Protocol

Protocol Title: Individualized Treatment for Relapsed/Refractory Multiple Myeloma Based on High Throughput Chemosensitivity and Genomics Data

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Individualized Treatment for Relapsed/Refractory Multiple Myeloma Based on High Throughput Drug Sensitivity and Genomics Data

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PROTOCOL SYNOPSIS

Protocol Title	*Individualized Treatment for Relapsed/Refractory Multiple Myeloma Based on High Throughput Drug Sensitivity and Genomics Data*
Protocol Number	*CC9944/RG1017011*
Trial Phase	*Feasibility*
Trial Type	*Pilot Study*
Clinical Indication	*Relapsed or Refractory Multiple Myeloma*
Study Objectives	*Test patient myeloma cells in a high throughput assay against individual drugs and drug combination to enable optimal choice of drug combinations for therapy.*
Study Design	*Feasibility Study. We will utilize a drug sensitivity assay to assess sensitivity of patient's myeloma cells to drugs and drug combinations. Bone marrow aspirate will be used for testing in the high-throughput assay and each patient will be classified as having an actionable response to assay or not. When available, mutational analysis using genomics will be utilized to choose drug(s).*
Population	*Multiple Myeloma*
Primary Endpoints	*Actionable result from high-throughput assay*
Secondary Endpoints	*Response rate per IMWG*
Type of control	*NA*
Investigation Drug	*See list of 49 drugs in section1.3*
Trial Blinding	*NA*
Treatment Groups	*1*
Efficacy Assessments	*Day 1 of every cycle*
Number of trial subjects	*40 subjects*
Estimated duration of trial	*6 years*
Duration of Participation	*2 years*

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1.0 INTRODUCTION TO THE PROTOCOL

1.1 Introduction

Multiple myeloma (MM) is a plasma cell neoplasm characterized by clonal proliferation of mature plasma cells in the bone marrow, with resulting end organ damage in the kidneys, bones, and hematopoietic system [1]. Front line therapies for active multiple myeloma generally consist of induction treatment with various combinations of a proteasome inhibitor, an alkylating agent, immunomodulatory agents, and glucocorticoids, followed by high-dose melphalan and autologous stem cell transplantation when disease is under control [2]. Unfortunately, despite major advances in the last 10 years in the treatment of multiple myeloma, the majority of patients will relapse and require salvage therapies, to which they eventually develop resistance. In particular, recent publications have described a median overall survival of 9 months for patients refractory to proteasome inhibitors and immunomodulatory agents [3]. These findings were recently confirmed in an analysis of real world data, where patients with ≥3 lines of prior therapy were shown to have a median overall survival of 7.9 months [4]. Although the introduction of CD38 monoclonal antibodies to the relapsed MM patient has improved outcomes, patients who are refractory to CD38 monoclonal antibodies, proteasome inhibitors, and immunomodulatory agents ("triple class refractory") continue to have poor outcomes, with a median OS of 8.6 months [5].

Traditionally, high risk multiple myeloma has been defined by biochemical markers of disease burden and chromosomal changes at the time of diagnosis. However, some patients with relapse early during treatment may have prognosis similar to high-risk MM, even in the absence of traditional high-risk markers, termed as functional high-risk MM. Functional high-risk multiple myeloma (FHRMM) is only defined through dynamic assessment of disease kinetics after treatment initiation. At its broadest level, FHRMM could potentially include all of the following types of patients: (1) Patients who achieve < VGPR to induction therapy, (2) patients with early relapse (i.e., within 12 months or less) following induction therapy and/or frontline ASCT. In a study of 1,320 patients, progression within 12 months of starting therapy for MM had a negative prognostic impact, with a median OS of 20 months for those patients (as compared to a median OS of 61 months for all other patients)[6] In a sub-analysis of the Myeloma XI trial, relapse within 12 months of autologous HCT had a negative prognostic impact, with a median OS of 26 months, compared to 91 months for all other patients [7]. Thus, there is a desperate need for new drugs and therapies to improve outcomes for these patients.

There has been longstanding interest in predicting response to cancer therapy through use of biomarkers, sensitivity assays, and genomics. Recently, using an advanced microfluidics platform, a group at H. Lee Moffitt Cancer Center pioneered an early chemosensitivity assay in multiple myeloma, and were able to predict sensitivity to bortezomib and melphalan in multiple myeloma cells lines and patient-derived primary myeloma cell populations [8]. Building on this approach, using patient specific mathematical models based on results of *ex*-vivo chemosensitivity assays, from a cohort of 52 patients, were able to successfully classify 96% as responders or non-responders [9, 10]. With respect to genomic signatures, Mitra et al recently were able to use machine learning algorithms with gene expression profiling to identify a 42-gene expression signature that could distinguish between good and poor responses in a myeloma cell panel [11]. The combination of chemosensitivity assays and genomic data thus are appealing as new potential ways to further individualize cancer therapy.

This clinical trial has a goal of attempting to identify new drugs that might be effective for patients with myeloma that is resistant to standard, approved therapies.

