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CLBH589DUS108T: Panobinostat with Carfilzomib and Dexamethasone for Relapsed/Refractory Multiple Myeloma: Correlation with In Vitro Chemosensitivity Testing

Principal Investigator: **Andrew Cowan, MD**
Professor of Medicine
University of Washington
825 Eastlake Ave East
Seattle, WA 98109
Tel: 206-667-4551
Email: ajcowan@seattlecca.org

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1. STUDY OVERVIEW

1.1. Primary Objective

To correlate in vitro drug sensitivity testing with clinical response we will determine rate of in vitro drug sensitivity to panobinostat, carfilzomib, and dexamethasone singly and in combination, doublets and triplets. We will determine parameters that predict that patients will achieve ≥ VGPR after clinical regimen.

1.2. Secondary Objective

To monitor response rates (partial response (PR), very good partial response (VGPR), and complete response) using the International Myeloma Working Group Uniform Response Criteria for Multiple Myeloma (Appendix C).

1.3. Exploratory Objective

Progression free survival and overall survival will be assessed for up to 3 years after last dose.

2. BACKGROUND AND STUDY RATIONALE

Several classes of drugs have proven efficacy in multiple myeloma and drug combinations have resulted in synergistic responses that have improved efficacy. The novel drug classes have included immunomodulatory drugs (IMiDS), proteasome inhibitors, monoclonal antibodies, and histone deacetylase inhibitors. These agents have been combined with each other and conventional cytotoxic agents and steroids to comprise highly effective regimens, with a very high proportion of response in newly diagnosed and relapsed patients. There are approved agents in each of the drug classes, and ongoing investigation of new agents.

Histone deacetylase inhibitors have been shown to be effective agents in hematologic malignancies. Their mechanism of action is alteration of chromatin structure, leading to growth arrest, apoptosis and differentiation. Both vorinostat and panobinostat were used in clinical trials in multiple myeloma. The latter agent, panobinostat, was extensively studied in phase I, II and III trials, leading to FDA approval. Rocilinostat (ACY1215) is a selective HDAC6 inhibitor and clinical trials in multiple myeloma are ongoing.

Panobinostat is a histone deacetylase inhibitor with activity against Class I, II and IV nonhistone and histone deacetylases. A dose escalation study of panobinostat in combination with bortezomib established a maximum tolerated dose of 20 mg of panobinostat administered three times a week in combination with bortezomib at 1.3 mg/m2/dose (San Miguel et al 2013). A phase 3 trial demonstrated that panobinostat plus bortezomib and dexamethasone was superior to placebo plus bortezomib and dexamethasone in patients with relapsed or refractory multiple myeloma in terms of progression free survival (San Miguel et al 2014). This result led to the FDA approval of panobinostat in patients with relapsed, refractory multiple myeloma. In a phase 3 trial, 60.7% of patients responded to the combination, including 27.6% complete responses (San- Miguel et al 2014).

Bortezomib was the first proteasome inhibitor that was approved for multiple myeloma, initially for relapsed patients, and subsequently, new diagnosis. It is highly active, and combinations of

bortezomib with many other agents have been extensively studied, including 3 and 4 drug regimens. One of the most active regimens that is highly utilized upfront is the combination of lenalidomide and bortezomib with dexamethasone (Richardson et al., 2010) with a ≥ VGPR rate of 74%. However, the combination of carfilzomib with lenalidomide and dexamethasone has also been highly effective in the upfront setting, with response rate of ≥ near complete remission (nCR) of 82% by 8 cycles, and 100% ≥ nCR by 16 cycles (Dytfeld et al., 2014). Moreover, the depth of response was remarkable, with 35% stringent complete responses (sCR) by 8 cycles and 78% by 16 cycles. Furthermore, carfilzomib has a much lower rate of neuropathy. Based on these features, carfilzomib was then studied in combination with panobinostat.

Panobinostat and carfilzomib were combined in a phase I/II trial for relapsed, refractory patients (Berdeja et al, 2015). Four combinations of carfilzomib and panobinostat were utilized in this phase I/II trial of the combination to assess toxicity and efficacy. The maximum tolerated dose used for the phase II expansion was 30 mg panobinostat and 20 then 45 mg/m2 carfilzomib (Berdeja et al, 2015).

Another comparison of additional doses of this combination demonstrated that panobinostat 20 mg on days 1,3,5,15,17,19 and carfilzomib 20 mg/m2 then 56 mg/m2/dose days 1,2,8,9,15,16 each cycle resulted in an overall response rate of 84% (34% ≥ VGPR, 50% ≥ PR) (Berdeja et al, 2015 abstract).

We now understand that there is a plethora of mutations associated with the development of multiple myeloma, and that there are gene expression signatures associated with prognosis. Each patient appears to have a unique pattern of mutations and gene expression. Given this heterogeneity, it might be predicted that individuals would have wide variability in response to standard drug combination s, and this is indeed the case. But when a new patient presents, or at the time of relapse, we do not yet have in hand the tools with which we might predict response with certainty. A test that could predict which patients might be expected to respond to specific agents might enable clinicians to optimize therapy for individual patients.

We have developed an in vitro assay (CLIA certified for AML thus far, including these drugs) for testing cancer cells that are adherent in 384 well plates.

We propose to assay in vitro sensitivity of purified multiple myeloma cells obtained just prior to clinical treatment on a clinical trial, then perform statistical analysis to determine if the test was predictive of response. The test will include 8 concentrations of the individual drugs, then combinations of the concentrations of each pair of drugs, and the triple drug combination. The assay will define combinations that lead to additive vs. synergistic cytotoxicity [analysis by isobolograms (Berenbaum, 1978; Steel and Peckham, 1979)]. We hope to define parameters of drug sensitivity to the individual drugs and/or drug combinations in vitro that correlate with clinical response.

