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CLINICAL TRIAL PROTOCOL

Phase I Study of Plitidepsin (Aplidin®) in Combination with Bortezomib and Dexamethasone in Patients with Relapsed and/or Refractory Multiple Myeloma

INVESTIGATIONAL MEDICINAL PRODUCT: **plitidepsin (Aplidin®)**
bortezomib (Velcade®)
dexamethasone

Protocol No.: APL-A-012-13
EudraCT No.: 2013-003835-31
NCT Code: 02100657

Version 3.0 (including Amendment #2): 8 October 2015

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dexamethasone

Eudra CT : 2013-003835-31

Version 3.0 (including Amendment #2): 8 October 2015

This study will be conducted in compliance with the protocol, GCP and applicable regulatory requirements.

STUDY CONTACTS	NAME AND ADDRESS	CONTACT DETAILS
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SYNOPSIS

TITLE	Phase I Study of Plitidepsin (Aplidin®) in Combination with Bortezomib and Dexamethasone in Patients with Relapsed and/or Refractory Multiple Myeloma
PROTOCOL CODE	APL-A-012-13
INVESTIGATORS / STUDY LOCATION	A complete list will be provided as a separate document.
STUDY OBJECTIVES **Primary**	• To determine the recommended dose (RD) of plitidepsin in combination with bortezomib and dexamethasone in patients with relapsed and/or refractory multiple myeloma (MM).
Secondary	• To determine the efficacy of the combination plitidepsin/ bortezomib/dexamethasone. • To evaluate the safety and tolerability of the combination in patients with relapsed and/or refractory MM. • To study the pharmacokinetics (PK) and pharmacodynamics (PDy) of plitidepsin in combination with bortezomib and dexamethasone.
STUDY DESIGN	This is a phase I dose finding study in patients with relapsed and/or refractory MM. Patients will be enrolled sequentially into three dose levels (DLs). The feasibility of administering plitidepsin in combination with bortezomib and dexamethasone and the RD of the combination will be determined. • Plitidepsin will be administered as a 3-hour (h) intravenous (i.v.) infusion on Day (D) 1 and 15, every four weeks (q4wk). • Bortezomib will be administered as a subcutaneous (s.c.) injection on D1, 4, 8 and 11, q4wk, for a maximum of eight cycles. • Dexamethasone will be taken orally on D1, 8, 15 and 22, q4wk.
STUDY POPULATION	Patients with relapsed and/or refractory MM. • **Refractory myeloma** is defined as disease that is non-responsive while on primary or salvage therapy, or progresses within 60 days of the last therapy. There are two categories of refractory myeloma: ✓ **Primary refractory myeloma** is defined as disease that is non-responsive in patients who have never achieved a minimal response (MR) or better, with any therapy. It includes patients who never achieve MR or better in whom there is no significant change in monoclonal protein (M-protein) and no evidence of clinical progression as well as primary, refractory disease progression (PD) where patients meet criteria for true PD. ✓ **Relapsed and refractory myeloma** is defined as disease that is non-responsive while on salvage therapy, or progresses within 60 days of the last therapy in patients who have achieved MR or better at some point previously

	before progressing. • **Relapsed myeloma** is defined as previously treated myeloma that progresses and requires the initiation of salvage therapy but does not meet the criteria for either **"primary refractory" or "relapsed-and-refractory"** myeloma categories.
INCLUSION CRITERIA	1) Patients must give written informed consent (IC) in accordance with institutional and local guidelines. 2) Age ≥ 18 years. 3) Patients must have a confirmed diagnosis of MM according to the Durie and Salmon criteria. 4) Patients must have relapsed and/or refractory disease. 5) Patients must have measurable disease defined as any of the following: a) Serum M-protein > 0.5 g/dL or > 0.2 g/24-h urine light chain (FLC) excretion. a) In patients who lack measureable M-protein in serum or urine, i.e. serum M-protein < 0.5 g/dL and urine M-protein < 0.2 g/24 h, serum free light chain (SFLC) levels are most informative. SFLC levels can be used only if the baseline SFLC ratio is abnormal (<0.26 or >1.65), indicating clonality. In addition, the baseline SFLC level must be ≥10 mg/dl of the appropriate involved light chain isotype. b) When applicable, measurable soft tissue plasmacytoma > 2 cm, by either physical examination and/or applicable radiological evaluation (i.e. magnetic resonance imaging [MRI], computed tomography [CT]-scan). 6) Prior autologous and/or allogeneic hematopoietic stem cell transplantation (HSCT) patients are allowed. Patients must not have acute/chronic graft-versus-host disease (GVHD) or be receiving immunosuppressive therapy at least 30 days before the onset of treatment with the study drug(s). 7) Patients must have received at least one previous treatment line, which can consist of: a) Induction regimen followed by high dose chemotherapy (CT) and peripheral blood stem cell collection. b) Induction regimen alone according to institutional guidelines. c) High doses of CT followed by non-myeloablative transplantation. d) CT followed by either single or tandem autologous stem cell transplantation (ASCT). e) CT followed by autologous and (if performed) subsequent non-myeloablative allogeneic stem cell transplantation. f) Previous line(s) of systemic CT and biological agents should have been completed at least 30 days and 15 days, respectively, prior to starting protocol treatment. 8) Previous treatment with bortezomib or another proteasome inhibitor (PI) is allowed provided patients achieved at least

	MR lasting a minimum of two months. 9) Patients must have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2. 10) Recovery to grade ≤ 1 from any non-hematological adverse event (AE) derived from previous treatment (excluding alopecia). 11) Laboratory data: a) Hemoglobin ≥ 8 g/dL. b) Absolute neutrophil count (ANC) ≥ 1,000 cells/mm^3 (1.0 x 10^9/L) (≥ 0.5 x 10^9/L if due to extensive bone marrow [BM] involvement –by ≥ 50% of plasma cells in BM biopsy). Screening of ANC should be independent of granulocyte- and granulocyte/macrophage-colony stimulating factor (GCSF and GM-CSF) support for at least one week and of pegylated G-CSF for at least two weeks. c) Platelet count ≥ 50,000/ mm^3 (50.0 x 10^9/L) for patients in whom < 50% of the BM nucleated cells are plasma cells. d) Platelet count ≥ 25,000/ mm^3 (25.0 x 10^9/L) for patients in whom ≥ 50% of BM nucleated cells are plasma cells. e) Serum total bilirubin < 1.5 x institutional upper limit of normal (ULN) (except when Gilbert syndrome is clearly documented and other liver function tests are within normal levels). f) AST (aspartate aminotransferase) and ALT (alanine aminotransferase) ≤ 3.0 x institutional ULN and AP (alkaline phosphatase) ≤ 2.5 x institutional ULN. g) Creatinine clearance (CrCl) > 30 mL/min, measured or calculated according to Cockcroft and Gault's formula. h) Albumin ≥ 2.5 g/dl. 12) Women of child-bearing potential must have a negative serum or urine pregnancy test within seven days prior to enrolment. In addition, all sexually active women of child-bearing potential and fertile male patients must agree to use adequate contraceptive methods throughout the study and during six months after treatment discontinuation. 13) Left ventricular ejection fraction (LVEF) above the lower limit of normal. 14) Patients must have a BM assessment within three weeks prior to enrolment.
EXCLUSION CRITERIA	1) Previous treatment with plitidepsin. 2) Active or metastatic primary malignancy other than MM. 3) Serious concomitant systemic disorders that would compromise the safety of the patient or the patient's ability to complete the study, including the following specific conditions: a) Uncontrolled psychiatric illness or medical illness that the Investigator feels will compromise the patient's tolerance of the study medication.

	b) Significant non-neoplastic liver disease. c) Uncontrolled endocrine diseases (i.e., requiring relevant changes in medication within the last month, or hospital admission within the last three months). d) Uncontrolled systemic infection. 4) Other relevant cardiac conditions: a) Symptomatic arrhythmia (excluding anemia-related grade ≤ 2 sinusal tachycardia) or any arrhythmia requiring ongoing treatment, and/or prolonged grade ≥ 2 QT-QTc; or presence of unstable atrial fibrillation (according to the National Cancer Institute Common Terminology Criteria for the Classification of Adverse Events [NCI-CTCAE] v4.0). Patients on treatment for stable atrial fibrillation are allowed, provided they do not meet any other cardiac or prohibited drug exclusion criterion. b) History or presence of unstable angina, myocardial infarction, valvular heart disease, cardiac amyloidosis or congestive heart failure within the last 12 months. c) Uncontrolled arterial hypertension (≥ 150/100 mmHg) despite optimal medical therapy. d) Previous treatment with doxorubicin at cumulative doses of > 400 mg/m². 5) History of hypersensitivity reactions to bortezomib, polyoxyl 35 castor oil or mannitol. 6) Myopathy or any clinical situation that causes significant and persistent elevation of creatine phosphokinase (CPK) (> 2.5 ULN) in two different determinations performed within one week of each other. 7) Sequelae of any grade ≥ 1 neuropathy (either bortezomib-related or not) according to NCI-CTCAE v4.0. 8) Any other major illness that, in the Investigator's judgment, will substantially increase the risk associated with the patients' participation in this study. 9) Pregnant and/or lactating women. 10) Known active human immunodeficiency virus (HIV) infection (HIV testing is not required unless infection is clinically suspected). 11) Active hepatitis B or C virus (HBV or HCV) infection. 12) Treatment with any Investigational Medicinal Product (IMP) in the 30 days before inclusion in the study. 13) Concomitant medications that include corticosteroids, CT, or other therapy that is or may be active against myeloma. Concurrent corticosteroids are allowed as an equivalent to a prednisone dose of ≤ 10 mg daily, administered as an antiemetic or as premedication for blood products. 14) Wash-out periods after the end of the previous therapy: a) Nitrosoureas must be discontinued six weeks prior to Cycle (C) 1, D1. b) Thirty days for other CTs and 15 days for other biological

	agents prior to C1 D1. c) Thirty days after the end of any prior radiation or radionuclide therapy (six weeks in the case of prior extensive external beam radiation, with more than 25% of BM distribution). 15) Plasma cell leukemia at the time of study entry. 16) Disease-related symptomatic hypercalcemia despite optimal medical therapy. 17) Limitation of the patient's ability to comply with the treatment or follow-up protocol. 18) Contraindication for the use of steroids.
EXPECTED NUMBER OF PATIENTS	Cohorts of 3-6 patients per dose level (up to three DLs) will be treated until the RD is defined. At the RD, at least six patients evaluable for determination of dose-limiting toxicities (DLTs) will be treated. A total of 20-30 patients are expected to participate. However, the number of patients may vary depending upon the tolerability of the combination and the number of DLs required to identify the RD.
EXPECTED NUMBER OF CENTERS	Five to 10 centers are expected to participate in the clinical trial. A complete list of centers will be provided as a separate document.
STUDY DRUGS FORMULATION	**Plitidepsin (Aplidin®)** is supplied as a lyophilized product in a glass vial containing 2 mg. The lyophilized powder is a concentrate for solution and contains plitidepsin as the active ingredient and mannitol as the inactive ingredient. The reconstitution solvent is supplied in ampoules, each containing 4 mL of polyoxyl 35 castor oil/ethanol/WFI (15/15/70% v/v/v). Plitidepsin vials and reconstitution ampoules should be stored in a locked area with limited access at 2 to 8ºC (36ºF to 46ºF) and protected from exposure to light. **Bortezomib (Velcade®)** is available for i.v. or s.c. injection use. Each single dose vial contains 3.5 mg of bortezomib as a sterile lyophilized powder. For further information on the drug product, please refer to the EU SmPC or the USP. **Dexamethasone** is administered orally as tablets that should be stored in well-closed containers.
TREATMENT SCHEDULE	**A treatment cycle** consists of oral dexamethasone administered on D1, 8, 15 and 22, q4wk at least one hour before a 3h i.v. infusion of plitidepsin on D1 and 15, q4wk, and immediately followed by a 3-5 second bolus s.c. injection of bortezomib on D1, 4, 8 and 11, q4wk. **Treatment cycles will be repeated q4wk**. Patients will receive a maximum of eight treatment cycles. If the patient responds to treatment or achieves stable disease (SD) during this time, treatment with plitidepsin and dexamethasone may continue in further cycles at the same plitidepsin dose upon Investigator's decision and agreement with the Sponsor.

CRITERIA FOR TREATMENT CONTINUATION

Before the administration of each dose (re-treatment), patients must fulfill the baseline criteria, as defined in the following table:

	Plitidepsin	**Bortezomib**
	Day 1[a] and 15[b]	**Day 1[a*], 4[b], 8[b] and 11[b]**
ANC	1.0 x 10^9/L (≥ 0.5 x10^9/L if due to extensive BM involvement)	1.0 x 10^9/L (≥ 0.5 x10^9/L if due to extensive BM involvement)
Platelets	≥ 50.0 x 10^9/L (≥ 25.0 x 10^9/L if ≥ 50% of BM nucleated cells are plasma cells)	≥ 50.0 x 10^9/L (≥ 25.0 x 10^9/L if ≥ 50% of BM nucleated cells are plasma cells)
Hemoglobin	≥ 8.0 g/dL	≥ 8.0 g/dL
Serum total bilirubin	≤ 1.5 x ULN[e]	≤ 1.5 x ULN[e]
AST/ALT/AP	≤ 2.5 x ULN	≤ 2.5 x ULN
Muscular toxicity (myalgia, muscular weakness, CPK increase)	< Grade 2	< Grade 2
Other non-hematological drug-related AEs (except for increased GGT, non-optimally treated nausea and vomiting, hypertension, alopecia)[c]	< Grade 2	< Grade 2
ECG, ECHO/MUGA[d]	Baseline values	Baseline values

[a]If a patient does not meet the requirements for treatment continuation on D1 of the following cycle, the infusion of study drugs will be withheld until recovery or for a maximum of 14 days. After this period, if the delay is due to toxicity assessed as related to a study drug, a dose reduction by one DL is mandatory; up to a maximum of two individual dose reductions are allowed. Patients needing additional dose reductions must be withdrawn from the study.

[a*]From C2 onwards, Hematology and Biochemistry A will only be collected on D1 and 15, hence only re-treatment criteria other than Hematology/Biochemistry A will apply on D4, 8 and 11.

[b]If a patient does not meet the requirements for treatment continuation on Day 15 (plitidepsin) or D4, 8 or 11 (bortezomib), the administration of plitidepsin or bortezomib, respectively, will be omitted. Patients requiring frequent dose omissions may have a dose reduction by one DL upon Investigator and Sponsor's agreement. Under no circumstances will more than two dose reductions be allowed.

[c]Any grade accepted for increased GGT.

[d]To be performed every three months unless more frequent assessments are clinically indicated.

[e]Except when Gilbert syndrome is clearly documented and other liver function tests are normal.

AEs, adverse event(s); ALT, alanine aminotransferase; ANC, absolute neutrophil count; AP, alkaline phosphatase; AST, aspartate aminotransferase; BM, bone marrow; CPK, creatine phosphokinase; D, day; DL, dose level; ECG, electrocardiogram; ECHO/MUGA, echocardiogram/multiple-gated acquisition scan; GGT, γ-glutamyltranspeptidase; L, liter; ULN, upper limit of normality.

The following guidelines must be followed before the administration of each bortezomib dose:

Severity of PN signs and symptoms*	Recommended modification of bortezomib dose and regimen
Grade 1 (paresthesia; weakness and/or loss of reflexes) without pain or loss of function	Reduce current bortezomib dose by one DL (1.3 to 1.0 mg/m^2) or, for patients receiving a twice-weekly schedule, change to a once-per-week schedule using the same dose.
Grade 1 with pain or grade 2 (with no pain but limiting instrumental ADLs**)	For patients receiving twice-weekly bortezomib, reduce current dose by one DL or change to a once-per-week schedule using the same dose. For patients receiving bortezomib on a once-per-week schedule, reduce current dose by one DL, or consider temporary discontinuation; upon resolution to grade ≤ 1, restart once-per-week dosing at a lower DL in cases of favorable benefit/risk ratio.
Grade 2 with pain or grade 3 (limiting selfcare and ADL***) or grade 4	Discontinue bortezomib

*Based on posology modifications in phase II and III MM studies and post-marketing experience. Grading based on NCI-CTCAE v 4.0.
**Instrumental ADL: refers to preparing meals, shopping for groceries or clothes, using telephone, managing money, etc.
***Self care ADL: refers to bathing, dressing and undressing, feeding self, using the toilet, taking medicinal products, and not bedridden.
ADL, activities of daily living; DL, dose level; MM, multiple myeloma; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for the Classification of Adverse Events; PN, peripheral neuropathy.

The criteria for treatment continuation with dexamethasone are to be implemented independently from those of plitidepsin and bortezomib. Dexamethasone treatment will therefore not be delayed in parallel to plitidepsin/bortezomib; if a patient experiences grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations), or drug-related grade ≥ 3 fatigue, or grade ≥ 2 mood disturbances or agitation or grade ≥ 3 fluid retention or grade 4 clinically documented infection, the dose of dexamethasone will be reduced by 50%, up to a maximum of two consecutive dose reductions (20 mg D1, 8, 15 and 22, and 20 mg D1 and 15 of each 28-day cycle). After two dose reductions, dexamethasone will be discontinued.

DOSE ESCALATION SCHEME

Dose escalation for plitidepsin and bortezomib will follow the rules shown in the table:

DL	Number of Patients	Plitidepsin dose (mg/m^2)	Bortezomib dose (mg/m^2)	Dexamethasone dose (mg)
-1	0-6	3.0	1.0	40.0
1	3-6	4.0	1.0	40.0
2	3-6	4.0	1.3	40.0
3	3-6	5.0	1.3	40.0

DL, Dose level.

Cohorts of three patients will be treated at each DL (up to three DLs):

- The first cohort of three patients will start at DL1.
- If no DLTs are observed in any of the three patients at a given DL, three additional patients will be treated at the next DL.

	• If a DLT is observed in one of three patients treated at a given DL, three additional patients will be entered at that same DL. • If DLTs are observed in two of three patients, no additional patients will be treated at that DL and the immediately lower DL will be expanded to at least six patients. • If two out of six patients present DLTs at DL1, the next patients enrolled will be treated at DL -1. If two out of six patients (≥33%) present DLTs at this DL, the study will be stopped and that DL will be considered the maximum tolerated dose (MTD). • The RD is defined as the DL at which fewer than two out of six patients (33% of patients) experience DLTs during the first cycle. • At the RD, at least six evaluable patients for the determination of DLTs will be treated. • At DL1, one patient must have completed the first cycle before accrual of the second and third patients. The second and third patients may be treated simultaneously. • Intermediate dose escalation or de-escalation is allowed. Intrapatient dose escalation is not allowed.
DOSE LIMITING TOXICITY	**Dose limiting toxicities (DLTs)** will be defined according to the following criteria: *Hematological Toxicity*: • Grade 3-4 neutropenia associated with fever or lasting > 7 days, considered related to the study drug(s) by the Investigator. • Grade 3-4 thrombocytopenia accompanied by grade 3/4 hemorrhage. • For patients with extensive BM infiltration (≥ 50% of BM nucleated cells are plasma cells), DLT is defined as grade 4 thrombocytopenia with grade 3/4 hemorrhage or grade 4 neutropenia lasting more than seven days or with fever. *Non-hematological Toxicity* • Grade 3/4 nausea and vomiting refractory to antiemetic therapy. • Grade ≥ 3 muscular AEs (myalgia, muscular weakness, muscle cramps, myopathy). • Grade ≥ 3 ALT/AST lasting more than one week. • Grade ≥ 3 bilirubin increase. • Grade ≥ 3 CPK increase. • Cardiac Toxicity: ✓ Symptomatic or treatment-requiring grade ≥ 1 cardiac arrhythmia related to plitidepsin. ✓ Grade ≥ 1 left ventricular systolic dysfunction related to plitidepsin. • Neuropathic pain and peripheral sensory neuropathy related to bortezomib will be considered a DLT if they result in a definitive bortezomib discontinuation according to International Myeloma Working Group (IMWG) guidelines.

	• Any other grade ≥ 3 toxicity considered related to study treatment by the Investigator.
DEFINITION OF RECOMMENDED DOSE	The RD will be the highest DL at which fewer than two out of six (33%) patients experience DLTs during the first 28-day cycle.
PHARMACOKINETICS	Pharmacokinetic (PK) analyses will be evaluated by standard non-compartmental analysis (population pharmacokinetic modeling may be performed if appropriate). Samples for PK analysis will be obtained during Cycle 1 exclusively.
EFFICACY EVALUATION CRITERIA	Patients are evaluable for efficacy if they receive at least one complete treatment cycle (two plitidepsin infusions, four bortezomib injections, four doses of dexamethasone), or the equivalent doses over two cycles and have, at least, one disease assessment. Efficacy will be evaluated according to IMWG criteria: • Overall response rate (ORR), including stringent complete response (sCR), complete response (CR), very good partial response (VGPR) and partial response (PR). • Minimal response (MR). • Stable disease (SD). • Clinical benefit rate, including ORR plus MR plus SD. • Duration of response (DOR). • Time to progression (TTP). • Progression–free survival (PFS). • Event-free survival (EFS).
SAFETY EVALUATIONS	Patients are evaluable for safety if they receive at least one (complete or incomplete) dose of plitidepsin. AEs will be graded according to NCI-CTCAE v 4.0.
STATISTICAL METHODS	• The RD will be descriptively determined according to the evaluation of DLTs. • ORR and clinical benefit rate will be calculated with binomial exact 95% confidence intervals (CI). • DOR, TTP, PFS and EFS will be calculated by Kaplan-Meier estimates with 95% CI. • Safety evaluations will be performed in a descriptive fashion.
DURATION OF STUDY	The total duration of the study will be approximately 24 months.
REPLACEMENT OF PATIENTS	Patients must be replaced if: • They are withdrawn from the study due to not being evaluable for the primary endpoint, due to hypersensitivity reactions or reasons other than drug-related AEs meeting DLT criteria (e.g., consent withdrawal, not meeting the eligibility criteria, non-

	compliance with follow-up, early PD, or unrelated AEs). • They require radiation therapy or other anticancer procedure within four weeks after the first dose, unless they previously had another drug-related AE included in the definition of DLT. • There is a protocol deviation/s precluding conclusions on the safety of the study therapy.
PLANNED STUDY PERIODS (individually per patient)	Patients will be evaluated at scheduled visits in three study periods: • **Pre-treatment:** from signature of the IC to the first study drug infusion. • **Treatment:** from first infusion of study drugs to end of treatment (EOT). • **Follow-up:** after EOT, patients will be followed q4wk until resolution of all toxicities, if any. Patients who discontinued treatment without disease progression will be followed every three months until disease progression, other antitumor therapy, death or until the date of study termination (clinical cut-off), whichever occurs first. Patients will be considered to be on-study from the signature of the IC to the end of the follow-up period. Patients will be considered to be on-treatment for the duration of their treatment and until the day of EOT, immediately before the start of the follow-up period. EOT is defined as 30 days after the day of last treatment, unless the patient starts a new antitumor therapy or dies (whichever occurs first), in which case the date of administration of this new therapy or the date of death will be considered the EOT date. An EOT visit will be performed within 30 days (± five days) after last treatment, unless the patient starts any subsequent new antitumor therapy outside this clinical study, in which case the EOT visit should be performed immediately before the start of the new therapy, whenever possible. Patients will receive the study drugs while it is considered to be in their best interest. Specifically, treatment will continue until: • Confirmed PD. • Life-threatening, unmanageable or unacceptable drug-related AEs, including the need for more than two dose reductions, except in cases of obvious patient benefit in continuing the treatment at the Investigator's criterion. • Intercurrent illness of sufficient magnitude to preclude safe continuation of the study. • Patient refusal and/or non-compliance with study requirements. • A major protocol deviation that may affect the balance of the risk/benefit ratio for the participating patient. • Treatment delay > 14 days due to toxicity (except in case of patient's clear clinical benefit, with the Sponsor's approval). • Pregnancy. • Investigator's decision.

PLANNED STUDY PERIODS (for the whole study)	**Planned start date:** First quarter of 2014 (1Q14). **Planned enrolment period:** approximately 15 months. **Total duration of the study:** approximately 24 months. **Planned end-of-study date (clinical cut-off):** six months after the patient's treatment discontinuation (last patient-last visit), or nine months after accrual of the last evaluable patient, whichever occurs first.

