper over at least 1 month; resume avelumab therapy following steroids taper (for initial Grade 3). If worsens, persists > 3 to 5 days, or recurs after improvement: Add infliximab 5mg/kg (if no contraindication). Note: infliximab should not be used in cases of perforation or sepsis.

15.3.2 Dermatological irAEs

Grade of Rash (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 1 to 2 Covering ≤ 30% body surface area	Continue avelumab therapy Symptomatic therapy (for example, antihistamines, topical steroids)	If persists > 1 to 2 weeks or recurs: Withhold avelumab therapy Consider skin biopsy Consider 0.5-1.0 mg/kg/day prednisone or equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy following steroids taper. If worsens: Treat as Grade 3 to 4.
Grade 3 to 4 Grade 3: Covering > 30% body surface area; Grade 4: Life threatening consequences	Withhold avelumab for Grade 3. Permanently discontinue for Grade 4 or recurrent Grade 3. Consider skin biopsy Dermatology consult 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections	If improves to Grade ≤ 1: Taper steroids over at least 1 month; resume avelumab therapy following steroids taper (for initial Grade 3).

15.3.3 Pulmonary irAEs

Grade of Pneumonitis (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 1 Radiographic changes only	Consider withholding avelumab therapy Monitor for symptoms every 2 to 3 days Consider Pulmonary and Infectious Disease consults	Re-assess at least every 3 weeks If worsens: Treat as Grade 2 or Grade 3 to 4.
Grade 2 Mild to moderate new symptoms	Withhold avelumab therapy Pulmonary and Infectious Disease consults Monitor symptoms daily; consider hospitalization 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	Re-assess every 1 to 3 days If improves: When symptoms return to Grade ≤ 1, taper steroids over at least 1 month, and then resume avelumab therapy following steroids taper If not improving after 2 weeks or worsening: Treat as Grade 3 to 4.
Grade 3 to 4 Grade 3: Severe new symptoms; New/worsening hypoxia; Grade 4: Life-threatening	Permanently discontinue avelumab therapy. Hospitalize. Pulmonary and Infectious Disease consults. 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to Grade ≤ 1: Taper steroids over at least 1 month If not improving after 48 hours or worsening: Add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)

15.3.4 Hepatic irAEs

Grade of Liver Test Elevation (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin > ULN to 1.5 x ULN	Continue avelumab therapy	Continue liver function monitoring If worsens: Treat as Grade 2 or 3 to 4.
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or total bilirubin > 1.5 to ≤ 3 x ULN	Withhold avelumab therapy Increase frequency of monitoring to every 3 days.	If returns to Grade ≤ 1: Resume routine monitoring; resume avelumab therapy. If elevation persists > 5 to 7 days or worsens: Treat as Grade 3 to 4.
Grade 3 to 4 AST or ALT > 5 x ULN and/or total bilirubin > 3 x ULN	Permanently discontinue avelumab therapy Increase frequency of monitoring to every 1 to 2 days 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist/ hepatologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	If returns to Grade ≤ 1: Taper steroids over at least 1 month If does not improve in > 3 to 5 days, worsens or rebounds: Add mycophenolate mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines.

15.3.5 Renal irAEs

Grade of Creatinine Increased (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 1 Creatinine increased > ULN to 1.5 x ULN	Continue avelumab therapy	Continue renal function monitoring If worsens: Treat as Grade 2 to 3 or 4.
Grade 2 to 3 Creatinine increased > 1.5 and ≤ 6 x ULN	Withhold avelumab therapy Increase frequency of monitoring to every 3 days 1.0 to 2.0 mg/kg/day prednisone or equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy	If returns to Grade ≤1: Taper steroids over at least 1 month, and resume avelumab therapy following steroids taper. If worsens: Treat as Grade 4.
Grade 4 Creatinine increased > 6 x ULN	Permanently discontinue avelumab therapy Monitor creatinine daily 1.0 to 2.0 mg/kg/day prednisone or equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy Nephrology consult	If returns to Grade ≤1: Taper steroids over at least 1 month.