3. ELIGIBILITY

3.1. Inclusion Criteria

1. Age 18 or older
2. Diagnosis of multiple myeloma refractory to or relapsed after ≥ 1 line of prior therapy (IMWG criteria)

3. Measurable disease, as indicated by one of the following:

a. Serum M-protein ≥ 1.0 g/dL

b. Elevated involved free light chain ≥ 10 mg/dL as per IMWG criteria, and abnormal ratio

c. Urine Bence Jones protein >200mg/24 hr

4. Adequate blood counts

a. ANC ≥ 750/uL

b. Platelet count ≥ 75,000/uL

c. Hemoglobin ≥ 7 g/dL

5. Adequate renal and hepatic function:

a. Creatinine ≤ 2.0 mg/dL or calculated creatinine clearance ≥ 30mL/min

b. Total bilirubin ≤ 1.5xULN unless elevation is thought to be due to Gilbert's syndrome

c. SGOT (AST) and SPGT (ALT) ≤ 2.5x ULN

6. Patients must avoid consumption of grapefruit, pomegranates, starfruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medications, due to potential CYP3A4 interaction with the study medications. Orange juice is allowed.

7. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, should have a pregnancy test prior to the initiation of treatment and use highly effective methods of contraception during and for 3 months post study treatment. Highly effective contraception methods include total abstinence, female sterilization, male sterilization, use of oral, injected, or implanted hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS) or other forms of hormonal contraception that have comparable efficacy. Women using hormonal contraceptives should additionally use a barrier method of contraception. Women are considered post-menopausal and not of child-bearing potential if they have had 12 months of natural amenorrhea or have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy, or tubal ligation at least 6 weeks ago.

8. Sexually active males must use a condom during intercourse while taking study drug and for 6 months after stopping treatment. Males should not father a child in this period. A condom is required to be used also by vasectomized men as well as during intercourse with a male partner. Female partners of sexually active men should also use an effective contraception

during treatment and for 6 months after their male partner has stopped taking the drug.

3.2. Exclusion Criteria

1. Another bone marrow malignancy
2. Another cancer with expected survival of < 2 years
3. Active viral, bacterial, or fungal infection progressing on current treatment
4. Clinically significant uncontrolled heart disease and/or recent cardiac event **within 6 months prior to enrollment**, such as:
    a. History of angina pectoris, symptomatic pericarditis, or myocardial infarction
    b. History or presence of any significant, uncontrolled, or persistent cardiac arrhythmias, e.g. ventricular, supraventricular, nodal arrhythmias or conduction abnormality. Stable atrial fibrillation within 6 months prior to randomization is permitted.
    c. Presence of unstable atrial fibrillation (ventricular response rate > 100 bpm).
    NOTE: patients with stable atrial fibrillation can be enrolled provided they do not meet other cardiac exclusion criteria.
    d. Resting heart rate < 50 bpm
    e. Complete left bundle branch block (LBBB), bifascicular block
    f. Congenital long QT syndrome
    g. Any clinically significant ST segment and/or T-wave abnormalities
    h. Corrected QT (QTcF) > 450 msec for males and > 470 msec for females using Fridercia's correction on screening Electrocardiogram (ECG)
    i. History of documented congestive heart failure (New York Heart Association functional classification III-IV)
    j. Uncontrolled hypertension defined by a Systolic Blood Pressure (SBP) ≥ 150 mmHg and/or Diastolic Blood Pressure (DBP) ≥ 100 mmHg with or without antihypertensive medication.
    NOTE: initiation or adjustment of antihypertensive medication(s) is allowed prior to screening.
    k. Other clinically significant heart disease or vascular disease
5. Currently taking medications that have known or definite risk of prolonging the QT interval or inducing Torsades de pointes (TdP). The medication must be discontinued or switched to a safe alternative medication prior to starting treatment. Specific exception is allowed for

patients on long-standing medications that have risk of prolonging QT interval or inducing TdP if screening ECG does not indicate a prolonged QT abnormality. For a list of drugs known to risk prolonging QT interval or inducing TdP, please refer to the published list of drugs at https://crediblemeds.org/pdftemp/pdf/CombinedList.pdf

6. Impairment of gastrointestinal (GI) Function or GI disease that may significantly alter the absorption of panobinostat or dexamethasone (e.g. ulcerative disease uncontrolled nausea, vomiting, malabsorption syndrome, obstruction, or stomach and/or small bowel resection)

7. Unresolved diarrhea ≥ CTCAE grade 2 or a medical condition associated with chronic diarrhea (such an irritable bowel syndrome, inflammatory bowel disease)

8. Major surgery ≤ 14 days prior to starting study treatment or side effects of surgery that have not recovered to < CTCAE grade 2

4. PATIENT REGISTRATION

Patients who fulfill eligibility criteria and who signed consent will be enrolled by the study coordinator.

5. DRUG INFORMATION

5.1. Panobinostat

1. Mechanism of Action

Panobinostat (LBH589) is a deacetylase inhibitor (DACi) belonging to a structurally novel cinnamic hydroxamic acid class of compounds. It is a potent class I/II pan-DAC inhibitor (pan-DACi) that has shown anti-tumor activity in pre-clinical models and cancer patients. Deacetylases (DAC) target lysine groups on chromatin and transcription factors and various non-histone proteins such as p53, tubulin, HSP90 and Rb. Panobinostat is formulated as an oral capsule. Inhibition of DAC provides a novel approach for cancer treatment. Histones are part of the core proteins of nucleosomes, and acetylation and deacetylation of these proteins play a role in the regulation of gene expression. Highly charged deacetylated histones bind tightly to the phosphate backbone of DNA, inhibiting transcription, presumably, by limiting access of transcription factors and RNA polymerases to DNA. Acetylation neutralizes the charge of histones and generates a more open DNA conformation. This conformation allows transcription factors and associated transcription apparatus access to the DNA, promoting expression of the corresponding genes. The opposing activities of two groups of enzymes, histone acetyltransferase (HAT) and HDAC control the amount of acetylation. In normal cells a balance exists between HAT and DAC activity that leads to cell specific patterns of gene expression. Perturbation of the balance produces changes in gene expression

Several lines of evidence suggest that aberrant recruitment of HDAC and the resulting modification of chromatin structure may play a role in changing the gene expression seen in transformed cells. For example, silencing of tumor suppressor genes at the level of chromatin is common in human tumors (Herman 1994, Pratt 1994, Szyf 1994, Herman 1995, Merlo 1995, Herman 1996,

Cameron 1999) and HDAC complexes have been shown to be crucial to the activity of the AML-specific fusion proteins PLZF-RAR-α, PML-RAR-α, and AML1/ETO (Gelmetti 1998, Grignani 1998, Lin 1998, Redner 1999). DAC inhibitors (DACi) have been shown to induce differentiation, cell cycle arrest or apoptosis in cultured tumor cells, and to inhibit the growth of tumors in animal models (Yoshida 1987, Yoshida 1988, Itazaki 1990, Yoshida 1990, Sugita 1992, Yoshida 1992, Medina 1997). In addition, DACi have been shown to induce expression of p21, a key mediator of cell cycle arrest in G1 phase and cellular differentiation (Biggs 1996, Nakano 1997, Sowa 1997, Sambucetti 1999).