1.2 Preclinical Data

The in vitro high throughput drug sensitivity assay used in this initial trial was comprised of both investigational drugs and FDA approved drugs. Testing was performed in the Quellos High Throughput Core Facility, University of Washington Medicine, Seattle WA. Thus far, we have tested a total of 7 MM/plasma cell leukemia patient blood or marrow samples using magnetic bead enrichment methodology with to improve CD138 positive plasma cell fraction in most cases. The assay is based on an assay used to test over 70 AML and 4 ALL patient samples thus far. Eight 384-well accommodate duplicate testing of 160 drugs and combination at 8 concentrations each plus controls. Cells were added to matrix protein coated non-tissue culture-treated 384-well plates at a density of 1-5,000 cells per well in 50µL of complete media (containing pen/strep and 10mM HEPES buffer) using a Thermo Scientific Matrix WellMate, and incubated overnight to allow adhesion. Compounds (50nL) were added (ranging from 5 pM to 100 µM) to patient samples using the CyBio CyBi-Well Vario and incubated at 37°C, 5% CO2 for 96 hours. The final solvent concentration, (DMSO) in the assay was 0.1%. CellTiter-Glo (Promega) was dispensed into the individual wells with the WellMate following the manufacturer's recommended procedures and, following 20 minutes incubation on an orbital shaker, luminescence was measured on a Perkin Elmer

EnVision Multi-label plate reader to assess viable cells. Measurements were corrected for background luminescence and percentage cell viability is reported as relative to the DMSO solvent control. IC50 values were calculated by fitting data using least squares method to the standard four-parameter logistic model where:

"Y" = ("Ymin"+("Ymax"/(1+(("X"/"IC50")Slope), and Y = % viability, Ymin = minimal % viability, Ymax = maximal % viability, X = compound concentration, IC50 = concentration of compound exhibiting 50% inhibition of cellular viability, Slope = the slope of the resultant curve. Curve fitting was performed using idbs XLFit software (Microsoft Excel).

Thus far, we have purified and tested six multiple myeloma patient samples in the high throughput drug sensitivity assy. The first assay was full synergy testing against a triple drug combination with testing performed against one, two or three of the drugs, as well as double and triple drug combinations. The next assay for one patient was against ~10 drugs known or suspected to exhibit sensitivity for multiple myeloma, and the third assay was against a wide variety of 150-300 drugs and targeted inhibitors active in hematologic malignancy or cancer. For one bone marrow sample, the purification method of CD138 selection was particularly successful and 19.6 million multiple myeloma cells were obtained, 94% purity (Figure 1), much more than the 8 million needed to perform the assay in duplicate. We have demonstrated sensitivity to several drugs used in multiple myeloma (Figure 2), and to other drugs not typically used in myeloma such as venetoclax, dinaciclib, vinblastine, and some investigational drugs.

Figure 1. Flow cytometry analysis of CD138+ multiple myeloma cells purified by magnetic beads

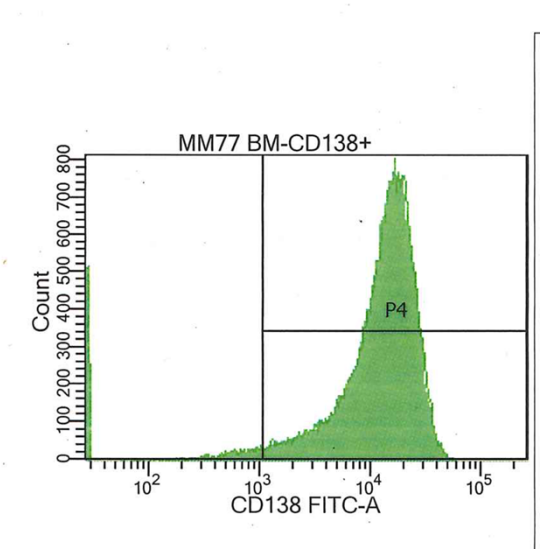
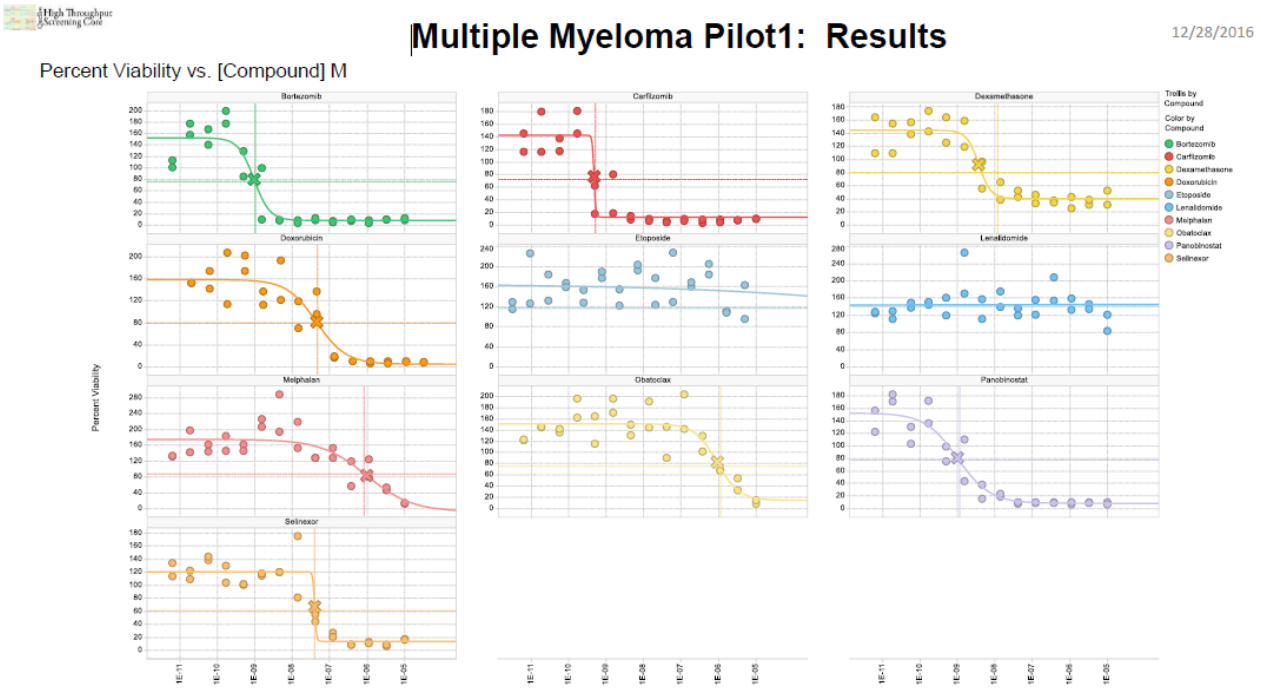