15.3.6 Cardiac irAEs

Myocarditis (All grades)	**Initial Management**	**Follow-up Management**
New onset of cardiac signs or symptoms and / or new laboratory cardiac biomarker elevations (e.g. troponin, CK-MB, BNP) or cardiac imaging abnormalities suggestive of myocarditis.	Withhold avelumab therapy. Hospitalize. In the presence of life threatening cardiac decompensation, consider transfer to a facility experienced in advanced heart failure and arrhythmia management. Cardiology consult to establish etiology and rule-out immune-mediated myocarditis. Guideline based supportive treatment as per cardiology consult.* Consider myocardial biopsy if recommended per cardiology consult.	If symptoms improve and immune-mediated etiology is ruled out, re-start avelumab therapy. If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consult, manage as immune-mediated myocarditis.
Immune-mediated myocarditis	Permanently discontinue avelumab. Guideline based supportive treatment as appropriate as per cardiology consult.* 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections.	Once improving, taper steroids over at least 1 month. If no improvement or worsening, consider additional immunosuppressants (e.g. azathioprine, cyclosporine A).
*Local guidelines, or e.g. ESC or AHA guidelines ESC guidelines website: https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines AHA guidelines website: http://professional.heart.org/professional/GuidelinesStatements/searchresults.jsp?q=&y=&t=1001		

15.3.7 Endocrine irAEs

Endocrine Disorder (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 1 or Grade 2 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Continue avelumab therapy Endocrinology consult if needed Start thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for Type I diabetes mellitus) as appropriate. Rule-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.
Grade 3 or Grade 4 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Withhold avelumab therapy Consider hospitalization Endocrinology consult Start thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for type I diabetes mellitus) as appropriate. Rule-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Resume avelumab once symptoms and/or laboratory tests improve to Grade ≤ 1 (with or without hormone replacement/suppression). Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.
Hypopituitarism/Hypophysitis, all grades (secondary endocrinopathies)	If secondary thyroid and/or adrenal insufficiency is confirmed (i.e. subnormal serum FT4 with inappropriately low TSH and/or low serum cortisol with inappropriately low ACTH): • Refer to endocrinologist for dynamic testing as indicated and measurement of other hormones (FSH, LH, GH/IGF-1, PRL, testosterone in men, estrogens in women) • Hormone replacement/suppressive therapy as appropriate • Perform pituitary MRI and visual field examination as indicated If hypophysitis confirmed: • Continue avelumab if mild symptoms with normal MRI. Repeat the MRI in 1 month • Withhold avelumab if moderate, severe or life-threatening symptoms of hypophysitis and/or abnormal MRI. Consider hospitalization. Initiate corticosteroids (1 to 2 mg/kg/day prednisone or equivalent) followed by corticosteroids taper during at least 1 month. • Add prophylactic antibiotics for opportunistic infections.	Resume avelumab once symptoms and hormone tests improve to Grade ≤ 1 (with or without hormone replacement). In addition, for hypophysitis with abnormal MRI, resume avelumab only once shrinkage of the pituitary gland on MRI/CT scan is documented. Continue hormone replacement/suppression therapy as appropriate.

15.3.8 Other irAEs

NOTE: This pertains to "other" irAEs (i.e., not described above).

Grade of other irAEs (NCI-CTCAE v5)	Initial Management	Follow-up Management
Grade 2 or Grade 3 clinical signs or symptoms suggestive of a potential irAE	Withhold avelumab therapy pending clinical investigation	If irAE is ruled out, manage as appropriate according to the diagnosis and consider re-starting avelumab therapy If irAE is confirmed, treat as Grade 2 or 3 irAE.
Grade 2 irAE **or** **first occurrence of Grade 3 irAE**	Withhold avelumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Taper steroids over at least 1 month and resume avelumab therapy following steroids taper.
Recurrence of same Grade 3 irAEs	Permanently discontinue avelumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Taper steroids over at least 1 month.
Grade 4	Permanently discontinue avelumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent and/or other immunosuppressant as needed Add prophylactic antibiotics for opportunistic infections Specialty consult.	If improves to Grade ≤ 1: Taper steroids over at least 1 month
Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks for reasons other than hormonal replacement for adrenal insufficiency **Persistent Grade 2 or 3 irAE lasting 12 weeks or longer**	Permanently discontinue avelumab therapy Specialty consult	

15.4 APPENDIX D: PERIPHERAL BLOOD AND URINE COLLECTION AND STORAGE

Blood Processing Core Figg Lab

Refer to Sections **3.7** (Study Calendar) and **5.1** for research blood and urine collection time points.