Tumor growth inhibition and apoptosis in response to DACi treatment may also be mediated through changes in acetylation of non-histone proteins (e.g., HSP90, p53, HIF-1α, α-tubulin). For example, the chaperone protein HSP90 has been shown to be acetylated in cells treated with DACi (Yu 2002, Fuino 2003, Nimmanapalli 2003). Acetylation of HSP90 inhibits its ability to bind newly synthesized client proteins, thus preventing proper client protein folding and function. In the absence of HSP90 function, misfolded proteins are targeted for degradation in the proteasome. Many proteins that require HSP90 association are critical to cancer cell growth, including ErbB1, ErbB2, AKT, Raf, KDR, and BCR-ABL. Acetylation of HSP90 in cells treated with DACi inhibits the chaperone function of HSP90, leading to degradation of the client proteins and eventual cell death.

In Feb 2015, panobinostat (Farydak®) has received approval from the FDA for the treatment of patients with multiple myeloma who have received at least 2 prior regimens, including bortezomib and an immunomodulatory agent.

2. Pharmacokinetics

After oral administration, panobinostat is rapidly absorbed with no observed lag phase. Maximum plasma concentrations were generally reached within 1 hour after oral dosing. The absolute bioavailability was 30% (data on file) and the mean (SD) half-life of panobinostat was comparable following i.v. and oral dosing ~15.0 (5) hours. Moderate drug accumulation was observed with oral three-times-a-week schedule but not with the weekly i.v. schedule (1.4-fold drug accumulation with oral three-times-a-week dosing), consistent with the terminal half-life of 15 hours and dosing interval.

Different degrees of renal impairment (mild, moderate and severe) did not alter panobinostat plasma exposure, whereas, mild hepatic dysfunction increased panobinostat plasma exposure by 43% and moderate hepatic impairment marginally increased panobinostat plasma exposure by 105% in cancer patients.

In vitro experiments suggested that the hepatic oxidative metabolism of panobinostat is mediated primarily by cytochrome P450 (CYP) 3A4, and to a lesser extent by CYP2D6 and CYP2C19. In addition to monooxygenation, hydrolysis of the hydroxamic sid chain (M43.5) were also found to be mediated (at least in-part) by the CYPs. These same metabolic pathways were also observed in the recent human ADME and mass balance study [CLBH589B218].

3. Toxicities and Adverse Events

Clinical development of panobinostat focuses on the oral formulation. The clinical program for the i.v. formulation is completed with no further company-sponsored studies currently planned. As of 31 December 2013, 36 clinical studies, including clinical pharmacology (CP), Phase I and Phase II trials, as well as two randomized Phase III studies have either been completed or are ongoing. A total of 2428 patients were enrolled, 235 for i.v. and 2193 for oral, who received at least one dose of panobinostat either as a single agent or in combination with other agents.

Patients were treated with panobinostat either TIW QW (666 patients) or TIW QOW (96 patients) in single agent oral panobinostat clinical trials. These patients comprise the pooled safety population experiencing AEs during study treatment. The most frequent non-hematologic toxicity included GI events (diarrhea, nausea, vomiting), mostly of Grade 1-2, in both groups. Blood and lymphatic system disorders were the second most often reported specific system organ class, with dose-related thrombocytopenia being the most frequent AE. Fatigue, mostly Grade 1-2, was also common among patients treated for TIW QW and TIW QOW.

Thyroid function, as monitored by the measurement of TSH and free T4, did not reveal overt hyper- or hypo- thyroidism, with fluctuations in TSH values being within normal limits.

AEs regardless of causality for TIW QW dosing were reported in 664 patients, 99.7% of the safety population for this dosing schedule. The most commonly reported AEs across doses were gastrointestinal (GI), i.e., diarrhea in 406 patients (61.0%) and nausea in 372 patients (55.9%). Thrombocytopenia was the third most frequent AE in 359 patients (53.9%) with the highest frequency in the 40 mg dose level (137 patients; 84%). Fatigue also was commonly seen across dose levels in 326 patients (48.9%) overall. Of note hypothyroidism was reported in 12.9% of patients treated at the dose level of 40 mg, mostly deriving from study [CLBH589E2214] in HL patients who are known to have an increased risk for hypothyroidism.

Grade 3-4 AEs regardless of causality were reported in 534 patients, 80.2% of the safety population for the QW schedule. The most commonly reported Grade 3-4 AEs across doses were thrombocytopenia in 272 patients (40.8%), neutropenia in111 patients (16.7%), anemia in 103 patients (15.5%), fatigue in 82 patients (12.3%) and febrile neutropenia in 46 patients (6.9%). There were more Grade 3-4 hematologic AEs at higher dose levels, with the highest incidences at 40 and 60 mg. The highest incidence of febrile neutropenia was seen at the dose level of 60 mg (27.4%) compared to the other dose levels where the incidence was $\leq$ 3.7%. This could be because this dose level was only tested in leukemia patients, in whom febrile neutropenia is a common AE. Grade 3-4 thrombocytopenia, Grade 3-4 neutropenia and Grade 3-4 anemia accounted for 75.7% (272/359), 82.8% (111/134) and 52.8% (103/195) of their respective all grade events. Grade3-4 diarrhea, Grade 3-4 vomiting and Grade 3-4 nausea accounted for less than 10% of their respective all grades events. For the TIW QOW dosing schedule, AEs regardless of causality were reported in 96 patients, which is 100% of the safety population. The most commonly reported AEs (all grades) across doses were diarrhea in 65 patients (67.7%), nausea in 60 patients (62.5%), fatigue in 54 patients (56.3%), vomiting in 42 patients (43.8%), thrombocytopenia in 41 patients (42.7%), pyrexia in 35 patients (36.5%) and anorexia in 33 patients (34.4%).