SCHEDULE OF ASSESSMENTS

PROCEDURE	Pretreatment (days)	Treatment: C1[4]		Treatment: further cycles		End of treatment visit	Follow-up
		D1	D15	D28=1	D15		
Written IC	Before any procedures	-	-	-	-	-	-
Demographic data	-14 to 0	-	-	-	-	-	-
Medical history	-14 to 0	-	-	-	-	-	-
Primary diagnosis/Prior treatment(s)	-14 to 0	-	-	-	-	-	-
Assessment of signs and symptoms	-14 to 0	•	-	•	-	-	-
Complete physical examination (1) and clinical neurological assessment	-14 to 0	•	-	•	-	•	-
ECOG PS (1)	-14 to 0	•	-	•	-	•	-
Concomitant treatments (2)	-14 to 0	Throughout the study					-
Hematology (3,4)	-7 to 0	•	•	•	•	•	-
Coagulation panel	-14 to 0	-	-	•	-	•	-
Biochemistry-A (3, 4, 5)	-7 to 0	•	•	•	•	•	-
Biochemistry-B	-7 to 0	-	-	•	-	•	-
Creatinine and measured or calculated CrCl	-7 to 0	-	-	•	-	•	-
Urinalysis (dipstick, sediment)	-14 to 0	-	-	-	-	•	-
Viral serology	-14 to 0	Repeat if clinically indicated				-	-
Pregnancy test (if applicable) (6)	-7 to 0	Every 4 weeks				•	-
ECG (7)	-14 to 0	Before and after each plitidepsin infusion				•	-
LVEF	-14 to 0	Every 12 weeks				•	-
PK	NA	C1 only		-		-	-
AEs (NCI-CTCAE v4.0) (8)	NA	Throughout the study					•
Disease Assessments							
Serum protein (9)	-14 to 0	•	-	•	-	•	•
Urine protein	-14 to 0	•	-	•	-	•	•
Serum beta-2 microglobulin	-14 to 0	-					
C-Reactive Protein	-14 to 0	Every 8 weeks				•	•
Bone Marrow Assessment (10)	-21 to 0	When serology indicates CR				If clinically indicated	If clinically indicated
Clinical and radiological tumor assessment in the presence of soft tissue plasmacytoma (11)	-14 to 0	If response is observed (to confirm CR) or if clinical symptoms suggest new plasmacytomas				If clinically indicated	
Skeletal evaluation	-28 to 0	If response is observed (to confirm CR) or if clinical symptoms suggest new bone lytic lesion				If clinically indicated	

1. ECOG PS and vital signs must be repeated on D1 prior to drug infusion.
2. A detailed description of all concomitant treatment (drug name, start and end dates, reason for administration, etc.), especially transfusion requirements, should be recorded.
3. Repeat on C1 D1 prior to first infusion.

4. Repeat on D1, 4, 8, 11 and 15 during the first four weeks and on D1 and 15 thereafter. Repeat at least every other day if non-febrile neutropenia is present and every day in the presence of febrile neutropenia or grade 4 thrombocytopenia.

5. Repeat at least every other day in the presence of grade 3/4 vomiting or any other drug-related SAE.
6. For women of child-bearing potential a serum or urine HCG analysis should be done.
7. It should allow rhythm definition (at least 30 seconds of duration) and include:
   - PR interval.
   - QT interval (raw and corrected by heart rate using Bazett's formula).
   - QRS complex and the maximum height of QRS complex in leads II.
8. Serious Adverse Events (SAEs) will be collected from the time of signature of the Informed Consent Form.
9. Protein electrophoresis, serum Ig determination, M-protein measurement and IF and SFLC determination.
10. BM evaluation is mandatory for all patients at screening, while on treatment if clinically indicated and if there is CR. BM evaluation must be repeated eight weeks later in patients with non-secretory MM, to confirm response or as clinically indicated.
11. In patients with non-secretory or oligosecretory MM associated with soft tissue plasmacytoma, assessments may be done every two cycles (whenever possible) to confirm response or as clinically indicated.

Complete physical examination: weight, BSA and vital signs including HR, ABP and temperature.

Hematology: Differential WBC count, hematocrit, hemoglobin and platelet count.

Coagulation: PT, INR, APTT.

Biochemistry A: AP, AST, ALT, LDH, bilirubin, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}), glucose, CPK, CPK-MB fraction.

Biochemistry B: total proteins and albumin.

Viral serology: HBV and HCV; CMV in patients who have undergone allogeneic BM transplantation.

Serum protein: Protein electrophoresis, serum Ig determination and M-protein measurement and IF, SFLC.

Urine protein: 24-h urine protein electrophoresis measurement and IF, UFLC and M-protein measurement.

BM: morphology, cytometry (if available), cytogenetics (if available).

Clinical and radiological tumor assessment: CT-scan or MRI of all involved measurable/evaluable involved sites of soft tissue plasmacytoma. For soft tissue plasmacytoma assessment, tumor measurement will be the sum of the cross-diameters of the measurable target lesions.

Skeletal evaluation: X-ray of skull, vertebral column, pelvis and proximal long bones or MRI.

ABP, blood pressure; AEs, adverse event(s); ALT, alanine aminotransferase; AP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BM, bone marrow; BSA, body surface area; C, cycle; CMV, cytomegalovirus; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase isoenzymes found in cardiac muscle (it will be performed only if CPK is > ULN); CR, complete response; CRP, C-reactive protein; CT-scan, computed tomography scan; CR, complete response; CrCl, creatinine clearance; D, day; ECG, electrocardiogram; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBV, hepatitis B virus; HCG, human chorionic gonadotropin; HCV, hepatitis C virus; HR, heart rate; IC, informed consent; IF, immunofixation; INR, International Normalized Ratio; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MM, multiple myeloma; MRI, Magnet Resonance Imaging; NA, not applicable; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for the Classification of AEs; PK, pharmacokinetics; PT, pro-thrombin time; SAE, serious adverse event; SFLC, serum free light-chain; UFLC, urine free light chain; ULN, upper limit of normality; WBC, white blood cell.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ABP	Arterial Blood Pressure
ADA	After Drug Administration
ADL	Activities of Daily Living
AE(s)	Adverse Event(s)
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
APTT	Activated Partial Thromboplastin Time
ASCO	American Society of Clinical Oncology
ASCT	Autologous Stem Cell Transplantation
ASH/FDA	American Society of Hematology/Food and Drug Administration
AST	Aspartate Aminotransferase
ATC-WHO	Anatomical Therapeutic Chemical Drug Classification by the World Health Organization
AUC	Area Under the Curve
BM	Bone Marrow
BSA	Body Surface Area
CBP	CREB-binding Protein
CI	Confidence Interval
c.i.v.i.	Continuous Intravenous Infusion
C_{max}	Maximum Plasma Concentration
CMV	Cytomegalovirus
CNS	Central Nervous System
CPK	Creatine Phosphokinase
CPK-MB	Serum CPK Isoenzymes (Found In Cardiac Muscle)
CR	Complete Response
CrCl	Creatinine Clearance
CRF	Case Report Form
e-CRF	Electronic Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
CT	Chemotherapy
CT-scan	Computed Tomography scan
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
D	Day
DI	Dose Intensity
DL	Dose Level
DLT	Dose Limiting Toxicity
DOR	Duration of Response
DTIC	Dimethyl Triazeno Imidazol Carboxamide
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG PS	Eastern Cooperative Oncology Group Performance Status
EFS	Event-free Survival
EMA	European Medicines Agency
EOI	End of Infusion

EOT	End-of-treatment
FLC	Free Light Chains
GCP	Good Clinical Practice
GCs	Glucocorticoids
G-CSF	Granulocyte Colony Stimulating Factor
GGT	Gammaglutamyl Transferase
GM-CSF	Granulocyte/Macrophage Colony Stimulating Factor
GMT	Greenwich Mean Time
h	Hour(s)
Hb	Hemoglobin
HBV	Hepatitis B virus
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C virus
HDT	High Dose Chemotherapy
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSCT	Hematopoietic Stem Cell Transplantation
IB	Investigator's Brochure
IC	Informed Consent
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IF	Immunofixation
IHC	Immunohistochemistry
IL	Interleukin
IMWG	International Myeloma Working Group
IMIDs	Immunomodulatory Drugs
IMP	Investigational Medicinal Product
INR	International Normalized Ratio for blood clotting time
i.p.	Intraperitoneal
IRB	Institutional Review Board
ITT	Intention-to-treat
i.v.	Intravenous
KPS	Karnofsky Performance Status
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
MedDRA AE	Medical Dictionary for Regulatory Activities for Adverse Events
MM	Multiple Myeloma
MOA	Mechanism of Action
MP	Melphalan Prednisone
MPT	Melphalan Prednisone Thalidomide
MR	Minimal Response
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
MTS	Tetrazolium Salt
MUGA scan	Multiple Uptake Gated Acquisition Scan
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria
ORR	Objective Response Rate
OS	Overall Survival

PD	Progressive Disease
PDy	Pharmacodynamics
PFS	Progression-free survival
PI	Proteasome Inhibitor
PI3K	Phosphatidylinositol-3 kinase
PK	Pharmacokinetics
PN	Peripheral Neuropathy
PR	Partial Response
PS	Performance Status
PT	Pro-thrombin Time
PVC	Polyvinyl Chloride
qRT-PCR	Real Time Polymerase Chain Reaction
Q3wk	Every Three Weeks
Q4wk	Every Four Weeks
RD	Recommended Dose
RR	Response Rate
SAE(s)	Serious Adverse Event(s)
SAR	Serious Adverse Reaction
s.c.	Subcutaneous
sCR	Stringent Complete Response
SD	Stable Disease
SFLC	Serum Free Light Chains
SPC	Summary of Product Characteristics
SVT	Supra-Ventricular Tachycardia
TBI	Total Body Irradiation
TTP	Time To Progression
$T_{1/2}$	Half-life
VBAP	Vincristine, Carmustine, Doxorubicin, Prednisone
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
UFLC	Urine Free Light Chains
ULN	Upper Limit of Normal
US	United States
VGPR	Very Good Partial Response
VMCP	Vincristine, Melphalan, Cyclophosphamide, Prednisone
vs.	*Versus*
Vss	Volume of Distribution at Steady State
WBC	White Blood Cells
WFI	Water For Injection
wk	Week(s)

1. BACKGROUND

1.1 Overview of the Disease

Multiple myeloma (MM) is a malignant plasma-cell disorder characterized by the production of a monoclonal protein from plasma cells in the bone marrow (BM). Information from the National Cancer Institute indicates that in the US, an estimated 22,350 new cases of MM will be diagnosed in 2013, and 10,710 people will die from the disease (1). The incidence of MM in Europe is 4.5-6.0/100,000 a year with a median age of diagnosis of between 63 and 70 years and a mortality rate of 4.1/100,000/year (2). In the Western hemisphere, about 1% of cancer-related deaths are due to myeloma. MM may be staged according to either the Durie-Salmon system (Appendix 4) based on the amount of abnormal monoclonal immunoglobulin in the blood or urine, blood calcium levels, the amount of bone damage shown by X-ray and blood hemoglobin levels (3), or the newer staging system, the International Staging System that relies on the levels of albumin and beta-2-microglobulin in the blood (4) (Appendix 4). In both systems, all stages are further subclassified by creatinine level either less than 2.0 mg/dL or greater than or equal to 2.0 mg/dL. Impaired renal function worsens prognosis regardless of the stage.

The disease primarily affects individuals later in life with a median age of 63-70 years. From the time of diagnosis, survival without treatment is between 6 and 12 months and extends to 3 years with chemotherapy (CT). MM is treatable but rarely curable. Most patients receive multiple treatments over the course of their disease, and the precise sequence of therapy and used regimens can be quite variable. With standard dose CT, patients have a median survival of 24–30 months. Twenty five percent of patients survive 5 years or longer, and the 10-year survival rate is approximately 3% (1). Failure of standard therapy to cure these diseases has led to the study of higher doses of chemotherapeutic agents. These conditioning regimens may involve ablative/reduced or non-myeloablative intensity and the rescue of the immune system following CT may involve autologous or allogeneic stem-cell transplantation.

1.2 Current Treatment for Multiple Myeloma

Patients considered candidates for a CT-based intervention are further divided into those who are and those who are not eligible for high-dose CT (HDT) followed by stem cell rescue, based on age, performance status (PS), and co-morbid medical conditions. HDT for MM was introduced in 1983 (5) and showed for the first time that a substantial percentage of complete remissions could be induced. Morbidity and mortality however, were high, but were strongly reduced later by the application of autologous stem cell rescue.

BM was the source of stem cells in the first studies, peripheral blood stem cells (PBSC) are now routinely applied as autologous rescue. In 1996, a randomized study was published which showed that autologous transplantation was superior to conventional treatment regarding response rate (RR), event-free survival (EFS) and overall survival (OS) (6). In this study, patients younger than 65 years were randomized at diagnosis to receive vincristine, carmustine, doxorubicin, prednisone/vincristine, melphalan, cyclophosphamide, prednisone (VBAP/VMCP) or high dose melphalan 140 mg/m^2 and total body irradiation (TBI) 8 Gy supported with autologous BM collected after 2 courses of VBAP/VMCP.

In summary, and according to several guidelines, HDT with autologous hematopoietic stem cell transplantation (HSCT) should be part of the primary treatment strategy in newly diagnosed patients up to the age of 65 years with adequate PS and organ function. It may also be considered in patients > 65 years with good PS. Allogeneic HSCT with human leukocyte antigen (HLA)-matched sibling donors may also be considered in patients up to the age of 50 years who have achieved at least a partial remission after initial therapy. Reduced-intensity conditioning allografting may be considered in patients up to the age of 70 with a HLA-matched sibling donor.

Until recently, there was general agreement that the standard of care for patients who are not eligible for transplantation was the treatment with melphalan and prednisone (MP), despite the low overall response rate (approximately 50%), with few complete responders, and the modest improvements in 5-year survival (7). Other combination chemotherapies have been used in this setting and, although they induced a more rapid response and a higher overall response rate, the differences did not translate into a survival advantage compared to that achieved with MP. However, new knowledge about the pathobiology and pathogenetics of MM, and the introduction of novel agents, such as thalidomide, bortezomib, arsenic trioxide, and more recently lenalidomide, with novel mechanisms of action (MOA) are defining new standards of care.

Novel agents such as immunomodulatory drugs (IMIDs) (thalidomide, lenalidomide, pomalidomide) and proteasome inhibitors (PIs) (bortezomib, carfilzomib, etc…) have doubled the duration of survival. However, new active agents are still needed for double refractory patients to IMIDs and PIs. With a progression-free survival (PFS) of 6 months and median survival of a year, treatment in this population is still challenging, an optimal combination of chemotherapeutic agents has not been defined and new therapies are needed in this setting. In this regard, plitidepsin has shown meaningful activity in patients failing treatment with IMIDs and PIs. Moreover, synergism between plitidepsin and bortezomib and thalidomide has been shown (unpublished data).

PIs are of particular interest, as they act on the ubiquitin-proteasome system, which is responsible for regulation and degradation of the majority of intracellular proteins. Proteasome inhibition leads to cell cycle disruption, activation of apoptosis pathways and ultimately, cell death. In MM cells, among others, PIs have been shown to target the unfolded protein response, a signaling pathway allowing the appropriate folding of proteins. A small study showed overall response rates (ORR) (better than minimal response) of 50% after re-treatment with bortezomib. Greater responses (56%) were seen in patients with a free-interval of at least six months when compared to patients re-treated within six months (33%). In this study, 75% of patients received bortezomib re-treatment in combination with dexamethasone. Hence, patients who relapse after bortezomib treatment may receive an additional course of bortezomib-based therapy if they had an initial response to the drug lasting at least six months and had no intervening therapies. The prognosis for patients who relapse after treatment with bortezomib plus either lenalidomide or thalidomide is poor, with a median EFS of only a few months and OS of six months.

In summary, therapeutic options are still limited and new active drugs are needed in the double-refractory population. Novel agents may give rise to new feasible combinations based on their activity and safety profiles and plitidepsin combinations with IMIDs and/or PIs are warranted.

1.3 Plitidepsin

The Sponsor is committed to the development of new drugs in an effort to broaden the spectrum of current antitumor therapies. Chemically, plitidepsin is a (now fully synthetic) natural occurring depsipeptide originally extracted from the Mediterranean Sea tunicate *Aplidium albicans*. Although the main MOA by which plitidepsin inhibits cell growth and/or induces cell death remains to be fully characterized, its major effects can be at least partially attributed to a cell cycle block in the G0/G1 phases and the induction of apoptosis (8-10) via activation of the JNK pathway; this activation leads to a decreased production of intracellular glutathione, an increase in reactive oxygen species and an alteration of the mitochondrial membrane potential, ultimately leading to both caspase-dependent and independent apoptosis. In addition to the pro-apoptotic properties of plitidepsin, the molecule has demonstrated antiangiogenic properties in several pre-clinical models via direct activity on vascular endothelial growth factor (VEGF)-stimulated angiogenesis. In fact, plitidepsin has been demonstrated to reduce the secretion of VEGF and its receptor type 1 (VEGFR-1) from MOLT-4 human leukemia cells in vitro (11). It seems that the majority of the pharmacological activity of plitidepsin can be attributed to a combination of these cellular effects *in vivo*.

Antitumor activity has been displayed by plitidepsin in *in vitro* and *in vivo* models. In addition, this observation has been sustained in early clinical trials as a single agent, showing clinical responses in patients with hematological malignancies as well as solid tumors. The toxicity of plitidepsin in normal hematopoietic tissue is several folds lower than in tumor cells. More importantly, this observation translates into a lack of clinically significant hematological toxicity in clinical trials to date, even in leukemia/lymphoma patients with limited BM reserve capacity. Consequently, plitidepsin may display a positive profile for combination with other agents in CT regimens, avoiding overlapping toxicity.

Please refer to the Investigator's Brochure (IB) for full information on plitidepsin.

1.3.1 Name and Chemical Information

Aplidin® is the trade name for plitidepsin [leucine, 1-(1,2-dioxopropyl) prolyl-N-methyl-leucylthreonyl-4-amino-3-hydroxy-6-methylheptanoyl-4-hydroxy-2,5-dimethyl-3-oxohexanoyl-N, 0-dimethyltyrosylprolyl, O-lactone)], a cyclic depsipeptide originally isolated from a Mediterranean marine tunicate, *Aplidium albicans*, which is currently manufactured by total synthesis.

1.3.2 Non-clinical Data

1.3.2.1 Mechanism of Action in Cell Lines

The major effects of plitidepsin can be specifically attributed to the induction of apoptosis secondary to oxidative stress and activation of JNK activity. Exposure of cultured human cervical cancer (HeLa) cells to plitidepsin induced oxidative stress resulting in cellular apoptosis (12) and a rapid and sustained activation of JNK, p38 MAPK and ERK in human breast cancer cells (MDA-MB-231) (13). In fact, genetically engineered mouse embryo fibroblast (MEFs) which did not express any JNK isoforms, were at least an order of magnitude less sensitive to plitidepsin (10). JNK has been shown as a critical component in plitidepsin-induced cytotoxicity through a decrease in the intracellular reduced glutathione (GSH) levels which, in turn, increases the levels of reactive oxygen species (13). The effects of plitidepsin on a pleiotropic regulatory protein Rac1, would contribute to the sustained activation of JNK (8, 14). Additionally,

a cell cycle inhibitor, p27 (kip1), has been shown to determine plitidepsin sensitivity *in vitro*, on a panel of mouse sarcoma cells from resected tumors (15). Data on human renal cell carcinoma lines (A-498 and ACHN) confirmed the oxidative operating mechanism and recent studies on human melanoma cell lines (SK-MEL-28 and UACC-257) have again involved a Rac1/JNK pathway in the apoptotic cell arrest induced by plitidepsin (13, 14), thus demonstrating a common mechanism in cells of different tumor origins. More recent studies have shown plitidepsin to be able to increase levels of cell membrane phospholipid oxidation and deoxyribonucleic acid (DNA) oxidation *in vitro* (8).

Apart from its pro-apoptotic properties, plitidepsin has also demonstrated antiangiogenic effects. The addition of the drug reduces the active secretion of VEGF and the expression of its receptor (VEGFR-1) on human leukemia (MOLT-4) cells *in vitro* (11), suggesting that the block of cell growth might be mediated by dual inhibition of the VEGF autocrine loop. Supporting these findings, plitidepsin has been shown to be highly cytotoxic on acute myelogenous leukemia cells, both on regular cultures (K-562, HEL and HL-60) and on blasts obtained from patients (16). Furthermore, plitidepsin was able to reduce the secretion of VEGF in a dose-dependent manner, thus confirming previous observations. At the functional level, plitidepsin inhibited spontaneous and growth factor-induced angiogenesis, prevented proliferation, migration and invasiveness, and hampered formation of capillary-like tridimensional structures, in *in vivo* and *in vitro* models (17).

In summary, it appears that the pharmacological activity of plitidepsin can be attributed, at least in part, to a combination of pro-apoptotic and antiangiogenic effects *in vivo*.

1.3.2.2 Plitidepsin as Single Agent: In vitro and In vivo Data

In vitro studies demonstrated antiproliferative activity against a broad spectrum of tumor types, namely bladder, breast, stomach, prostate thyroid and lung cancer, melanoma, neuroblastoma (with IC_{50} values ranging from 10^{-7} to 10^{-9} M), and leukemia, myeloma and lymphoma (with IC_{50} values ranging from 10^{-8} to 10^{-9} M) (18).

In addition, an animal model in MM has been explored (19). At clinically achievable concentrations, plitidepsin exhibited potent *in vitro* activity against primary MM tumor cells and a broad spectrum of human MM cell lines, including cells resistant to conventional (e.g., dexamethasone, alkylating agents, and anthracyclines) or novel (e.g., thalidomide and bortezomib) anti-MM agents. Plitidepsin was active against MM cells in the presence of proliferative/antiapoptotic cytokines or BM stromal cells and had additive or synergistic effects with some of the established anti-MM agents. The anti-MM effect of plitidepsin was associated with suppression of a constellation of proliferative/antiapoptotic genes and up-regulation of several potential regulators of apoptosis.

In conclusion, plitidepsin showed consistent cytotoxic activity against a broad selection of human-derived solid tumor cell lines such as lung, breast, thyroid, prostate, stomach, bladder, and kidney, as well as human malignant cell lines of hematological origin.

In the hollow fiber *in vivo* model, in which athymic rats were treated with i.v. plitidepsin, tumors of the bladder, stomach, and prostate were shown to be susceptible to the drug. In xenograft models, activity was noted against human renal and pancreatic tumors when injected to athymic mice.

Additionally, the antitumor and antiangiogenic effects of plitidepsin were evaluated in the 5T33MM syngeneic orthotopic model of MM (20). *In vitro*, plitidepsin inhibited DNA synthesis and induced an arrest in transition from G0/G1 to S phase. Furthermore, plitidepsin induced apoptosis by lowering the mitochondrial membrane potential. For the *in vivo* experiment, i.p.-injected plitidepsin was well tolerated by the mice and reduced serum paraprotein concentration by 42% ($p<0.001$), while BM invasion with myeloma cells was decreased by 35% ($p<0.001$). Plitidepsin also reduced the myeloma-associated angiogenesis to basal values. This antiangiogenic effect was confirmed *in vitro* and may be explained by inhibition of endothelial cell proliferation and vessel formation. In summary, these data indicate that plitidepsin is well tolerated *in vivo* and its antitumor and antiangiogenic effects support the use of the drug in MM (21).

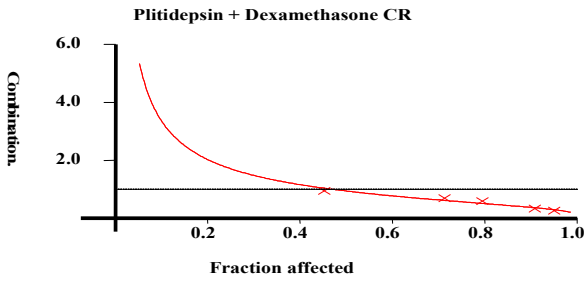
1.3.2.3 Plitidepsin in Combination: In vitro and In vivo Data

Clinical experience in the management of MM patients supports the concept that drug combinations induce higher RRs than single agents. The ability of plitidepsin to increase the activity of other established anticancer agents was assessed in several human tumor cell lines. The plitidepsin/dexamethasone combination selected for further development in solid tumors and preclinical data are summarized below.

Plitidepsin-Dexamethasone Combination: Non-clinical Data

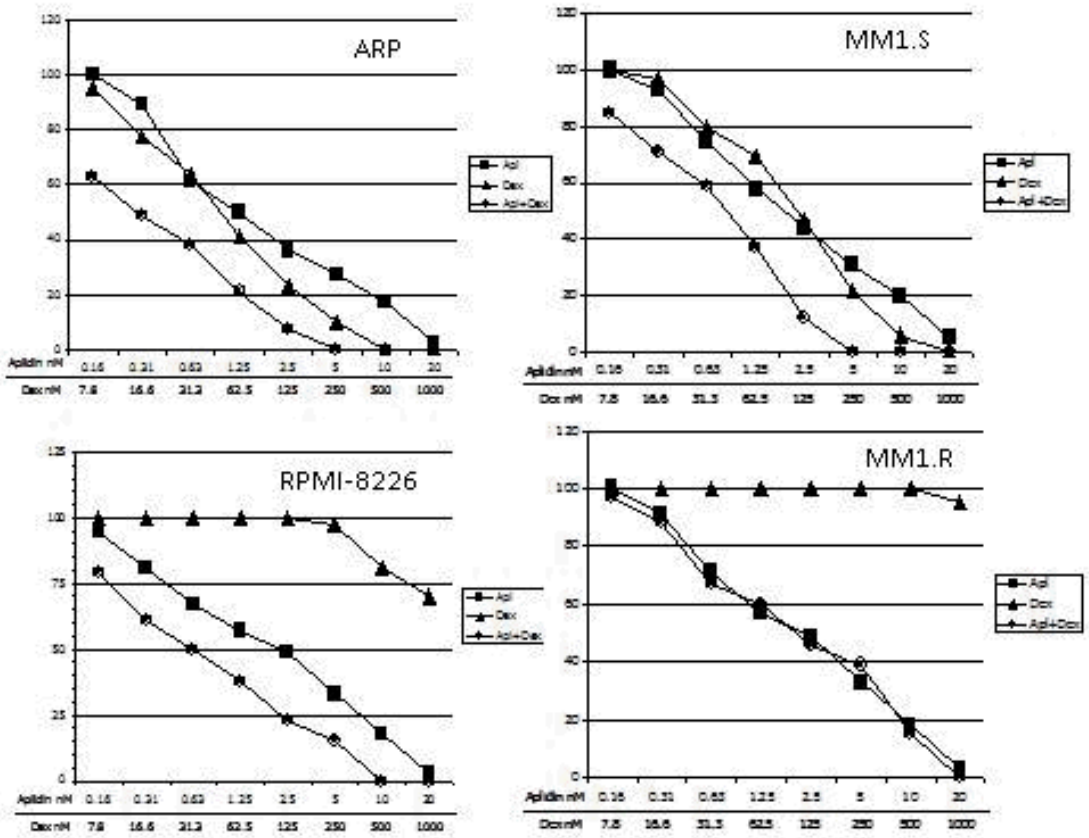
The plitidepsin/dexamethasone combination was explored with viability analyses in MM1.S and U266-LR7 cells. The experimental design included both the non-constant ratio (for suboptimal drug doses) and the constant ratio. The effects of single and combined treatments after 72 hours (h) were evaluated by MTT assays. The results clearly showed that plitidepsin increased the anti-MM effect of dexamethasone. The combination was additive but tended to be synergistic at higher doses (19) (Figure 1).