Figure 2. High throughput assay results (some selected drugs shown). Note sensitivity to bortezomib, carfilzomib, panobinostat and doxorubicin, but not to etoposide or lenalidomide.

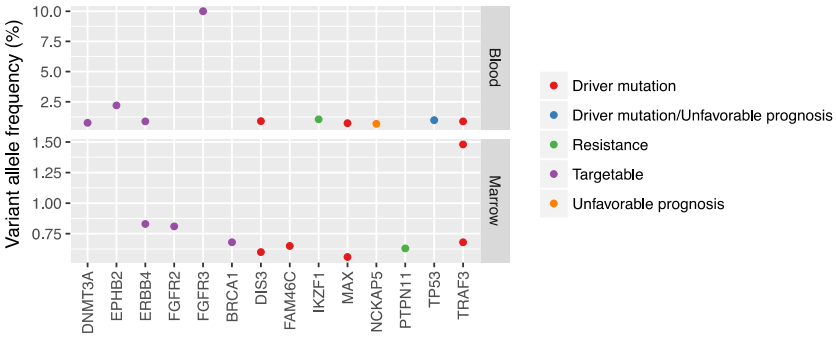


The novel aspect of the assay is that the cell survival is measured on a substratum to which the cells are adherent, a property which confers drug resistance for all hematologic malignancies. Recent studies demonstrate the importance of the microenvironment in drug sensitivity testing [12, 13]. Our adherence strategy, although it does not substitute for microenvironment, is a component previously demonstrated sufficient to reproduce drug resistance in AML, where this strategy has previously been tested [14].

Treatment response of targeted molecular therapy may correlate with mutations in tumor DNA; therefore we will perform next generation sequencing of $CD138^{+}$ plasma cells isolated from bone marrow as well as circulating tumor DNA extracted from plasma. Since a recent study demonstrated that up 75% of patients with multiple myeloma will exhibit spatial genomic heterogeneity of their cancer, we expect that by sequencing circulating tumor DNA, which can arise from multiple sites, we will detect a greater range of mutations that will be more representative of the disease [15]. Specifically, we will investigate if the results of the *in vitro* high throughput drug sensitivity assay as well as patient treatment response correlate with detected DNA mutations.

To compare the genomic heterogeneity between bone marrow and circulating tumor DNA samples, we performed next generation sequencing on 7 patients with newly diagnosed and relapsed myeloma using a custom 63 gene panel which includes recurrently mutated genes, genes with known therapeutic targets, and genes with prognostic value. Our results demonstrate the presence of single nucleotide variants unique to each tissue compartment as well as mutations present in both (figure 3).

Figure 3. Targeted next generation sequencing of bone marrow and circulating tumor DNA in a representative patient with relapsed multiple myeloma.



1.3 List of Assay Drugs

The current assay includes 12 investigational drugs, and 37 FDA approved drugs. Several of these drugs are tested in combination, mirroring frequently used anti-Myeloma regimens. Table 1 is a list of drugs used in the 49-drug panel and their mechanisms of action.

Table 1. The Assay Drugs and their mechanisms of action

Drug Name	Class	FDA Approved?
Bortezomib	Proteasome inhibitor	Y
Ixazomib	Proteasome inhibitor	Y
Oprozomib	Proteasome inhibitor	N
Carfilzomib	Proteasome inhibitor	Y
Thalidomide	IMID	Y
Lenalidomide	IMID	Y
Pomalidomide	IMID	Y
Iberdomide	CELMOD	N
Mezigdomide	CELMOD	N
Cyclophosphamide	Alkylator	Y
Doxorubicin	Anthracycline	Y
Daunorubicin	Anthracycline	Y
Idarubicin	Anthracycline	Y
Etoposide	Topoisomerase II inhibitor	Y
Cisplatin	Platinum	Y
Carboplatin	Platinum	Y
Dexamethasone	Glucocorticoid	Y
Prednisone	Glucocorticoid	Y
Vinblastine	Vinca alkaloid	Y
Vincristine	Vinca alkaloid	Y
Vemurafenib	BRAF inhibitor	Y
Melphalan	Alkylator	Y
Venetoclax	BCL2 inhibitor	Y
Encorafenib	BRAF inhibitor	Y
Binimetinib	MAPK inhibitor	Y