In the TIWQOW schedule, Grade 3-4 AEs regardless of causality were reported in 81 patients, 84.4% of the safety population. The most commonly reported Grade 3-4 AEs across doses were thrombocytopenia in 35 patients (36.5%), neutropenia in 25 patients (26.0%), fatigue in 14 patients (14.6%), diarrhea in 11 patients (11.5%), anemia in 10 patients (10.4%), and febrile neutropenia in 8 patients each (8.3%).

Overall, the most frequent Grade 3-4 AEs regardless of causality for both schedules (TIWQW and TIWQOW) were ascribed to the same SOC, namely blood and lymphatic system disorders.

5.2. Carfilzomib

1. Mechanism of Action

Carfilzomib is a modified peptide epoxyketone, an analog of the natural products epoxomicin and eponemycin, that irreversibly binds to the chymotrypsin-like activity of the 20S proteasome. The peptide portion selectively binds in the proteasome with high affinity and the epoxy ketone interacts with the threonine-containing active sites to irreversibly inhibit enzyme activity. The cellular consequences of this inhibition include the accumulation of proteasome substrates and induction of apoptosis. (Demo 2007).

2. Pharmacokinetic

Intensive PK sampling with single- and multiple-dose PK characterization was evaluated in Studies PX-171-005 and PX-171-007. Following repeated doses of carfilzomib at 15 and 20 mg/m2 , systemic exposure and half-life were similar on Days 1 and 15 or 16 of Cycle 1, suggesting there was no systemic carfilzomib accumulation. At doses between 20 and 36 mg/m2 , there was a dose-dependent increase in exposure. The mean steady-state volume of distribution of a 20 mg/m2 dose of carfilzomib was 28 L. When tested in vitro, the binding of carfilzomib to human plasma proteins averaged 97% over the concentration range of 0.4 to 4 micromolar. Peptide fragments and the diol of carfilzomib were the predominant metabolites measured in human plasma and urine, suggesting that peptidase cleavage and epoxide hydrolysis were the principal pathways of metabolism. Cytochrome P450-mediated mechanisms played a minor role in overall carfilzomib metabolism.

A pharmacokinetic study was conducted in which 43 multiple myeloma patients who had various degrees of renal impairment (mild, moderate, and severe) had no effect on the clearance or exposure of carfilzomib. No pharmacokinetic studies were performed with carfilzomib in patients with hepatic impairment.

In an in vitro study using human liver microsomes, carfilzomib showed modest direct and time-dependent inhibitory effect on human cytochrome CYP3A4/5. In vitro studies indicated that carfilzomib did not induce human CYP1A2 and CYP3A4 in cultured fresh human hepatocytes. Cytochrome P450-mediated mechanisms play a minor role in the overall metabolism of carfilzomib. Carfilzomib is not expected to inhibit CYP3A4/5 activities and/or affect the exposure to CYP3A4/5 substrates.

3. Toxicities and Adverse Events

Safety data for single-agent carfilzomib have been analyzed for 526 patients with advanced multiple myeloma who took part in one of 4 phase II studies. The most common adverse events of any grade included fatigue (55.5%), anemia (46.8%), and nausea (44.9%). Grade 3/4 non-hematologic AEs were uncommon (<10% Grade 3 and <1% Grade 4). Thrombocytopenia (23.4%), anemia (22.4%), lymphopenia (18.1%), and pneumonia (10.5%) were the most common AEs Grade 3 or over. Common serious AEs (SAEs) included pneumonia (9.9%), acute renal failure (4.2%), pyrexia (3.4%), CHF (3.4%), dyspnea (2.1%), hypercalcemia (2.1%), and pathological fracture (2.1%). AEs related to study treatment included fatigue (41.4%), nausea (35.2%), thrombocytopenia (28.3%), anemia (26.8%), diarrhea (22.4%), and dyspnea (20.3%). there were 37 deaths on study or within 30 days of last dose across all 4 studies. Of the 37 deaths 7 were considered possibly related by the investigator, these included: cardiac arrest (n=2), hepatic failure (n=1), dyspnea (n=1), multi-organ failure (n=1), cardiac disorder (n=1), and unknown (n=1).A total of 8 cardiac-related deaths were deemed possibly related to carfilzomib per sponsor assessment. The frequency of Grade 3 or over peripheral neuropathy observed with carfilzomib is low (1.3%) (Siegel 2013). Carfilzomib does not induce neurodegeneration *in vitro* via a proteasome-independent mechanism (Arastu-Kapur 2011)

5.3. Dexamethasone

1. Mechanism of Action

Dexamethasone is a synthetic adrenocortical steroid with glucocorticoid activity. It is one of the most active glucocorticoids and has both anti-inflammatory and immunosuppressant activity.

Unbound dexamethasone crosses cell membranes and binds to specific cytoplasmic glucocorticoid receptors; this complex in turn binds to DNA elements, resulting in a modification of transcription and protein synthesis. Anti-inflammatory responses include inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue.

2. Pharmacokinetics

Dexamethasone is quickly absorbed after oral administration, achieving peak plasma concentrations after one hour. Binding to plasma proteins occurs more quickly than for most other corticosteroids. The biological half-life is approximately 190 minutes.

The drug penetrates tissue and cerebrospinal fluids. The metabolism of dexamethasone occurs in most tissues but is primarily done in the liver. Inactive metabolites are excreted through renal functions.

3. Toxicities/Adverse Events

Dexamethasone has been in use for decades; as such, the toxicities and expected adverse events are well-documented.