Figure 1. MM1.S cell line, 48h incubation.



Similar results were obtained in a study by Medina et al. (internal report, March 2008), where the plitidepsin/dexamethasone combination (at a fixed ratio of 1/50) was tested in four MM cell lines (MM1.S, ARP, RPMI-8226 and the dexamethasone-resistant MM1.R). As shown in Figure 2, the plitidepsin/dexamethasone combination resulted in a significant increase in cell toxicity in the cell lines MM1.S, ARP and RPMI-8226 compared to either drug alone (plitidepsin or dexamethasone). In contrast, only plitidepsin had an effect on the dexamethasone-resistant cell line, MM1.R.

Figure 2. Activity of the combination plitidepsin/dexamethasone in MM cell lines.



The plitidepsin/dexamethasone combination was synergistic in the cell lines MM1.S, ARP, and RPMI-8226 at all (nanomolar) tested drug concentrations, while it was additive in MM1.R at high concentrations.

Molecular Rationale Based on the MOA of Dexamethasone: Similarities with Plitidepsin Activity

The pleiotropic molecular effects elicited by plitidepsin treatment preclude reaching conclusions on which pathways are the most important for its antitumor activity; however, they offer a potentially unique MOA and a major therapeutic advantage. In particular, they may account for the synchronized targeting of different specific proliferative/antiapoptotic pathways in MM tumor cells. In fact, gene expression profiling data in tumor cells provide a framework for designing a combinatorial therapy to potentiate each individual antitumor effect. It is likely that their pleiotropic and synergistic effects *in vitro* over MM cells may neutralize the pathways that enable tumors to evade cell death and to become resistant to anticancer treatment.

Glucocorticoids (GCs), such as dexamethasone, induce apoptosis in the hematological lineage, while supporting the survival of several non-hematological tissues, such as the mammary gland, ovary, liver or fibroblasts (22-24). GCs exert their action through interaction with the intracellular GC receptor (GR), a ligand regulated transcription factor that positively or negatively alters the expression of specific target genes. In turn, GR either induces gene transcription by binding to specific DNA elements in the promoter-enhancer regions of responsive genes or reduces gene transcription by transrepression (24-27). Thus, dexamethasone acts over genes responsible for the

induction of apoptosis in lymphoid cells, in what seems a plitidepsin complementary pathway, therefore enhancing plitidepsin cytotoxicity (28-31).

Like plitidepsin, GCs may induce apoptosis by directly regulating both the extrinsic and intrinsic apoptosis pathways. Death receptors (CD95 and TRAIL) and downstream effectors have been found deregulated by each of the drugs in the extrinsic pathway. Regarding the intrinsic and mitochondria-mediated pathways, which lead to the release of pro-apoptotic molecules upon depolarization of the mitochondrial membrane potential, the apoptotic response is tightly regulated by the interaction between pro- and anti-apoptotic Bcl-2 family members. Additionally, both, plitidepsin and dexamethasone, upregulate pro-apoptotic genes (TRAIL-Rl/DR4 and TRAIL-R2/DR5, Bax, Bak, Bad, Fas, FasL, TRAIL, Noxa, PIG3, Bim, Bik and Puma, Bcr-Abl [in CML], c-Myc, and HDAC3), while they downregulate pro-survival genes (c-FLIP, MCl-I, Bel-X, and Bcl-2) (25, 32).

On the other hand, both agents have been found to disrupt the cellular redox state (e.g., ROS), and damage mitochondria in cells undergoing apoptosis, as an effect of the depolarization of mitochondrial membrane, which will enhance the expression of death receptors and ligands resulting again in the activation of the caspase cascade.

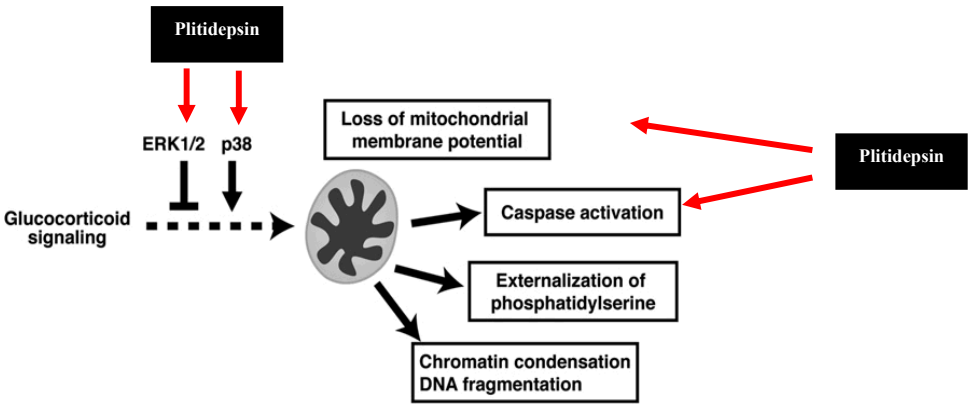
Moreover, plitidepsin-induced suppression of caspase inhibitors (FLIP, survivin) may contribute to the increased sensitivity of plitidepsin-treated MM cells to caspase-dependent apoptosis by dexamethasone. Both agents deregulate Hsp90 complexes such as receptor FKBP5, HSPs and DNAJs.

Other molecules and pathways involved in the antiproliferative effect of both dexamethasone and plitidepsin, could yet contribute to the synergism of the combination. Plitidepsin downregulates genes with a documented role in oncogenic transformation in MM: Myb, Myc or Ras families, frequently mutated in MM cells, effect also seen after dexamethasone administration (33). Likewise, both had a synergistic effect on NF-kappaB (survival transcription factor) and its inhibitor (34, 35).

Besides, a synergistic effect may occur on the cell cycle, where the arrest induced by the combination may be mediated by their joint and coordinated regulation of the expression of CDKI (p21WAF1/CIP1, p27 KIP), INK4 family of proteins (p15INK4b, p18INK4c, p19INK4d), cycA, cycD1 and D2, suppression of CDK4, suppression of p107, or hypophosphorylation of Rb (24).

Plitidepsin treatment also reduces Erk (an extracellular signal-regulated kinase, ERK 1/2) activation but increases activation of p38MAPK (Figure 3), enhancing GC sensitivity by the induction of apoptosis. Similarly, MAPK pathway activation by plitidepsin may improve the effects of GC.

Figure 3. Plitidepsin-dexamethasone molecular model of apoptosis.



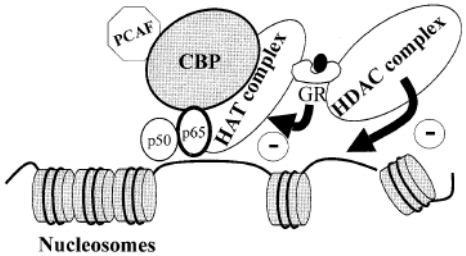
Combined treatment with plitidepsin and dexamethasone suppresses genes involved in cytokine-induced proliferative/antiapoptotic signaling pathways and oncogenic transformation triggering MM proliferation: IGF-1R, IL-6R, gp130, CXCR-4 etc.

In addition to the documented effects induced by both drugs at the genomic level, some of the effects of dexamethasone seem to be non-specific, i.e., a non-genomic activity, with a quick activation of protein kinases, including the MAPK cascade, phosphatidylinositol-3 kinase (PI3K) and Akt. These non-specific effects could explain the short-term rapid synergism of the combination, since plitidepsin has also shown a rapid effect over the same pathways.

GR reduces gene transcription by interaction with proinflammatory transcription factors such as AP-1 (Fos-Jun heterodimers) and NF-kB (p65-p50 heterodimers). Both require the coactivator CREB binding protein (CBP) for maximal activity. Therefore, these data also suggest that alterations in chromatin structure may be important in modulating GC actions.

On this regard, the direct inhibition of CBP-associated histone acetyltransferase (HAT) activity and the active recruitment of a histone deacetylase complex 2 (HDAC2) induced in cell lines treated with dexamethasone appear crucial (36, 37). Both complexes are closely related to chromatin remodeling and consequently, to the modulation of gene expression induced by dexamethasone (Figure 4).

Figure 4. Glucorticoids and the chromatin complex.



Glucocorticoids induce the acetylation of specific lysine residues (K5 and K16) in histone H4. The consequences of this epigenetic activity are wide, e.g. preventing other transcription factors, such as activator protein 1 (AP-1) and nuclear factor κB (NF-κB), from activating their target genes by inhibition of acetylation of specific lysine residues in histone H4. This may also have an antiangiogenic potential (reducing VEGF expression).

Therefore, histone H4 K5 acetylation can be considered as a marker of dexamethasone transactivation. Dexamethasone predominantly targeted acetylation on histone H4 K5 and K16 in all subjects. However, the GR complex inhibits acetylation of K8 and K12 by acting both as a direct inhibitor of CBP-associated histone acetylation and by recruiting HDAC2 to the p65-CBP HAT complex. Thus, it has been described that both HAT and HDAC activities coexist within the same complex in the presence of p65 and GR and that they can each act independently. This mechanism for glucocorticoid repression is novel and it establishes that inhibition of histone acetylation brings an additional level of control of inflammatory/antiproliferative/apoptotic gene expression. This further suggests that pharmacological manipulation of specific histone acetylation status is a potentially useful approach for the treatment of dexamethasone-sensitive diseases. On this regard, the effects of dexamethasone have been shown to improve in the presence of trichostatin A (TSA), a classical potent in vitro HDAC inhibitor, and in the presence of SAHA, recently approved for *in vivo* use at the clinical setting. HDACs were also pointed out as playing a role in dexamethasone repression.

Further analysis of the epigenetic activity with dexamethasone revealed an induction of phosphorylation of Histone 3 at Ser10 (inhibited in response to TNFα) and histone 3 methylation at Lysine 4 (H3K4 methylation). These two markers of the histone code are targets for rapid hyperacetylation upon treatment with the HDAC inhibitor sodium butyrate or with TSA. Moreover, most, if not all, available Lys in the H3 tail becomes acetylated when they are marked with K4 methylation or S10 phosphorylation. Such specificity suggests that the activation of HATs after treatment with dexamethasone together with an HDAC inhibitor is not random.

1.3.2.4 Toxicology

Plitidepsin, given by intravenous (i.v.) injection in daily (d) x1 (dx1), dx5 or 3-cycle dx5 regimens, produced toxicological effects typical of cytotoxic antitumor agents. Tissues containing cells with a high turnover were especially targeted. In the dx1 and dx5 studies, the principal organs affected were the reticuloendothelial system and the gastrointestinal tract in all species, testes in the mouse and rat, and pancreas in the dog. Additional affected organs were: epididymides, pancreas, heart, mammary gland and skeletal muscle in the rat and mouse, and liver, thymus and testes in the dog. In all three species, toxic effects were dose-related and generally fully or partially reversible. Most toxicities were reversible at the maximum tolerated dose (MTD) level at the end of an acute toxicity evaluation.

Of interest is the observation that the toxicity of plitidepsin in normal hematopoietic tissue (IC_{50}: 150-2250 nM) was 1-3 orders lower than in tumor cells (IC_{50}: 0.2-27 nM) (38). These and other data (39) indicate that plitidepsin might be a potential compound in multidrug CT regimens, assuming that it does not increase hematotoxicity significantly, which is often the dose-limiting toxicity of these regimens.

1.3.2.5 Safety Pharmacology

Cardiovascular System

The cardiovascular (CV) safety pharmacology evaluation of plitidepsin involved both *in vitro* and *in vivo* studies.

Briefly, no inhibition of the hERG tail current was found after a 15-min exposure of HEK293 cells stably transfected with hERG cDNA to a concentration of 1μM of plitidepsin. Moreover, increasing concentrations of plitidepsin (10, 100 and 1000 nM)

did not produce changes in the action potential morphology of isolated cardiac Purkinje fibers from dogs.

The effect of plitidepsin on CV parameters [arterial blood pressure (ABP), heart beating rate (HBR) and electrocardiogram (ECG) variables - PR interval, QRS duration, RR interval, and QT interval] was evaluated in conscious, telemetered dogs. Plitidepsin infusions at doses up to 0.03 mg/kg (0.6 mg/m^2) did not affect ABP. HBR increased from 2 to 48 h after administration of 0.03 mg/kg (0.6 mg/m^2) of plitidepsin. There were no significant effects on corrected QT (QTcF) at the studied plitidepsin doses. No ECG abnormalities were observed during or up to 8 h after discontinuation of the plitidepsin infusion.

Respiratory System

Intravenous administration of up to 0.50 mg/kg (3 mg/m^2) of plitidepsin to male rats did not significantly affect either respiratory rate or tidal volume. Significant decreases in tidal volume and respiratory rate were detected at 24 h post-dosing with 1.50 mg/kg (9 mg/m^2).

Neurotoxicity

Intravenous administration of up to 0.50 mg/kg (3 mg/m^2) of plitidepsin to male rats did not produce gross behavioral or physiological state changes at any observation period. In rats treated with 1.5 mg/kg (9 mg/m^2) occasional cage dispersion, increased cutaneous blood flow, diarrhea, and vocalization were observed.

1.3.3 Clinical Data

1.3.3.1 Phase I Trials

Phase I clinical studies with plitidepsin were started in 1998. These studies were conducted according to classical phase I study design standards including pharmacokinetic (PK) evaluations. All single-agent phase I studies were conducted in the EU and Canada and explored five different infusion times or administration schedules of plitidepsin: 3-h infusion on D1 and 15 every four weeks (q4wk), 24-h infusion on D1 and 15 q4wk, 1-h infusion on D1, 8 and 15 q4wk, 24-h infusion on D1, 8 and 15 q4wk and 1-h infusion on D1-5 every three weeks (q3wk).

Phase I combination studies were conducted in Europe and the US to evaluate plitidepsin in combination with other antineoplastic drugs. Three of these studies, which evaluated plitidepsin combined with cytarabine, bortezomib or docetaxel, were early terminated following a decision by the Sponsor. RDs are currently available for the following plitidepsin combinations: carboplatin, dacarbazine (DTIC), sorafenib, bevacizumab and gemcitabine.

Up to the cut-off date of 31 March 2013, 214 adult patients and 38 pediatric patients have been treated with single-agent plitidepsin and 114 adult patients with plitidepsin in combination with other drugs in these phase I studies, for a total of 366 patients. Results obtained in single-agent phase I trials in adult patients are summarized in Table 1.

Table 1. Single-agent plitidepsin phase I trials in adult patients.

	24 h i.v. d1,8,15 q4w	3 h i.v. d1,15 q4w	1 h i.v. d1,8,15 q4w	24 h c.i.v.i. d1,15 q4w		1 h i.v. d1-5 q3w
				Plitidepsin alone	Plitidepsin + L-carnitine	
No of patients	35	27	48	47	20	37
MTD (mg/m^2)	4.50	6	3.60	6	8	1.35
DLTs	**MTD** G4 CPK inc. + muscular weakness G3 trans. inc. **RD** G3 CPK inc. + renal impairment G3 SVT	**MTD** G3 CPK inc. G4 CPK inc. + MOF **RD** G3 CPK increase G3/4 ALT/AST inc. G3 asthenia	**MTD** G3 muscular pain + G2 CPK elevation G4 CPK inc. **RD** G3 AP inc. **At 2.7 mg/m^2** G3 AST, AP, bilirubin inc. + fatigue	**MTD** G4 CPK inc. G2 CPK inc. for > 15 days **RD** G4 CPK inc.	**MTD** G3 asthenia + G1 fever G4 CPK inc. **RD** G4 CPK inc. and G3 asthenia + myalgia G4 CPK inc.	**At 1.5 mg/m^2** G3 emesis + skin rash G3 myalgia **MTD** G3 fatigue + skin rash G3 fatigue and diarrhea **RD** none
RD (mg/m^2)	3.75	5	3.2	5	7	1.2
DI at the RD (mg/m^2/week)	2.8	2.5	2.4	2.5	3.5	2.0

c.i.v.i., continuous intravenous infusion; D, day; DI, Dose intensity; DLT, dose-limiting toxicity; inc., increase; i.v., intravenous; MOF, multiorgan failure; MTD, maximum tolerated dose; NCI-CTCAE, National Cancer Institute Common Toxicity Criteria for the Classification of Adverse Events; RD, recommended dose; SVT, supraventricular tachycardia; trans., transaminase.

Of note, the proposed recommended dose (RD) for further developments as single agent after the extensive phase I program consistently delivered a similar dose intensity, around 2.5 mg/m^2/week and a similar pattern of dose-limiting toxicities (DLTs), without unexpected toxicities regardless of the schedule.

Of relevance, a phase I study of plitidepsin in combination with bortezomib and dexamethasone in patients with relapsed or refractory MM was started in 2008. This study was terminated early, following a Sponsor's decision. No MTD or RD were reached.

Safety Overview in Adult Single-Agent Phase I Trials

The primary DLTs found in the dose-finding phase I studies with single-agent plitidepsin were musculoskeletal AEs. The most common were myalgia, muscle weakness and increase in serum CPK (non-cardiac fraction) levels.

In the 77 patients treated at the RD, myalgia occurred in 35 patients (46%) and muscle weakness in 12 patients (16%); these events were generally mild and only reached grade 3 in two (3%) patients each. General symptoms (e.g., fatigue) were also reported during treatment with plitidepsin. Grade 1/2 vomiting occurred in about half of the patients treated at the RD. Grade 3/4 emesis was far less common, occurring in only two patients (3%). Therefore, according to current guidelines, single-agent plitidepsin should be considered as a high-emetogenic non-cisplatin agent, and appropriate standard prophylaxis is indicated.

Mild infusion site reactions were found, particularly (although not exclusively) when plitidepsin was administered through a peripheral line. As a result, use of a central line is suggested to administer plitidepsin although a peripheral line may be used for the fortnightly schedule only whenever a central venous line is deemed unsuitable for any reason (e.g., coagulation problems, technical difficulties, patient's refusal, etc.).

Transient and reversible transaminase increases (particularly ALT) were dose-limiting in some single-agent phase I studies and in most phase I combination studies. AP

increases were far less common, whereas bilirubin increases were extremely rare and almost universally were disease-related.

In contrast to many other cytotoxic agents, single-agent plitidepsin did not induce clinically significant bone marrow (BM) toxicity, stomatitis or alopecia within the dose range explored. Of note, neither grade 3/4 neutropenia nor grade 3/4 thrombocytopenia were reported in any phase I trials with single agent plitidepsin.

Overall, clinical data suggest that the safety profile of plitidepsin is acceptable and administration is safe for the treatment of cancer patients.

Almost all deaths that occurred during treatment or follow-up were due to progression of the underlying malignancy and were unrelated to plitidepsin. One drug-related death was reported (0.4%) among the 214 adult patients treated with single-agent plitidepsin in phase I clinical trials. This patient had a metastatic renal carcinoma with prior nephrectomy and was receiving plitidepsin at 6 mg/m^2 as a 3-h infusion on D1,15 q4wk. Fatal multiorgan failure with disseminated intravascular coagulation, acute renal failure, acute hepatic failure, myositis with CPK elevation and secondary myocardial events appeared. The patient did not improve despite intensive supportive measures, such as hemodialysis.

A total of 29 (14%) of the 214 adult patients treated with single-agent plitidepsin during the phase I program had at least one serious adverse event (SAE) that was possibly, probably or definitely drug-related (SAR).

Safety Overview in Adult Combination Phase I Trials

As of the cut-off date, only one combination (plitidepsin/gemcitabine) was still ongoing; patient accrual into this study had been completed, and data verification was ongoing at the cut-off.

The most common DLTs found with the plitidepsin combination schedules were transaminase increases, which occurred when plitidepsin was combined with carboplatin, sorafenib, bevacizumab, gemcitabine or DTIC. DLTs consisting of hematological abnormalities were uncommon and only comprised grade 4 thrombocytopenia alone or concomitantly with neutropenia for > 5 days (with plitidepsin and carboplatin or gemcitabine) and grade 4 febrile neutropenia and pancytopenia (with plitidepsin and DTIC). Other DLTs were grade 3 hand-foot syndrome (with plitidepsin and sorafenib) and grade 3 fatigue and myalgia (with plitidepsin and bevacizumab).

Efficacy Data in Phase I Trials

Overall, one confirmed PR and three unconfirmed PRs were found in phase I studies of plitidepsin as single agent in adult patients. Thirty patients with varied tumor types also experienced clinical benefit as SD lasting more than three months.

Antitumor activity observed with the plitidepsin combinations includes: one complete response in a patient with NHL and one confirmed PR and three SD > 3 months with tumor reduction in patients with Hodgkin's lymphoma in patients treated with plitidepsin and gemcitabine, one confirmed PR, two unconfirmed PRs and four SD > 3 months in patients with melanoma treated with plitidepsin and DTIC, one 3-month SD in one melanoma patient treated with plitidepsin and carboplatin, and one confirmed PR in one patient with MM treated with plitidepsin and bortezomib. These combinations only induced SD for > 3 months in other tumor types, the most common being

colorectal carcinoma (n=5 patients) and renal cancer (n=4) (with sorafenib, bevacizumab or gemcitabine).

1.3.3.2 Phase II Trials

Two schedules were selected for further evaluation in phase II studies on the basis of the findings from phase I studies: a fortnightly schedule with a 3-h i.v. infusion on D1 and 15, q4wk at the RD of 5 mg/m^2 (a 24-h i.v. infusion was also tested at the same RD and at 7 mg/m^2 if supplemented with L-carnitine) and a weekly schedule (1-h i.v. infusion on D1, 8 and 15, q4wk at the RD of 3.2 mg/m^2).

The results from completed phase II trials confirm the preliminary safety profile described from the subset of patients treated at the proposed RD in phase I trials.

Evidence suggests that the use of shorter infusion times may help reduce the incidence of some types of drug-related events, including CPK elevations, gastrointestinal (anorexia, nausea and vomiting), constitutional (fatigue) and injection site reactions. In addition, shorter infusion times are more convenient for the patient and significantly reduce costs and treatment complexity. No significant differences were found in the dose intensity and the safety profile of the treatment administration schemes. Therefore, the clinical development of 24-h continuous i.v. infusion schedules has been discontinued in favor of the 1-h and 3-h infusion schedules.

The cardiac safety of plitidepsin does not seem to be of special concern. The most common cardiac adverse event (AE) observed to date in the clinical trials are harmless rhythm alterations. No cardiac AEs have resulted in a fatal outcome. In addition, no life-threatening ventricular arrhythmias have occurred. Relevant predisposing factors are mostly related with the patient's baseline characteristics and disease, but not to drug exposure or treatment characteristics.

Severe hypersensitivity reactions have been found with plitidepsin, even after prophylactic premedication with antihistamines and glucocorticoids. These reactions are rapidly reversible and to date none has had a fatal outcome. They may be due to the Polyoxyl 35 castor oil l present in the formulation, but a direct relationship between plitidepsin itself and hypersensitivity may not be excluded yet.

Phase II Trial of Plitidepsin in Combination with Dexamethasone

The safety and efficacy of plitidepsin in patients with relapsed and/or refractory MM have been investigated in a phase II study and final results have been reported (40). This was an open-label study of plitidepsin given as a 3-h infusion every two weeks; the addition of dexamethasone was allowed in patients with suboptimal response to plitidepsin alone (defined as PD after three cycles or SD after four cycles of plitidepsin). The primary endpoint was the objective response rate (ORR = CR+ PR + minimum response [MR]) according to strict Bladé criteria (41) in the intent-to-treat (ITT) population according to Myeloma Response Criteria. Secondary endpoints were time to progression (TTP) and safety.

The safety profile of the plitidepsin/dexamethasone combination was acceptable and did not significantly change after dexamethasone addition. In particular, the incidence of grade 3-4 drug-related hematological toxicity was very low, with only 11% and 21% of patients experiencing grade 3-4 neutropenia and thrombocytopenia, respectively (similar figures were obtained for plitidepsin alone).

Response was firstly evaluated using the Myeloma Response Criteria. Two PRs and four MRs were found in 47 evaluable patients treated with single-agent plitidepsin (ORR = 12.8%). Twenty-four other patients had SD, which lasted for >3 months in four patients. Nineteen patients who showed PD or SD after receiving four cycles of single-agent plitidepsin had dexamethasone added to treatment. Two PRs and two MRs were found in 18 evaluable patients in this cohort (ORR = 22.2%), while SD for >3 months occurred in eight patients. When response was assessed as per Investigator's criteria, the ORR was 12.8% for single-agent plitidepsin (3 PRs and 3 MRs in 47 evaluable patients) and 27.8% for plitidepsin combined with dexamethasone (3 PRs and 2 MRs in 18 evaluable patients). Overall, these results suggest that plitidepsin administered alone or in combination with dexamethasone showed clinical activity in patients with relapsed/refractory MM.