AZD 5991	MCL-1 inhibitor	N
Milademetan	MDM2 inhibitor	N
Crenigacestat	Gamma secretase inhibitor	N
Nirogacestat	Gamma secretase inhibitor	N
BGB-11417	BCL2 inhibitor	N
Erdafitinib	FGFR inhibitor	Y
Pemigatinib	FGFR inhibitor	N
Infagratinib	FGFR inhibitor	Y
Futibatinib	Tyrosine kinase inhibitor	Y
Bendamustine	Alkylator	Y
Daratumumab	CD38 monoclonal antibody	Y
Isatuximab	CD38 monoclonal antibody	Y
Elotuzumab	SLAMF7 monoclonal antibody	Y
Ibrutinib	BTK inhibitor	Y
Acalabrutinib	BTK inhibitor	Y
Zanabrutinib	BTK inhibitor	Y
Panobinostat	HDAC inhibitor	N
Trametinib	MEK inhibitor	Y
Palbociclib	CDK 4/6 inhibitor	Y
Afatinib	EGFR inhibitor	Y
Crizotinib	ALK inhibitor	Y
Marizomib	Proteasome inhibitor	N
Selinexor	Selective inhibitor nuclear export	Y
Eltanexor	Selective inhibitor nuclear export	N

2.0 OVERVIEW OF CLINICAL TRIAL

2.1 Study Objectives

2.1.1 Primary Objectives: The study will utilize an in vitro high-throughput drug sensitivity assay and patient mutational analysis, as available, to choose specific drugs or drug combinations for the treatment of patients with relapsed or refractory multiple myeloma. The primary objective is to assess the frequency of obtaining an actionable result from the assay and to estimate feasibility as defined as a frequency of at least 50%.

Our primary aim is to utilize an in vitro high-throughput drug sensitivity assay and patient mutational analysis to guide choice of specific drugs or drug combinations for the treatment of patients with multiple relapsed and/or refractory multiple myeloma. The primary objective of this study is to test patient cells in a high-throughput assay against individual drugs and drug combinations within 21 days, to enable optimal choice of drug combinations for therapy. In addition, mutational analysis that reveals susceptibility to specific agents may also be considered in choice of treatment.

2.1.2 Secondary Objective: The secondary objective is to assess the response rate among patients treated after physicians are presented with the testing results.

2.1.3 Exploratory Objective: The exploratory objective is to identify new agents or drug combinations that exhibit activity in multiple myeloma that could be tested in prospective trials.

2.2 Study Population

Subjects enrolled in this study will have relapsed or refractory multiple myeloma (defined as having progressed or been resistant to at least 3 lines of therapy) or having functional high risk relapsed multiple myeloma (defined as having achieved less than a very good partial response to first line therapy, or early relapse after autologous HCT (12 months or less)).

2.3 Study Design

This is a feasibility study of 15 patients to assess the success in utilizing a high-throughput drug sensitivity assay for determination of drug choice in patients with relapsed or refractory multiple myeloma. The sample size is determined by funding and time constraints, rather than testing of specific hypotheses. Information from this study will enable us to design a larger study with more standard therapeutic objectives.

Although efficacy is not the primary endpoint, the ultimate objective of this type of individualized therapy is to increase response rates – and ultimately, survival. In this population of multiple myeloma patients, response rates are typically not greater than 10%. As few as 3 responses in 15 patients would therefore be a successful outcome with respect to efficacy and would provide 80% confidence that the true response rate exceeds 10%.

After the initial 15 patients are enrolled on this study, we propose an expansion cohort of an additional 25 patients for a total of 40 patients on trial.

2.4 Estimated Accrual

We estimate that we will accrue approximately 40 patients to this study over 6 years.

3.0 ENDPOINTS

3.1 Study Endpoints

3.1.1 Primary Endpoint: The primary endpoint of the study is obtainment of an actionable assay result.

3.1.2 Secondary Endpoint: The secondary endpoint of the study is the overall response rate to the therapy chosen after performing the assay, as assessed by the IMWG response criteria.

3.1.3 Exploratory Endpoint: The exploratory endpoint of the study is determining aggregate data on drug sensitivity to generate hypotheses for new drugs and combinations that may be effective in treating multiple myeloma.

4.0 STATISTICAL CONSIDERATIONS

The primary objective of this study is to assess the feasibility of this approach in terms of obtaining an actionable response from the proposed assay in at least 50% of patients examined. A total of 15 patients will be administered the assay, so that 8 or more patients who obtain assay results that are deemed actionable will result in viewing the proposed approach as feasible. If the true probability of an actionable result is 30%, the probability of 8 or more “successes” among 15 patients is .05; if the true probability is 70%, the probability of 8 or more successes is .95.

A secondary objective is to estimate the response among all patients who received the assay as well as among patients who obtained an actionable result from the assay. While this study is not designed to assess potential efficacy given the relatively small number of patients to be examined, we will estimate the response rate among the groups as stated; a rough benchmark for an informal comparison is a 10% response rate.

If at any time a 7th patient fails to receive an actionable result from the assay, consideration will be given to terminating the trial.

If this study is deemed to be feasible as defined above, a subsequent study will be designed to more formally assess potential efficacy using this high-throughput assay.