Allergic reactions: Anaphylactoid reaction, anaphylaxis, angioedema

Cardiovascular: Bradycardia, cardiac arrest, cardiac arrhythmias, cardiac enlargement, circulatory collapse, congestive heart failure, fat embolism, hypertension, myocardial rupture following recent myocardial infarction, edema, pulmonary edema, syncope, tachycardia, thromboembolism, thrombophlebitis, vasculitis

Dermatologic: Acne, allergic dermatitis, dry scaly skin, ecchymoses and petechiae, erythema, impaired wound healing, increased sweating, rash, striae, suppression of reactions to skin tests, thin fragile skin, thinning scalp hair, urticarial

Endocrine: Decreased carbohydrate and glucose tolerance, development of cushingoid state, hyperglycemia, glycosuria, hirsutism, hypertrichosis, increased requirements for insulin or oral hypoglycemic agents in diabetes, manifestations of latent diabetes mellitus, menstrual irregularities, secondary adrenocortical and pituitary unresponsiveness

Fluid and electrolyte disturbances: Congestive heart failure in susceptible patients, fluid retention, hypokalemic alkalosis, potassium loss, sodium retention

Gastrointestinal: Abdominal distention, elevation in serum liver enzyme levels (usually reversible upon discontinuation), hepatomegaly, increased appetite, nausea, pancreatitis, peptic ulcer with possible perforation and hemorrhage, perforation of the small and large intestine (particularly in patients with inflammatory bowel disease), ulcerative esophagitis

Metabolic: Negative nitrogen balance due to protein catabolism

Musculoskeletal: Aseptic necrosis of femoral and humeral heads, loss of muscle mass, muscle weakness, osteoporosis, pathologic fracture of long bones, steroid myopathy, tendon rupture, and vertebral compression fractures

Neurological/Psychiatric: Convulsions, depression, emotional instability, euphoria, headache, increased intracranial pressure with papilledema (pseudotumor cerebri) usually following discontinuation of treatment, insomnia, mood swings, neuritis, neuropathy, paresthesia, personality changes, psychic disorders, vertigo

Ophthalmic: Exophthalmos, glaucoma, increased intraocular pressure, posterior subcapsular cataracts

Other: Abnormal fat deposits, decreased resistance to infection, hiccups, increased or decreased motility and number of spermatozoa, malaise, moon face, weight gain.

6. STUDY DESIGN AND TREATMENT PLAN

6.1. Study Design

This is a phase 2, open labeled, non-randomized trial that will correlate in vitro drug sensitivity testing with clinical outcomes of multiple myeloma patients receiving panobinostat with carfilzomib and dexamethasone. Enrollment is projected as 40 patients over 3 years at a single site.

6.2. Chemosensitivity Assay

Multiple myeloma cells will be procured from the patient blood (if circulating) and bone marrow. Myeloma cells will be isolated after density depletion, and there will be enrichment by magnetic bead separation (CD138+ column). Cells are added to matrix protein coated non-tissue culture-treated 384-well plates at a density of 5,000 cells per well in 50µL of complete media using a Thermo Scientific Matrix WellMate, and incubated overnight to allow attachment. Compounds (50nL) are 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. CellTiter- Glo (Promega) is dispensed into the individual wells with the WellMate. Following 20 minutes incubation on an orbital shaker. luminescence is measured on a Perkin Elmer EnVision Multi-label plate reader. Percentage cell viability is reported as relative to the DMSO solvent control. IC50 values are calculated by fitting data using least squares method to the standard four-parameter logistic model. Curve fitting is performed using IDBS XLFit software (Microsoft Excel).

The results are obtained within 1 week following the specimen collection, and the drug sensitivity curves and effective concentrations that kill 50% of the cells (EC50) are available.

6.3. Treatment

Panobinostat will be dosed at 20mg PO every other week on days 1, 3, 5, 15, 17, and 19 of a 28-day cycle. Carfilzomib will be administered at 20 mg/m² IV on days 1 and 2; then 45 mg/m² IV on days 8, 9, 15, and 16 of the first 28-day cycle. For all subsequent 28-day cycles, carfilzomib will be administered at 45 mg/m² IV on days 1, 2, 8, 9, 15, and 16. Dexamethasone will be dosed at 20mg PO on days of carfilzomib.

Dosing (4-week cycles)	**Week 1 (Day 1-7)**							**Week 2 (Day 8-14)**							**Week 3 (Day 15-21)**							**Week 4 (Day 22-28)**
Panobinostat	1		3		5			Rest period							15		17		19			Rest period
Carfilzomib	1	2						8	9						15	16						Rest period
Dexamethasone	1	2						8	9						15	16						Rest period

NOTE: Adjustments of ± 1 day on days 1, 8, and 15 will be permitted for administrative/scheduling flexibility, as long as infusions are given on two consecutive days each week and there are 6-8 days between the start of each week. Treatment will be initiated in approximately 28-day cycles (+ 14 days).

6.4. Treatment Courses

Patients will be treated until disease progression, patient withdrawal, PI or sponsor decision, or a maximum of 12 cycles.

6.5. Dose Reductions/Modifications

For patients who do not tolerate the protocol-specified dosing schedule, dose modifications (dose interruptions, dose delays, dose reductions, or changes to the dosing scheme) are permitted in order to allow the patient to continue the study treatment. Patients can return to a previous dose level after a dose reduction has been made, per investigator discretion.

1. Panobinostat

Panobinostat dose reduction levels for toxicity:

Dose Level	Dose
Starting dose	20mg TIW, Days 1, 3, and 5, and 15, 17, and 19
1st reduction	15mg TIW, Days 1, 3, and 5, and 15, 17, and 19
2nd reduction	10mg TIW, Days 1, 3, and 5, and 15, 17, and 19
3rd reduction	10mg BIW, Days 1 and 5, and 15 and 19

If permanent discontinuation of panobinostat is required, patients must discontinue all components of study treatment. If a dose of panobinostat is missed, it can be taken up to 12 hours after the scheduled dose. If more than 12 hours have passed, then missed dose should be skipped and treatment continues with next scheduled dose. If vomiting occurs, dose should not be repeated but should be taken at the next usual scheduled time.

2. Carfilzomib

Carfilzomib dose reduction levels for toxicity:

Dose level	Dose
Starting dose	45 mg/m²
1st reduction	36 mg/m²
2nd reduction	27 mg/m²
3rd reduction	Permanently discontinue study treatment

If permanent discontinuation of carfilzomib is required, patients must discontinue all components of study treatment.

3. Dexamethasone

Dexamethasone dose reduction levels for toxicity:

Dose level	Dose
Starting Dose	20mg on days 1, 8, 15 and 20mg on days 2, 9, 16
1st dose reduction	20mg on days 1, 8, 15 and 4mg on days 2, 9, 16
2nd dose reduction	4mg on days 1, 8, 15 and 4mg on days 2, 9, 16

The above dose reduction table is a suggested reduction order. Dexamethasone dose reduction does not have to exactly follow the above dose reduction order. If patients have an intolerance to dexamethasone, the dose can be reduced to any level at any point in the study, per the treating physician's discretion. Study records will document the actual dexamethasone doses. Patients unable to tolerate the minimum dose level of dexamethasone may discontinue dexamethasone and continue study treatment.