1.3.3.3 Phase III Program

An ongoing multicenter, open-label, randomized, Phase III clinical trial started in June 2010 is comparing the efficacy and safety of plitidepsin combined with dexamethasone vs. dexamethasone alone in patients with relapsed/refractory MM previously treated with at least three but not more than six therapeutic regimens. This is the first plitidepsin pivotal trial and is expected to randomize up to 250 patients worldwide to receive either plitidepsin 5 mg/m^2 i.v. as a 3-h infusion on D1 and 15 q4wk plus dexamethasone 40 mg orally on D1, 8, 15 and 22, q4wk, or dexamethasone alone at the same dose and schedule.

To 31 March 2013, a total of 84 patients have been included into this trial. A total of 129 SAEs have been reported in 54 patients, regardless of treatment arm. Of these, 44 SAEs reported in 25 patients have been considered to be related to or with an unknown relationship with the treatment. The most common related SAEs were pneumonia (n=7, including one case of primary atypical pneumonia), hyperbilirubinemia (n=3), transaminase increases, sepsis, myopathy, CPK increase, psychotic disorders and renal failure (n=2 each). All three episodes of hyperbilirubinemia were grade 3 and involved one patient each; of these, one had tumor lesions in the liver and another one had diffuse fatty changes in the liver. No further safety data are available yet. Of note, an interim analysis of unblinded data from 79 treated patients conducted by an Independent Data Monitoring Committee (IDMC) found no safety reasons to terminate the study.

On 9 December 2012, the evaluation by the IDMC of efficacy and safety data from the 60 evaluable patients included in the first stage resulted in a recommendation to continue the trial unmodified, as the study met the established efficacy threshold of 30% pre-specified in the protocol. No safety issues were reported. Therefore, patient accrual was resumed.

1.3.4 Summary of Pharmacokinetic Results

After non-compartmental analysis, plitidepsin was found to be widely distributed, with apparent volumes of distribution in steady state (V_{ss}) of about 500 to 1350 L based on plasma, and from about 100 to 225 L based on whole blood, suggesting that blood cells are an important distribution compartment. Concentrations were about 3-fold higher in whole blood than in plasma. This initial characterization was updated after analyzing data from phase II clinical trials, during which samples were collected at later time points than in phase I trials. Furthermore, patients included in phase II trials had samples taken for PK evaluation during both the first and third treatment cycle, allowing for a more extensive analysis. Population methodology was used, as several

phase II studies had sparse sampling. The final model was a three-compartment disposition model with linear elimination within the dose ranges clinically explored. In this analysis, plitidepsin showed a prolonged terminal half-life of 88 h (almost double than initial calculations performed by non-compartmental methods of analysis of phase I data). Additionally, a population PK/PDy model was developed to evaluate the relationship between the pharmacokinetics of plitidepsin and ALT increases, as a measure of hepatocyte injury. The main conclusion of this analysis was that the time course of the ALT elevation depends on dose and schedule but not on infusion duration.

1.4 Bortezomib

1.4.1 Scientific Background

Bortezomib (Velcade®; Millennium Pharmaceuticals Inc, Cambridge, MA, USA and Johnson & Johnson Pharmaceutical Research and Development LLC, La Jolla, CA, USA) is a potent, reversible, and specific inhibitor of the proteasome (PI) and represents a first-in-class anti-neoplastic cytotoxic agent that differs from conventional cytotoxic agents by a favorable side effect profile, including its lack of significant myelosuppression, hair loss and mucositis.

Bortezomib is a modified dipeptidyl boronic acid derived from leucine and phenylalanine; its chemical name is N-pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid and has a molecular weight of 384.25 daltons (42, 43).

Bortezomib was the first clinically-validated boronate-based dipeptide PI approved for use in relapsed/refractory MM. It was approved in the USA in 2005 for the treatment of patients with MM who have received at least one prior therapy, and in 2008 for front-line treatment of patients with MM in combination with melphalan and prednisone. Bortezomib reversibly binds the 20S subunit, inhibiting the ChT-active site and also significantly the C-L active site; however, it has minimal effect on T-L activity. Bortezomib targets numerous pathways by inhibiting the proteasome and controlling key transcription factors. The apoptotic activity of bortezomib results from inhibition of NFB activity, disruption of cyclin-dependent kinase activity, stabilization of c-Jun N-terminal kinases leading to Fas upregulation, stabilization of p53, and a shift of the pro-apoptotic and antiapoptotic balance in the Bcl-2 family of proteins.

Due to the non-optimal AE profile observed to date with the i.v. administration of bortezomib, alternative schemes of administration are currently being investigated. One study (44) showed weekly administration of bortezomib to be feasible; more importantly, weekly administration, when compared with the standard scheme of administration, considerably reduced neuropathic toxicity without interference with activity. Furthermore, subcutaneous (s.c.) administration yields the same activity whilst being much less toxic. Based on this information, we plan to administer weekly bortezomib subcutaneously in combination with biweekly plitidepsin along with the standard low dose weekly dexamethasone.

Inhibitors of the 26S proteasome act through multiple mechanisms to suppress tumor survival pathways, arrest tumor growth, tumor spread and angiogenesis. Unlike conventional chemotherapeutics, bortezomib represents a novel class of anti-cancer agent because it has the ability to affect a combination of cellular regulatory mechanisms. This multiple mechanistic approach potentially represents a more effective anti-cancer strategy compared to the anti-tumor activity afforded by conventional CT (42).

1.4.2 Mechanism of Action

The mechanisms of anti-tumor activity that have been established for bortezomib involve many pathways thought to be integral to cancer treatment strategies (45-48). The following mechanisms have been demonstrated in *in vitro* and *in vivo* experiments:

- Inhibits activation of NF-kB in cells and in tumor microenvironment
- Reduces adherence of myeloma cells to BM stromal cells
- Blocks production and intracellular signaling of IL-6 in myeloma cells
- Blocks production and expression of pro-angiogenic mediators
- Overcomes defects in apoptotic regulators, such as Bcl-2 overexpression and alterations in tumor suppressor p53
- Activity is cell-cycle independent
- Stabilizes cell cycle regulatory proteins
- Unaffected by drug efflux pumps

1.4.3 Preclinical Experience

Pre-clinical research with PIs has demonstrated their ability to induce apoptosis and inhibit tumor growth, supporting their potential role in the treatment of various tumor types, especially hematological malignancies.

PK and PDy studies have been conducted in the rat and cynomolgus monkey. Upon i.v. bolus administration, bortezomib displays a rapid distribution phase ($t_{1/2\alpha}$ <10 min) followed by a longer elimination phase ($t_{1/2\beta}$ 5–15 h). Bortezomib has a large volume of distribution (range 5–50 L/kg). Its plasma PK profile is well described by a two-compartment model. The PDy action of bortezomib is well established and can be measured through an *ex vivo* assay (20S proteasome activity) (49). This assay was used to determine the duration of drug effect in lieu of the PK data in the early preclinical toxicology studies as well as to set a guide for dose escalation in humans. Following dosing with bortezomib in the rat and cynomolgus monkey, proteasome inhibition in peripheral blood had a half-life less than 24 h, with proteasome activity returning to pretreatment baseline within 24 h in monkey and within 48 to 72 h in rat after a single dose of bortezomib. Further, intermittent but high inhibition (>70%) of proteasome activity was better tolerated than sustained inhibition. Thus, a twice-weekly clinical dosing regimen was chosen in order to allow return of proteasome activity towards baseline between dose administrations (46, 47, 50, 51).

1.4.4 Clinical Pharmacology

The clinical pharmacology program has been designed and partially carried out to investigate the disposition characteristics, and the pharmacodynamics of bortezomib (52, 53). Conclusions from the completed investigations are:

- Upon i.v. bolus administration, bortezomib displays a rapid distribution phase ($t_{1/2\alpha}$< 30 min) followed by a longer elimination phase $t_{1/2\beta}$> 10 h) and a large volume of distribution, all consistent with a 2-compartment PK model.
- The high volume of distribution, rapid distribution phase, prolonged biological effect ($t_{1/2\sim24}$ h), and high potency (K_i = 0.6 nM with slow off rate),

along with *in vitro* metabolic studies suggest that de-boronation and proteolytic cleavage of bortezomib at the cellular level represent the majority of the catabolism of this compound. This conclusion is based on extensive preclinical evaluation of the disposition characteristics, PK and the PDy of bortezomib. Inhibition of 20S proteasome activity occurs in a dose-related manner. The maximum pharmacodynamic effect on circulating whole blood 20S activity occurs within 1 h of dosing. The relationship between bortezomib plasma concentrations and proteasome inhibition is well described by a simple E_{max} model.

1.4.5 Phase I Clinical Experience

Data from four phase I studies designed to evaluate the MTD of bortezomib and DLTs in a variety of doses and dose schedules have been analyzed. The MTD of bortezomib, regardless of individual protocol definition, appeared to be dependent on the treatment schedule employed and the patient population treated. MTDs and DLTs in these studies were as follows:

- The MTD of bortezomib administered twice per week for 2 weeks followed by a 10-day rest period to patients with advanced solid tumors was determined to be 1.3 mg/m^2. DLTs of fatigue, diarrhea and peripheral neuropathy (PN) were observed at 1.56 mg/m^2/dose (54).
- The MTD of bortezomib administered once per week for 4 weeks followed by a 14-day rest period to patients with solid tumors was 1.6 mg/m^2. This was the least dose-intensive schedule but had the highest individual doses administered (55).
- The MTD of bortezomib administered twice per week for four weeks in eight dose cycles followed by a 14-day rest period to patients with hematological malignancies was determined to be 1.04 mg/m^2. A DLT (hyponatremia) and more frequently grade 3 thrombocytopenia was observed at a bortezomib dose of 1.04 mg/m^2 and 1.38 mg/m^2 (56).

Neurotoxicity was observed in phase I studies, particularly a painful sensory PN, that was dose-related, and more prevalent among patients previously treated with neurotoxic agents (e.g., platinum, thalidomide, vincristine and taxane-containing regimens) and dose-limiting in patients with refractory solid tumors.

A relatively low incidence of significant myelosuppression in Phase I, febrile neutropenia, infections and transfusion-dependent thrombocytopenia or anemia, mucositis or alopecia was notable; this has also been borne out in the phase II data evaluated to date. Effects on the liver, kidney, and heart were rarely found. Decreases in platelet count have been observed on treatment during both phase I and II studies and appear to be related to dose. Clinically significant thrombocytopenia can occur and appears to be influenced by baseline platelet count. Platelet count tends to recover during the rest period. Patients should be carefully monitored throughout treatment with bortezomib for hematological abnormalities.

Although demonstration of efficacy was not a primary objective in the phase I clinical studies, anti-tumor activity was observed in patients with squamous cell carcinoma of the nasopharynx, bronchoalveolar carcinoma of the lung, renal cell carcinoma, prostate cancer, lymphoma, Waldenström's macroglobulinemia and MM.

1.4.6 Phase II Clinical Experience

The safety and efficacy of bortezomib were evaluated in an open-label, single-arm, multicenter phase II study of 202 patients with relapsed and refractory MM who had received at least 2 prior lines of treatment and were progressing on most recent therapy (SUMMIT) (57). Patients with relapsed and refractory myeloma have an expected survival of 6-9 months.

The 202 patients had multiple poor prognostic factors at study entry including elevated beta-2-microglobulin, poor hematopoietic reserve, evidence of organ dysfunction, abnormal renal function and chromosomal abnormalities. The median number of prior lines of therapy was six.

An i.v. bolus injection of bortezomib 1.3 mg/ m^2 dose was administered twice weekly for 2 weeks without routine pre-medication, followed by a 10-day rest period (21 day treatment cycle) for a maximum of eight treatment cycles. Patients who experienced benefit from bortezomib treatment were allowed to continue treatment in an extension study. Patients who experienced PD after at least 2 cycles, or had SD after at least 4 cycles with bortezomib were allowed, at their physicians' discretion, to have high dose dexamethasone (40 mg) added to their bortezomib treatment. Response rates to bortezomib alone were determined by an independent response committee (IRC) based on Bladé's criteria (58).

Complete remission required 100% reduction in M-protein and included patients with positive or negative immunofixation (IF+ or IF-, respectively). All 202 patients were evaluable for time to event analyses. A total of 193 patients were evaluated for response (nine patients with non-measurable disease could not be evaluated for response by the IRC). In SUMMIT, bortezomib demonstrated an overall response rate (CR+PR+MR) of 35% with 59% patients experiencing improved or stable disease (SD). A total of 10% of patients experienced a complete remission (4% IF- and 6% IF+). The median time to response was 38 days.

The median survival of all patients enrolled in SUMMIT was 16 months. RR was independent of the number or type of previous therapies. In addition, the rate of response remained consistent regardless of the patients' gender, race, body surface area, performance status, myeloma type or chromosome 13-deletion status. The median time to progression for all 202 patients enrolled in SUMMIT was 7 months. Median time to progression on their last previous therapy was 3 months and when using patients from SUMMIT as their own controls, the median time to progression was twice as long on bortezomib relative to the last therapy. Effects on serum and/or urine monoclonal paraprotein and plasma cells from BM aspirate and biopsy were also evaluated. Overall, 70% of patients had either reduction or stable serum and/or urine paraprotein levels. A total of 69% of patients included in the analysis for BM biopsy results had a 50% decrease in plasma cells, thereby demonstrating that treatment with bortezomib reduces the number of or clears myeloma cells from the BM. BM aspirate results were consistent with those obtained by biopsy.

Responders (CR+PR) in SUMMIT also had an increase in mean hemoglobin and decreased overall transfusion requirements; stable renal function; stable or improved Karnofsky Performance Status (KPS) and increased mean non-myeloma immunoglobulin levels (IgM, IgA and IgG). The mean IgM returned to the normal range by the end of treatment; 28% (15/53) of patients had increases of $\geq$ 2 fold in one of their non-myeloma immunoglobulins. An association between RR and improvement

in quality of life was apparent. Patients who responded to treatment experienced an improvement in EORTC-C30 Global and Physical parameters, including a decrease in disease symptoms, pain and fatigue.

In SUMMIT, 74 patients were administered dexamethasone in combination with bortezomib and were assessed for response. Eighteen percent (13/74) achieved an improved response (MR or PR) with combination treatment.

The CREST study was a randomized open-label, single-arm, multicenter study which enrolled 54 patients with MM that progressed or relapsed on or after front-line therapy (CREST) (59). Bortezomib was administered twice weekly for 2 weeks followed by a 10-day rest period for a maximum of eight treatment cycles as second line therapy.

Patients were prospectively randomized to receive 1.0 or 1.3 mg/m^2/dose. A total of 28 patients received 1.0 mg/m^2/dose and 26 patients were administered 1.3 mg/m^2/dose. Patients who experienced benefit from bortezomib treatment were allowed to continue treatment in an extension study. Patients who experienced PD after at least two cycles or SD after at least four cycles with bortezomib alone were allowed, at their physician's discretion, to have high dose dexamethasone (40mg) added to their treatment.

Both dose groups were similar with regards to demographic and baseline characteristics. The median age for all patients was 63 years; 57% had a KPS score of 90 to 100 with only 13% of patients with a score of $\leq$ 70; 19% had a hemoglobin level <100 g/l and no patients had a platelet count < 50 x 10^9/L. The median duration of time between diagnosis of MM and the first dose of bortezomib was 2.0 years and patients had received a median of one prior treatment line (median of three prior therapies), including steroids (98% of patients), alkylating agents (72% of patients), anthracyclines (54% of patients), prior stem cell transplant (48% of patients) and thalidomide (30% of patients). The median time to progression for all treated patients was 11 months. Eighty percent of patients were alive at 1 year.

In CREST, the combination of bortezomib and dexamethasone was administered to 28 patients, 16 patients in the 1.0mg/m^2 group and 12 patients in the 1.3mg/m^2 group. A total of 9 patients (32%) had an improved response (CR, PR or MR) with combination treatment (4 of the 16 patients receiving 1.0 mg/m^2 and 5 of the 12 patients in the 1.3 mg/ m^2 group). Two of these 9 patients achieved a CR while receiving dexamethasone in combination with bortezomib therapy. A total of 51 patients entered the extension study.

Initial data indicate that bortezomib can be administered to patients with relapsed and/or refractory MM for longer than six months with similar tolerability to that of the first six months of treatment. Patients were able to maintain their response or had an improved response with additional cycles of bortezomib therapy.

The phase III study (M34101-039), also referred to as the APEX study, was designed to determine whether bortezomib provided benefit (TTP, RR, and survival) to patients with relapsed and/or refractory MM relative to treatment with high-dose dexamethasone (60). The study was also designed to determine the safety and tolerability of bortezomib relative to high-dose dexamethasone, and whether treatment with bortezomib was associated with superior clinical benefit and quality of life relative to high-dose dexamethasone. A total of 669 patients were enrolled and 663 patients received study drug (bortezomib: 331; dexamethasone: 332). Patients randomized to bortezomib received 1.3 mg/m^2 i.v. push twice weekly on Day 1, 4, 8, and 11 of a 3-week cycle for

up to eight treatment cycles as induction therapy, followed by 1.3 mg/m^2 bortezomib weekly on Day 1, 8, 15, and 22 of a 5-week cycle for three cycles as maintenance therapy. Patients randomized to dexamethasone received oral dexamethasone 40 mg once daily on Day 1 to 4, 9 to 12, and 17 to 20 of a 5-week cycle for up to four treatment cycles as induction therapy, followed by dexamethasone 40 mg once daily on days 1 to 4 followed of a 4-week cycle for five cycles as maintenance therapy. The EBMT response criteria, as described by Bladé were utilized to determine disease response.

There was a 78% increase in TTP for the bortezomib arm. Median TTP was 6.2 months for the bortezomib arm and 3.5 months for the dexamethasone arm ($p < 0.0001$). CR + PR were 38% with bortezomib vs. 18% with dexamethasone ($p < 0.0001$). CR was 6% with bortezomib vs. <1% with dexamethasone ($p < 0.0001$). The CR + near CR (nCR) rate was 13% with bortezomib vs. 2% with dexamethasone. In patients who had received only one prior line of treatment (bortezomib: 132; dexamethasone: 119), CR + PR were 45% with bortezomib vs. 26% with dexamethasone ($p = 0.0035$). With a median 8.3 months of follow-up, OS was significantly longer ($p = 0.0013$) for patients on the bortezomib arm vs. patients on the dexamethasone arm.

The probability of survival at one year was 80% for the bortezomib arm vs. 66% for the dexamethasone arm, which represented a 41% decreased relative risk of death in the first year with bortezomib ($p = 0.0005$). In patients who had received only one prior line of treatment, the probability of survival at one year was 89% for the bortezomib arm vs. 72% for the dexamethasone arm, which represented a 61% decreased relative risk of death in the first year with bortezomib ($p = 0.0098$) (61).

1.4.7 Safety Issues

The data described below reflect exposure to bortezomib in 256 patients with MM (57) (58). The median total dose administered across all 256 patients was 46 mg, median duration of treatment was 131 days, and median number of doses administered was 22 (six cycles). The most commonly reported AEs were nausea (62%), fatigue (54%), diarrhea (48%), constipation (41%), thrombocytopenia (41%), pyrexia (36%), vomiting (34%), and anorexia (30%).

Events reported as PN, peripheral sensory neuropathy and PN aggravated were reported in 35% of patients. PN was grade 3 for 13% of patients and grade 4 for < 1% of patients. New onset or worsening of existing neuropathy was noted throughout the cycles of treatment. Five percent (5%) of patients discontinued bortezomib due to neuropathy. Notably, more than 80% of patients had signs or symptoms of PN at baseline evaluation. The incidence of grade 3 neuropathy was low (2 of 60 patients, 3%) in patients without baseline neuropathy. Symptoms may improve in some patients upon discontinuation of bortezomib; complete resolution of PN has been reported.

Transient and uncomplicated thrombocytopenia was reported during treatment with bortezomib in 42% of patients. The thrombocytopenia observed was characterized by a dose-related decrease in platelet count during the bortezomib dosing period (Days 1 to 11) with a return to baseline in platelet count during the resting period (Days 12 to 21) in each treatment cycle. Thrombocytopenia was assessed as bortezomib-related in 38% of patients and grade 3-4 in intensity in 29% of patients. Three percent of patients experienced grade 4 thrombocytopenia. All episodes of grade 4 events were bortezomib-related. Four percent of patients discontinued bortezomib treatment due to thrombocytopenia of any grade.

Notably, infusion reactions, infusion site reactions, alopecia, mucositis, febrile neutropenia and sepsis were rarely reported. Acute development or exacerbation of congestive heart failure has been seen in subjects with risk factors for or existing heart disease.

Thirteen percent of patients experienced at least one episode of grade 4 toxicity, with the most common events being thrombocytopenia (3%) and neutropenia (2%). A total of 124 (48%) of the 256 patients experienced SAEs during the studies. The most commonly reported SAEs included pyrexia (7%), pneumonia (7%), diarrhea (5%), vomiting (5%), dehydration (5%) and nausea (4%).

AEs leading to treatment discontinuation were reported in 28% of patients. The reasons for discontinuation were evenly distributed across the most common types of toxicity and included PN (5%), thrombocytopenia (4%), PD (3%), diarrhea (2%), and fatigue (2%). The majority of patients discontinuing treatment due to AEs were not responding to therapy.

The addition of dexamethasone did not appear to adversely affect the safety profile of bortezomib.

1.5 Study Rationale and Drug Selection

MM is still an incurable disease. As front-line treatment to reduce tumor burden, hematopoietic stem cell transplantation as well as emergent drugs used in newly diagnosed patients offer the best chance for long-term survival. However, while many studies have shown the benefits of this approach, most patients will relapse. Thus, additional therapeutic options are needed for these patients.

In 1999, plitidepsin was found to have substantial *in vitro* anti-tumor activity in cells isolated from patients with advanced myeloma, and preliminary clinical data are confirmatory of activity against refractory and relapsed MM: a 3-h i.v. plitidepsin infusion at a dose of 5 mg/m^2, q2w induces objective responses (around 20%) with an acceptable toxicity profile, and with a remarkable lack of neurological and hematological toxicity.

Bortezomib, a novel PI, has been shown to induce clinically significant responses with manageable toxic effects in patients with relapsed and/or refractory MM. In the pivotal study, the ORR, including complete responses, was 35%. The median duration of responses was 12 months, and there was an increase by a factor of 2 to 4 in the TTP with bortezomib therapy as compared to the last therapy. In the CREST trial patients with refractory MM were randomized to receive 1.0 or 1.3 mg/m^2 of bortezomib twice weekly for 2 weeks, every 3 weeks, for a maximum of eight cycles. Dexamethasone was permitted in patients with progressive or SD after 2 or 4 cycles, respectively. Patients who received bortezomib at a dose of 1.3 mg/m^2 had increased CR+PR rates (50% vs. 33%), prolonged median duration of response and median TTP (11 months vs. 7 months). Thus, the dose of 1.3 mg/m^2, twice per week in 3-week cycles, for eight cycles, seems to be the optimal dose for bortezomib as single agent or in the combination with dexamethasone.

The combination of plitidepsin plus bortezomib has been studied *in vitro*. The degree of cytotoxicity was determined by a MTS (tetrazolium salt) assay. Data from the MTS assay was expressed as the fraction of cells affected by the dose (Fa) in drug-treated cells as compared to single agent treated cells (control). *In vitro* results of this combination showed synergy at the higher dose of the concentration range tested.

The combination was further tested in a human plasmacytoma (MM-1s) subcutaneously implanted in NOD SCID mice. The bortezomib combination showed a trend towards a better outcome than the single agents, although differences were not very evident, probably due to the low doses of plitidepsin and bortezomib used in the experiment.

The use of bortezomib is limited by its toxicity, such as PN and thrombocytopenia, which restricts the clinical dosing regimen to a biweekly (D1, D4) schedule, allowing proteasome activity to recover between doses. In an effort to improve tolerability in patients with relapsed and/or refractory MM, a recent analysis evaluated the feasibility of a single bortezomib dose in the setting of combination therapy. The majority of response parameters, including ORR and PFS, were similar, while the safety profile improved (with a PN incidence of 8% vs. 28% in patients receiving twice-weekly dosing) (44). A separate phase III study examined the impact of s.c. bortezomib on safety and efficacy parameters; in addition to a more convenient administration, s.c. bortezomib offered similar efficacy but lower toxicity (62).

Based on the positive results observed with plitidepsin and bortezomib both as single agents and in combination with dexamethasone against MM, their different MOA, and the potential synergism of the triple combination, the efficacy of plitidepsin plus bortezomib and dexamethasone in patients with relapsed and/or refractory MM deserves to be addressed in clinical trials.

2. OBJECTIVES

2.1 Primary Objective

To determine the recommended dose (RD) of plitidepsin in combination with bortezomib and dexamethasone in patients with relapsed and/or refractory MM.

2.2 Secondary Objectives

- To determine the efficacy of plitidepsin in combination with bortezomib and dexamethasone.
- To evaluate the safety and tolerability of the combination in patients with relapsed and/or refractory MM.
- To study the pharmacokinetics (PK) and pharmacodynamics (PDy) of plitidepsin in combination with bortezomib and dexamethasone.

3. OVERALL STUDY DESIGN

This is a multi-center, uncontrolled, single arm, phase I study, designed to establish the optimal dose of the combination plitidepsin, bortezomib and dexamethasone in patients with refractory and/or relapsed MM. Patients will be enrolled sequentially into three dose levels. The feasibility of administering plitidepsin with bortezomib in combination with dexamethasone and the RD of the combination will be determined. Patients will be evaluated at scheduled visits in three study periods: pre-treatment, treatment and follow-up.