Contact: For questions, please contact Dr. Figg's Clinical Pharmacology Program (CPP) at 240-760-6180; additionally, for pre-notification of planned samples (at least 24 hours in advance, the Friday before is preferred) email NCIBloodcore@mail.nih.gov. After sample collection, please page 102-11964 for immediate pick-up. For any questions regarding sample processing, you may contact NCIBloodcore@mail.nih.gov or at 240-760-6180.

Venipuncture

- Approximately 40 ml of peripheral blood will be collected into EDTA (lavender) and Streck (brown/black) tubes. The amount of blood collected will be dictated by the number of experiments to be performed. Although, above tubes are ideal for the collection, they may be substituted at the discretion of the investigator.
- cfDNA
    1. Collect blood in two 10 ml Streck Tubes
    2. Keep at room temperature until processing (Samples are stable for up to five days at room temperature and can be batched with other samples drawn on different days)
    3. All steps for plasma separation should be done in a clean space, preferably in a separate room dedicated for this procedure, but minimally without the presence of molecular biology reagents such as PCR products, plasmids or any other low complexity DNA, which will contaminate the plasma. The centrifuge used for this should not be used if the centrifuge is also used for bacteria, plasmid purification etc. Use of a 1% bleach solution to wipe down areas before and after processing (inside and outside the workspace) can greatly cut down on contamination issues. (Clorox Healthcare Bleach Germicidal Wipes - Wipe - 6" Width x 5" Length - 150 / Canister - 1 Each – White Item # 129202)
    4. In hood: transfer blood to 15 mL conical tube
    5. Centrifuge 15 mL conical tubes for 10 minutes at 1500 ± 150xg (=1500RCF)
    6. Transfer supernatant to a fresh 15 ml tube without disturbing the leukocyte layer
    7. Centrifuge plasma a second time for 10 min at 3000 ± 150xg (=3000 RCF)
    8. Transfer supernatant to a fresh 15 ml centrifuge tube without disturbing the cellular layer. Leave a residual volume of about 0.3 ml (~7 mm) on the bottom of the 15 ml tube to avoid cellular contamination.
    9. After transferring the plasma to a new 15 ml centrifuge tube as described, gently mix plasma and record total plasma volume (typically ~ 4 ml plasma per 10 ml blood).
    10. Make 1 mL aliquots into standard cryovials
    11. Barcode as plasma
    12. Enter sample note: cfDNA
    13. Store plasma at -80°C freezer, ship without thawing
- gDNA
    1. Collect blood in one 10 ml Lavender EDTA tube

2. Keep at room temperature until processing
3. Follow standard procedures for DNA isolation per Figg Lab
4. Store at -80°C freezer

- PBMC

1. Collect blood in one 10 ml Lavender EDTA tube
2. Keep at room temperature until processing
3. Follow standard procedures for PBMC pellet preparation per Figg Lab
4. Store at -80°C freezer

Urine (spot) Sample Collection

- Approximately 45 mL of urine will be collected into a standard urine collection cup for further analysis. The amount of urine collected will be dictated by the number of experiments to be performed.
- Transfer to two 50 ml screw-cap conical tubes
- Freeze immediately at -20°C or lower
- Maintain in -80°C freezer for storage until shipment

Labeling of Samples

All specimens are to be labeled per the local site's standard procedures. The following information, if not provided on the specimen label, must be linked to the specimen label and provided on the inventory sheet:

- Patient study ID #
- Sample type
- Date/time of draw (DD/MMM/YY 24:00)
- Time point (ex. C1D1 pre, C1D1 24hr post)
- Any collection issues (short draw, delayed processing, etc.)
- Protocol title/number
- Institute name
- Contact information
- Do not include the patient name, medical record number, or initials.