7. STUDY PROCEDURES

7.1. 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 will be reviewed to determine eligibility for the study

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

b. ECOG performance status (Appendix A)

c. Concurrent medical conditions.

d. Hematology: CBC with differential and platelet count

e. Serum chemistries: Electrolytes (sodium, potassium, magnesium, phosphorus, 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

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

g. Serum protein electrophoresis, serum free light chains, and 24-hour urine protein electrophoresis as defined by disease characteristics

3. ECG: 12 lead ECG

4. Serum Pregnancy Test: for women of child-bearing potential. Post-menopausal women must have been amenorrhoeic for ≥ 12 months in order to be considered "of non-childbearing potential."

5. A research bone marrow aspirate and core biopsy sample, to occur either at the same time as or independent of a standard of care bone marrow evaluation. If the aspirate and core biopsy contain an inadequate number of plasma cells, another bone marrow aspirate and core biopsy 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 assay. To avoid the risk of another invasive procedure to procure a new sample, samples from locations other than the bone marrow that were procured under other protocols (including, but not limited to, 1757, 7606, 9673) can be used for the chemosensitivity assay if samples are available and viably cryopreserved.

7.2. On Study Evaluations/Procedures

1. Adverse events will be collected from the time therapy is started through 30 days after the last day therapy in administered.

2. Hematology: CBC with differential and platelet count and peripheral blood smear at frequency per standard of care. Bone marrow aspirations by standard practice.

3. Serum chemistries: Electrolytes (sodium, potassium, magnesium, phosphorus, chloride, and bicarbonate), BUN, creatinine, glucose, and liver function tests (AST, ALT, ALP, total bilirubin, LDH) per standard of care.

4. Serum protein electrophoresis, serum free light chains, and 24-hour urine protein electrophoresis as defined by disease per standard practice.

5. ECG: 12-lead ECGs will be performed every 3rd cycle and at the end of treatment. The patient should be resting for approximately 10 minutes prior to each ECG collection timepoint.
6. Serum Pregnancy Test

7.3. Follow-Up Evaluations

1. Response data. All reports for bone marrow evaluations done as standard of care following this treatment, including morphology, flow cytometry, cytogenetics/FISH and mutation studies, will be collected.

2. Subsequent complete blood count, renal function, liver function, SPEP, free light chains, 24hr urine tests obtained for clinical reasons for a period of up to one month post the last dose of chemotherapy, as needed to define toxicity or duration of response.

3. Reports on each patient's course post stem cell transplant, if applicable, will be reviewed to determine response and duration of remission.

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

8. CRITERIA FOR RESPONSE

Response will be evaluated using International Myeloma Working Group Uniform Response Criteria for Multiple Myeloma, which is outlined in Appendix C.

9. DOCUMENT RETENTION

Case report forms, diagnostic pathology reports, and laboratory test results will be kept in a secure location that protects confidential information or electronically with secure access. Follow-up information regarding survival and information about time of relapse and subsequent therapy will also be maintained. Once the last patient has finished the long-term follow-up and all data has been

reviewed, study documents may go to long-term storage, where they will be retained for at least 2 years after the investigation is completed, according to local and federal regulations.

10. REGULATORY AND REPORTING REQUIREMENTS

10.1. Adverse Event Monitoring and Reporting

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade (mild, moderate, severe) or (grade 1-4)
2. Its relationship to the study drug(s) (suspected/not suspected)
3. Its duration (start and end dates or if continuing at final exam)
4. Action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)
5. Whether it constitutes a serious adverse event (SAE)

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the Panobinostat Investigator Brochure (IB) or will be communicated between IB updates in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

The principal investigator is responsible for monitoring the safety of patients who enroll in this study. All non-hematologic toxicities ≥ grade 3 occurring after any administration of the study drug through 30 days after the last administration of therapy will be recorded and followed until resolution. The

descriptions and grading scales found in the most current available NCI CTCAE version will be used for adverse event reporting.

10.2. Serious Adverse Events

A serious adverse event (SAE) is any adverse event that occurs that results in any of the following outcomes:

- Death.
- Life threatening adverse event.
- Hospitalization or prolongation of hospitalization*
- Persistent of significant disability/incapacity.
- A congenital anomaly/birth defect.
- Requires surgical intervention to prevent one of the outcomes listed above.

For the purposes of this study, hospitalizations or prolongation of hospitalizations for protocol-scheduled procedures, blood product transfusions, or for social reasons (i.e. awaiting transport home) will not be considered a SAE.

10.3. Expedited Reporting Requirements

Every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Secura Bio within 24 hours of learning of its occurrence. The form must be faxed within 24 hours to securabio@parexel.com.

Additionally, any Panobinostat study drug misuse or abuse must be reported to securabio@parexel.com.

In accordance with FHCRC/UW Cancer Consortium IRB policy, all adverse events (AEs; whether occurring on-site or off-site), which in the opinion of the principal investigator are (1) unexpected, and (2) related or possibly related to the research, and (3) serious or suggests that the research places research participants or others at a greater risk of physical or psychological harm than was previously known or recognized, will be submitted to the IRB within 10 calendar days of learning of the problem.

AEs that do not meet the requirement for expedited reporting will be reported to the IRB as part of the annual renewal of the protocol.

10.4. Pregnancy Reporting Requirements

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Secura Bio within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be reported by the investigator to Secura Bio and should include the period of gestation at the time of exposure, dates of drug exposure, an assessment of the possible relationship to the panobinostat. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

10.5. Concomitant Medications

Concomitant medications are all medications or treatments other than the study drugs that are taken or received by the subject. Concomitant medications will be assessed and recorded from the time of first dose of study drug through the end of treatment.

11. DATA AND SAFETY MONITORING PLAN

The Principal Investigator will carry out ongoing trial oversight and will meet frequently with the study team to review recently acquired data and adverse events. The data recorded within the research charts and protocol database is compared with the actual data that is available from the medical record and/or clinical histories. All investigators on the protocol have received formal training in the ethical conduct of human research. The Principal Investigator will receive monitoring support as described below. Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC 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), FHCRC Scientific Review Committee (SRC) and the FHCRC/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 patients. 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. STATISTICAL CONSIDERATIONS

The primary objective of this study is to compare the in vitro sensitivity of various single agents as well as combinations of these agents between patients who respond to their treatment and patients who do not respond to their treatment. Towards this end, we will use the IC50 as a measure of "sensitivity", and in doing so; we will then compare the mean sensitivity in responders to the mean sensitivity in non-responders. We plan to enroll a total of 40 patients, and we expect a response rate of approximately 30%, thereby yielding roughly 12 responders and 28 non-responders. With 40

patients and this projected response rate, we'll have 90% power to detect a statistically significant difference (at the one-sided significance level of .05) if the true mean IC50 for responders and non-responders are 1.03 standard-deviation units apart.