This study was amended to accrue 25 additional patients. If the success rate is 50%, this will likely give a total of 20 patients to estimate the clinical response rate. If the true response rate is 30%, comparing with the benchmark 10%, with 20 patients, we will have 81% power to show the true response rate is statistically significantly higher than the benchmark at one-sided 0.05 level using one-sample proportional test. Such tests reject the null hypothesis with 6 or more response out of 20 patients. As an example, if 6 responses were seen, then the exact 90% confidence interval would be (0.14,0.51)

5.0 SUBJECT ELIGIBLITY

5.1 Inclusion Criteria:

- Patients ≥ 18 years of age
- Diagnosis of multiple myeloma or plasma cell leukemia with documented relapsed or refractory disease according to IMWG criteria, in any one of the following categories:
    - 3 prior lines of therapy including an IMiD and a PI
    - Less than a very good partial response (VGPR) to initial therapy
    - Early relapse (< 12 months) after autologous HCT or after 1st line of therapy
- Collection of a bone marrow, fluid, or tissue sample that is expected to have enough cells to run the assay
- Measurable disease defined by one of the following:
    - Serum monoclonal protein ≥ 0.5 g/dL by SPEP
    - ≥ 200 mg/monoclonal protein in urine on 24 hr UPEP
    - Involved serum free light chain ≥ 10 mg/dL and abnormal involved:uninvolved ratio
    - Plasmacytomas that are palpable per exam or measurable per standard radiologic review
    - Circulating plasma cells ≥ 2,000 if diagnosis of plasma cell leukemia
- ECOG Performance Status 0-3
- Female patients of child bearing potential and non-vasectomized male patients agree to practice appropriate methods of birth control
- Ability to understand purpose and risks of the study and provide signed and dated informed consent, and authorization to use protected health information
- Expected Survival is > 100 days
- Adequate organ function as determined by the investigator

5.2 Exclusion Criteria:

- Mucosal or internal bleeding, or platelet transfusion refractory
- Any medical conditions that would impose excessive risk to the patient, or would adversely affect his/her participation in the study
- Known active infection requiring antibiotics within 7 days of initiation of assay-guided treatment, unless considered controlled in the opinion of the investigator
- Other malignancy with life expectancy < 1 year due to the other malignancy
- Pregnant or breast feeding women
- Serious psychiatric illness, alcoholism, or drug addiction
- Active hepatitis B or C infection
- HIV with detectable viral load (VL) by PCR (note that HIV patients with undetectable VL on anti-viral medications are eligible)
- Previous treatments for MM within 2 weeks of initiation of assay-guided treatment
- Prior autologous or allogeneic SCT within 12 weeks of initiation of assay-guided treatment
- Prior allogeneic HCT with active GVHD on therapeutic dosing of immunosuppression or prednisone >20 mg daily equivalent

- Prior major surgical procedure or radiation treatment within 2 weeks of initiation of assay-guided treatment (not including limited radiation used for palliation of bone pain)

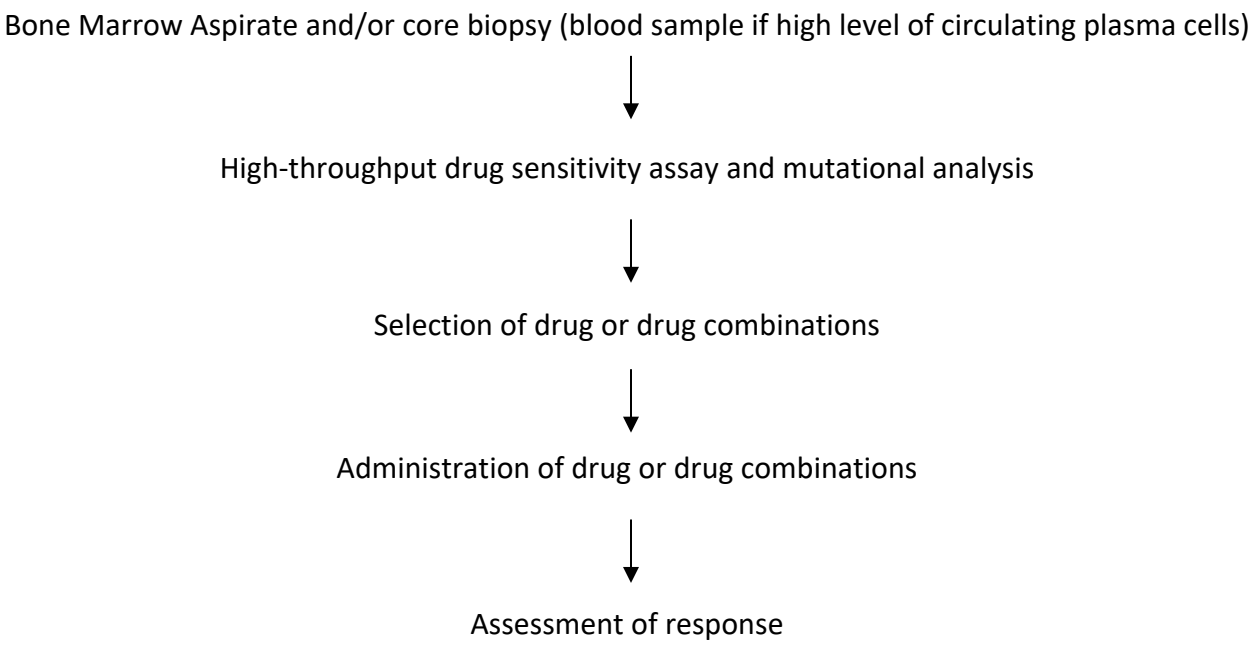
6.0 SUBJECT REGISTRATION

Subjects will be registered by the Study Coordinator and entered into the institutional clinical trial management system. A complete, signed, study consent and HIPAA consent are required for registration. See Appendix D for the registration form.