15.5 APPENDIX E: CD138+ CELL SORTING: CALVO LAB

The following is example protocol which may be modified as needed:

1. Pour all the bone marrow aspirate into 50 ml tube. Add PBS to about 24 ml as diluted BM
2. Prepare six 15ml tubes. Add 3 ml Ficoll into each tube. SLOWLY add 4 ml diluted BM on the wall of each tube with Ficoll. Try not to disturb the bottom Ficoll. Spin at 2200rpm for 20min.
3. Carefully pipette 1ml top clean solution as diluted BM plasma into 1 cryovial. Collect 2 vials and label on cryovial with patient ID number on the first row, "diluted BM plasma" as second row, and date as third row. Then transfer the medium part BMMCs after Ficoll from each tube combined into one 50ml tube. Spin at 1250rpm for 10min.
4. Discard supernatant. Resuspend the pellet with 10ml PBS and transfer all the suspension into a new 50 ml tube through the cell strainer on the top of the tube. Use 20 ul for counting cell number.
5. Spin at 1250 rpm for 10min. Discard supernatant.
   If only total BMMCs is needed (i.e. MM patient is in CR), resuspend the pellet in 0.5ml PBS, and transfer all the suspension into 1 new 1.5 ml Eppendorf tube. Spin at 5000 rmp for 5min. Aspirate supernatant, and store it at -20ºC freezer. I usually label on 1.5ml Eppendorf tube with patient ID number on the first row, "total BMMCs" as second row, cell number as third row, and date as fourth row.
   If CD138+ selection is needed, resuspend the pellet with 2ml "MACS + BSA" buffer. Calculate the volume of "MACS + BSA" buffer and CD138+ microbeads. Use 80 ul "MACS + BSA" buffer and 20 ul CD138+ microbeads for 20 million total BMMCs. Scale up accordingly. If the total BMMCs number is below 20 million, use 80 ul buffer and 20 ul beads as minimum. Spin at 1250 rpm for 10min. Discard supernatant. Resuspend the pellet at the calculated volume of buffer and beads. Mix the pellet well by tapping the tube. Put in 4ºC refrigerator for 20 min incubation. Tap the tube gently every 5 minutes.
6. After incubation, add 2 ml "MACS + BSA" buffer in the tube. Spin at 1250 rpm for 10 min. Meanwhile, set up two LS columns for selection. Label three 15 ml tubes as "-"; "+1"; "+2". Put "-" tube under first LS column. Add 0.5 ml "MACS + BSA" buffer on the column to wash. Repeat adding 0.5 ml buffer two times, i.e. in total, wash 3 times.
7. Discard supernatant of the 50 ml tube. Resuspend the pellet in 0.5 ml "MACS + BSA" buffer. Transfer all to the top of LS column until no elution out, repeat to use 0.5 ml "MACS + BSA" buffer to wash the 50 ml tube and transfer on the top of first column for three times, i.e. in total 2 ml.
8. Until no elution out of column, put the "-" 15 ml tube under second LS column. Pull out first LS column and insert it into the new "+1" 15 ml tube. Add 1 ml "MACS + BSA" buffer on top of the column. Insert syringe and quickly plunge it down. Transfer all the elution from "+1" tube on the top of second LS column. Until no elution out, repeat to use 0.5 ml "MACS + BSA" buffer to wash the "+1" tube and transfer on the top of second column for three times, i.e. in total 2 ml.
9. Until no elution out of column, pull out first LS column and insert it into the new "+2" 15 ml tube. Add 1 ml "MACS + BSA" buffer on top of the column. Insert syringe and quickly plunge it down. Use 20 ul resuspension from "+2" tube to count CD138+ cell number and 20 ul resuspension from "-" tube to count CD138- cell number.
10. Spin both "-" and "+2" tubes at 1250 rpm for 10 min. Resuspend in 0.5 ml "MACS + BSA" buffer and transfer to new 1.5 ml Eppendorf tubes as CD138- and CD138+ cells respectively.
11. Spin at 5000 rmp for 5 min. Aspirate supernatant and store it in -20ºC freezer. Label on 1.5 ml Eppendorf tube with patient ID number on the first row, "CD138-" or "CD138+" as second row, cell number as third row, and date as fourth row.