This power calculation was done using the two-sample t-test, but when the data are analyzed we will potentially use either a transformation or use the rank-sum test as appropriate.

If there is sufficient evidence to suggest that the true probability of death exceeds 5%, the study will be suspended pending a detailed review by a DSMB. Sufficient evidence will be taken to be an observed proportion with a lower one-sided 80% confidence limit in excess of 5%. These proportions will be examined after every 10^{th} patient is enrolled and evaluable. Any of the following would trigger such a rule: 2 of the first 10 (or fewer) patients, 3 of the first 30 or fewer, 4 of the first 40 or fewer. If the true probability of death is .01, then the probability of such a trigger occurring after 20 or 40 patients is approximately .007 and .009, respectively. If the true probability is .16, the probability of such a trigger after 20 or 40 patients is approximately .70 and .93, respectively (probabilities estimated from 5,000 simulations).

13. REFERENCES

Arastu-Kapur S, Anderl JL, Kraus M, et al. (2011) Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res;17(9): 2734–4

Berdeja JG, Hart LL, Mace JR, et al (2015) Phase I/II study of the combination of panobinostat and in patients with relapsed/refractory multiple myeloma. Haematologica.;100(5):670-6.

Berdeja JG, Gregory TB, Faber EA et al. (2015) A Phase I/II Study of the Combination of Panobinostat(PAN) and Carfilzomib (CFZ) in Patients (pts) with Relapsed or Relapsed/Refractory Multiple Myeloma (MM): Comparison of Two Expansion Cohorts. ASH abstract 1825, 2015

Berenbaum MC. (1978) A method for testing for synergy with any number of agents. J Infect Dis;137:122-30.

Biggs JR, Kudlow JE and Kraft AS (1996) The role of the transcription factor Sp1 in regulating the expression of the WAF1/CIP1 gene in U937 leukemic cells. J Biol Chem; 271:901-6.

Demo SD, Kirk CJ, Aujay MA, et al. (2007) Anti-tumor activity of PR-171, a novel irreversible inhibitor of the proteasome. Cancer Res; 67(13):6383-91.

Fuino L, Bali P, Wittmann S, et al (2003) Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. Mol Cancer Ther; 2:971-84.

Gelmetti V, Zhang J, Fanelli M, et al (1998) A. Aberrant recruitment of the nuclear receptor corepressor-histone deacetylase complex by the acute myeloid leukemia fusion partner ETO. Mol Cell Biol; 18:7185-92.

Grignani F, DeMatteis S, Nervi C, et al (1998) Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase complex in promyelocytic leukemia. Nature (Lond); 391:815-8.

Herman JG, Latif F, Weng Y, et al (1994) Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci U S A; 91:9700-4.

Herman JG, Merlo A, Mao L, et al (1995) Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res; 55:4525-30.

Herman JG, Jen J, Merlo A, et al (1996) Hypermethylation-associated inactivation indicates a tumor suppressor role for p15 (INK4B). Cancer Res; 56:722-7.

Herman JG, Umar A, Polyak K, et al (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A; 95:6870-5.

Itazaki H, Nagashima K, Sugita K, et al (1990) Isolation and structural elucidation of new cyclotetrapeptides, trapoxin A and B, having detransformation activities as antitumor agents. J Antibiotics; 12:1524-32.

Lin RJ, Nagy L, Inoue S, et al (1998) Role of the histone deacetylase complex in acute promyelocytic leukemia. Nature (Lond); 391:811-4.

Merlo A, Herman JG, Mao L, et al (1995) 5’ CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDKN2/MTS1 in human cancers. Nat Med; 1:686-92.

Nakano K, Mizuno T, Sowa Y, et al (1997) Butyrate activates the WAF1/Cip1 gene promoter through SP1 sites in a p53-negative human colon cell line. J Biol Chem; 272:22199-206.

Nimmanapalli R, Fuino L, Bali P, et al (2003) Histone Deacetylase Inhibitor LAQ824 Both Lowers Expression and Promotes Proteasomal Degradation of Bcr-Abl and Induces Apoptosis of Imatinib Mesylate-sensitive or -refractory Chronic Myelogenous Leukemia-Blast Crisis Cells. Cancer Res; 63:5126-35.

Redner RL, Wang J and Liu JM (1999) Chromatin remodeling and leukemia: new therapeutic paradigms. Blood; 94:417-28.

Richardson PG, Schlossman RL, Alsina M, et al (2013) PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma. Blood; 122(14):2331-2337.

Richardson PG[1], Weller E, Lonial S, et al (2010) Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood; 116(5):679-86

Sambucetti LC, Fischer DD, Zabludoff S, et al (1999) Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. J Biol Chem. 3;274(49):34940-7.

San-Miguel JF, Hungria VT, Yoon SS, et al. (2014) Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. Lancet Oncol;15(11):1195-206.

San-Miguel J, Richardson P, Günther A, et al. (2013) Phase Ib study of panobinostat and bortezomib in relapsed or relapsed and refractory multiple myeloma. J Clin Oncol 31: 3696–3703.

Siegel D, Martin T, Nooka A, et al (2013) Integrated safety profile of single-agent carfilzomib: experience from 526 patients enrolled in 4 phase II clinical studies Haematologica; 98(11): 1753–1761

Siegel DS, Martin T, Wang M, et al. (2012) A phase 2 study of single-agent carfilzomib (PX-171- 003-A1) in patients with relapsed and refractory multiple myeloma. Blood;120 (14):2817-2825.

Sowa Y, Orita T, Minamikawa S, et al (1997) Histone deacetylase inhibitor activates the WAF1/Cip1 gene promoter through the Sp1 sites. Biochem Biophys Res Commun; 241:142-50.

Steel GG, Peckham MJ. (1979) Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys; 5: 85–93.