7.0 TREATMENT PLAN

7.1 Treatment Plan Overview

SCHEMA



7.2 Pretreatment Evaluation

7.2.1 Screening Evaluations/Procedures

1. Signed, written informed consent: Consent must be completed prior to performing any study-related procedures.

2. The following standard of care evaluations, if done, may be reviewed to assist in determining eligibility for the study, unless required per the inclusion/exclusion criteria:

a. Medical history: Detailed documentation of disease and treatment history with outcomes

b. ECOG performance status (Appendix A)

c. Concurrent medical conditions.

d. Serum chemistries: Electrolytes (sodium, potassium, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, Lactate Dehydrogenase (LDH)

e. Initial standard of care diagnostic bone marrow reports, including hematopathology, cytogenetics / FISH, and flow cytometry.

f. A research bone marrow aspirate and/or core biopsy sample, to occur either at the same time as or independent of a standard of care bone marrow evaluation.

7.3 Drug Sensitivity Assay

A bone marrow sample will be sent to the research laboratory for testing in the high throughput assay, and results will be available in about a week. If the aspirate contains an inadequate number of plasma cells, another bone marrow aspirate may be obtained with patient approval. Investigator discretion will be used for analyzing the adequacy of the cell counts. If multiple myeloma is present in a location other than the bone marrow, a sample of fluid or a biopsy from that location (for example, plasmacytoma biopsy, pleural fluid, blood) may be sent to the research laboratory for testing in the high throughput assay.

Both blood and bone marrow will be collected for future correlative analyses. $CD138^{+}$ cells will be isolated from bone marrow aspirate by magnetic bead purification (Miltenyi) and DNA will be extracted using the QIAamp DNA Blood Mini kit (QIAGEN). Additionally, plasma will be extracted from blood collected in cell free DNA tubes (Streck) and cell free DNA will be extracted using the QIAamp MinElute cfDNA kit. DNA extractions, sequencing, and mutational analysis will be performed in future analyses.

Treatment with any chemotherapeutic agent necessary to control myeloma burden is permitted during the time of testing and drug procurement. These agents must be stopped prior to initiation of assay guided therapy.

7.4 Drug Choice

A drug or combination of 2-3 drugs tested in combination will be chosen from only the FDA approved drugs listed in Table 1 based on availability, insurance clearance, degree of apparent in vitro response (lowest absolute EC50 or lowest EC50 relative to other patients for individual drugs), prior publication of drug or drug combination use in other multiple myeloma patients and difference in class of drug compared to the drugs to which the patient has previously not responded. In addition, mutational analysis that reveals mutations in genes that confer susceptibility to specific agents may also be considered in choice of treatment.

If an inhibitor is available that has the same target (e.g.-a specific kinase) as the one tested, this drug may be substituted. For combination regimens, we intend to only utilize regimens for which there are clinical data in humans. Additional FDA approved drugs or combinations of drugs may be added to the assay panel in the future if there is evidence to suggest efficacy in myeloma patients. However, the protocol will be amended to include these drugs prior to their use in any assay-guided treatment regimen.

7.5 Drug Dosing

Individual drugs will be used in doses that have been utilized in humans with anti-cancer response, at standard or maximum tolerated dose. Drug combinations will be used at standard doses or doses for which there is published experience.

7.6 Discussion about individual regimens

In addition to the consent discussion for this study, there may be a general treatment discussion which will occur only as required per standard practice by treating physicians.

8.0 SUBJECT EVALUATION

8.1 On-Study Clinical Evaluations

1. The on-study period begins at time of confirmation that the amount of cells available are enough to run through the assay as per PI discretion. Patients leave the study at the time assay results are distributed to the participant and/or the treating physician. Outcome data for any study directed therapies may be collected for a period up to 2 years. Adverse events will be collected from the time of consent until distribution of assay results.

2. Hematology: CBC with differential and platelet count and peripheral blood smear at frequency per standard of care. Serum protein electrophoresis (SPEP) and serum free light chain assay at beginning of each cycle, per standard of care.

3. Serum chemistries: Electrolytes (sodium, potassium, chloride, and bicarbonate), BUN, creatinine, glucose,

and liver function tests (AST, ALT, ALP, total bilirubin, LDH) per standard of care.

4. Grade 3 and higher adverse events will be recorded using the NCI CTCAE, Version 5.0

8.2 Follow-Up

1. Response data. All reports for bone marrow evaluations done as standard of care following the provision of assay results, including the collection of morphology, flow cytometry, and cytogenetics/FISH.

2. Subsequent complete blood count, renal function, and liver function tests obtained for clinical reasons for a period of up to one month post the last consolidation chemotherapy cycle, as needed to define toxicity or duration of response.

3. Disease free and overall survival data will be assessed by contacting the referring MD or the patient every three months for the first two years.

9.0 ADVERSE EVENTS

9.1 Adverse Event

According to ICH guidelines (Federal Register. 1997; 62(90):25691-25709), and ICH E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:

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

Abnormal laboratory values for laboratory parameters specified in the study should not be recorded as an adverse event unless an intervention is required (repeat testing to confirm the abnormality is not considered intervention), the laboratory abnormality results in a serious adverse event or the adverse event results in study termination or interruption/discontinuation of assay-guided treatment.

Medical conditions present at screening (i.e., before the assay-guided treatment is administered) are not adverse events and should not be recorded on adverse event pages of the CRFs. These medical conditions should be adequately documented on the subject chart. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods should be reported and recorded as adverse events.

9.2 Serious Adverse Event

An adverse event should be classified as an SAE if it meets one of the following criteria:

Fatal	Adverse event results in death.
Life threatening:	The adverse events placed the subject at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization:	If required or prolongation inpatient hospitalization for unexpected adverse events. Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Hospitalization for febrile neutropenia or infection or bleeding or thromboembolism or fracture/impending fracture or pain control is not considered unexpected for this diagnosis. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
Disabling/incapacitating	Resulted in a substantial and permanent disruption of the subject's ability to carry out normal life functions.

Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the molecule or treatment plan regimen before conception or during pregnancy.
Medically significant:	The adverse event did not meet any of the above criteria, but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above.

Grade 3 or 4 hematologic toxicity will not be considered SAE as it is a consequent of the disease and its treatment. Skeletal or thromboembolic events will not be considered SAE as they are expected in patients with plasma cell disorders.

9.3 Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the prior medical condition of the subject or treatment given to the subject. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported in preclinical or clinical studies rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

9.4 Monitoring and Recording Adverse Events

All SAEs will be assessed by the investigator or qualified designee and recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious, noting all criteria that apply
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug, a study procedure, or other causality
- The action taken due to the adverse event
- The outcome of the adverse event

9.5 Grading Adverse Event Severity

All SAEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event.

9.6 Attribution of an Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related, unrelated if it is assessed as unlikely related or unrelated.

9.7 Adverse Event Recording Period

AEs will be monitored and recorded in study specific case report forms (CRFs) from the time of signing consent through the time at which assay results are provided to the patient and treating physician.

9.8 Adverse Event Reporting Requirements

9.8.1 Reporting to IRB

The investigator or designee will report events to the FHCC IRB in accordance with the policies of the IRB.

10.0 CRITERIA FOR ENDPOINT EVALUATIONS

10.1 Criteria for Feasibility

We define feasibility in terms of obtaining an actionable response from the proposed assay in at least 50% of patients examined. A total of 15 patients will be administered the assay, so that 8 or more patients who obtain assay results that are deemed actionable will result in viewing the proposed approach as feasible. If the true probability of an actionable result is 30%, the probability of 8 or more "successes" among 15 patients is .05; if the true probability is 70%, the probability of 8 or more successes is .95.

10.2 Criteria for Response

Responses to treatment will be on the basis of the standard myeloma response criteria [16].

10.2.1 Definition of response by Standard Criteria

Response will be defined by criteria of Kumar et al, Appendix C.

10.2.2 Duration of Response

Time to progression will be noted for patients.

11.0 DATA AND SAFETY MONITORING PLAN

Ongoing trial oversight is carried out by the principal investigator and the primary research coordinator. These individuals will meet regularly to review recently acquired data, and adverse events.

Institutional support of trial monitoring will be in accordance with the FHCC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCC Scientific Review Committee (SRC) and the FHCC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

12.0 DATA MANAGEMENT/CONFIDENTIALITY

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique subject number to protect subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents.

13.0 STOPPING RULES, ETHNIC AND GENDER DISTRIBUTION CHART

13.1 Stopping Rules

The study will be paused for review if we have difficulty in demonstrating feasibility. Operationally, this will be triggered if the 7th patient fails to receive assay-guided results, indicating that the objective of 9 of 15 patients receiving actionable results may not be met.

Patients must be successfully enrolled (i.e., meet the eligibility criteria and provide enough cells for the assay) to count towards the stopping rule.

13.2 Ethnic and Gender Distribution Chart

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	4	6	10
Not Hispanic or Latino	14	16	30
Ethnic Category Total of All Subjects*	18	22	40
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	5	5	10
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	4	5	9
White	9	12	21
More Than One Race	0	0	0
Racial Categories: Total of All Subjects*	18	22	40

14.0 INVESTIGATOR OBLIGATIONS

The PI is responsible for the conduct of the clinical trial at the site and is responsible for personally overseeing the treatment of all study subjects. The PI must assure that all study site personnel, including sub-Investigators and other study staff members, adhere to the study protocol and to all applicable regulations and guidelines regarding clinical trials both during and after studycompletion.

All subjects are informed of the nature of the program, its possible hazards, and their right to withdraw at any time, and each subject signs a form indicating their consent to participate prior to receiving any study-related procedures.

15.0 ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

15.1 Pre-Study Documentation

The following documentation must be received by the study team prior to initiation of the trial: curricula vitae of the PI and all Sub-Investigators; copy of the correspondence from the IRB/EC indicating approval of the protocol and Informed Consent Forms, signed by the IRB/EC chairperson or designee; copy of the Informed Consent Forms that were reviewed and approved by the IRB/EC.

15.2 Study Site Training

Before initiation of the study, the PI or designated representatives will review and discuss the following items with the study staff: the protocol, study procedures, record keeping and administrative requirements, drug accountability, AE reporting, Good Clinical Practice guidelines, CRF/eCRF completion guidelines, monitoring requirements, and the ability of the site to satisfactorily complete the protocol. Additional documents with instructions for study compliance and CRF/eCRF completion will be provided.