15.6 APPENDIX F: IMWG RESPONSE CRITERIA

NOTE: The following are taken from the International Myeloma Working Group (IMWG) response criteria, 2016 (adapted).[1]

IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative (Sustained MRDnegCR)	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g., MRD-negative at 5 years)
Flow MRD-negative (Flow MRDnegCR)	Flow MRD-negative Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative (Seq MRDnegCR)	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging plus MRD-negative (Img MRDnegCR)	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue

Standard IMWG response criteria	
Stringent complete response (sCR)	Complete response as defined below plus normal FLC ratio and absence of clonal cells in bone marrow biopsy by immune-histochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)
Complete response (CR)	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 (VGPR)	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response (PR)	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; 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

Standard IMWG response criteria	
Minimal response (MR)	≥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 (SD)	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 (PD)	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

15.7 APPENDIX G: PATIENT REPORTED OUTCOME INSTRUMENT – PROMIS

NOTE: The English versions of the paper short forms are shown here for illustration purposes; Spanish versions are available and will be used for Spanish-speaking subjects, if applicable. The use of electronic versions (e.g., iPad) is preferred.

Please respond to each question or statement by marking one box per row.

	Physical Function **In the past 7 days…**	**Without any difficulty**	**With a little difficulty**	**With some difficulty**	**With much difficulty**	**Unable to do**
PFA11	Are you able to do chores such as vacuuming or yard work?……..	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA21	Are you able to go up and down stairs at a normal pace?……..	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA23	Are you able to go for a walk of at least 15 minutes?…….	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA53	Are you able to run errands and shop?…….	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFC7r1	Are you able to run five miles (8 km)? ….	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFM17	Are you able to remove a heavy suitcase (50 lbs/25 kg) from an overhead bin on an airplane or bus? …….	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

	Anxiety **In the past 7 days…**	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
EDANX01	I felt fearful…….……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDANX40	I found it hard to focus on anything other than my anxiety……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDANX41	My worries overwhelmed me……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDANX53	I felt uneasy……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	Depression **In the past 7 days...**	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
EDDEP04	I felt worthless……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP06	I felt helpless……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP29	I felt depressed……...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP41	I felt hopeless…….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	Fatigue **During the past 7 days…**	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
HI7	I feel fatigued……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN3	I have trouble <u>starting</u> things because I am tired……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	In the past 7 days…					
FATEXP41	How run-down did you feel on average?..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP40	How fatigued were you on average?……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP35	How much were you bothered by your fatigue on average? …….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP49	To what degree did your fatigue interfere with your physical functioning? …….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	Sleep Disturbance **In the past 7 days…**	**Very poor**	**Poor**	**Fair**	**Good**	**Very good**
Sleep109	My sleep quality was……	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
	In the past 7 days…	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
Sleep116	My sleep was refreshing……....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep20	I had a problem with my sleep……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep44	I had difficulty falling asleep……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	Ability to Participate in Social Roles and Activities **In the past 7 days…**	**Never**	**Rarely**	**Sometimes**	**Usually**	**Always**
SRPPER11_CaPS	I have trouble doing all of my regular leisure activities with others ……	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SRPPER18_CaPS	I have trouble doing all of the family activities that I want to do	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SRPPER23_CaPS	I have trouble doing all of my usual work (include work at home) ……	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SRPPER46_CaPS	I have trouble doing all of the activities with friends that I want to do ……	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

	Pain Interference **In the past 7 days…**	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
PAININ9	How much did pain interfere with your day to day activities?…….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PAININ22	How much did pain interfere with work around the home?…….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PAININ31	How much did pain interfere with your ability to participate in social activities?…….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PAININ34	How much did pain interfere with your household chores?…….	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Pain Intensity

In the past 7 days…

Global07	How would you rate your pain on average……..	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10
		No pain										**Worst pain imaginable**

	Cognitive Function **In the past 7 days…**	**Never**	**Rarely (Once)**	**Sometimes (Two or three times)**	**Often (About once a day)**	**Very Often (Several times a day)**
PC8r	I have had trouble concentrating……..	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PC11r	I have had trouble remembering where I put things, like my keys or my wallet……	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PC25r	I have had to work really hard to pay attention or I would make a mistake……	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PC-CaPS25r	I have had difficulty multi-tasking…….	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1