The **pre-treatment period** includes screening and baseline visits. After providing written IC to participate in the study, patients will be screened for study eligibility during a screening period of up to 28 days. Baseline assessment consists of a detailed

history of pre-existing diseases, a complete physical examination and clinical neurological assessment, Eastern Cooperative Oncology Group Performance Status (ECOG PS) (Appendix 1), electrocardiogram (ECG), left ventricular ejection fraction (LVEF) and laboratory tests (including hematology and biochemistry), urinalysis, hepatitis B and C virus screening and serum pregnancy tests for women of child bearing potential (see Table: Schedule of Assessments).

Disease-specific markers will be analyzed: beta-2-microglobulin, C-reactive protein (CRP), IgG, IgA, IgM, immunofixation (IF) from blood and urine, serum free light chains (SFLC), a representative BM aspirate and/or BM biopsy, and a skeletal survey: X-rays of the skull, vertebral column, pelvis and proximal long bones or MRI. In case of extramedullary soft tissue plasmacytoma, CT-scan or MRI of any site involved will be performed.

During the **treatment period**, all patients are to attend study center visits on Day 1, 4, 8, 11 and 15 on an every four-week basis to assess safety and toxicity. All patients are to attend an end-of-treatment visit 30 (±5) days after the last dose of study therapy.

Prior to each administration of study drug, a short medical history focusing on plitidepsin-, bortezomib- and dexamethasone-associated side effects will be performed as well as cardiac markers, kidney and liver function tests. Complete blood count and biochemistry will be carried out before each plitidepsin administration as described in Section 4. Other disease-modifying treatments (e.g., alpha interferon) are strictly prohibited.

Tests for disease assessment will be performed during the treatment period described in Section 4.6.

A cycle is defined as 28 days, plus any additional days required for dosing delays due to any reason. All patients will remain in the study until PD, excessive side effects or withdrawal of consent (see Section 4.4). Patients who complete eight cycles can continue to receive plitidepsin exclusively if they have shown clinical benefit (respond to treatment or achieve SD) and upon Investigator's decision and agreement with the Sponsor. Patients who achieve a stringent complete response (sCR), a complete response (CR), very good partial response (VGPR), partial response (PR), minimal response (MR) or stable disease (SD) as defined by response criteria may be taken off the study if eligible to proceed to high dose CT and autologous stem cell transplantation (ASCT). Treatment may continue in case of obvious patient's benefit according to the Investigator and upon discussion with the Sponsor. Patients who discontinue plitidepsin treatment must stop taking bortezomib and dexamethasone in the study setting.

After completion of the treatment period or in case of discontinuation, patients are to attend follow-up visits. Patients will be followed for AEs during 30 days after the last administration of study drug and until their resolution. In addition, patients will be followed every three months to assess disease status. Patients taken off-study due to reasons other than PD, will be followed for disease status every three months until PD, initiation of another anticancer therapy, study termination or death, whichever comes first.

Detailed visit-by-visit study procedures are contained in Section 4.

3.1 Study Endpoints

3.1.1 Primary Endpoint

The RD will be the highest dose level (DL) at which fewer than two out of six patients (33%) experience DLTs during the first cycle.

3.1.2 Secondary Endpoints

- Overall response rate (ORR) (including sCR, CR, VGPR and PR).
- Minimal response (MR).
- Stable disease (SD).
- Clinical benefit rate, (including ORR plus MR and SD).
- Duration of response (DOR).
- Time to progression (TTP).
- Progression-free survival (PFS).
- Event-free survival (EFS).
- Safety and tolerability.
- PK and PDy of plitidepsin in combination with bortezomib and dexamethasone.

3.2 Number of Patients

Cohorts of 3-6 patients per DL (in up to three DLs) will be treated until the RD is defined. At the RD, at least six patients evaluable for the determination of DLTs will be treated. The number of patients may vary depending upon the tolerability to plitidepsin administered in combination with bortezomib and the number of DLs required to identify the RD, but a total of 20-30 patients are expected.

3.3 Selection of Patients

Patients with relapsed and/or refractory MM will be included.

- **Refractory myeloma** is defined as disease that is non-responsive while on primary or salvage therapy, or progresses within 60 days of the last therapy. There are two categories of refractory myeloma:
  - ✓ **Primary refractory myeloma** is defined as disease that is non-responsive in patients who have never achieved a MR or better, with any therapy. It includes patients who never achieved MR or better in whom there is no significant change in monoclonal protein (M-protein) and no evidence of clinical progression as well as primary refractory, disease progression (PD) where patients meet criteria for true PD.
  - ✓ **Relapsed and refractory myeloma** is defined as disease that is non-responsive while on salvage therapy, or progresses within 60 days of last therapy in patients who have achieved MR or better at some point previously before progressing.
- **Relapsed myeloma** is defined as previously treated myeloma that progresses and requires the initiation of salvage therapy but does not meet the criteria for either **"primary refractory"** or **"relapsed-and-refractory"** myeloma categories.

3.3.1 Inclusion Criteria

A patient is eligible for enrolment if all of the following inclusion criteria are met:

1) Patients must give written IC in accordance with institutional and local guidelines.
2) Age ≥ 18 years.
3) Patients must have a confirmed diagnosis of MM according to the Durie & Salmon criteria (Appendix 3).
4) Patients must have relapsed and/or refractory disease.
5) Patients must have measurable disease defined as any of the following:
    a) Serum M-protein > 0.5 g/dL or > 0.2 g/24-h urine light chain (UFLC) excretion.
    b) In patients who lack measureable M-protein in serum or urine, i.e. serum M-protein < 0.5 g/dL and urine M-protein < 0.2 g/24 h, serum FLC (SFLC) levels are most informative. SFLC levels can be used only if the baseline SFLC ratio is abnormal (<0.26 or >1.65) indicating clonality. In addition, the baseline SFLC level must be ≥10 mg/dL of the appropriate involved light chain isotype.
    c) When applicable, measurable soft tissue plasmacytoma > 2 cm, by either physical examination and/or applicable radiological evaluation (i.e. MRI, CT-scan).
6) Prior autologous and/or allogeneic hematopoietic stem cell transplantation (HSCT) patients are allowed. Patients must not have acute/chronic graft-versus-host disease (GVHD) or be receiving immunosuppressive therapy at least 30 days before the onset of treatment with the study drug(s).
7) Patients must have received at least one previous treatment line, which can consist of:
    a) Induction regimen followed by high dose chemotherapy (CT) and peripheral blood stem cell collection.
    b) Induction regimen alone according to institutional guidelines.
    c) High doses of CT followed by non-myeloablative transplantation.
    d) CT followed by either single or tandem autologous stem cell transplantation (ASCT).
    e) CT followed by autologous and (if performed) subsequent non-myeloablative allogeneic stem cell transplantation.
    f) Previous line(s) of systemic CT and biological agents should have been completed at least 30 days and 15 days, respectively, prior to starting protocol treatment.
8) Previous treatment with bortezomib or another PI is allowed provided patients achieved at least MR lasting a minimum of two months.
9) Patients must have an ECOG PS ≤ 2.
10) Recovery to grade ≤ 1 from any non-hematological AE derived from previous treatment (excluding alopecia).

11) Laboratory data:

a) Hemoglobin ≥ 8 g/dL.

b) Absolute neutrophil count (ANC) ≥ 1,000 cells/mm^3 (1.0 x 10^9/L) (≥ 0.5 x 10^9/L if due to extensive BM involvement –by ≥50% of plasma cells in BM biopsy). Screening of ANC should be independent of granulocyte- and granulocyte/macrophage-colony stimulating factor (GCSF and GM-CSF) support for at least one week and of pegylated G-CSF for at least two weeks.

c) Platelet count ≥ 50,000/ mm^3 (50.0 x 10^9/L) for patients in whom < 50% of the BM nucleated cells are plasma cells.

d) Platelet count ≥ 25,000/ mm^3 (25.0 x 10^9/L) for patients in whom ≥ 50% of BM nucleated cells are plasma cells.

e) Serum total bilirubin < 1.5 x institutional upper limit of normal (ULN) (except when Gilbert syndrome is clearly documented and other liver function tests are within normal levels).

f) AST (aspartate aminotransferase) and ALT (alanine aminotransferase) and ≤ 3.0 x institutional ULN and AP (alkaline phosphatase) ≤ 2.5 x institutional ULN.

g) Creatinine clearance > 30 mL/min, measured or calculated according to Cockcroft and Gault's formula (Appendix 2).

h) Albumin ≥ 2.5 g/dL.

12) Women of child-bearing potential must have a negative serum or urine pregnancy test within seven days prior to enrolment. In addition, all sexually active women of child-bearing potential and fertile male patients must agree to use adequate contraceptive methods throughout the study and during six months after treatment discontinuation.

13) Left ventricular ejection fraction (LVEF) above the lower limit of normal.

14) Patients must have a BM assessment within three weeks prior to enrolment.

3.3.2 Exclusion Criteria

A patient will not be eligible for this study if any of the following exclusion criteria are met:

1) Previous treatment with plitidepsin.
2) Active or metastatic primary malignancy other than MM.
3) Serious concomitant systemic disorders that would compromise the safety of the patient or the patient's ability to complete the study, including the following specific conditions:

a) Uncontrolled psychiatric illness or medical illness that the Investigator feels will compromise the patient's tolerance of the study medication.

b) Significant non-neoplastic liver disease.

c) Uncontrolled endocrine diseases (i.e., requiring relevant changes in medication within the last month, or hospital admission within the last three months).

d) Uncontrolled systemic infection.

4) Other relevant cardiac conditions:

   a) Symptomatic arrhythmia (excluding anemia-related grade ≤ 2 sinusal tachycardia) or any arrhythmia requiring ongoing treatment, and/or prolonged grade ≥ 2 QT-QTc; or presence of unstable atrial fibrillation (according to CTCAE v4.0). Patients on treatment for stable atrial fibrillation are allowed, provided they do not meet any other cardiac or prohibited drug exclusion criterion.

   b) History or presence of unstable angina, myocardial infarction, valvular heart disease, cardiac amyloidosis or congestive heart failure within the last 12 months.

   c) Uncontrolled arterial hypertension (≥ 150/100 mmHg) despite optimal medical therapy.

   d) Previous treatment with doxorubicin at cumulative doses of > 400 mg/m².

5) History of hypersensitivity reaction to bortezomib, polyoxyl 35 castor oil or mannitol.

6) Myopathy or any clinical situation that causes significant and persistent elevation of creatine phosphokinase (CPK) (> 2.5 ULN) in two different determinations performed within one week of each other.

7) Sequelae of any grade ≥ 1 neuropathy (either bortezomib-related or not) according to CTCAE v4.0.

8) Any other major illness that, in the Investigator's judgement, will substantially increase the risk associated with the patient's participation in this study.

9) Pregnant and/or lactating women.

10) Known active human immunodeficiency virus (HIV) infection (HIV testing is not required unless infection is clinically suspected).

11) Active hepatitis B or C virus (HBV or HCV) infection.

12) Treatment with any Investigational Medicinal Product (IMP) in the 30 days before inclusion in the study.

13) Concomitant medications that include corticosteroids, CT, or other therapy that is or may be active against myeloma. Concurrent corticosteroids are allowed provided as an equivalent of a prednisone dose of ≤ 10 mg daily, administered as antiemetic or as premedication for blood products.

14) Wash-out periods after the end of previous therapy:

   a) Nitrosoureas must be discontinued six weeks prior to Cycle (C)1 Day (D)1.

   b) Thirty days for other chemotherapies and 15 days for other biological agents prior to C1 D1.

   c) Thirty days after the end of any prior radiation or radionuclide therapy (six weeks in the case of prior extensive external beam radiation, with more than 25% of BM distribution).

15) Plasma cell leukemia at the time of study entry.

16) Disease-related symptomatic hypercalcemia despite optimal medical therapy.

17) Limitation of the patient's ability to comply with the treatment or follow-up protocol.
18) Contraindication for the use of steroids.

4. PLAN OF THE STUDY

4.1 Duration of the Study Individually per Patient

Patients will be evaluated at scheduled visits in three study periods:

- **Pre-treatment:** from signature of the IC to the first study drug infusion.
- **Treatment:** from first infusion of study drugs to end of treatment (EOT).
- **Follow-up:** after EOT, patients will be followed q4wk until resolution of all toxicities, if any. Patients who discontinued treatment without disease progression will be followed every three months until disease progression, other antitumor therapy, death or until the date of study termination (clinical cut-off), whichever occurs first.

Patients will be considered to be on-study from the signature of the IC to the end of the follow-up period. Patients will be considered to be on-treatment for the duration of their treatment and until the day of EOT, immediately before the start of the follow-up period. EOT is defined as 30 days after the day of last treatment, unless the patient starts a new antitumor therapy or dies (whichever occurs first), in which case the date of administration of this new therapy or the date of death will be considered the EOT date. An EOT visit will be performed within 30 days (± five days) after last treatment, unless the patient starts any subsequent new antitumor therapy outside this clinical study, in which case the EOT visit should be performed immediately before the start of the new therapy, whenever possible.

4.2 Duration of the Study (for the Whole Study)

- **Planned start date:** First quarter of 2014 (1Q14).
- **Planned enrolment period:** approximately 15 months.
- **Total duration of the study:** approximately 24 months.
- **Planned end-of-study date (clinical cut-off):** six months after the patient's treatment discontinuation (last patient-last visit), or nine months after accrual of the last evaluable patient, wichever occurs first.

4.3 Subject Participation

After ensuring that the patient meets all eligibility criteria and has given IC, patients can be enrolled in the study by contacting a designated study monitor at PharmaMar, and completing the electronic screening form. Registration will be confirmed and checked by PharmaMar. No patient can be included in the study until the electronic screening form has been completed and received by Pharma Mar. A patient number will be provided to the site of enrollment. This patient number should be used on all future documentation and correspondence referring to this patient. Should eligibility criteria problems arise, final decisions will be taken between PharmaMar and the Investigator.

Patients will be evaluated at scheduled visits in up to three study periods: pre-treatment, treatment and follow-up. Patients will receive study medication while it is considered to

be in their best interest (see Section 4.4 Discontinuations). In responding patients, treatment may continue upon Investigators' discretion.

Patients will be considered to be on-study for the duration of their treatment and during the 30 days following treatment discontinuation. Treatment discontinuation is defined as the day of the last dose of study drug administration.

4.4 Discontinuations

A discontinuation occurs when an enrolled patient ceases to participate in the study, regardless of the circumstances. The primary reason for patient's discontinuation will be recorded on the electronic Case Report Form (e-CRF). The final evaluation required by the protocol will be performed thirty days (±5) after the last dose of study therapy.

If the patient is discontinued from the study before completion, every effort should be made to complete the assessments scheduled during the post-treatment follow-up period. The Investigator is ultimately responsible for the patient's safety and wellbeing. Study treatment should be discontinued if this is considered to be in the patient's best interest. Plitidepsin, bortezomib and dexamethasone are to be permanently discontinued for patients meeting any of the following criteria:

- Confirmed PD.
- Life-threatening, unmanageable or unacceptable drug-related AEs, including the need for more than two dose reductions, except in cases of obvious patient benefit in continuing the treatment at the Investigator's criterion.
- Intercurrent illness of sufficient magnitude to preclude safe continuation of the study.
- Patient refusal and/or non-compliance with study requirements.
- A major protocol deviation that may affect the balance of the risk/benefit ratio for the participating patient.
- Treatment delay > 14 days due to toxicity (except in case of patient's clear clinical benefit, with the Sponsor's approval).
- Pregnancy.
- Investigator's decision.

Study discontinuation could also occur due to administrative reasons, a Sponsor's decision or unforeseen reasons. Patients withdrawn from the study must not re-enter this study at any time.

4.5 Replacement of Patients

Patients must be replaced if:

- They are withdrawn from the study due to not being evaluable for the primary endpoint, due to hypersensitivity reactions or reasons other than drug-related AEs meeting DLT criteria (e.g., withdrawal of consent, not meeting the eligibility criteria, non-compliance with follow-up, early PD, or unrelated AE).

- They require radiation therapy or other anti-cancer procedure within four weeks after the first dose, unless they previously suffered an AE included in the definition of DLT.
- There is a protocol deviation resulting in the impossibility of evaluating the safety of the study therapy.

4.6 Screening and Baseline Assessments

Table 2. Screening and baseline assessments.

Screening and baseline	Investigations	Time
General		
1. Medical history and physical examination	• Written IC signed by the patient • Demographic data. • Medical history including: ✓ Date of MM diagnosis, relapse(s) or evidence(s) of refractoriness to prior treatments. ✓ Documentation of PD prior to inclusion. ✓ M-protein determinations. ✓ Previous specific treatments (surgery, radiotherapy, CT, immunotherapy etc.) with dates, best response and TTP. • Concomitant treatments. • Transfusional requirements. • Clinical assessment of signs and symptoms (tumor-related or not). • Complete physical examination, including weight, height and vital signs assessment (HR, ABP and temperature). • Complete clinical neurological assessment. • Baseline ECOG PS (Appendix 1).	Prior to any study-specific procedure. Within 14 days prior to inclusion. To be repeated on C1 D1 prior to infusion.
2. Hematology	• Differential WBC count. • Hematocrit, hemoglobin. • Platelets.	Within 7 days prior to inclusion. Repeat prior to the first infusion.
3. Coagulation	• PT, INR, APTT.	Within 14 days prior to inclusion.
4. Biochemistry	• AP, AST, ALT, LDH, bilirubin, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}), glucose, CPK, CPK-MB fraction, total proteins, albumin. • Creatinine and CrCl measured or calculated (Appendix 2).	Within 7 days prior to inclusion.
5. Urine	• Dipstick, sediment.	Within 14 days prior to inclusion.
6. Viral serology	• HBV and HCV. • CMV in patients who have undergone allogeneic BM transplantation.	Within 14 days prior to inclusion.
7. Pregnancy test	• Serum or urine in women of child bearing potential.	Within 7 days prior to enrolment.

Screening and baseline	Investigations	Time
General		
8. Heart function	• ECG: It should allow a rhythm definition (at least 30 second of duration). ✓ PR interval. ✓ QT interval (raw and corrected by HR using Bazett's formula). ✓ QRS complex and the maximum height of QRS complex in derivation II]. • LVEF by ECHO or MUGA scan.	Within 14 days prior to inclusion.
Disease assessment		
9. Serum protein	• Protein electrophoresis. • Serum Ig determination and M-protein measurement and IF • SFLC.	Within 14 days prior to inclusion.
10. Urine protein	• 24-h urine protein electrophoresis • UFLC. • Urine M-protein measurement and IF.	
11. Serum beta-2-microglobulin and CRP		Within 14 days prior to inclusion.
12. Bone marrow	• BM morphology. • BM cytometry (if available). • BM cytogenetics (if available).	Within 21 days prior to treatment.
13. Clinical and radiological tumor assessment in case of soft tissue plasmacytoma	• CT-scan or MRI of all measurable/evaluable involved sites.	Within 14 days prior to inclusion.
14. Skeletal evaluation	• X-ray of skull, vertebral column pelvis and proximal long bones or MRI.	Within 4 weeks prior to inclusion.
15. Other relevant tests	• Where indicated, according to the clinical context.	Within 14 days prior to inclusion.

ABP, blood pressure; ALT, alanine aminotransferase; AP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BM, bone marrow; C, cycle; CMV, cytomegalovirus; CPK, creatine phosphokinase, CPK-MB fraction, CPK isoenzymes found in cardiac muscle (it will be performed only if CPK is > ULN); CrCl, creatinine clearance; CRP, C-reactive protein; CT, chemotherapy; CT-scan, computed tomography scan; D, day; ECG, electrocardiogram; ECHO, echocardiogram; ECOG PS, Eastern Cooperative Oncology Group performance status; FLC, free light chains; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HR, heart rate; IF, immunofixation; INR, international normalized ratio for blood clotting time; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MM, multiple myeloma; MUGA scan, multiple uptake gated acquisition scan; MRI, magnetic resonance imaging; PD, disease progression; PT, prothrombin time; SFLC, Serum FLC; TTP, time to progression; UFLC, urine FLC; ULN, upper limit of normality; WBC, white blood cell counts.

4.7 Treatment Period Assessments

Table 3. Treatment period assessments.

Treatment period	Investigations	Time
General		
1. Clinical examination	• ECOG PS.	D1 of each cycle.

Treatment period	Investigations	Time
	• Physical examination, including weight, BSA and vital signs (HR, ABP, temperature). • Intercurrent adverse events. • Assessment of patient's signs and symptoms (disease related or not). • Complete neurological assessment. • Concomitant treatments (especially transfusion requirements)[a].	Throughout the study
2. Hematology	• Differential WBC count. • Hematocrit, hemoglobin. • Platelets.	D1, 4, 8, 11 and 15[b] during the first 4 weeks; D1 and 15 thereafter.
3. Coagulation	• PT, INR, APTT.	D1 of each cycle from C2 (an early 2-day window is allowed).
4. Biochemistry	• Serum chemistry A*.	D1, 4, 8, 11 and 15 during the first 4 weeks; D1 and 15 thereafter[c].
	• Serum chemistry B**.	Day 1 of each cycle from C2 (an early 2-day window is allowed).
5. Creatinine and CrCl	• Calculated or measured (Appendix 2).	D1 of each cycle from C2 (an early 2-day window is allowed).
6. Viral serology	• HBV and HCV. • CMV in patients who have undergone allogeneic BM transplantation.	When clinically indicated
7. Pregnancy test	• Serum or urine HCG.	Every 4 weeks (an early 2-day window is allowed).
8. AEs	• Evaluation of AEs (NCI-CTCAE v4.0).	Throughout the study
9. Heart function	• ECG: it should allow a rhythm definition (at least 30 second of duration). ✓ PR interval. ✓ QT interval (raw and corrected by heart rate using Bazett's formula) ✓ QRS complex and the maximum height of QRS complex in derivation II. • LVEF by ECHO or MUGA scan.	Before and after each plitidepsin infusion. Every 12 weeks.
10. PK	• As in Section 7.	C1.
Disease assessment[d]		
11. Serum Protein	• Protein electrophoresis • Serum Ig determination and M-protein quantitation and IF. • SFLC.	D1 of each cycle (an early 2-day window is allowed).
12. Urine protein	• 24-h urine protein electrophoresis. • Urine M-protein quantitation and IF. • UFLC.	D1 of each cycle (an early 2-day window is allowed).
13. BM[e]	• BM morphology. • BM cytometry (if available). • BM cytogenetic (if available).	When serology indicates CR. When clinically indicated.
14. Clinical and radiological tumor assessment in the presence of soft tissue plasmacytoma[f]	• CT-scan or MRI of all measurable/evaluable involved sites.	If response is observed (to confirm CR) or when clinical symptoms suggest new plasmacytomas.

Treatment period	Investigations	Time
15. Skeletal evaluation	• X-ray of skull, vertebral column pelvis and proximal long bones or MRI (if clinically indicated).	If response is observed (to confirm CR) or when clinical symptoms suggest new lytic bone lesion.
16. Other relevant tests	• Where indicated, according to the clinical context.	When clinically indicated

a. Detailed description of the concomitant treatment (drug, start and end date, reason for administration, etc.)
b. At least every other day if non febrile grade 4 neutropenia is present and every day in the presence of febrile neutropenia or grade 4 thrombocytopenia.
c. At least every other day in the presence of grade 3-4 vomiting or any other drug-related SAE.
d. Disease assessment is to occur at baseline to document the sites of disease; disease response is to be assessed primarily by non-invasive procedures, if possible. If CR is suspected, then invasive procedures required for disease response assessment (e.g. BM, skeletal survey, etc) are to be performed.
e. BM evaluation is mandatory in all patients with CR. In patients with non-secretory MM, it must be repeated eight weeks later to confirm response. BM evaluation must be repeated in all cases where there is any clinical indication.
f. In case of non-secretory or oligosecretory MM associated with soft tissue plasmacytoma assessments may be done every two cycles (if possible) to confirm response or as clinically indicated.

* Serum chemistry-A: AST, ALT, bilirubin, AP, LDH, Na^{+}, K^{+}, Ca^{++}, Mg^{++}, glucose and CPK, CPK-MB fraction.
**Serum chemistry-B: total proteins, albumin.
ABP, blood pressure; ALT, alanine aminotransferase; AP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BM, bone marrow; C, cycle; CMV, cytomegalovirus; CPK, creatine phosphokinase, CPK-MB fraction, CPK isoenzymes found in cardiac muscle (it will be performed only if CPK is > ULN); CrCl, creatinine clearance; CRP, C-reactive protein; CT, chemotherapy; CT-scan, computed tomography scan; D, day; ECG, electrocardiogram; ECHO, echocardiogram; ECOG PS, Eastern Cooperative Oncology Group performance status; FLC, free light chains; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HR, heart rate; IF, immunofixation; INR, international normalized ratio for blood clotting time; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MM, multiple myeloma; MUGA scan, multiple uptake gated acquisition scan; MRI, magnetic resonance imaging; NCI-CTCAE, National Cancer Institute Common Criteria for the Classification of Adverse Events; PD, disease progression; PT, prothrombin time; SFLC, Serum FLC; TTP, time to progression; UFLC, urine FLC; ULN, upper limit of normality; WBC, white blood cell counts.

4.8 Evaluations at the End of Treatment

4.8.1 End-of-treatment Visit

Table 4 lists the procedures that are required during the end-of treatment (EOT) visit. EOT is defined as 30 days after the last treatment day unless the patient starts a new antitumor therapy or dies (whichever comes first), in which case the date of administration of this new therapy or the date of death will be considered the EOT date. This visit needs to be performed in all avaluable patients 30 (±5) days after the last dose of study therapy.

Please refer to Section 6.2.2 and 6.2.3 for detailed instructions on Adverse Event Reporting and Monitoring.

Table 4. End of treatment visit.

End of treatment visit	Investigations	Time
1. Clinical examination	• ECOG PS. • Complete physical examination, including weight and vital signs (HR, ABP, temperature) • Concomitant treatments (including transfusion requirements).	Thirty (±5) days after the last dose of study therapy.
2. Hematology	• Differential WBC count. • Hematocrit, hemoglobin. • Platelets.	Thirty (±5) days after the last dose of study therapy.
3. Coagulation	• PT, INR, APTT.	Thirty (±5) days after the last dose of study therapy.

End of treatment visit	Investigations	Time
4. Biochemistry	• AP, AST, ALT, LDH, bilirubin, electrolytes (Na^+, K^+, Mg^{++}, Ca^{++}), glucose, CPK CPK-MB fraction, total proteins, albumin.	Thirty (±5) days after the last dose of study therapy.
5. Urine	• Dipstick, sediment.	Thirty (±5) days after the last dose of study therapy.
6. Pregnancy test	• Serum or urine.	For women of child bearing potential, 30 (±5) days after the last dose of study therapy.
7. Creatinine and creatinine clearance	• Calculated or measured.	Thirty (±5) days after the last dose of study therapy.
8. AEs	• Evaluation of AEs (NCI-CTCAE v4).	Thirty (+5) days after the last dose of study therapy.
9. Heart function	• ECG: it should allow a rhythm definition (at least 30 second of duration). ✓ PR interval. ✓ QT interval (raw and corrected by heart rate using Bazett's formula). ✓ QRS complex and the maximum height of QRS complex in derivation II]. • LVEF by ECHO or MUGA scan.	Thirty (±5) days after the last dose of study therapy.
Disease assessment		
10. Serum Protein	• Protein electrophoresis. • Serum Ig quantitation and M-protein quantitation and IF. • SFLC	Thirty (±5) days after the last dose of study therapy.
11. Urine Protein	• 24-h urine protein electrophoresis. • Urine M-protein quantitation and IF. • UFLC	Thirty (±5) days after the last dose of study therapy.
12. C-reactive protein		Thirty (±5) days after the last dose of study therapy.
13. BM	• BM morphology. • BM cytometry (if available). • BM cytogenetic (if available).	If indicated, thirty (±5) days after the last dose of study therapy.
14. Radiological assessment in case of soft tissue plasmacytoma	• CT-scan or MRI of all measurable/evaluable sites.	If indicated, thirty (±5) days after the last dose of study therapy.
15. Skeletal evaluation	• X-ray of skull, vertebral column pelvis and proximal long bones or MRI.	If indicated, thirty (±5) days after the last dose of study therapy.
16. Other relevant tests	• Where indicated, according to the clinical and laboratory context.	Thirty (±5) days after the last dose of study therapy.

ABP, blood pressure; ALT, alanine aminotransferase; AP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BM, bone marrow; C, cycle; CMV, cytomegalovirus; CPK, creatine phosphokinase, CPK-MB fraction, serum CPK isoenzymes found in cardiac muscle (it will performed only if CPK is > ULN); CrCl, creatinine clearance; CRP, C-reactive protein; CT, chemotherapy; CT-scan, computed tomography scan; D, day; ECG, electrocardiogram; ECHO, echocardiogram; ECOG PS, Eastern Cooperative Oncology Group performance status; FLC, free light chains; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HR, heart rate; IF, immunofixation; INR, international normalized ratio for blood clotting time; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MM, multiple myeloma; MUGA scan, multiple uptake gated acquisition scan; MRI, magnetic resonance imaging; NCI-CTCAE, National Cancer Institute Common Criteria for the Classification of Adverse Events; PD, disease progression; PT, prothrombin time; SFLC, Serum FLC; TTP, time to progression; UFLC, urine FLC; ULN, upper limit of normality; WBC, white blood cell counts.

4.8.2 Follow-up Visits

Patients who have not progressed at the end of treatment will have a complete disease assessment performed every three months until PD has been documented, a new therapy has been started, study termination or death. In this manner, the first follow-up assessment should occur at three months after the end-of-treatment visit. All subsequent follow-up assessments should be performed in three-month intervals.

Additional parameters and/or increased frequency of observations should be performed at the Investigator's discretion and according to the nature of the AEs observed. In case of death, when available, autopsy data should be provided.

The reason and date of removal for all patients will be documented on the e-CRF. The Investigator will attempt to complete all discharge procedures at the time a patient is discontinued from treatment.

5. STUDY MEDICATION

5.1 Plitidepsin

Plitidepsin is supplied as a lyophilized product in a glass vial containing 2 mg. The lyophilized powder is a concentrate for solution. The reconstitution solvent is supplied in ampoules, each containing 4 mL of polyoxyl 35 castor oil/ethanol/WFI (15/15/70% v/v/v). Plitidepsin vials and reconstitution ampoules should be stored in a locked area with limited access at 2 to 8ºC (36ºF to 46ºF) and protected from exposure to light.

Upon reconstitution of the 2 mg plitidepsin vial with 4 mL of reconstitution solvent, the reconstituted solution will be clear, colorless, essentially clear from visible particles, and contain 0.5 mg/mL of plitidepsin. This solution should be immediately diluted with sodium chloride (0.9%) solution for infusion or glucose (5%) solution for infusion for administration as an intravenous infusion.

The required volume of the reconstituded solution should be determined based on the dose established for each individual patient:

Volume (ml) = [Body Surface Area (BSA) (m^2) x xIndividual dosage (mg/m^2)]/0.5 mg/mL

For detailed instructions on the reconstitution and dilution refere to the latest plitidepsin IB and the "Preparation Guide for Infusion" document.

5.1.1 Plitidepsin Recommendations for Safe Handling

Similar to other antineoplastic potentially toxic agents, caution should be exercised when handling plitidepsin and when preparing solutions. Personnel should be trained to reconstitute the drug. The use of mask, goggles and gloves is recommended. Should plitidepsin premix solution, or infusion solution come into contact with the skin or mucous membranes, the areas should be immediately and thoroughly washed with water (mucous membranes) and water and soap (skin).

5.1.2 Required Prophylactic Medication

Patients must receive the following prophylactic medication before the administration of plitidepsin (Table 5).

Table 5. Prophylactic medication for plitidepsin treatment.

Agent	Dose	Route	Day
Ondansetron or equivalent	8.0 mg	intravenous	30 minutes before plitidepsin
Diphenhydramine hydrochloride or equivalent	25 mg	intravenous	
Ranitidine	50 mg	intravenous	

In addition to the above, and if necessary, 10 mg metoclopramide every 8 h may be administered after the infusion, or the duration of treatment with 5-HT3 antagonists and/or dexamethasone will be extended.

5.2 Bortezomib

Bortezomib (Velcade®) is available for use as i.v. infusion and s.c. injection. Each single dose vial contains 3.5 mg of bortezomib as a sterile lyophilized powder. For further information on bortezomib, please refer to the EU SmPC and/or USP.

5.2.1 Prophylactic Medication

Prophylactic antiemetic medication for bortezomib will be given according to the Investigator's criteria. Herpes virus infection prophylaxis must be given while patients are on bortezomib therapy.

5.3 Dexamethasone Drug Formulation

Dexamethasone is administered orally as tablets. Dexamethasone tablets should be stored in well-closed containers. Dexamethasone will be administered orally at a dose of 40 mg on D1, 8, 15 and 22 q4wk.

5.4 Treatment Schedule

Oral dexamethasone will be administered at least one hour before the administration of plitidepsin infusion. Plitidepsin will be administered as a 3-h i.v. infusion; one minute after the end of the plitidepsin infusion, bortezomib should be administered as a 3-5 second bolus s.c. injection (Table 6).

Treatment cycles will be repeated every 4 weeks.

Patients will receive a maximum of eight treatment cycles. If the patient responds to treatment or achieves SD during this time, treatment with plitidepsin and dexamethasone may continue in further cycles at the same plitidepsin dose upon Investigator's decision and agreement with the Sponsor.

Table 6. Study drug administration schedule.

Agent	Dose	Route	Day
Dexamethasone	40 mg	Oral	D1, 8, 15 and 22*
Plitidepsin	As assigned by PharmaMar	Intravenous	D1 and 15 q4wk*
Bortezomib		Subcutaneous	D1, 4, 8 and 11 q4wk*

* Patients will be allowed a maximum of eight treatment cycles. If the patient responds to treatment or achieves stable disease (SD) during this time, treatment with plitidepsin exclusively may continue at the same dose upon Investigator's decision and agreement with the Sponsor.
D, day; q4wk, every four weeks.

5.5 Criteria for Treatment Continuation and Re-treatment

Table 7 lists the minimum requirements needed to re-expose the patient to the study drug(s). Other factors might be considered critical by the Investigator, which should be

appropriately documented in the patient's record and on the e-CRF, and discussed with the Sponsor.

Table 7. Criteria for plitidepsin and bortezomib treatment continuation.

	Plitidepsin	**Bortezomib**
	Day 1[a] and 15[b]	**Day 1[a*], 4[b], 8 [b] and 11 [b]**
ANC	1.0 x 10^9/L (≥ 0.5 x10^9/L if due to extensive BM involvement)	1.0 x 10^9/L (≥ 0.5 x10^9/L if due to extensive BM involvement)
Platelets	≥ 50.0 x 10^9/L (≥ 25.0 x10^9/L if due to extensive BM involvement)	≥ 50.0 x 10^9/L (≥ 25.0 x10^9/L if due to extensive BM involvement)
Hemoglobin	≥ 8.0 g/dl	≥ 8.0 g/dl
Serum total bilirubin	≤ 1.5 x ULN[e]	≤ 1.5 x ULN[e]
AST/ALT/AP	≤ 2.5 x ULN	≤ 2.5 x ULN
Muscular toxicity (myalgia, muscular weakness, CPK increase)	< Grade 2	< Grade 2
Other non-hematological drug-related AEs (except for increased GGT, non-optimally treated nausea and vomiting or hypertension, alopecia)[c]	< Grade 2	< Grade 2
ECG, ECHO/MUGA[d]	Baseline	Baseline

[a]If a patient does not meet the requirements for treatment continuation on D1 of the following cycle, the infusion of study drug(s) will be withheld until recovery or for a maximum of 14 days. After this period, if the delay is due to toxicity related to the study drug(s), a dose reduction by one DL is mandatory; up to a maximum of two individual dose reductions are allowed. Patients needing additional dose reductions must be withdrawn from the study.

[a*]From C2 onwards, Hematology and Biochemistry A will only be collected on D1 and 15, hence only re-treatment criteria other than Hematology/Biochemistry A will apply on D4, 8 and 11.

[b]If a patient does not meet the requirements for treatment continuation on D15 (plitidepsin) or D4, 8 and 11 (bortezomib), the administration of plitidepsin or bortezomib, respectively, will be omitted. Patients requiring frequent dose omissions may have a dose reduction by one DL upon the Investigator and the Sponsor's agreement. No more than two dose reductions are allowed under any circumstances.

[c]Any grade accepted for increased GGT.

[d]To be performed every three months unless more frequent assessments are clinically indicated.

[e]*Except when Gilbert syndrome is clearly documented and other liver function tests are normal.*AEs, adverse event(s); ANC, absolute neutrophil count; AP, alkaline phosphatase; AST/ALT, aspartate aminotransferase/alanine aminotransferase; BM, bone marrow; CPK, creatine phosphokinase; ECG, electrocardiogram; ECHO/MUGA, echocardiogram/multiple-gated acquisition scan; GGT, γ-glutamyltranspeptidase; ULN, upper limit of normality.

The following guidelines must be followed before the administration of each bortezomib dose (63):

Table 8. Recommended posology modifications for bortezomib-related neuropathy.

Severity of PN signs and symptoms*	**Recommended modification of bortezomib dose and regimen**	**Supportive data**
Grade 1 (paresthesia; weakness and/or loss of reflexes) without pain or loss of function	Reduce current bortezomib dose by one DL (1.3 to 1.0 mg/m^2) or, for patients receiving a twice-weekly schedule, change to a once-per-week schedule using the same dose.	*Prior PN was the only risk factor associated with bortezomib-related PN in newly diagnosed patients treated with VMP.* *Baseline PN was a risk factor for the development of grade ≥ 3 PN in relapsed/refractory MM patients treated with single-agent bortezomib.* *A VMP regimen using bortezomib 1.3*

Severity of PN signs and symptoms*	Recommended modification of bortezomib dose and regimen	Supportive data
		mg/m^2 once weekly from the start of therapy showed reduced neurotoxicity a delivered a similar cumulative dose of bortezomib to that in the VISTA trial, and resulted in similar efficacy.
Grade 1 with pain or grade 2 (with no pain but limiting instrumental ADLs**)	For patients receiving twice-weekly bortezomib, reduce current dose by one DL or change to a once-per-week schedule using the same dose. For patients receiving bortezomib on a once-per-week schedule, reduce current dose by one DL, or consider temporary discontinuation; upon resolution to grade ≤ 1, restart once-per-week dosing at a lower DL in cases of favorable benefit/risk ratio.	*Early reduction of bortezomib from 1.3 mg/m^2 twice weekly to once weekly in patients receiving VMP showed reduced neurotoxicity, delivered similar cumulative dose of bortezomib to that of the VISTA trial and resulted in similar efficacy. Dose reduction strategies including dose reduction from 1.3 to 1.0 mg/m^2 changing from twice-weekly to once-weekly dosing, and withholding of bortezomib resulted in improvement or resolution of PN in most patients with bortezomib-related PN.*
Grade 2 with pain or grade 3 (limiting selfcare and ADL***) or grade 4	Discontinue bortezomib	*Discontinuation as part of PN management strategy resulted in improvement or resolution of clinically significant neuropathy in 71% of patients in an analysis of two phase II bortezomib studies.*

*Based on posology modifications in phase II and III MM studies and post-marketing experience. Grading based on NCI-CTCAE v 4.0.
**Instrumental ADL: refers to preparing meals, shopping for groceries or clothes, using telephone, managing money, etc;
***Self care ADL: refers to bathing, dressing and undressing, feeding self, using the toilet, taking medicinal products, and not bedridden.
ADL, activities of daily living; DL, dose level; MM, multiple myeloma; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for the Classification of Adverse Events; PN, peripheral neuropathy; VMP, Velcade/Melphalan/Prednisone.

The criteria for treatment continuation with dexamethasone are to be implemented independently from those of plitidepsin and bortezomib. Dexamethasone treatment will therefore not be delayed in parallel to plitidepsin/bortezomib; if a patient experiences grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations), or drug-related grade ≥ 3 fatigue, or grade ≥ 2 mood disturbances or agitation or grade ≥ 3 fluid retention or grade 4 clinically documented infection, the dose of dexamethasone will be reduced by 50%, up to a maximum of two consecutive dose reductions (20 mg D1, 8, 15 and 22, and 20 mg D1 and 15 of each 28-day cycle). After two dose reductions, dexamethasone will be discontinued.

5.6 Definition of Dose-limiting Toxicity

For this protocol DLTs are defined as AEs and laboratory abnormalities related to study agent with an attribution of possible, probable, or definite during the first treatment cycle and fulfilling one of the following criteria:

Hematological Toxicity:

- Grade 3-4 neutropenia associated with fever or lasting > 7 days, considered related to the study drug(s) by the Investigator.
- Grade 3-4 thrombocytopenia accompanied by grade 3/4 hemorrhage.
- For patients with extensive BM infiltration (≥ 50% of BM nucleated cells are plasma cells), DLT is defined as grade 4 thrombocytopenia with grade 3/4

hemorrhage or grade 4 neutropenia lasting more than seven days or with fever.

Non-hematological Toxicity:

- Grade 3/4 nausea and vomiting refractory to antiemetic therapy.
- Grade ≥ 3 muscular AEs (myalgia, muscular weakness, muscle cramps, myopathy).
- Grade ≥ 3 ALT/AST lasting for more than one week.
- Grade ≥ 3 bilirubin increase.
- Grade ≥ 3 CPK increase.
- Cardiac toxicity:
  - ✓ Symptomatic or treatment-requiring grade ≥ 1 cardiac arrhythmia related to plitidepsin.
  - ✓ Left ventricular systolic dysfunction grade ≥ 1 related to plitidepsin.
- Neuropathic pain and peripheral sensory neuropathy related to bortezomib will be considered as DLT if they result in a definitive bortezomib discontinuation according to IMWG guidelines (Table 7).
- Any other grade ≥ 3 toxicity considered related to study treatment by the Investigator.

5.7 Definition of Recommended Dose

To define the RD for phase II trials, patients will be evaluated for DLTs during the first 28-day cycle. The RD will be the highest DL at which fewer than two out of six (33%) of evaluable patients experience a DLT during the first 28-day cycle.

5.8 Dose Escalation

Treatment will start with the 60% of the RD of plitidepsin given as single agent. Dose escalation for plitidepsin and bortezomib will follow the rules shown on Table 9.

Table 9. Dose escalation rule for plitidepsin and bortezomib combination.

Dose Level	No. Patients	Plitidepsin dose (mg/m^2)	Bortezomib dose (mg/m^2)	Dexamethasone (mg)
-1	0-6	3.0	1.0	40.0
1	3-6	4.0	1.0	40.0
2	3-6	4.0	1.3	40.0
3	3-6	5.0	1.3	40.0

Cohorts of three patients will be treated at each DL (up to three DLs).

- The first cohort of three patients will start at DL1.
- If no DLTs are observed in any of the three patients at a given DL, three additional patients will be treated at the next DL.
- If a DLT is observed in one of three patients treated at a given DL, three additional patients will be entered at that same DL.

- If DLTs are observed in two of three patients, no additional patients will be treated at that DL and the immediately lower DL will be expanded to at least six patients.
- If two out of six patients present a DLT at DL 1, the next patients enrolled will be treated at DL-1. If two of six patients (≥ 33%) present DLTs at this DL, the study will be stopped and that DL will be considered the MTD.
- The RD is defined as the DL at which fewer than two out of six patients (33%) experience DLTs during the first cycle.
- At the RD, at least six evaluable patients for determination of DLTs will be treated.
- At DL1, one patient must have completed the first cycle before accrual of the second and third patients. The second and third patients may be treated simultaneously.
- Intermediate dose escalation or de-escalation is allowed. Intrapatient dose escalation is not allowed.

5.9 Dose Modifications Based on Adverse Events

Dose modifications will be based on AEs and laboratory abnormalities graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE version 4.0). Patients will be assessed for toxicity before each plitidepsin infusion or bortezomib injection. If several AEs or laboratory abnormalities are seen simultaneously, the dose will be modified according to the greatest reduction required for the most severe event observed. Reductions apply to treatment given in the preceding cycle and are based on AEs observed since the prior dose. Dose reductions levels are the following:

- Dose adjustments will initially involve the drug to which an AE is suspected to be related to, whenever possible. If causality cannot be attributed to a specific drug, the two drugs (bortezomib and plitidepsin) will be reduced one DL.
- DL reductions for plitidepsin should follow Table 9 (5.0 mg/m^2 →4.0 mg/m^2→ 3.0 mg/m^2).
- ***For patients receiving twice-weekly bortezomib, reduce current dose by one DL (from 1.3 mg/m^2 to 1.0 mg/m^2) or change to a once-per-week schedule (D1, 8, 15 and 22, q4wk) using the same dose. For patients receiving bortezomib on a once-per-week schedule (D1, 8, 15 and 22, q4wk), reduce current dose by one DL.*** No additional dose reductions will be allowed due to bortezomib-related hematological toxicity.
- Patients for whom bortezomib is discontinued may continue to receive the other agents if, in the opinion of the Investigator, the patient may continue to benefit from treatment.
- Dexamethasone doses are to be reduced by 50%, up to a maximum of two consecutive dose reductions (20 mg D1, 8, 15 and 22, and 20 mg D1 and 15 of each 28-day cycle), if a patient experiences grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations), or drug-related grade ≥ 3

fatigue, or grade ≥ 2 mood disturbances or agitation or grade ≥ 3 fluid retention or grade 4 clinically documented infection. These dose reductions are to be implemented independently from plitidepsin dose reductions, if required. After two dose reductions, dexamethasone will be discontinued.

- If the patient suffers from vomiting after dexamethasone, it should not be re-administered and will be considered as omitted.
- Patients with toxicities that are manageable with supportive treatment may not require dose reductions (e.g., nausea/vomiting may be treated with antiemetics, diarrhea may be treated with loperamide rather than by dose reductions).
- Patients requiring dose reductions should not have the dose re-escalated with subsequent treatments.
- Patients will be withdrawn from the study if they fail to recover to baseline values from a treatment-related toxicity within 14 days, unless the Investigator and the responsible medic at PharmaMar agree that the subject should remain in the study because of evidence that the patient may continue to benefit from it. The appropriate reduced dose will be determined after discussion between the principal Investigator and the responsible medic at PharmaMar.

Patients with bortezomib-related neuropathic pain and/or peripheral sensory neuropathy are to be managed according to the guidelines on Table 8 (63).

5.10 Permitted Treatment and Medication

5.10.1 Transfusions

5.10.1.1 Guidelines for Platelet Transfusions

Thrombocytopenia can occur as a consequence of BM infiltration by myeloma cells or may be related to study drug administration. The clinical significance of the thrombocytopenia should be assessed in light of its etiology (bortezomib treatment, underlying disease or both), the state of the myeloma (stable *versus* worsening disease), and whether the patient is bleeding or being prepared for a surgical procedure.

The use of any platelet product should be considered in the following circumstances:

- As preparation for an invasive surgical procedure, transfuse in order to maintain a platelet count >50.0 x 10^9/L to prevent bleeding.
- If the patient has an active infection, high fever, rapid decrease in platelet count to ≤ 20.0 x 10^9/L and/or coagulopathy, transfuse to maintain a platelet count to > 20.0 x 10^9/L as prophylaxis for spontaneous bleeding.
- If the patient is actively bleeding or has a platelet count below 10.0 x 10^9/L, transfuse in order to maintain a platelet count > 10.0 x 10^9/L.

5.10.1.2 Guidelines for Red Cell Transfusions

The use of any red cell product should be considered in the following circumstances:

- If the patient has a hemoglobin < 7.0 g/dL, transfuse to maintain a hemoglobin > 8.0 g/dL in order to reduce the risk of inadequate oxygenation.

- If the patient is asymptomatic and has a hemoglobin value between ≥ 7.0 and ≤ 8.0 g/dL, the Investigator may consider transfusion on a per-patient basis in order to maintain a hemoglobin > 8.0 g/dL.
- If the patient is actively bleeding or has symptomatic cardiac or pulmonary disease or other extenuating circumstances where oxygenation is impaired, the Investigator may elect to transfuse on a per-patient basis. In these instances, the trigger hemoglobin value may be > 8.0 g/dL
- The use of erythropoietin (e.g. Eprex®/Erypo®) is allowed.

5.10.2 Other Therapies

- Therapies for the treatment of preexisting and/or emergent medical conditions not specifically forbidden as per protocol elsewhere.
- Antiemetics (as in Section 5.1.2 and according to institutional or ASCO guidelines) (64).
- Use of G-CSF/GM-CSF according to institutional or ASCO guidelines (65) and after C1.
- Palliative local radiation may be applied. The irradiated lesion will then not be considered an area of measurable/evaluable disease.
- Systemic and/or local therapies for symptomatic relief, particularly in the case of diarrhea or skin toxicity.
- Patients who develop grade 2 or greater muscular toxicity may be empirically treated with oral L-carnitine at a total daily amount of 3 g, divided into 3 doses, until it decreases to grade ≤ 1.
- Adequate analgesic medication, including opioids for symptomatic pain relief if indicated.
- Drugs that prolong QT interval and/or induce Torsades de Pointes may be used with caution (Appendix 5).
- Patients receiving aminobisphosphonates before study entry may continue to receive them during the study according to ASCO guidelines.

5.11 Prohibited Medication

- Concomitant administration of any other antineoplastic therapy.
- Other investigational agents.
- Immunosuppressive therapies, including systemic corticosteroids, unless given as an equivalent to a prednisone dose of ≤ 10 mg daily administered as an antiemetic or as pre-medication for blood prodcuts.
- Other disease-modifying treatment (e.g., alpha interferon) is strictly prohibited.
- Primary prophylaxis with colony-stimulating factors such as G-CSF and GM-CSF (i.e., within the first cycle).

5.12 Packaging and Labeling

The following information will appear on the label:

- Name of the Sponsor
- Study number and patient number
- Dosage and route of administration
- Quantity of contents of container
- Batch number and packaging number
- Expiration date and storage conditions
- Local legal requirements as appropriate

5.13 Drug Accountability

Proper drug accountability will be done by the study monitor. The responsible institution will keep records to allow a comparison of quantities of drug received and used at each site.

All unused drug supplied by PharmaMar will be properly destroyed at the investigational site (documentation of this procedure must be provided) or returned to the drug repository with the agreement of PharmaMar.

6. STUDY ASSESSMENTS

6.1 Efficacy

Response or disease progression will be assessed on D1 of each therapy cycle according to the International Myeloma Working Group (IMWG) criteria (66) with evaluation of serological myeloma specific markers: M-protein, serum FLC and IF from blood and urine. When serological markers indicate CR, a BM aspirate or biopsy will be performed. Also, a skeletal survey will be performed to confirm no increase in size or number of lytic bone lesions (development of a compression fracture does not exclude response).

The efficacy analysis will include ORR, clinical benefit rate (ORR + MR + SD), PFS, TTP, EFS and DOR. They will be analyzed in all eligible patients who received at least one treatment cycle.

Patients are evaluable for efficacy if they receive at least one complete treatment cycle (two plitidepsin infusions, four bortezomib injections, four doses of dexamethasone), or the equivalent doses over two cycles and have, at least, one disease assessment.

6.1.1 Response Criteria for Multiple Myeloma

6.1.1.1 Stringent Complete response (sCR)

These patients have a normal FLC ratio and have no clonal cells by BM immunohistochemistry (IHC) or immunofluorescence. Clonal cells detected in the BM by IHC or immunofluorescence, are considered to be present if there is a kappa/lambda ratio of > 4:1 or <1:2 after examination of a minimum of 100 plasma cells.

6.1.1.2 Complete response (CR)

These patients have absence of M-protein in serum and urine by IF with no current evidence of soft tissue plasmacytoma. In addition, BM aspirate and biopsy must

demonstrate less than 5% clonal plasma cells. In patients who lack measurable M-protein in the serum and urine being monitored using the FLC levels, the definition of CR requires a normalization of the FLC ratio in addition to the above criteria.

6.1.1.3 Very good partial response (VGPR)

These patients have serum and urine M-protein detectable by IF but not on electrophoresis or at least a 90% reduction in serum M-protein with a urine M-protein <100 mg/24 h. In patients who lack measurable M-protein in the serum and urine being monitored using the FLC levels, the definition of VGPR requires > 90% decrease in the difference between involved and uninvolved FLC levels.

6.1.1.4 Partial response (PR)

These patients have ≥ 50% reduction in serum M-protein and reduction of 24-h urinary M-protein by 90% or to < 200 mg/24 h. In patients who lack measurable M-proteins in the serum and urine, the definition of PR requires ≥ 50% decrease in the difference between involved and uninvolved FLC levels. If the FLC levels were also immeasurable at baseline, a 50% reduction in BM plasma cells is acceptable as long as the original BM contained at least 30% plasma cells. PR also requires a 50% reduction in size of any soft tissue plasmacytomas if present at baseline.

6.1.1.5 Minimal response (MR)

These patients have ≥ 25% but ≤ 49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50-89%. In addition, if present at baseline, 25-49% reduction in the size of soft tissue plasmocytomas is also required. No increase in size or number of lytic bone lesions.

6.1.1.6 Stable Disease (SD)

These patients do not meet the criteria for sCR, CR, VGPR, PR, MR or PD.

6.1.1.7 Progressive disease (PD)

These patients present with a 25% increase from the lowest response value in any of the following: serum M-protein (absolute increase must be ≥ 0.5 g/dL), urine M-protein (absolute increase must be ≥ 200 mg/24h), BM plasma cell percentage (absolute increase must be ≥10%), or difference in the kappa and lambda FLC (absolute increase must be >10 mg/dL). As discussed earlier, the FLC criteria should only be used for patients with immeasurable M-protein in the serum and urine. PD is also diagnosed when there is an increase in the size or development of new bone lesions or soft tissue plasmacytomas or the development of a serum calcium >11.5 mg/dL with no other cause.

6.1.1.8 Overall Response Rate (ORR)

It includes sCR plus CR plus VGPR plus PR.

6.1.1.9 Cinical Benefit Rate

It includes ORR plus MR plus SD.

Note: 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 the 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.

All response categories (CR, sCR, VGPR and PR) require two consecutive assessments made at any time before the institution of any new therapy; sCR, CR, VGPR, PR and SD categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed.

Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments need not to be confirmed.

For PD, serum M-component increases of ≥ 1 g/100 ml are sufficient to define relapse if starting M-component is ≥ 5 g/100ml.

6.1.2 Additional Efficacy Endpoints

According to the American Society of Hematology/Food and Drug Administration ASH/FDA clinical endpoints in MM:

- DOR is defined as the time from the first observation of response to the time of PD, with censoring of deaths due to causes other than PD.
- TTP is defined as the duration from treatment start to PD, with censoring of deaths due to causes other than PD.
- PFS is defined as the duration from treatment start to PD or death (regardless of the cause of death), whichever comes first.
- EFS is defined as the duration from treatment start to PD or death (regardless of the cause of death), whichever comes first. It may include additional "events" that are considered to be of importance besides death and PD, including serious drug toxicity.

The duration of sCR, CR, VGPR and PR should be reported. Observation curves will be estimated using the Kaplan-Meier method. ORR and clinical benefit rate will be calculated with binomial exact 95% CI.

6.1.3 Efficacy Assessment Determinations

6.1.3.1 Myeloma Protein Measurements

Serum

Serum quantitation of immunoglobulins and M-protein, and assessment of M-protein by IF and FLC must be determined for all patients at screening. The same determinations will be performed in patients with secretory MM on D1 of every cycle. For patients with "non-secretory myeloma" FLC will be assessed at the same times. If there is evidence of PD, these evaluations must be repeated one to three weeks later in order to confirm PD.

Urine

Quantitation of M-protein and assessment of M-protein by IF from 24-h urine samples must be determined for all patients at screening. For patients with positive results, the same determinations will be done from 24-h urine samples collected on D1 of every cycle. If there is evidence of PD, this evaluation is to be repeated with a second measurement one to four weeks later in order to confirm PD. In patients with IF-negative non-secretory MM, these tests will not be repeated after screening.

6.1.3.2 Bone Marrow

A representative BM aspirate or a BM biopsy must be obtained at screening. During the treatment period, BM assessment is mandatory in the presence of CR. In patients with non-secretory myeloma, collection and evaluation of BM is required eight weeks later to confirm CR. BM examination has to be performed in all cases where it is judged as clinically necessary. BM studies will include cytogenetics and cell cycle analysis by flow cytometry, if available.

6.1.3.3 Skeletal Survey and Other Radiographs

A complete bone survey including examination of the skull, vertebral column, pelvis and proximal long bones, should be done at screening. In this period, it is important to document sites of myelomatous disease, especially in extramedullary areas. This may require clinical examination, CT-scanning or MRI evaluations.

During the treatment phase, a skeletal survey must take place in the presence of CR or if clinical symptoms suggest new lytic bone lesions. In the case of non-secretory or oligosecretory MM associated with soft tissue plasmacytoma, CT or MRI assessments may be performed every two cycles (whenever possible) and to confirm response if clinically indicated.

For soft tissue plasmacytomas, tumor assessment will consist of the sum of the cross-diameters of the measurable lesion

Radiographic examination of any location must be performed when clinical symptoms suggest a new bone lytic lesion.

6.1.3.4 Beta-2-Microglobulin and C-Reactive Protein

Beta-2-microglobulin will be measured at screening and C-reactive protein will be measured at screening, every eight weeks during the treatment phase and in the last final visit (30±5 days) after the last dose of the study therapy.

6.2 Safety Assessment

Individual patients will be evaluated from treatment start until 30 days after the last IMP administration.

Patients will be evaluable for safety if they receive at least one (complete or incomplete) dose of plitidepsin.

Safety will be evaluated by the occurrence of clinical and laboratory toxicities and changes from baseline in physical examination findings, vital signs, and, if applicable, chest X-ray and ECG findings. AEs will be graded according to NCI-CTCAE v 4.0

6.2.1 Adverse Event Definitions

6.2.1.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject which does not necessarily have a causal relationship with the study treatment.

For the purpose of this protocol, PD or worsening of the underlying MM should not be reported as AEs.

6.2.1.2 Serious Adverse Event

A serious adverse event (SAE) is any adverse experience occurring at any dose that:

- results in death (is fatal),
- is life-threatening,
- requires or prolongs inpatient hospitalization,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect, or
- is medically significant.
- Any suspected transmission via a medicinal product of an infectious agent.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as an important medical event that may not be immediately life-threatening or result in hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the above definition.

6.2.1.3 Death

Death as such is the outcome of a SAE and should, whenever possible, not be used as the SAE term itself. The cause of death should be recorded as the SAE term instead. When available, the autopsy report will be provided by the Sponsor.

Grade 5 should be used for events leading immediately and directly to death, and grade 4 should be used with outcome death for events leading to death after a longer time period, and that may also be linked to additional morbidities.

6.2.1.4 Life Threatening Event

A life threatening event is defined as any event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

6.2.1.5 Hospitalization /Prolongation of Hospitalization

Any event requiring hospitalization (or prolongation of hospitalization) that occurs or worsens during the course of a patient's participation in a clinical study must be reported as a SAE. Prolongation of hospitalization is defined as any extension of an inpatient hospitalization beyond the stay anticipated/required for the initial admission, as determined by the Investigator or treating physician.

Hospitalizations that do not meet criteria for SAE reporting are:

- Reasons described in the protocol (e.g., drug administration, protocol-required investigations). Hospitalization or prolonged hospitalization for a complication of therapy administration or procedure will be reported as a SAE.
- Hospitalization or prolonged hospitalization for technical, practical or social reasons, in the absence of an AE.
- Pre-planned hospitalizations (i.e., planned before study entry). Any surgery or procedure planned before study entry must be documented on the e-CRF.

Only if the pre-planned surgery needs to be performed earlier due to a worsening of the condition, should this event (worsened condition) be reported as a SAE.

Other situations that MUST NOT be considered as hospitalizations are:

- An emergency visit due to an accident where the patient is treated and discharged.
- When the patient is held 24 h for observation and is finally not admitted.
- Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (i.e. laser eye surgery, arthroscopy etc…).

6.2.1.6 Unexpected/Unlisted Adverse Event

An AE is considered unexpected when the nature or severity of which is not consistent with the applicable product information. The reference safety information for the evaluation of expectedness will be:

- The current IB for the study drug without marketing authorization.
- The summary of product characteristics (SPC) for MPs with a marketing authorization.

6.2.1.7 Adverse Reaction

All untoward and unintended response to an IMP related to any dose administered. This definition also covers medication errors and uses outside what is foreseen in the protocol, including overdose, lack of efficacy, misuse and abuse of the product. Any event involving adverse drug reactions (ADR), illnesses with onset during the study or exacerbations of pre-existing illnesses should be recorded.

6.2.1.8 Adverse Events Related to the Study Drug

An AE is considered associated with the use of the drug if the causality assessment is related to the study drug or unknown according to the definitions listed below.

6.2.1.9 Expedited Reporting

The Sponsor will assume responsibility for appropriate expedited reporting of serious unlisted/unexpected and related adverse events (SUSAR/SUA), including misuse, overdose, abuse, medication error and those considered of special interest to the Competent Authorities. The Sponsor will also report all SAEs, including misuse, overdose, abuse, medication error, which are unlisted/unexpected and related to the study drug(s) [IMP(s)] to the Investigators and to the IEC/IRBs according to current legislation unless otherwise required and documented by the IEC/IRB.

6.2.1.10 Causality Assessment

The Investigator must provide an assessment of causality for each of the Investigation Medicinal Product (IMP) (including combination and comparator products) according to the following criteria:

- Related to study drug(s).
- Not related to study drug(s) but related to:

  ✓ Disease under study.

- ✓ Other illness (must be specified).
- ✓ Previous/concomitant treatment/therapy.
- ✓ Unknown but not related to study drug.

- Unknown.

If the causality assessment is unknown and the Investigator cannot rule out relationship to the study drug, then "unknown" should be chosen. If the causality assessment is "unknown but not related to the study drug", this should be clearly documented on the study records.

6.2.2 Adverse Event Reporting Procedures

6.2.2.1 Reporting of Adverse Events

The Sponsor will collect AEs from the onset of the treatment period and until 30 days after administration of the last dose of study drug. All AEs suspected to be related to the study drug or the combination of the drugs must be followed after the time of therapy discontinuation until the event or its sequelae resolve or stabilize to an acceptable level to the Investigator and the Sponsor.

All AEs, including misuse, overdose, abuse and medication error must be recorded in English using medical terminology on the source document and the e-CRF. Investigators must assess the severity (grade) of the event following NCI-CTCAE v4.0 and assign a relationship to study therapy, pursue and obtain information adequate to determine the outcome and to assess whether it meets the criteria for classification as a SAE requiring immediate notification to PharmaMar or its designated representative. The Investigator must provide any information as requested by the Sponsor in addition to that on the e-CRF.

Abnormal laboratory tests obtained during the study are AEs, but they should only be recorded on the AE section of the e-CRF in some cases (please refer to e-CRF instructions for comprehensive information).

6.2.2.2 Reporting Serious Adverse Events

All SAEs (as defined above) regardless of treatment group or suspected relationship to the study drug must be reported within 24h to Pharmacovigilance by fax (+34 91 846 6004) or phone (+34 91 823 4569). Out of office hours (Greenwich Mean Time, GMT) assistance on SAE reporting can be obtained by calling the Pharmacovigilance Service phone +34 91 823 47 42. It is preferable that SAEs be reported by completing the electronic AE/SAE summary forms. An initial SAE report by phone must be followed by a completed electronic "SAE Form" from the investigational staff within one working day.

The Sponsor will collect SAEs from the time of signing of the IC. If the patient is definitively included in the study, and the SAE occurs after registration has been confirmed, this information will also be recorded on the AE and SAE summary sections of the e-CRF. The Sponsor will collect SAEs until 30 days after the administration of the last dose of study drug. Beyond this period of time, only those SAEs suspected to be related will be reported.

The Sponsor will evaluate any safety information that is spontaneously reported by an Investigator beyond the time frame specified in the protocol. All SAEs suspected to be related to a study drug must be followed after the time of therapy discontinuation until

the event or its sequelae resolve or stabilize to an acceptable level to the Investigator and the clinical monitor or his/her designated representative.

The cause of death of a subject in a clinical study, whether the event is expected or associated with the investigational agent, is considered a SAE and should therefore be reported using the SAE Form.

6.2.2.3 Pregnancy

FDA and EMA regulations require that pregnancy be reported as a SAE. Hence, the following events will also be handled and reported as SAEs:

- Any occurrence of a pregnancy.
- Possible exposure of a pregnant woman (this could involve a partner of a male patient or a pregnant female who came in contact with the medication).
- All reports of elevated/questionable or indeterminate beta human chorionic gonadotrophins (betahCGs).

Pregnancy and suspected pregnancy (including a positive pregnancy test regardless of age or disease state) of a female patient or the female partner of a male patient occurring while the patient is on study drug, or within 30 days of the patient's discontinuation visit, is considered an immediately reportable event. The study drug must be discontinued immediately and the patient instructed to return any unused portion of the study drug to the Investigator. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Drug Safety Department at PharmaMar immediately by facsimile using the SAE Report Form.

The Investigator will follow the pregnancy until completion/termination, and must notify the outcome of the pregnancy to the Drug Safety Department at PharmaMar as a follow-up to the initial SAE report in patients who receive study medication.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous or therapeutic abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus]), the Investigator should follow the procedures for reporting SAEs (i.e., report the event to the Drug Safety Department at PharmaMar by facsimile within 24 h of the Investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, regardless of causality, as SAEs. In addition, any death of an infant after 30 days of birth that the Investigator suspects as related to the in utero exposure to the study drug should also be reported to the Drug Safety Department at PharmaMar by facsimile within 24 h of the Investigators' knowledge of the event. If the female is found not to be pregnant, any determination regarding the patient's continued participation in the study will be determined by the Investigator and the PharmaMar Clinical Research Physician.

6.2.3 Adverse Events Monitoring

Safety review will be performed at Pharma Mar S.A. once the SAE forms have been received electronically or by fax and the CRFs have been completed electronically by the Investigator.

Periodic safety review of clinical data will be performed; however, no formal Data Safety Monitoring Board has been appointed for this study. AEs will be monitored by

the Investigators and by the study team at Pharma Mar S.A. The personnel in charge of this process are defined in the section "Study Contacts" of this protocol. In general, a clinical oncologist, together with a member of the Pharma Mar S.A. Pharmacovigilance Department will review the safety data of this study on an ongoing basis.

SAEs will be collected, assessed and reported as per the applicable Regulations by the Pharmacovigilance Department. Periodic safety reviews of SAE reports are to be conducted by the clinical oncologist every 3-6 months, depending on recruitment.

As per the applicable regulations, Pharma Mar S.A. will report to the IECs/IRBs, Investigators and Competent Authorities:

- Expeditedly: all serious, related, unlisted/unexpected AEs or critical safety finding from this and any other clinical trial with the IMP(s) and,
- Periodically: all relevant safety information generated in all clinical trials with the IMP(s) within the Development Safety Update Report.

Non-serious AEs will be assessed during monitoring visits by the monitor, who will discuss them with the Investigators.

Any protocol deviation will also be discussed with the Investigator during monitoring visits.

6.2.3.1 Discontinuations

The reason for a patient's discontinuation from the study will be recorded on the e-CRF. A discontinuation occurs when an enrolled patient ceases to participate in the study, regardless of the circumstances, prior to completion of the protocol. The Investigator must determine the primary reason for discontinuation. Withdrawal due to an AE should be distinguished from withdrawal due to insufficient response according to the definition of AE noted earlier. A discontinuation must be reported immediately to the clinical monitor or his/her designated representative if it is due to a SAE. The final evaluation required by the protocol will be performed at the time of study discontinuation. The Investigator will record the reason for study discontinuation, provide or arrange for appropriate follow-up (if required) for such patients, and document the course of the subject's condition.

7. PHARMACOKINETIC STUDIES

All patients included in the study will be sampled for PK. All sample collection dates and times will be recorded on the electronic CRF.

Samples will be obtained on C1D1 for the PK analysis of plitidepsin and bortezomib. Additionally, patients treated in the expanded cohort at the RD will be sampled for plitidepsin analysis on C1D15. Time points for blood sample collection for the determination of whole blood concentrations of plitidepsin and plasma concentrations of bortezomib are detailed on Table 10. Sample volumes will vary according to the drug being measured: bortezomib, plitidepsin or both. Figure 5 summarizes the PK sampling schedule.

Drug administration on the PK sample collection day should be as follows: after collection of the pre-dose PK sample, oral dexamethasone will be administered at least one hour before the administration of plitidepsin infusion. Plitidepsin should be

administered as a 3-h i.v. infusion. One minute after the end of the plitidepsin infusion, bortezomib should be administered as a 3-5 second bolus s.c. injection. Those patients belonging to the RD expansion cohort will have whole blood samples taken for the measurement of plitidepsin on D15 at the same time points as those on D1.

Figure 5. PK sampling schedule on Day 1.

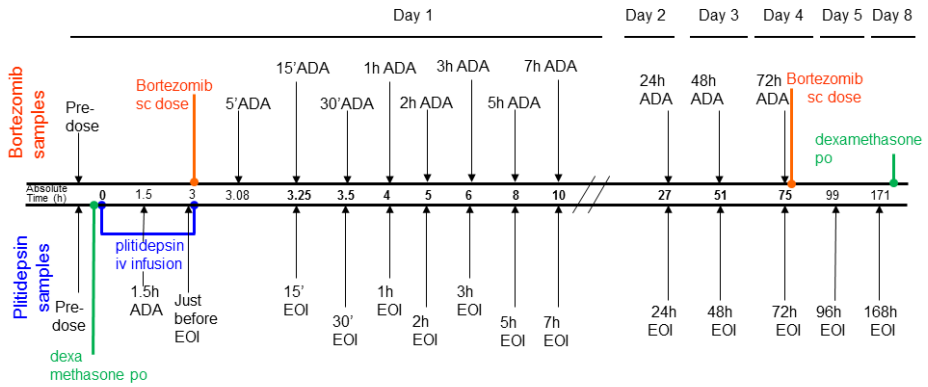


Table 10. Sampling schedule for the determination of bortezomib and plitidepsin.

Sample Number	Day	Absolute time from the start of plitidepsin infusion (h)	Time points for plitidepsin samples	Time points for bortezomib samples
1	1	0	Pre-dose	Pre-dose
2	1	1.5	1.5 ADA	--
3	1	3	Just before EOI	--
4	1	3.08	--	5 min EOI
5	1	3.25	15 min after EOI	15 min EOI
6	1	3.5	30 min after EOI	30 min EOI
7	1	4	1h after EOI	1h EOI
8	1	5	2h after EOI	2h EOI
9	1	6	3h after EOI	3h EOI
10	1	8	5h after EOI	5h EOI
11	1	10	7h after EOI	7h EOI
12	2	27	24h EOI	24h EOI
13	3	51	48h EOI	48h EOI
14[#]	4	75	72h EOI	72h EOI
15	5	99	96h EOI	--
16[##]	8	171	168h EOI	--

[#] This sample has to be taken BEFORE the administration of the second dose of bortezomib.
[##] This sample has to be taken BEFORE the administration of the second dose of dexamethasone.
ADA, after drug administration; EOI, end of infusion.

Should information obtained during the evaluation allow improvement of the schedule, sampling times may be changed while maintaining or decreasing the total sample number and volume. Accurate recording of actual dosing and sampling times is much more important than the strict adherence to the scheduled times.

Samples intended for the measurement of bortezomib and plitidepsin will have a volume of 8 mL. A blood volume of 4 mL will be enough for samples for the measurement of only bortezomib or only plitidepsin.

The exact recording of the time of drug administration and sampling times is crucial on those days when sampling for PK testing takes place. The infusion rate of plitidepsin will be established so that it ensures that the total dose is infused in 3 h. The drug will be infused at a constant rate throughout the 3-h period and the infusion rate should not be modified once the infusion begins in order to obtain reliable PK information. If a variation in the infusion time does eventually occur, it is very important to reflect this modification on the e-CRF, writing clearly the beginning and the end times of the infusion. The pre-established infusion rate should not be changed to maintain the scheduled duration of infusion; it would just be enough to record the actual duration on the e-CRF and in the PK sampling sheet.

Blood samples for PK will be obtained through a peripheral vein located in the contralateral side to that of plitidepsin administration. In any case, the sampling vein has to be different to that from which drugs are being administered. Even the last sample must never be collected from the catheter used for the drug administration.

If the blood sample is obtained from a catheter, the first milliliter (mL) of blood will be discarded to avoid the dilution of the sample with the solution used to keep it clean. Heparin (10 U/mL in normal saline solution) or a slow drip of normal saline solution (10 mL/h) can be used to keep the catheter permeable between extractions.

Samples should be placed in a sodium heparin tube and gently inverted several times to ensure proper mixing. After sampling, test tubes containing the blood samples will either be processed immediately or can be placed in an ice bath at 0-4 ºC for a maximum of 30 minutes prior to centrifugation. Before centrifugation, a whole blood aliquot will be taken from plitidepsin samples for single plitidepsin measurement; bortezomib samples will be centrifuged for plasma single bortezomib determination). Samples for the measurement of both plitidepsin and bortezomib should have an aliquot of whole blood (4 mL) taken before centrifugation and the remaining sample should be centrifuged at 4ºC at 1200 x g for 10 min; this procedure will yield approximately 2 mL of plasma to be used for plasma bortezomib determination.

Once all samples from a patient have been collected, they should be shipped to the central PK laboratory for analysis as soon as possible, ideally the next shipping day. Samples from several patients can be sent in the same shipment; however, the time span between the moment the last PK sample for a patient has been collected and the shipment of all samples from this patient to the central laboratory should not exceed 2 months.

A manual of full instructions for sample extraction, labeling, storage, and shipment will be provided as a separate document (Procedure Manual for the Collection, Storage and Shipment of Plasma Samples for Pharmacokinetics).

7.1 Pharmacokinetic calculations

PK will be elucidated using standard non-compartmental methods. The following parameters will be calculated: maximum drug concentration (C_{max}), area under the plasma concentration-time curve (AUC), total plasma clearance (Cl), volume of distribution at steady state (V_{ss}) and half-life ($t_{1/2}$).

C_{max} will be derived directly from experimental data. The terminal rate constant (k) will be estimated by log-linear regression analysis of the terminal phase of the plasma concentration *versus* time curve. AUC_{inf} will be determined using the log-linear trapezoidal method with extrapolation to infinity using the terminal rate constant k

(C_{last}/k, where C_{last} is the last measured analyte concentration). $T_{1/2}$ will be calculated from the equation 0.693/k; Cl will be determined by dividing the total administered dose (mg) by the AUC_{inf}. V_{ss} will be calculated as V_{ss} = Cl x MRT_{inf}, where MRT_{inf} is the mean residence time, which is determined as MRT_{inf} = ($AUMC_{inf}$/AUC_{inf}) - (1⁄2 x duration of infusion), where $AUMC_{inf}$ is the area under the first moment curve with extrapolation to infinity.

If considered appropriate by PharmaMar, compartmental analysis on the study results will also be performed, and population kinetics analysis will be done with pooled results of the different studies.

PK parameters will be tabulated and graphically displayed per dose level.

8. STATISTICAL METHODS

8.1 Assessment of the Primary Endpoint

For the determination of the RD for the combination plitidepsin, bortezomib and dexamethasone, eligible patients will be fully evaluable unless:

- They are withdrawn from the study due to not being evaluable for the primary endpoint, due to hypersensitivity reactions or for reasons other than a drug-related AEs meeting DLT criteria of (e.g., withdrawal of consent, not meeting the eligibility criteria, non-compliance with follow up, early PD, or unrelated AE).
- They require radiation therapy or other anticancer procedure within three weeks after the first dose, unless they previously had another drug-related AE included in the definition of DLT.
- There is a protocol deviation resulting in an impossibility of concluding anything regarding the safety of the study therapy.

The RD for the combination plitidepsin, bortezomib and dexamethasone will be descriptively determined according to the evaluation of DLTs in the aforementioned population.

8.2 Efficacy

The efficacy population is defined as all eligible patients without protocol deviations with an effect on the risk/benefit ratio of the clinical study, which may jeopardize the efficacy evaluation and who have received at least one treatment cycle (two plitidepsin infusions, four bortezomib injections, four doses of dexamethasone), or the equivalent doses over two cycles and have, at least, one disease assessment. Patients from this population who have inadequate post-baseline data to assess efficacy according to the response criteria in Section 6.1.1 will be considered treatment failures and will be included in the ORR and clinical benefit calculations. For evaluation of ORR and clinical benefit, rates will be calculated with binomial exact confidence intervals at 95%.

Follow-up time will be calculated from the date of the first infusion to the date of the last documented exam. The DOR will be analyzed for all patients in whom a response has been observed and will be calculated from the date of the first documentation of response to the date of PD. Deaths due to causes other than PD will be censored.

TTP will be calculated from the date of the first infusion to the date of documented PD or death due to PD. PFS will be calculated from the date of the first infusion to the date of documented PD or death. EFS will be calculated from the date of the first infusion to the date of documented PD or death (may include additional events besides death and PD considered of importance). If any patient is lost to follow-up before PD or receives another anti-tumor therapy, the TTP, PFS or EFS will be censored on the date of the last tumor assessment. If there were no tumor assessments, the patient will be censored on the date of the first drug administration.

Median time to onset and duration of response, time to PD, PFS, EFS and estimated rate of patients free of progression will be calculated by Kaplan-Meier estimates with 95% confidence intervals.

8.3 Toxicity and Adverse Events

Analysis of safety will be performed on patients who receive at least one or part of one plitidepsin infusion.

Safety evaluations will be based on the incidence, intensity, and type of AEs, and clinically significant changes in the patient's physical examination findings, vital signs, clinical laboratory results and neurotoxicity score. Safety variables will be tabulated and presented for all patients who receive any amount of plitidepsin, bortezomib and dexamethasone. Exposure to study drugs and reasons for discontinuation of study treatment will be tabulated. Analyses will be performed in a descriptive fashion.

AEs will be coded using the MedDRA coding system. All AEs occurring during the study will be listed in by-patient data listings. Treatment-emergent events will be tabulated, where treatment-emergent is defined as any AE that occurs after administration of the first dose of study drug and up to 30 days after the last dose of study drug, any event that is considered drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered drug-related by the Investigator. Events that are considered related to treatment will also be tabulated. Deaths, SAEs and events resulting in study discontinuation will be tabulated.

Changes from baseline in clinical laboratory parameters will be summarized across time throughout the study, and the frequency of clinically significant abnormal laboratory values will be tabulated. Shift tables for each cycle will be produced for selected laboratory parameters, to include at least hemoglobin, WBC count, neutrophils, lymphocytes, platelets, AST, ALT, bilirubin, creatinine, AP, CPK and electrolytes. These tables will summarize, by cycle and DL, the number of patients with each baseline NCI-CTCAE grade and changes to the maximum NCI-CTCAE grade in the cycle.

Changes in vital sign parameters will be summarized over time in a similar fashion to laboratory parameters, and any abnormal values will be tabulated.

In order to most clearly enumerate toxicity rates and to further define the safety profile of plitidepsin, bortezomib and dexamethasone, additional safety analyses may be done at any time without prejudice.

All toxicities will be graded according to NCI-CTCAE version 4.0, whenever an NCI-CTCAE grading exists. Otherwise, severity will be noted. As a convention, the term "grade" will always be used. Toxicities will be described according to the worst NCI-

CTCAE grade or, for toxicities which do not form the subject of NCI-CTCAE classification, according to the worst severity. NCI-CTCAE grading will be programmed and automatically evaluated *versus* normal laboratory parameters by each center.

Only events reported by the Investigator as 'not related to plitidepsin +/- bortezomib +/- dexamethasone' will be excluded from the study analysis of drug related events. A second set of tables including all events will also be presented.

8.4 Other Analyses

Non-continuous variables will be described in frequency tables using counts and percentages. Continuous variables will be described by median, minimum and maximum values.

8.5 Baseline and Demographic Data

Baseline data such as demographics, serum calcemia, renal function (Cr, CrCl), anemia, bone involvement, serum Ig quantitation and M-protein quantitation and IF from the beginning of the last CT until inclusion in the current study, prior anticancer therapy, biological values, prior relevant history, signs and symptoms, ECG and concomitant medication (ATC-WHO coded) will be described.

8.6 Treatment Administration

Cumulative dose, dose intensity and relative dose intensity, cycle delays and dose modifications will be described.

8.7 Protocol Deviations

A protocol deviation is defined as any departure from what is described in the protocol of a clinical trial approved by an IEC/IRB and Competent Authorities. Therefore, this applies to deviations related to patient inclusion and clinical procedures (e.g., assessments to be conducted or parameters to be determined), and also to other procedures described in the protocol that concern GCP guidelines or ethical issues (e.g., issues related to obtaining the patients' IC, data reporting, Investigator's responsibilities etc.).

Deviations with no effect on the risk/benefit ratio of the clinical study (such as minimal delays in assessments or visits) will be distinguished from those that might have an effect on this risk/benefit ratio, such as:

- Deviations that might affect the clinical study objectives, such as those involving the inclusion/exclusion criteria (which could mean that the patient is not eligible for the study) and those having an effect on patient evaluability.
- Deviations that might affect the patient's well-being and/or safety, such as an incorrect dosing of the IMP(s) due to not following dose adjustment specifications or an incorrect preparation of the medication.
- Deviations related to the GCP guidelines compliance as described in the protocol and regulations in force, such as deviations when obtaining IC or not following the terms established for reporting SAEs, etc.

The investigators may suggest the authorization of certain protocol deviations to the Sponsor, especially if they are related to inclusion/exclusion criteria or if they may have

an effect on the patient's evaluability. As a general rule, NO deviations that may have an effect on the risk/benefit ratio of the clinical study will be authorized.

All protocol deviations considered particularly relevant (related to ethical issues, fulfillment of GCP guidelines and study procedures) will be notified to the pertinent IEC/IRB and to the relevant authorities, as established by local regulations.

8.8 Pharmacokinetics

PK parameters will be tabulated and selected parameters will be graphically displayed per dose level.

PK interactions between plitidepsin and bortezomib will be evaluated comparing the PK parameters obtained in this study with those obtained from single agent plitidepsin studies and with literature data for bortezomib.

The potential influence on selected PK parameters of selected demographic and clinical dichotomous variables (gender, laboratory test results above/below selected cut-off values, etc) will be evaluated by a Student's t test or a Mann-Whitney's U test as appropriate.

For multinomial variables, analysis of variance will be used. For selected continuous demographic and clinical variables (age, laboratory test results…), the relationship with selected PK parameters will be graphically explored and assessed using correlation and regression methods.

The potential influence on efficacy and safety of the selected PK parameters will be graphically explored. For dichotomous outcomes, a Student's T test or a Mann-Whitney's U test, as appropriate, will be used for assessment. For multinomial outcomes, analysis of variance will be used. Continuous outcomes will be assessed using correlation and regression methods.

Other tests may be applied if the results of the above evaluations suggest that they may yield additional relevant information.

8.9 Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original statistical analysis plan will be provided in the final clinical study report.

9. ADMINISTRATIVE REQUERIMENTS

9.1 Ethics

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki (Appendix 6) and will be consistent with GCP and other applicable regulatory requirements.

Study personnel involved in conducting this study will be qualified by education, training and experience to perform their respective task(s).

The study will be conducted in compliance with the protocol. The protocol, any amendments and the patient IC will receive IRB/IEC approval/favorable opinion prior to initiation. The decision of the IEC/IRB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of the study.

The Investigator and/or the Sponsor is/are responsible for keeping the IEC/IRB informed of significant new information about study drug.

All protocol amendments will be agreed upon by the Sponsor and the Investigator.

Administrative changes of the protocol are minor corrections and/or clarifications that have no impact on the way the study is to be conducted.

9.2 Monitoring, Auditing and Inspecting

The study will be monitored by regular site visits and telephone calls to the Investigator by the clinical study monitor and/or the CRO clinical study monitor (if applicable).

During site visits, the study monitor should review original patient records, drug accountability records and document retention (study file). Additionally, the monitor should observe study procedures and will discuss any problem with the Investigator.

The Investigator should allocate adequate time for these visits. The Investigator should also ensure that the monitor is given direct access (as per ICH GCP Guideline, Sections 4.9.7 and 6.10) to the patient's source documents (i.e., hospital or private charts, original laboratory records, appointment books, etc.), which support data entered in the e-CRFs, as defined in the ICH GCP Guideline, Sections 1.51. and 1.52.

The necessary systems and procedures will be implemented to assure the quality of every aspect of the study.

At any time during the course or at the end of the study, the Clinical Quality Assurance Department of PharmaMar or external auditors contracted by the Sponsor may conduct an onsite audit visit to the centers (ICH guideline glossary Section 1.6).

Participation in this study implies acceptance of potential inspections by national or foreign Health Authorities.

9.3 Patient Informed Consent

Before agreeing to participate in this study, all patients will be provided with full written information about the study in a "Patient Information Sheet" with language that is non-technical and easily understood. The Patient Information Sheet will include all elements required by ICH, GCP and other applicable regulatory requirements and will be submitted for approval to the IEC/IRB along with the protocol. A statement of document approval should be provided before commencement of the study.

The Investigator, or designated person, must provide the patient with a copy of the Patient Information Sheet and consent form and should allow the necessary time for the patient to inquire about the details of the study; then, IC must be freely signed and personally dated by the patient and by the person who conducted the IC discussion before commencement of the study. The patient should receive a copy of the signed IC and any other written information provided to patients prior to participation in the study.

During a patient's participation in the study, any updates to the IC form and any updates to the written information will be provided to the patient.

If there is a need to obtain new consent from the patients, the Investigator, or designated person, should inform the patient of any new information relevant to the patient's willingness to continue to participate in the study, before obtaining the written consent.

9.4 Confidentiality/Patients Identification

The collection and processing of personal data from patients enrolled in this study will be limited to data that are necessary to investigate the efficacy, safety, quality, and utility of the IMP(s) used in this study. It is the Investigator's responsibility that sufficient information pertaining to the patient's identity be retained.

The study monitor, auditor at PharmaMar, IRB/IEC, or regulatory authorities should have direct access to all requested study related records and agree to keep the identity of study patients confidential.

Data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

PharmaMar shall comply with Directive 95/46/EEC of the European Parliament and of the Council of 24 October 1995, on the protection of individuals with regards to the processing of personal data and on the free movement of such data.

9.5 Case Report Forms

Electronic CRFs will be used to record all data for each patient. It is the responsibility of the Investigator to ensure that the e-CRFs are properly and completely filled in. Electronic CRFs must be completed for all patients who have given IC and have been admitted to the study.

The patient's source documentation is the physician's patient records, and as such, they should be maintained at the study site.

Data collected in the e-CRF will be entered into a database at PharmaMar which complies with the Spanish Act implementing Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data.

9.6 Insurance

The Sponsor will provide insurance or indemnity in accordance with the applicable regulatory requirements.

9.7 Records Retention

The Investigator/Institution should maintain study documents according to ICH Topic E6, Section 8, and as required by the applicable regulatory requirements.

Essential documents should be retained according to ICH guidelines, or for a longer period if required by the applicable regulations.

9.8 Use of Information and Publication

Before the Investigators of this study submit a paper or abstract for publication or otherwise publicly disclose information concerning the study IMP(s), PharmaMar must be provided with at least 60 days to review and approve the proposed publication or disclosure to assure protection of confidential and proprietary data. If PharmaMar determines that patentable subject matter is disclosed in such proposed publication or disclosure, the publication or disclosure shall be withheld during the period of time that it is considered convenient.

If the study is part of a multicenter study, the first publication of the study shall be done as a multicenter publication, in collaboration with all investigators and institutions contributing data, analysis and comments. However, if such a multicenter publication is not submitted within 12 months after conclusion, abandonment or termination of the study at all sites, the present study may be published individually in accordance with the procedure established before.

The order of the co-authors will reflect the relative contribution of each one of them to the study development and analysis. In general, the first author will be the Clinical Investigator who recruits the highest number of patients with information finally available for data analysis. Relevant PharmaMar personnel who have fully participated in the study must be considered for co-authorship of the publication.

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Appendix 1. Performance Status

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 2. Cockcroft and Gault's Formula for Calculating Creatinine Clearance

$$\text{Creatinine clearance (mL/min)} = \frac{[(140\text{-age (years)}) \times \text{weight (Kg)}]}{72 \times \text{serum creatinine (mg/dL)}} \times G^{1}$$

$$\text{Creatinine clearance (mL/min)} = \frac{[(140\text{-age (years)}) \times \text{weight (Kg)}]}{72 \times \text{serum creatinine (μmol/L)}} \times G^{1} \times 0{,}00113$$

[1]G(Gender)= 0.85 if Female; 1 if Male.

DW Cockcroft, H Gault. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31-41.

Appendix 3. Multiple Myeloma Diagnosis Criteria

Durie Salmon Diagnosis Criteria

Standards for diagnosis currently require confirmation of one major and one minor criteria or three minor criteria in a patient displaying symptoms of myeloma.

Major criteria:

- A biopsy-proven plasmacytoma.
- A BM sample showing 30% plasma cells.
- Elevated monoclonal immunoglobulin levels in the blood or urine.

Minor criteria:

- A BM sample showing 10-30% plasma cells.
- Minor monoclonal immunoglobulin levels in blood or urine.
- Imaging studies revealing holes in bones due to tumor growth.
- Antibody levels (not produced by the cancer cells) in the blood are abnormally low.

Appendix 4. Durie Salmon Staging Criteria

Stage	Durie-Salmon Criteria	International Staging System (ISS) Criteria
I	All of the following: • Hemoglobin value >10 g/dL • Serum calcium value normal or ≤12 mg/dL • Bone X-ray, normal bone structure (scale 0) or solitary bone plasmacytoma only • Low M-component production rate • IgG value <5 g/dL; IgA value <3 g/dL • Bence Jones protein <4 g/24 h	Beta-2-M<3.5 mg/L Albumin ≥3.5 g/dL Median survival: 62 months
II	Neither stage I nor stage III	Neither stage I nor stage III* Median survival: 44 months
III	One or more of the following: • Hemoglobin value <8.5 g/dL • Serum calcium value >12 mg/dL • Advanced lytic bone lesions (scale 3) • High M-component production rate • IgG value >7 g/dL; IgA value >5 g/dL • Bence Jones protein >12 g/24 h	Beta-2-M > 5.5 mg/L Median survival: 29 months

Durie-Salmon sub classifications (either A or B)
A: Relatively normal renal function (serum creatinine value <2.0 mg/dL)
B: Abnormal renal function (serum creatinine value ≥2.0 mg/dL)
*There are two categories for stage II: serum ß2-microglobulin < 3.5 mg/L but serum albumin < 3.5 g/dL; or serum ß2-microglobulin 3.5 to < 5.5 mg/L irrespective of the serum albumin level.

Appendix 5. Lists of Drugs that Prolong the Qt Interval and/or Induce Torsades de Pointes Ventricular Arrhythmia

List 1: Drugs that prolong the QT interval and/or induce Torsades De Pointes Source: www.torsade.org
Drugs that are generally accepted by authorities to have a risk of causing Torsades de Pointes.

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
Amiodarone (Pacerone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low
Amiodarone (Cordarone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low
Arsenic trioxide (Trisenox®)	Anti-cancer/Leukemia	
Bepridil (Vascor®)	Anti-anginal/heart pain	Females>Males
Chloroquine (Arelan®)	Anti-malarial/malaria infection	
Chlorpromazine (Thorazine®)	Anti-psychotic/ Anti-emetic/schizophrenia/ nausea	
Cisapride (Propulsid®)	GI stimulant/heartburn	Restricted availability; Females>Males
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection	
Disopyramide (Norpace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males
Dofetilide (Tikosyn®)	Anti-arrhythmic/abnormal heart rhythm	
Domperidone* (Motilium®)	Anti-nausea/nausea	not available in the United States.
Droperidol (Inapsine®)	Sedative;Anti-nausea/anesthesia adjunct, nausea	
Erythromycin (Erythrocin®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males
Erythromycin (E.E.S. ®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males
Halofantrine (Halfan®)	Anti-malarial/malaria infection	Females>Males
Haloperidol (Haldol®)	Anti-psychotic/schizophrenia, agitation	
Ibutilide (Corvert®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	
Mesoridazine (Serentil®)	Anti-psychotic/schizophrenia.	
Methadone (Dolophine®)	Opiate agonist/pain control, narcotic dependence	Females>Males
Methadone (Methadose®)	Opiate agonist/pain control,narcotic dependence	Females>Males
Pentamidine (NebuPent®)	Anti-infective/pneumocystis pneumonia	Females>Males

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
Pentamidine (Pentam®)	Anti-infective/pneumocystis pneumonia	Females>Males
Pimozide (Orap®)	Anti-psychotic/Tourette's tics	Females>Males
Procainamide (Pronestyl®)	Anti-arrhythmic/abnormal heart rhythm	
Procainamide (Procan®)	Anti-arrhythmic/abnormal heart rhythm	
Quinidine (Quinaglute®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males
Quinidine (Cardioquin®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males
Sotalol (Betapace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection	
Thioridazine (Mellaril®)	Anti-psychotic/schizophrenia	

List 2: Drugs that in some reports may be associated with Torsades De Pointes but at this time lack substantial evidence for causing Torsades De Pointes

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
Alfuzosin (Uroxatral®)	Alpha1-blocker/Benign prostatic hyperplasia	
Amantadine (Symmetrel®)	Dopaminergic/Anti-viral/Anti-infective/ Parkinson's Disease	
Azithromycin (Zithromax®)	Antibiotic/bacterial infection	
Chloral hydrate (Noctec®)	Sedative/sedation/ insomnia	
Clozapine (Clozaril®)	Anti-psychotic/schizophrenia	
Dolasetron (Anzemet®)	Anti-nausea/nausea, vomiting	
Felbamate (Felbatrol®)	Anti-convulsant/seizure	
Flecainide (Tambocor®)	Anti-arrhythmic/abnormal heart rhythm	
Foscarnet (Foscavir®)	Anti-viral/HIV infection	
Fosphenytoin (Cerebyx®)	Anti-convulsant/seizure	
Gatifloxacin (Tequin®)	Antibiotic/bacterial infection	
Gemifloxacin (Factive®)	Antibiotic/bacterial infection	
Granisetron (Kytril®)	Anti-nausea/nausea and vomiting	
Indapamide (Lozol®)	Diuretic/stimulate urine & salt loss	
Isradipine (Dynacirc®)	Anti-hypertensive/high blood pressure	
Levofloxacin (Levaquin®)	Antibiotic/bacterial infection	
Lithium (Eskalith®)	Anti-mania/bipolar disorder	
Lithium (Lithobid®)	Anti-mania/bipolar disorder	
Moexipril/HCTZ (Uniretic®)	Anti-hypertensive/high blood	

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
	pressure	
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection	
Nicardipine (Cardene®)	Anti-hypertensive/high blood pressure	
Octreotide (Sandostatin®)	Endocrine/acromegaly, carcinoid diarrhea	
Ofloxacin (Floxin®)	Antibiotic/bacterial infection	
Ondansetron (Zofran®)	Anti-emetic/nausea and vomiting	
Quetiapine (Seroquel®)	Anti-psychotic/schizophrenia	
Ranolazine (Ranexa®)	Anti-anginal/chronic angina	
Risperidone (Risperdal®)	Anti-psychotic/schizophrenia	
Roxithromycin* (Rulide®)	Antibiotic/bacterial infection	*not available in the United States
Tacrolimus (Prograf®)	Immunosuppressant/Immune suppression	
Tamoxifen (Nolvadex®)	Anti-cancer/breast cancer	
Telithromycin (Ketek®)	Antibiotic/bacterial infection	
Tizanidine (Zanaflex®)	Muscle relaxant/	
Vardenafil (Levitra®)	phosphodiesterase inhibitor/vasodilator	
Venlafaxine (Effexor®)	Anti-depressant/depression	
Voriconazole (VFend®)	Anti-fungal/anti-fungal	
Ziprasidone (Geodon®)	Anti-psychotic/schizophrenia	

List 3: Drugs to be avoided for use in patients with diagnosed or suspected congenital long QT syndrome. (Drugs on Lists 1, 2, and 4 should also be avoided by patients with QT syndrome

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
Albuterol (Ventolin®)	Bronchodilator/Asthma	
Albuterol (Proventil®)	Bronchodilator/Asthma	
Amphetamine/dextroamphetamine (Adderall®)	CNS stimulant/ADHD	
Atomoxetine (Strattera®)	norepinephrine reuptake inhibitor /ADHD	
Cocaine (Cocaine)	Local anesthetic/	
Dexmethylphenidate (Focalin®)	CNS stimulant/ADHD	
Dextroamphetamine (Dexadrine®)	CNS stimulant/ADHD	
Dobutamine (Dobutrex®)	Catecholamine/heart failure and shock	
Dopamine (Intropine®)	Anti-arrhythmic/abnormal heart rhythm	
Ephedrine (Broncholate®)	Bronchodilator,	

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
	decongestant/Allergies, sinusitis, asthma	
Ephedrine (Rynatuss®)	Bronchodilator, decongestant/Allergies, sinusitis, asthma	
Epinephrine (Primatene®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	
Epinephrine (Bronkaid®)	catecholamine, vasoconstrictor/anaphylaxis, allergic reactions	
Fenfluramine (Pondimin®)	Appetite suppressant/dieting, weight loss	
Isoproterenol (Isupres®)	Catecholamine/allergic reaction	
Isoproterenol (Medihaler-Iso®)	Catecholamine/allergic reaction	
Levalbuterol (Xopenex®)	Bronchodilator/asthma	
Metaproterenol (Alupent®)	Bronchodilator/asthma	
Metaproterenol (Metaprel®)	Bronchodilator/asthma	
Methylphenidate (Ritalin®)	CNS stimulant/ADHD	
Methylphenidate (Concerta®)	CNS stimulant/ADHD	
Midodrine (ProAmatine®)	Vasoconstrictor/low blood pressure, fainting	
Norepinephrine (Levophed®)	Vasconstrictor, Inotrope/shock, low blood pressure	
Phentermine (Fastin®)	Appetite suppressant/dieting, weight loss	
Phentermine (Adipex®)	Appetite suppressant/dieting, weight loss	
Phenylephrine (Neosynephrine®)	Vasoconstrictor, decongestant/low blood pressure, allergies, sinusitis, asthma	
Phenylpropanolamine (Acutrim®)	Decongestant/allergies, sinusitis, asthma	
Phenylpropanolamine (Dexatrim®)	Decongestant/allergies, sinusitis, asthma	
Pseudoephedrine (PediaCare®)	Decongestant/allergies, sinusitis, asthma	
Pseudoephedrine (Sudafed®)	Decongestant/allergies, sinusitis, asthma	
Ritodrine (Yutopar®)	Uterine relaxant/prevent	

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
	premature labor	
Salmeterol (Serevent®)	Sympathomimetic/asthma, COPD	
Sibutramine (Meridia®)	Appetite suppressant/dieting, weight loss	
Terbutaline (Brethine®)	Bronchodilator/asthma	
Tolterodine (Detrol®)	Bladder Antispasmodic/	
Tolterodine (Detrol LA®)	Bladder Antispasmodic/	

List 4: Drugs that, in some reports, have been weakly associated with Torsades De Pointes and/or QT prolongation but that are unlikely to be a risk for Torsades De Pointes when used at the usual recommended dosages and in patients without other risk factors (e.g., concomitant QT prolonging drugs, bradycardia, electrolytes disturbances, congenital long QT syndrome, concomitant drugs that inhibit metabolism)

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
Amitriptyline (Elavil®)	Tricyclic Antidepressant/depression	
Amoxapine (Asendin®)	Tricyclic Antidepressant/depression	
Ciprofloxacin (Cipro®)	Antibiotic/bacterial infection	
Citalopram (Celexa®)	Anti-depressant/depression	
Clomipramine (Anafranil®)	Tricyclic Antidepressant/depression	
Desipramine (Pertofrane®)	Tricyclic Antidepressant/depression	
Doxepin (Sinequan®)	Tricyclic Antidepressant/depression	
Fluconazole (Diflucan®)	Anti-fungal/fungal infection	
Fluoxetine (Sarafem®)	Anti-depressant/depression	
Fluoxetine (Prozac®)	Anti-depressant/depression	
Galantamine (Reminyl®)	Cholinesterase inhibitor/ Dementia, Alzheimer's	
Imipramine (Norfranil®)	Tricyclic Antidepressant/depression	
Itraconazole (Sporanox®)	Anti-fungal/fungal infection	
Ketoconazole (Nizoral®)	Anti-fungal/fungal infection	
Mexiletine (Mexitil®)	Anti-arrhythmic/Abnormal heart rhythm	
Nortriptyline (Pamelor®)	Tricyclic Antidepressant/depression	
Paroxetine (Paxil®)	Anti-depressant/depression	
Protriptyline (Vivactil®)	Tricyclic Antidepressant/depression	

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments
Sertraline (Zoloft®)	Anti-depressant/depression	
Solifenacin (VESIcare®)	muscarinic receptor anatagonist/treatment of overactive bladder	
Trimethoprim-Sulfa (Sulfa®)	Antibiotic/bacterial infection	
Trimethoprim-Sulfa (Bactrim®)	Antibiotic/bacterial infection	
Trimipramine (Surmontil®)	Tricyclic Antidepressant/depression	

A note about Brand Names: Drugs are listed with up to 2 common brand names. There are many more brand names for some of the common drugs, such as pseudoephedrine and erythromycin. It is also important to look at the list of active drugs in medicines that contain a combination of drugs such as Zyrtec-D®, which contains pseudoephedrine.

Appendix 6. Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)

55th WMA General Assembly, Tokyo 2004 (Note of Clarification on paragraph 30 added)

59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research

on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There

may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the

patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    a. The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    b. Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.