15.3 Documentation

The documentation of clinical data must be stored by the PI according to legal requirements. The PI and study staff has responsibility for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be suitable for inspection by the FDA, and/or other applicable regulatory agencies/competent authorities at any time, and should consist of the following elements: subject files (complete medical records, laboratory data, supporting source documentation, and the Informed Consent); study files (the protocol with all amendments, copies of all pre-study documentation, and all correspondence between the Competent Authorities, IRB/EC, site, and PI); and drug accountability files, containing a complete account of the receipt and disposition of the study drug.

15.4 Data Collection

Electronic case report forms must be completed and submitted for each subject enrolled in the study. Any changes or corrections made to the CRF/eCRF must be subsequently reviewed and signed by the PI. All data fields in the CRF/eCRF must be completed to avoid queries.

15.5 Protocol Interpretation and Compliance

The procedures defined in the protocol are carefully reviewed by the PI and his/her staff prior to the time of study initiation to ensure accurate representation and implementation. Protocol amendments, if any, are reviewed and implemented promptly following IRB/EC and relevant Competent Authorities approval. The PI is responsible for submitting protocol amendments and other regulatory agencies according to national, state or local requirements. The PI is always available to answer protocol- or subject-related questions.

15.6 Study Monitoring and Data Collection

A representative from the Fred Hutchinson Cancer Center (FHCC) Clinical Research Support will visit the study center periodically to monitor adherence to the protocol, applicable FDA regulations and/or other regulatory agencies national, state or local requirements, and the maintenance of adequate and accurate clinical records. Electronic case report forms are reviewed to ensure that key safety and efficacy data are collected and recorded as specified by the protocol. The monitor is permitted to access subject medical records, laboratory data and other source documentation as needed to appropriately monitor the trial. The CRF/eCRF and related source documents will be reviewed in detail by the monitor at each site visit. Only original source documents are acceptable for review. This review includes inspection of data acquired as a requirement for participation in this study and other medical records as required to confirm information contained in the CRF/eCRF, such as past history, secondary diagnoses, and eligibility requirements. Other study records, such as correspondence with the PI and the Competent Authorities, and IRB/EC will also be inspected. All source data and study records must also be available for inspection by representatives of the FDA or other regulatory agencies.

15.7 Disclosure of Data/Publication

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Such medical information may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection on request by the FDA or other regulatory agencies and by the IRB/EC.

15.8 Ethical Considerations

The Investigator agrees to conduct this study in accordance with applicable United States FDA clinical trial regulations and guidelines, applicable United States FDA clinical trial regulations and guidelines, the ICH (E6) GCP guidelines, the European Union Directive 2001/20/EC for clinical trials conducted in the European Union, the IRB/EC and local legal requirements and with the Declaration of Helsinki (1989). The Investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

15.9 Informed Consent

All institutional, NCI, state and federal regulations concerning informed consent and peer judgment will be fulfilled. Written consent will be obtained from all patients entering the study.

15.10 Institutional Review Board/Ethics Committee

The PI will assure that an appropriately constituted IRB/EC that complies with the requirements of 21 CFR Section 56 or written assurance of compliance with ICH (E6) guidelines will be responsible for the initial and continuing review and approval of the clinical study. Before initiation of the study, the PI or designee will forward copies of the protocol and Consent Form to be used for the study to the IRB/EC for its review and approval.
The PI or designee will also assure that all changes in the research activity and all unanticipated problems involving risks to human subjects or others will be reported promptly to the IRB/EC, and that no changes will be made to the protocol without prior Sponsor and IRB/EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.
The Investigator or designee will be responsible for submitting periodic progress reports to the IRB/EC at intervals appropriate to the degree of subject risk involved in the study, but not less than once per year and at the completion or termination of the study.

15.11 Subject Privacy

The Investigator affirms and upholds the principle of the subject's right to privacy. The Investigator shall comply with applicable national and local privacy laws.

To verify compliance with this protocol, the Investigator permits the FHCC Clinical Research Support, monitors to review the subject's original medical records. Should access to such medical records require a waiver or authorization separate from the statement of Informed Consent, the Investigator will obtain such permission in writing from the subject before the subject is entered into the study.

16.0 REFERENCES

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17.0 APPENDICES

Appendix A: Performance Status Scales (ECOG and Karnofsky**)**
Appendix B: Response Criteria in Multiple Myeloma

17.1 [APPENDIX A: Performance Status]

ECOG Performance Status Scale

GRADE	SCALE
0	Fully active, able to carry out all pre-disease performance without restriction
1	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	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

KARNOFSKY PERFORMANCE STATUS SCALE

General	Index	Specific Criteria
Able to carry on normal activity; no special care needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	70	Care for self, unable to carry on normal activity or to do work
	60	Requires occasional assistance from others but able to care for most needs
	50	Requires considerable assistance from others and frequent medical care
Unable to care for self, requires institutional or hospital care or equivalent; disease may be rapidly progressing	40	Disabled; requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
	10	Moribund
	0	Dead

17.2 [Appendix B: Response Criteria in Multiple Myeloma]

STANDARD IMWG response criteria*[16]

Stringent complete response	Complete response as defined below plus normal FLC ratio ** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ to λ ratio ≤ 4:1, or ≥ 1:2 for κ and λ patients, respectively, after counting ≥ 100 plasma cells)
Complete response	Negative immunofixation on the serum and or and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥ 90% reduction in serum M-protein plus urine M-protein level < 100 mg per 24 hr
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≤90% or to <200 mg per 24 h
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50-89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in size of soft tissue plasmacytomas is also required
Stable disease	Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease	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 size 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