Sugita K, Koizumi K and Yoshida H (1992) Morphological reversion of sis-transformed NIH3T3 cells by trichostatin A. Cancer Res; 52:168-72

Vij R, Siegel DS, Jagannath S, et al. (2012) An openlabel, single-arm, phase 2 study of single agent carfilzomib in patients with relapsed and/or refractory multiple myeloma who have been previously treated with bortezomib. Br J Haematol;158(6):739-748

Yoshida M and Beppu T (1988) Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A. Exp Cell Res; 177:122-31.

Yoshida M, Hoshikawa Y, Koseki K, et al (1990) Structural specificity for biological activity of trichostatin A, a specific inhibitor of mammalian cell cycle with potent differentiation-inducing activity in Friend leukemia cells. J Antibiotics; 43:1101-6.

Yoshida M, Nomura S and Beppu T (1987) Effects of trichostatins on differentiation of murine erythroleukemia cells. Cancer Res; 47:3688-91.

Yoshida M and Sugita K (1992) A novel tetrapeptide, trapoxin, induces phenotypic change from transformed to normal in sis-oncogene-transformed NIH3T3 cells. Jpn J Cancer Res; 83:324-8.

Yu X, Guo Z, Marcu M, et al (2002) Modulation of p53, ErbB1, ErbB2, and Raf-1 Expression in Lung cancer Cells by Depsipeptide FR901228. J Natl Cancer Inst; 94:504-13.

APPENDICES

Appendix A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B: Schedule of Events

The following table is a representation of what may occur during participation on this trial, variations from this schedule will be done per physician discretion and will not be considered a protocol deviation.

Procedure	Screening	Treatment 28-day cycle (+ 14 days)	Follow-Up
Informed Consent	x		
Physical Exam w/ ECOG	x		
Medical History	x		
Pregnancy Test	x	x	
CBC	x	x	x
Serum Chemistries	x	x	x
Tumor Markers	x	x	x
Chemosensitivity Assay	x		
Bone Marrow Evaluations	x		x (only required if needed to determine response)
Electrocardiograms	x	x (every 3 months)	x
Study Agent Therapy		x	
AE Assessment		x	x
Concomitant Meds	x	x	x
Survival			x

Appendix C: International Myeloma Working Group Uniform Response Criteria for Multiple Myeloma

Response	IMWG Criteria
sCR	• CR as defined below, *plus* • Normal FLC ratio, *and* • Absence of clonal cells in bone marrow[3] by immunohistochemistry or immunofluorescence[4]
CR	• Negative immunofixation on the serum and urine, *and* • Disappearance of any soft tissue plasmacytomas, *and* • < 5% plasma cells in bone marrow[3]
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/24 h
PR	• ≥ 50% reduction of serum M-protein and reduction in 24 hours urinary M-protein by ≥ 90% or to < 200 mg/24h • If the serum and urine M-protein are not measurable,[5] 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 not measurable, and serum free light assay is also not measurable, ≥ 50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥ 30% • In addition to the above listed criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
MR	• ≥ 25% but < 49% reduction of serum M protein and reduction in 24-hour urine M protein by 50-89%, which still exceeds 200 mg per 24 hour • In addition to the above criteria, if present at baseline, 25-49% reduction in the size of soft tissue plasmacytomas is also required • No increase in size or number of lytic bone lesions (development of compression fracture does not exclude response)
No change/Stable disease	• Not meeting criteria for CR, VGPR, PR, MR, or progressive disease

Progressive Disease	• Increase of ≥ 25% from lowest response value in any one or more of the following:
	  ○ Serum M-component (the absolute increase must be ≥ 0.5 g/dL)[6], *and/or*
	  ○ Urine M-component (the absolute increase must be ≥ 200 mg/24 h), *and/or*
	• Only in patients without measurable serum and urine M-protein

Response	IMWG Criteria
	levels: the difference between involved and uninvolved FLC levels. The absolute increase must be > 10 mg/dL • Only in patients without measurable serum and urine M-protein levels and without measurable disease by FLC levels: bone marrow plasma cell percentage (absolute percentage must be ≥ 10%)[7] • Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas • Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.65 mmol/L) that can be attributed solely to the plasma cell proliferative disorder
Relapse	Clinical relapse requires one or more of: • Direct indicators of increasing disease and/or end organ dysfunction (CRAB features).[6] It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice • Development of new soft tissue plasmacytomas or bone lesions • Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion • Hypercalcemia (> 11.5 mg/dL) [2.65 mmol/L] • Decrease in hemoglobin of ≥ 2 g/dL [1.25 mmol/L] • Rise in serum creatinine by 2 mg/dL or more [177 mol/L or more]
Relapse from CR[5] (to be used only if the endpoint studied is DFS)[8]	Any one or more of the following: • Reappearance of serum or urine M-protein by immunofixation or electrophoresis • Development of ≥ 5% plasma cells in the bone marrow[7] • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcemia)

Overall Responders: PR, VGPR, CR, sCR Clinical Benefit Rate: Overall responders + MR

Adapted from Durie BGM, et al. Leukemia 2006; 20: 1467-1473; and Kyle RA, Rajkumar SV. Leukemia 2008;23:3-9.

Note: A clarification to IMWG criteria for coding CR and VGPR in patients in whom the only measurable disease is by serum FLC levels: CR in such patients is defined as a normal FLC ratio of 0.26–1.65 in addition to CR criteria listed above. VGPR in such patients is defined as a >90% decrease in the difference between involved and uninvolved free light chain (FLC) levels.
3 Confirmation with repeat bone marrow biopsy not needed.

Presence/absence of clonal cells is based upon the kappa/lambda ratio. An abnormal kappa/lambda ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is kappa/lambda of >

4:1 or < 1:2.

All relapse categories require two consecutive assessments made at any time before classification as relapse or disease progression and/or the institution of any new therapy. In the IMWG criteria, CR patients must also meet the criteria for progressive disease shown here to be classified as progressive disease for the purposes of calculating time to progression and progression-free survival. The definitions of relapse, clinical relapse and relapse from CR are not to be used in calculation of time to progression or progression free survival

For progressive disease, serum M-component increases of ≥1 gm/dL are sufficient to define relapse if starting M-component is ≥5 g/dL.

Relapse from CR has the 5% cut-off versus 10% for other categories of relapse.

For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease.