SPONSOR
Pharma Mar, S.A.
Avda de los Reyes, 1
Polígono Industrial "La Mina"
28770 Colmenar Viejo (Madrid), Spain
Phone: + 34 918 466 000
Fax: + 34 918 466 003

PharmaMar USA, Inc.
230 Park Avenue, suite 1000
New York, NY 10169
USA
Phone: +1 212 309 8737
Fax: +1 646 435 5846

CLINICAL TRIAL PROTOCOL

Phase II Trial of Plitidepsin (Aplidin®) in Combination with Bortezomib and Dexamethasone in Multiple Myeloma Patients Double Refractory to Bortezomib and Lenalidomide

INVESTIGATIONAL MEDICINAL PRODUCTS: plitidepsin (Aplidin®), bortezomib and dexamethasone.

Protocol No.: APL-B-022-15

EudraCT No.: 2015-003486-29

NCT Code: 03117361

Protocol version 1.0: 10 November 2015

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PharmaMar, S.A.
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28770 Colmenar Viejo (Madrid) Spain
Phone: + 34 91 846 6000
Fax: + 34 91 846 6003

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Eudra CT: 2015-003486-29

Protocol version 1.0: 10 November 2015

This trial will be conducted in compliance with the protocol, Good Clinical Practice and applicable regulatory requirements.

TRIAL CONTACTS	NAME AND ADDRESS	CONTACT DETAILS
Pharma Mar, S.A. Pharma Mar USA, Inc.	Avda. de los Reyes, 1 Polígono Industrial "La Mina" 28770 Colmenar Viejo Madrid Spain One Liberty Plaza 23rd floor, Suite 2335 New York 10006 USA	Phone: +34 91 846 6000 Fax: +34 91 846 6003 Phone: + 1 212 201 6770 Fax: +1 212 201 6771
Responsible Physician(s)	José Rodríguez Diaz-Pavón, MD, PhD Clinical Oncology Clinical Development Area Sara Martínez Gonzalez, MD Clinical Oncology Clinical Development Area	Phone: +34 91 846 6157 Fax: +34 91 823 4504 e-mail: jrodriguez@pharmamar.com Phone: +34 91 823 4647 Fax: +34 91 823 4504 E-mail: smgonzalez@pharmamar.com
Project Manager Clinical Trial Monitoring	Pilar García Paramio, PhD Clinical Operations Clinical Development Area Pharma Mar, S.A. Clinical Operations Clinical Development Area	Phone: +34 91 823 4597 Fax: +34 91 823 4529 E-mail: mpgarcia@pharmamar.com
Pharmacovigilance	Carmen Martinez Caballero, MD Pharmacovigilance (PhV) Clinical Development Pharma Mar, S.A.	Phone: +34 91 823 4569 Fax: +34 91 846 6004 E-mail: phv@pharmamar.com Out of office hours (GMT) PhV service phone: +34 91 823 47 42
Clinical Pharmacology	Salvador Fudio Muñoz, MD Clinical Pharmacology Clinical Development Pharma Mar, S.A.	Phone: + 34 91 823 4521 Fax: + 34 91 823 4504 E-mail: sfudio@pharmamar.com

Central Laboratory for Pharmacokinetic Samples	Soledad García Martinez DynaKin, S.L. Parque Tecnológico de Bizkaia Ed.801-B first floor 48160 Derio, Vizcaya Spain	Phone: +34 94 404 5504 Fax: +34 94 655 9463 E-mail: sgarcia@dynakin.com
Pharmacogenomics (PGx) contact	Miguel Aracil, Ph.D. Pharmacogenomics Department. Pharma Mar, S.A.	Phone: + 34 91 846 6098 Fax: + 34 91 846 6001 E-mail: maracil@pharmamar.com
Central Laboratory for PGx Samples	**RNA/DNA analysis:** Maria Luisa Villahermosa GENOMICA S.A.U. Parque Empresarial Alvento, Edificio B Via de los Poblados 1, 1ª planta 28033 Madrid Spain **IHC analysis:** Richard Fink Q2 Solutions Unit B1, Parkway West Industrial Estate Cranford Lane – Heston, Middlesex TW5 9QA, UK	Phone: +34 91 674 8990 Fax: +34 91 674 8991 E- mail: mlvillahermosa@genomica.com Phone: +44 208 377 3300 Fax: +44 208 377 3350 E-mail: Richard.S.Fink@QuestDiagnostics.com

PRINCIPAL INVESTIGATORS

A full list of Investigators will be available as a separate document.

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SYNOPSIS

TITLE	Phase II Trial of Plitidepsin (Aplidin®) in Combination with Bortezomib and Dexamethasone in Multiple Myeloma Patients Double Refractory to Bortezomib and Lenalidomide
PROTOCOL CODE	APL-B-022-15
INVESTIGATORS / TRIAL LOCATION	A complete list will be provided as a separate document.
TRIAL OBJECTIVES	**Primary objective:** To evaluate the efficacy of plitidepsin in combination with bortezomib and dexamethasone in patients with multiple myeloma (MM) double refractory to bortezomib and lenalidomide in terms of overall response rate (ORR), including stringent complete response (sCR), complete response (CR), very good partial response (VGPR) and partial response (PR). **Secondary objectives:** • To evaluate time-to-event efficacy endpoints of plitidepsin in combination with bortezomib and dexamethasone, i.e., duration of response (DOR), time to progression (TTP), progression-free survival (PFS) and event-free survival (EFS). • To evaluate overall survival (OS) and OS rate at 6 and 12 months (OS6 and OS12, respectively). • To evaluate the safety and tolerability of plitidepsin in combination with bortezomib and dexamethasone. • To study the pharmacokinetics (PK) and pharmacodynamics (PDy) of plitidepsin in combination with bortezomib and dexamethasone. • To obtain pharmacogenomic information (Pharmacogenomics [PGx]) on markers of response to plitidepsin and bortezomib treatment.
TRIAL DESIGN	This is a multicenter, open-label, single arm, non-comparative phase II study to evaluate the efficacy of plitidepsin in combination with bortezomib and dexamethasone in patients with MM double refractory to bortezomib and lenalidomide. • Plitidepsin will be administered as a 3-hour (h) intravenous (i.v.) infusion at a dose of 5 mg/m^2 on Day (D) 1 and 15, every four weeks (q4wk). • Bortezomib will be administered as a subcutaneous (s.c.) injection at a dose of 1.3 mg/m^2 on D1, 4, 8 and 11, q4wk. • Dexamethasone will be taken orally at a dose of 40 mg/day on D1, 8, 15 and 22, q4wk. Patients will be treated until PD, excessive side effects or withdrawal of consent. If patients respond to treatment or achieve stable disease (SD) and bortezomib toxicity precludes any further treatment, they may continue to receive plitidepsin and dexamethasone at the same dose upon Investigator's decision and agreement with the Sponsor.

	Once plitidepsin treatment is stopped, patients must be discontinued from the trial.
TRIAL POPULATION	Patients with MM double refractory to bortezomib and lenalidomide. **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.
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 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 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. c) 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). 5) 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 90 days before the onset of treatment with the trial drug(s). 6) Patients must have received previous treatment with bortezomib and lenalidomide and be refractory to both. 7) Patients must have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2.

	8) Recovery to grade ≤ 1 from any non-hematological adverse event (AE) derived from previous treatment (if present, alopecia and peripheral neuropathy must be grade <1). 9) 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 (G-CSF 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) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 3.0 x institutional ULN and alkaline phosphatase (AP) ≤ 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. 10) Evidence of non-childbearing status for women of childbearing potential (WOCBP): WOCBP must have a negative serum or urine pregnancy test within seven days prior to enrolment and must agree to use a highly effective contraceptive measure throughout the trial and during six months after treatment discontinuation. Male patients enrolled in the study should also use contraceptive methods during and after treatment discontinuation. 11) Left ventricular ejection fraction (LVEF) ≥ 45%. 12) 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 trial, including the following specific conditions: a) Uncontrolled psychiatric illness or medical illness that the Investigator feels will compromise the patient's tolerance of the trial 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. e) Acute infiltrative pulmonary and pericardial disease. 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, or equivalent. 5) History of hypersensitivity reactions and/or intolerance to bortezomib, polyoxyl 35 castor oil, mannitol, boron or dexamethasone. 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) 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 trial. 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 trial. 13) Concomitant medications that include corticosteroids, chemotherapy (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 trial 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 to use steroids.
INCLUSION CRITERIA FOR THE PGx SUB-STUDY	Only patients with available BM samples and who voluntarily sign the Informed Consent Form (ICF) for the PGx sub-study will be able to participate. Refusal to participate in the PGx sub-study will not affect patient participation in the trial APL-B-022-15.
EXPECTED NUMBER OF PATIENTS	Approximately 64 evaluable patients will be needed for the evaluation of the primary endpoint, ORR. An early futility analysis will be performed with the efficacy data collected from the first 20 evaluable patients. The futility analysis will commence once patient number 20 has completed two full treatment cycles. Patient recruitment will not be halted during the conduct of this futility analysis.
EXPECTED NUMBER OF CENTERS	A complete list of centers (10-15 are expected) will be provided as a separate document.
TRIAL 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** is available for i.v. or s.c. injection use. For further information on the drug product, please refer to the EU Summary of Product Characteristics (SmPC) or the USP (US Product Information). **Dexamethasone** is administered orally as tablets that should be stored in well-closed containers. For further information on the drug product, please refer to the EU SmPC or the USP.
TREATMENT SCHEDULE	**A treatment cycle** consists of oral dexamethasone administered on D1, 8, 15 and 22, q4wk, at a dose of 40 mg/day at least one hour before a 3h i.v. infusion of plitidepsin on D1 and 15, q4wk, at a dose

<table>
<tr><td></td><td>of 5 mg/m^2 and immediately followed by a 3-5 second bolus s.c. injection of bortezomib on D1, 4, 8 and 11, q4wk at a dose of 1.3 mg/m^2.
Treatment cycles will be repeated q4wk.
Patients will be treated until PD. If the patient responds to treatment or achieves SD, and if bortezomib toxicity precludes any further treatment, treatment may continue with plitidepsin and dexamethasone at the same dose upon Investigator’s decision and agreement with the Sponsor. Once plitidepsin treatment is stopped, patients must be discontinued from the trial.</td></tr>
<tr><td>CRITERIA FOR TREATMENT CONTINUATION</td><td>Re-treatment criteria for plitidepsin and dexamethasone will be monitored on D1 and D15 and re-treatment criteria for bortezomib will be monitored on D1, as defined in the table below:
<table>
<tr><th></th><th>Plitidepsin/Dexamethasone</th><th>Bortezomib</th></tr>
<tr><th></th><th>1[a] and 15[b]</th><th>Day 1[a]</th></tr>
<tr><td>ANC</td><td>1.0 x 10^9/L
(≥ 0.5 x10^9/L if due to extensive BM involvement)</td><td>0.75 x 10^9/L
(≥ 0.5 x10^9/L if due to extensive BM involvement)</td></tr>
<tr><td>Platelet count</td><td>≥ 50.0 x 10^9/L
(≥ 25.0 x 10^9/L if ≥ 50% of BM nucleated cells are plasma cells)</td><td>≥ 25.0 x 10^9/L</td></tr>
<tr><td>Hemoglobin</td><td>≥ 8.0 g/dL</td><td>≥ 8.0 g/dL</td></tr>
<tr><td>Serum total bilirubin</td><td>≤ 1.5 x ULN[e]</td><td>≤ 3.0 x ULN[e]</td></tr>
<tr><td>AST/ALT</td><td>≤ 3.0 x ULN</td><td>≤ 5.0 x ULN</td></tr>
<tr><td>AP</td><td>≤ 2.5 x ULN</td><td>≤ 5.0 x ULN</td></tr>
<tr><td>Muscular toxicity (myalgia, muscular weakness, CPK increase)</td><td>< Grade 2</td><td>< Grade 3</td></tr>
<tr><td>Other non-hematological drug-related AEs (except for increased GGT, non-optimally treated nausea and vomiting, hypertension, alopecia)[c]</td><td>< Grade 2</td><td>< Grade 3[f]</td></tr>
<tr><td>ECG</td><td>Baseline values</td><td>Baseline values</td></tr>
<tr><td>ECHO/MUGA[d]</td><td>Baseline values</td><td>Baseline values</td></tr>
</table>
[a]If a patient does not meet the requirements for plitidepsin/dexamethasone treatment continuation while meeting the requirements for bortezomib treatment continuation on D1 of the following cycle, the plitidepsin/dexamethasone dose will be omitted and bortezomib will be administered.
If a patient does not meet the requirements for plitidepsin/dexamethasone and bortezomib treatment continuation on D1 of the following cycle, plitidepsin/dexamethasone and bortezomib will be delayed until recovery or for a maximum of 14 days. After this period, if the delay is due to toxicity assessed as related to a trial drug, a dose reduction (according to the “Criteria for Dose Reduction” section) is mandatory.
[b]If a patient does not meet the requirements for plitidepsin/dexamethasone treatment continuation on D15 of the following cycle, the plitidepsin/dexamethasone dose will be omitted.</td></tr>
</table>

	[c]Any grade accepted for increased GGT. [d]To be performed every three months unless more frequent assessments are clinically indicated. [e]Except if Gilbert syndrome is clearly documented and other liver function tests are normal. **[f]If peripheral neuropathy (PN) occurs, follow the guidelines for bortezomib dose reduction available in the "Criteria for Dose Reduction" section.** 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-glutamyl transpeptidase; L, liter; ULN, upper limit of normality; PN, peripheral neuropathy.
CRITERIA FOR DOSE REDUCTION	**Plitidepsin** Under the following circumstances, patients may continue plitidepsin treatment after a 20-25% dose reduction, upon Investigator's decision and agreement with the Sponsor, if patient benefit is perceived: • Less than 50% compliance with the treatment schedule, and/or • Grade ≥ 3 febrile neutropenia, or • Grade 4 neutropenia and infection, or grade 4 neutropenia lasting > 7 days (except for patients with extensive BM involvement), and/or • Grade 4 thrombocytopenia (except for patients with extensive BM involvement), and/or • Grade 4 thrombocytopenia with grade ≥ 3 bleeding (except for patients with extensive BM involvement), and/or • Grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations). In the presence of muscular toxicity, plitidepsin will be reduced first; if toxicity persists, dexamethasone will then be reduced. • Any grade ≥ 3 clinically relevant non-hematological toxicity other than non-optimally treated nausea and vomiting, diarrhea lasting < 48 h and/or grade ≥ 3 asthenia/fatigue lasting < 5 days. Up to a maximum of two plitidepsin dose reductions (from 5.0 mg/m^2 to 4 mg/m^2 and from 4 mg/m^2 to 3.0 mg/m^2) will be allowed upon Sponsor's agreement, if patient benefit is perceived. After two dose reductions, trial treatment will be discontinued. **Dexamethasone** Under the following circumstances, and after recovery from toxicity, patients may continue dexamethasone treatment after a 50% dose reduction: • Grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations) (in the presence of muscular toxicity, plitidepsin will be reduced first; if toxicity persists, dexamethasone will then be reduced), or • Drug-related grade ≥ 3 fatigue, or • Grade ≥ 2 mood disturbances or agitation, or • Grade ≥ 3 fluid retention, or • Grade 4 clinically documented infection. • Grade ≥ 3 gastrointestinal disorders, despite optimal treatment.

- Acute pancreatitis (discontinue dexamethasone without previous dose reduction).
- Grade ≥ 3 hyperglycemia, despite optimal treatment.

Up to a maximum of two consecutive dose reductions (i.e., 20 mg D1, 8, 15 and 22, and 20 mg D1 and 15 of each 28-day cycle) will be allowed upon Sponsor's agreement, if patient benefit is perceived. After two dose reductions, dexamethasone will be discontinued.

Bortezomib

The following guidelines must be followed for bortezomib-related hematological and non-hematological toxicity:

Severity of toxicity	Recommended modification of bortezomib dose and regimen
≥ Grade 3 febrile neutropenia, or Grade 4 neutropenia, or Grade 4 thrombocytopenia, or Grade 3 thrombocytopenia with bleeding	If two or more doses are omitted consecutively or within the same cycle, then reduce bortezomib one dose level
Herpes Zoster reactivation (any grade)	Hold therapy until lesions are dry
≥ Grade 3 bortezomib-related non-hematological toxicity (as judged by the Investigator)	If two or more doses are omitted consecutively or within the same cycle, then reduce bortezomib one dose level

The following guidelines must be followed for bortezomib-related peripheral neuropathy:

Severity of PN signs and symptoms[a]	Recommended modification of bortezomib dose and regimen
Grade 1 (paresthesia; weakness and/or loss of reflexes) without pain or loss of function	For patients receiving twice-weekly bortezomib, change to once-per-week schedule (D1, 8, 15, 22, q4wk), then Reduce current dose by one DL (from 1.3 to 1.0 mg/m^2) (from 1.0 to 0.7 mg/m^2), then Discontinue bortezomib
Grade 1 with pain or grade 2 (with no pain but limiting instrumental ADLs[b])	For patients receiving twice-weekly bortezomib, change to a once-per-week (D1, 8, 15, 22, q4wk) schedule, then Reduce current dose by one DL (from 1.3 to 1.0 mg/m^2) (from 1.0 to 0.7 mg/m^2) or consider temporary discontinuation; upon resolution to grade ≤ 1, re-start once-per-week dosing (D1, 8, 15, 22, q4wk) at the same DL, then Discontinue bortezomib
Grade 2 with pain or grade 3 (limiting self-care and ADL[c]) or grade 4	Discontinue bortezomib

[a]Based on posology modifications in phase II and III MM studies and post-marketing experience. Grading based on NCI-CTCAE v 4.0.
[b]Instrumental ADL: refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[c]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. Up to a maximum of two bortezomib consecutive dose reductions will be allowed (from 1.3 mg/m^2 to 1.0 mg/m^2 and from 1.0 mg/m^2 to 0.7 mg/m^2) upon Sponsor's agreement, if patient benefit is perceived. After two dose reductions, bortezomib will be discontinued and treatment with plitidepsin/dexamethasone will be continued. Once a dose reduction of any trial drug has been implemented, the dose will not be re-escalated thereafter.
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 International Myeloma Working Group (IMWG) criteria on D1 of each treatment cycle, if response is observed or if clinical symptoms suggest new plasmacytomas and/or new bone lytic lesions: • 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). • OS and OS rate at 6 and 12 months (OS6 and OS12, respectively).
SAFETY EVALUATIONS	Patients are 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 electrocardiogram (ECG) findings. AEs will be graded according to NCI-CTCAE v 4.0. Treatment delays, dose reduction requirements and reasons for discontinuation will be monitored throughout the trial.
PHARMACOKINETIC EVALUATIONS	Samples for PK analysis will be obtained during Cycle 1 and Cycle 2. PK parameters will be calculated using population methods, after pooling data from this study with data obtained from other studies.
PHARMACOGENOMIC EVALUATIONS (PGx SUB-STUDY)	The analysis of potential predictive factors of response to plitidepsin and bortezomib treatment will be done on prior available BM samples obtained at the time of the baseline visit and after a patient who has signed a written IC to participate in the PGx sub-study, has responded to treatment. The response to trial treatment will be correlated with the expression levels of selected genes that could be potentially

	predictive factors of response to plitidepsin and bortezomib. Factors related to the mechanism of action (MOA) of plitidepsin and/or bortezomib and/or related to the pathogenesis of the disease will be included in the analysis. Their expression will be analyzed at the mRNA or protein level by real-time reverse transcriptase quantitative polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC), respectively; polymorphisms and mutations in the selected genes could also be analyzed, if relevant.
STATISTICAL METHODS	**Sample size considerations:** Patients will be treated to test the null hypothesis (H_0) that 20% or fewer patients achieve a response according to IMWG criteria ($p \leq 0.20$) *versus* the alternative hypothesis (H_1) that 40% or more patients achieve a response according to IMWG criteria ($p \geq 0.40$). The variance of the standardized test is based on the empirical estimate. The type I error rate (alpha) associated with this one-sided test is 0.025 and the type II error rate (beta) is 0.1; hence, statistical power is 90%. In order to test these hypotheses, it is necessary to recruit 64 evaluable patients. A futility analysis based on the primary endpoint (ORR) is planned for the time when the first 20 evaluable patients have been recruited. The analysis will commence once the last of the 20 patients has completed two full treatment cycles. Patient recruitment will not be halted during the conduct of this futility analysis. A spending function defined by the Gamma family with parameter (-2) has been selected. If there are two or fewer responders according to boundaries and sample size assumptions, then the alternative hypothesis could be rejected and recruitment might be stopped at that time. Otherwise, patient accrual will continue to a total of 64 patients. Overall, if ≥ 21 (i.e., 33%) patients achieve a response, then the null hypothesis can be rejected.
REPLACEMENT OF PATIENTS	Patients must be replaced if they are not evaluable for efficacy, the primary objective of the trial. Patients must have received at least one complete treatment cycle (two plitidepsin infusions, four bortezomib injections and four doses of dexamethasone), or the equivalent doses over two cycles and must have had at least one tumor assessment.
PLANNED TRIAL PERIODS (individually per patient)	Patients will be evaluated at scheduled visits in three trial periods: • **Pre-treatment:** from signature of the IC to the first trial drug infusion. • **Treatment:** from first infusion of trial 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 PD will be followed every three months until PD, other antitumor therapy, death or until the date of trial termination (clinical cut-off), whichever occurs first. Patients will be considered to be on-trial 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 trial, in which case the EOT visit should be performed immediately before the start of the new therapy, whenever possible. Patients will receive the trial 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 trial. • Patient refusal and/or non-compliance with trial 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 TRIAL PERIODS (for the whole trial)	**Planned start date:** 1Q2016 **Planned enrolment period:** approximately 24 months. **Total duration of the trial:** approximately 30 months. **Planned end-of-trial date (clinical cut-off):** six months after the last patient's treatment discontinuation (last patient-last visit), or nine months after accrual of the last evaluable patient, whichever occurs first. If there are patients still being treated at the planned cut-off date, the actual cut-off date will be the date when those patients have completed the ongoing treatment cycle and the corresponding EOT visit.

SCHEDULE OF ASSESSMENTS

PROCEDURE	Pretreatment (days)	Treatment: C1		Treatment: further cycles		End of treatment visit	Follow-up
		D1	D15	D28=1	D15		
Written ICs (general and PGx sub-study)	Before any procedures	-	-	-	-	-	-
Plitidepsin administration	-	●	●	●	●	-	-
Bortezomib administration	-	D1, 4, 8 and 11		D1, 4, 8 and 11		-	-
Dexamethasone administration	-	D1, 8, 15 and 22		D1, 8, 15 and 22		-	-
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 trial					-
Hematology (3)	-7 to 0	●†	●	●	●	●	-
Coagulation panel	-14 to 0	-	-	●	-	●	-
Biochemistry-A (4)	-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) (5)	-7 to 0	-	-	●	-	●	-
ECG (6)	-14 to 0	●	-	●	-	●	-
LVEF	-14 to 0	Every 12 weeks				●	-
PK	NA	C1 and C2 only		-		-	-
AEs (NCI-CTCAE v4.0) (7)	SAEs only	Throughout the trial					●
Disease Assessments							
Serum protein (8)	-14 to 0	●	-	●	-	●	●
Urine protein	-14 to 0	●	-	●	-	●	●
Serum beta-2 microglobulin	-14 to 0	-					
C-reactive protein	-14 to 0	Every 8 weeks				●	●
BM for PGx analysis (only if written IC given)	Available stored BM samples -21 to 0	If response is observed				-	-
BM assessment (9)	-21 to 0	When all parameters indicate CR				If clinically indicated	If clinically

PROCEDURE	Pretreatment (days)	Treatment: C1		Treatment: further cycles		End of treatment visit	Follow-up
		D1	D15	D28=1	D15		
Clinical and radiological tumor assessment in the presence of soft tissue plasmacytoma (10)	-14 to 0	If response is observed (to confirm CR) or if clinical symptoms suggest new plasmacytomas				If clinically indicated	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	

Day 0 = C1D1

[†]Repeat prior to the first infusion only if more than seven days have elapsed from the last measurement.

A 2-day window is allowed for hematology, biochemistry A and B, coagulation panel, creatinine, CrCl, serum protein, urine protein tests, physical examination, assessment of patients' signs and symptoms and neurological assessments. A 2-week window is allowed for radiological tumor (to confirm response) and LVEF (by ECHO or MUGA) assessments.

Note: windows for laboratory assessments only apply prior to the scheduled infusion (e.g. 2-day window = within 48 hours before the following scheduled infusion, etc.). Windows for radiological tumor and LVEF assessments (by MUGA or ECHO) apply either before or after the corresponding scheduled infusion.

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 D1 (prior to first infusion) and D15 of every cycle. Repeat 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.
4. Repeat at least every other day in the presence of grade 3/4 vomiting or any other drug-related SAE.
5. For women of childbearing potential a serum or urine HCG analysis should be done. During the on-treatment period, testing should be repeated every cycle, or at least, every four weeks.
6. To be done just before the first infusion of each cycle.
   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.
7. SAEs will be collected from the time of signature of the ICF.
8. Protein electrophoresis, serum Ig determination, M-protein measurement and IF and SFLC determination.
9. BM evaluation is mandatory for all patients at screening (BM morphology, BM cytometry, if available, BM FISH and BM cytogenetics), while on treatment if clinically indicated and if there is a CR. BM evaluation must be repeated eight weeks later in patients with non-secretory MM, to confirm response or as clinically indicated.
10. 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 including weight, BSA and vital signs (HR, ABP and temperature).

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

Coagulation panel: PT, INR, APTT.

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

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, arterial 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 increased); CR, complete response; CrCl, creatinine clearance; CRP, C-reactive protein; CT-scan, computed tomography scan; D, day; ECG, electrocardiogram; ECOG PS, Eastern

Cooperative Oncology Group Performance Status; FISH, fluorescence in situ hybridization; GGT, ɣ-glutamyl transpeptidase; HBV, hepatitis B virus; HCG, human chorionic gonadotropin; HCV, hepatitis C virus; HR, heart rate; IC, informed consent; ICF, informed consent form; 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; PGx, pharmacogenomics; PK, pharmacokinetics; PT, pro-thrombin time; SAE, serious adverse event; SFLC, serum free light-chain; UFLC, urine free light chain; 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	ɣ-glutamyl Transpeptidase
GM-CSF	Granulocyte/Macrophage Colony Stimulating Factor
GMT	Greenwich Mean Time
h	Hour(s)
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
IC_{50}	Half Maximal Inhibitory Concentration
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
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
OS6	Overall Survival Rate at 6 Months
OS12	Overall Survival Rate at 12 Months
PD	Progressive Disease
PDy	Pharmacodynamics
PFS	Progression-free Survival
PGx	Pharmacogenomics
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	Quantitative Reverse Transcriptase 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
SmPC	Summary of Product Characteristics
SVT	Supraventricular Tachycardia
TBI	Total Body Irradiation
TTP	Time To Progression
$T_{1/2}$	Half-life
UFLC	Urine Free Light Chains
ULN	Upper Limit of Normal
US	United States
USP	US Product Information
VBAP	Vincristine, Carmustine, Doxorubicin, Prednisone
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
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)
WOCBP	Woman/Women of Childbearing Potential

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 United States (US), an estimated 26,850 new cases of MM are expected to be diagnosed in 2015, and 11,240 people are expected to 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 1) 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 1). 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% [5]. 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 [6] 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) [7]. 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 [8]. 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 [9-11] 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 [12]. 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 [13] and a rapid and sustained activation of JNK, p38 MAPK and ERK in human breast cancer cells (MDA-MB-231) [14]. 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 [11]. 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 [14]. The effects of plitidepsin on a pleiotropic regulatory protein Rac1, would contribute to the

sustained activation of JNK [9, 15]. 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 [16]. 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 [14, 15], 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* [9].

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* [12], 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 [17]. 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 [18].

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 a half maximal inhibitory concentration [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) [19].

In addition, an animal model in MM has been explored [20]. 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 intravenous (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 [21]. *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 [22].

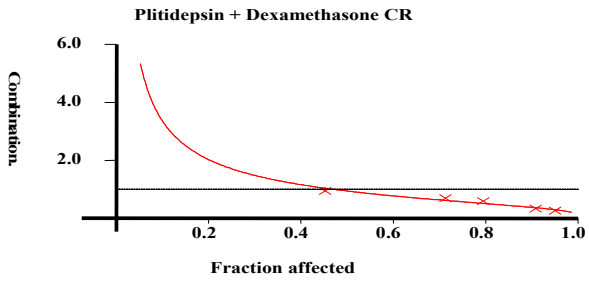
1.3.2.3 Plitidepsin in Combination: In vitro and In vivo Data

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

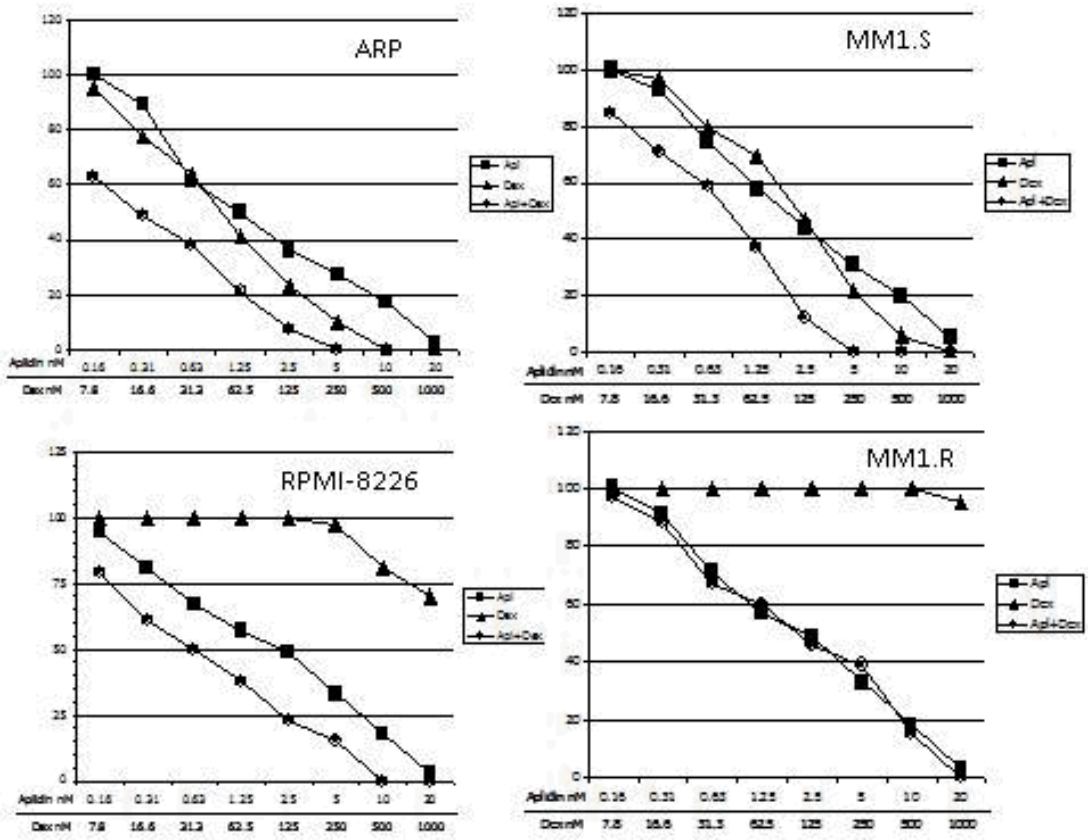
The plitidepsin/dexamethasone combination was explored with viability analyses in MM1.S and U266-LR7 cells. The experimental design included both the non-constant ratio (for suboptimal drug doses) and the constant ratio. The effects of single and combined treatments after 72 hours (h) were evaluated 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 [20] (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 [23-25]. 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 [25-28]. 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 [29-32].

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) [26, 33].

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.

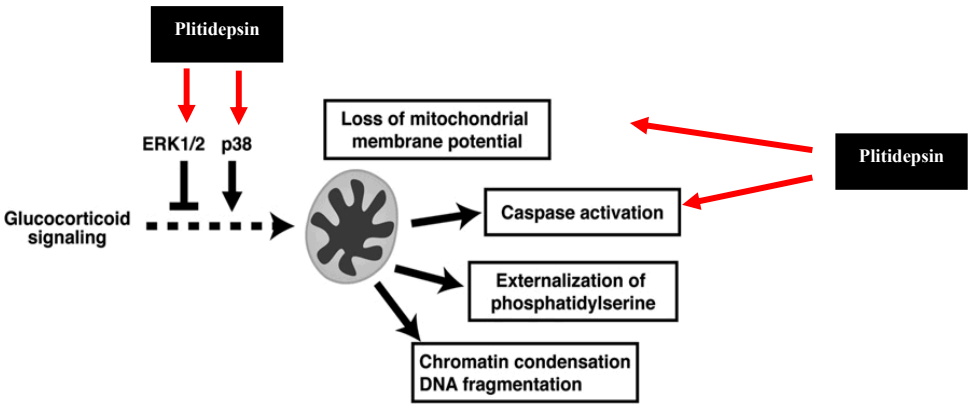
Moreover, plitidepsin-induced suppression of caspase inhibitors (FLIP, survivin) may contribute to the increased sensitivity of plitidepsin-treated MM cells to caspase-dependent apoptosis by dexamethasone. Both agents deregulate Hsp90 complexes such as receptor FKBP5, HSPs and DNAJs.

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 [34]. Likewise, both had a synergistic effect on NF-kappaB (survival transcription factor) and its inhibitor [35, 36].

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 [25].

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.



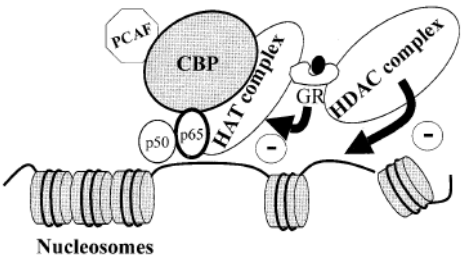
Combined treatment with plitidepsin and dexamethasone suppresses genes involved in cytokine-induced proliferative/antiapoptotic signaling pathways and oncogenic transformation triggering MM proliferation: IGF-1R, IL-6R, gp130, CXCR-4 etc.

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 [37, 38]. Both complexes are closely related to chromatin remodeling and consequently, to the modulation of gene expression induced by dexamethasone (Figure 4).

Figure 4. Glucocorticoids 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 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) [39]. These and other data [40] 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 terminated early following a decision by the Sponsor (for reasons other than safety). Recommended doses (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 2015, 215 adult patients and 38 pediatric patients have been treated with single-agent plitidepsin and 122 adult patients with plitidepsin in combination with other drugs in these phase I studies, for a total of 375 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; CPK, creatine phosphokinase; 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 (for reasons other than safety). 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 creatine phosphokinase (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 alanine aminotransferase - ALT) were dose-limiting in some single-agent phase I studies and in most phase I combination studies. Alkaline phosphatase (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 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 (Serious Adverse Reaction).

Safety Overview in Adult Combination Phase I Trials

At the time of writing, only one combination study (plitidepsin/bortezomib/dexamethasone) was still ongoing. To this date, dose escalation had proceeded as follows: the first dose level (4.0 mg/m^2 plitidepsin/40 mg dexamethasone/1.0 mg/m^2 bortezomib) included eight patients, three were evaluable for DLTs and none had DLTs; the next dose level (4.0 mg/m^2 plitidepsin/40 mg dexamethasone/1.3 mg/m^2 bortezomib) included four patients, three were evaluable for DLTs and none had DLTs; the following dose level (5.0 mg/m^2 plitidepsin/40 mg dexamethasone/1.3 mg/m^2 bortezomib) included six patients, five were confirmed to be evaluable for DLTs (eligibility was pending in the remaining patient) and none had DLTs.

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: grade 4 thrombocytopenia (on its own or concomitant with neutropenia for > 5 days) when plitidepsin was combined with carboplatin or gemcitabine) and grade 4 febrile neutropenia and pancytopenia, when plitidepsin was combined with 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 partial response (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 stable disease (SD) lasting more than three months.

Antitumor activity observed with the plitidepsin combinations includes:

- Patients treated with plitidepsin and gemcitabine: one complete response in a patient with NHL, one confirmed PR and three SD > 3 months with tumor reduction in patients with Hodgkin's lymphoma, two SD > 3 months in patients with head and neck cancer, one SD > 3 months in a patient with colorectal cancer, two SD > 3 months in patients with soft tissue sarcoma and one SD in a patient with duodenal cancer of the small intestine.
- Patients treated with plitidepsin and DTIC one confirmed PR, two unconfirmed PRs and four SD > 3 months in patients with melanoma.
- Patients treated with plitidepsin and carboplatin: six SD ≥ 3 months, two in colorectal carcinoma, one in a hepatocarcinoma, one in an adenocarcinoma of the gastroesophageal junction, one in a neuroendocrine gallbladder carcinoma and one in a malignant melanoma.
- Patients treated with plitidepsin and bortezomib: one confirmed PR in one patient with MM.
- Patients treated with plitidepsin and docetaxel: one SD > 3 months in a patient with head and neck cancer.
- Patients treated with plitidepsin and bevacizumab: four SD > 3 months, two in renal cancer, one in colorectal cancer and one in cancer of the cervix.
- Patients treated with plitidepsin and sorafenib: four SD > 3 months, two in renal cancer, one with pleural mesothelioma and one with colorectal cancer.

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 published [41]. This was an open-label study of plitidepsin given at a dose of 5 mg/m^2, as a 3-h infusion every two weeks; the addition of dexamethasone was allowed in patients with suboptimal response to plitidepsin alone (defined as disease progression [PD] after three cycles or SD after four cycles of plitidepsin). The primary endpoint was the objective response rate (ORR = complete response [CR]+ partial response [PR] + minimal response [MR]) according to strict Bladé criteria [42] 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 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 *versus* (*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.

As of the latest IB cut-off date (31 March 2015), a total of 248 patients have been included into this trial and 243 patients have been treated with plitidepsin/dexamethasone or dexamethasone alone. A total of 303 SAEs have been reported in 115 patients, regardless of treatment arm. Of these, 129 SAEs reported in 63 patients have been considered to be related to or with an unknown relationship with the study treatment. The most common

related SAEs were pneumonia (n=8), ALT increases (n=7), aspartate aminotransferase (AST) increases and CPK increase (n=6 each), hyperglycemia and sepsis (n=5 each). 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 Pharmacokinetic [PK]/Pharmacodynamic [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 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 [43, 44].

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 [45] 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 antitumor activity afforded by conventional CT [43].

1.4.2 Mechanism of Action

The mechanisms of antitumor activity that have been established for bortezomib involve many pathways thought to be integral to cancer treatment strategies [46-49]. 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 (half-life [$t_{1/2\alpha}$] <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) [50]. 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 [47, 48, 51, 52].

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 [53, 54]. 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 [55].
- 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 [56].
- 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 [57].

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, antitumor 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) [58]. 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 [59].

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 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) [60]. 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 (40 mg) 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 [61]. 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 ($p = 0.0098$) [62].

1.4.7 Safety Issues

The data described below reflect exposure to bortezomib in 256 patients with MM [58] [59]. 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 Trial Rationale and Drug Selection

MM is still an incurable disease. As front-line treatment to reduce tumor burden, HSCT 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.

Rationale for the double refractory patient population to bortezomib and lenalidomide:

Most MM patients will receive PIs and/or IMIDs, either as frontline therapy prior to consolidation with HSCT or as initial therapy in patients who are not eligible for HSCT. Furthermore, many patients nowadays receive maintenance treatment with one or both types of agents. As a result, resistance to these drugs, two of the most commonly used being bortezomib and lenalidomide, is an increasingly observed problem that must be

addressed through the introduction of novel agents and/or combinations within these two drug families.

Second generation PIs and IMIDs, such as carfilzomib [63] and pomalidomide [64], are now becoming available, which are more potent, less toxic and have more convenient administration schedules. However, despite observed responses of 25%, most of them PRs, when used as single agents or in combination with dexamethasone, efficacy in the double refractory population to bortezomib and lenalidomide is still low, with a median PFS of 3.5 to 4 months [64].

More recently, promising efficacy results have been observed in a phase II study (PANORAMA II) carried out in a patient population double refractory to bortezomib and lenalidomide when bortezomib and dexamethasone were combined with a histone deacetylase inhibitor, panobinostat [65]. This study, together with a randomized phase III study of the combination led to the approval of panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and refractory MM. These results would suggest that double-targeting the proteasome and the aggresome may overcome the resistance to PIs and IMIDs [66, 67]. Despite the promising efficacy data from these trials, generally low efficacy and substantial hematological (over 60% grade 3-4 thrombocytopenia) and gastrointestinal toxicity were observed with this combination in a population with yet a dismal prognosis.

Promising efficacy results are also being observed with other agents under development with differentiated MOAs, such as kinesin spindle protein inhibitors [68, 69] or monoclonal antibodies (both for use as single agents or in combination) that target specific antigens on the membrane of MM cells (e.g., SLAM7 or CD38) [70]. Specifically, promising activity is being reported with daratumomab, a monoclonal antibody that targets CD38.

Despite the developments mentioned above, novel agents with differentiated MOAs and showing synergism with the majorly established drugs, such as bortezomib, are urgently needed. Moreover, these agents should be able to overcome resistance to widely used drugs, such as bortezomib and lenalidomide, when given as part of a combination regimen, both during induction and later treatment phases. Given that in the double refractory population setting patients will have developed substantial toxicity (e.g., poor BM reserve, neuropathy…), the searching for agents and/or drug combinations with activity in this setting and with a safe toxicity profile is a must.

Rationale for the combination of plitidepsin with bortezomib and dexamethasone:

In 1999, plitidepsin was found to have substantial *in vitro* antitumor 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 [41].

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 a pivotal trial, the ORR, including complete responses, was 35%, the median response duration 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 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.

A phase I clinical study of plitidepsin in combination with bortezomib and dexamethasone is currently ongoing and has indicated a favorable safety profile (out of 11 evaluable patients, no DLTs have been reported to date).

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) [45]. A separate phase III trial 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 [71].

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 MM double refractory to bortezomib and lenalidomide deserves to be addressed in clinical trials.

Rationale for the Pharmacogenomic (PGx) Sub-study:

Several studies have shown that plitidepsin induces apoptosis in a cell type- and dose-dependent manner, and these effects are related to the induction of early oxidative stress, the activation of Rac1 GTPase and the inhibition of protein phosphatases, which in conjunction cause the sustained activation of JNK and p38 MAPK. The final consequence is the triggering of the mitochondrial apoptotic pathway with caspase-9 and caspase-3 activation [72]. Additional effects may be mediated by indirect effects on the cell microenvironment, mainly mediated by antiangiogenic properties [12, 17, 18] and indirect effects on monocyte-derived cells, including follicular dendritic cells [73].

Recently plitidepsin has been shown to interact with GTP-bound eEF1A2. eEF1A2 is commonly depleted in plitidepsin-resistant cells and its restitution to normal levels

resensitize them to the compound. These results show that eEF1A2 is the primary target of plitidepsin mediating its antitumor activity [74]. Interestingly eEF1A2 is overexpressed in tumors, including multiple myeloma [75].

Therefore, in the PGx study a panel of markers potentially related to the mechanism of action of plitidepsin (e.g., angiogenesis, endoplasmic reticulum stress, oxidative stress apoptosis…) will be analyzed and correlated with the clinical outcome.

2. OBJECTIVES

Primary Objective:

To evaluate the efficacy of plitidepsin in combination with bortezomib and dexamethasone in patients with MM double refractory to bortezomib and lenalidomide in terms of overall response rate (ORR), including stringent complete response (sCR), complete response (CR), very good partial response (VGPR) and partial response (PR).

Secondary Objectives:

- To evaluate time-to-event efficacy endpoints of plitidepsin in combination with bortezomib and dexamethasone, i.e., duration of response (DOR), time to progression (TTP), progression-free survival (PFS) and event-free survival (EFS).
- To evaluate overall survival (OS) and OS rate at 6 and 12 months (OS6 and OS12, respectively).
- To evaluate the safety and tolerability of plitidepsin in combination with bortezomib and dexamethasone.
- To study the pharmacokinetics (PK) and pharmacodynamics (PDy) of plitidepsin in combination with bortezomib and dexamethasone.
- To obtain pharmacogenomic information (Pharmacogenomics [PGx]) on markers of response to plitidepsin and bortezomib treatment.

3. OVERALL TRIAL DESIGN

This is a multi-center, open-label, single arm, non-comparative phase II trial, designed to evaluate the efficacy of plitidepsin in combination with bortezomib and dexamethasone in patients with MM double refractory to bortezomib and lenalidomide. Plitidepsin will be administered as a 3-hour (h) i.v. infusion at a dose of 5 mg/m^2, on Day (D) 1 and 15, q4wk, bortezomib will be administered as a s.c. injection at a dose of 1.3 mg/m^2 on D1, 4, 8 and 11, q4wk and dexamethasone will be taken orally at a dose of 40 mg/day on D1, 8, 15 and 22, q4wk.

Patients will be evaluated at scheduled visits in three trial periods: pre-treatment, treatment and follow-up.

The **pre-treatment period** includes screening and baseline visits. After providing written informed consent (IC) to participate in the trial, patients will be screened for trial 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 2), 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 childbearing potential (WOCBP) (see Table: Schedule of Assessments).

Disease-specific markers will be analyzed: beta-2-microglobulin, C-reactive protein (CRP), IgG, IgA, IgM, 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 magnetic resonance imaging (MRI). In case of extramedullary soft tissue plasmacytoma, computed tomography (CT)-scan or MRI of any site involved will be performed.

During the **treatment period**, all patients are to attend trial 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 (EOT) visit 30 (±5) days after the last dose of trial therapy.

Prior to each administration of trial 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 counts and biochemistry tests 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 4.7.

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 trial until PD, excessive side effects or withdrawal of consent (see Section 4.4). If patients respond to treatment or achieve SD and bortezomib toxicity precludes any further treatment, they may continue to receive plitidepsin and dexamethasone at the same dose upon Investigator's decision and agreement with the Sponsor. Once plitidepsin treatment is stopped, patients must be discontinued from the trial. Patients who achieve a sCR, a CR, VGPR, PR, MR or SD as defined by response criteria may be taken off the trial 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 trial 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 trial drug and until their resolution. In addition, patients will be followed every three months to assess disease status. Patients taken off the trial due to reasons other than PD, will be followed for disease status every three months until PD, initiation of another anticancer therapy, trial termination or death, whichever comes first.

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

3.1 Trial Endpoints

Primary Endpoint

ORR (including sCR, CR, VGPR and PR).

Secondary Endpoints

- Time-to-event efficacy endpoints of plitidepsin in combination with bortezomib and dexamethasone, i.e., DOR, TTP, PFS and EFS.
- OS and OS rate at 6 and 12 months (OS6 and OS12).
- Safety and tolerability of plitidepsin in combination with bortezomib and dexamethasone.
- PK and PDy of plitidepsin in combination with bortezomib and dexamethasone.
- PGx information on markers of response to plitidepsin and bortezomib treatment.

3.2 Number of Patients

Approximately 64 evaluable patients will be needed for the evaluation of the primary endpoint, ORR.

An early futility analysis will be performed with the efficacy data collected from the first 20 evaluable patients. The futility analysis will commence once patient number 20 has completed two full treatment cycles. Patient recruitment will not be halted during the conduct of this futility analysis.

3.3 Selection of Patients

Patients with MM double refractory to bortezomib and lenalidomide 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, 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.

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 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 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.

c) When applicable, measurable soft tissue plasmacytoma ≥ 2 cm, by either physical examination and/or applicable radiological evaluation (i.e. MRI, CT-scan).

5) 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 90 days before the onset of treatment with the trial drug(s).
6) Patients must have received previous treatment with bortezomib and lenalidomide and be refractory to both.
7) Patients must have an ECOG PS ≤ 2.
8) Recovery to grade ≤ 1 from any non-hematological AE derived from previous treatment (if present, alopecia and peripheral neuropathy must be grade <1).
9) 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 (G-CSF 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) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and ≤ 3.0 x institutional ULN and alkaline phosphatase (AP) ≤ 2.5 x institutional ULN.
   g) Creatinine clearance > 30 mL/min, measured or calculated according to Cockcroft and Gault's formula (Appendix 4).
   h) Albumin ≥ 2.5 g/dL.
10) Evidence of non-childbearing status for WOCBP: WOCBP must have a negative serum or urine pregnancy test within seven days prior to enrolment and must agree to use a highly effective contraceptive measure throughout the trial and during six months after treatment discontinuation. Valid methods to determine the childbearing potential, adequate contraception and requirements for WOCBP partners are described in Appendix 5. Male patients enrolled in the study should also use contraceptive methods during and after treatment discontinuation.

11) Left ventricular ejection fraction (LVEF) ≥ 45%.
12) 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 trial 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 trial, including the following specific conditions:
    a) Uncontrolled psychiatric illness or medical illness that the Investigator feels will compromise the patient's tolerance of the trial 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.
    e) Acute infiltrative pulmonary and pericardial disease.
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 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, or equivalent.
5) History of hypersensitivity reaction and/or intolerance to bortezomib, polyoxyl 35 castor oil, mannitol, boron or dexamethasone.
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) Grade ≥ 1 neuropathy (either bortezomib-related or not) according to NCI-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 trial.
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 trial.
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 D1.
    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 trial 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 to use steroids.

3.3.3 Inclusion Criteria for the Pharmacogenomic (PGx) Sub-study

Only patients with available BM samples and who voluntarily sign the Informed Consent Form (ICF) for the PGx sub-study will be able to participate. Refusal to participate in the PGx sub-study will not affect patient participation in the trial APL-B-022-15.

4. PLAN OF THE TRIAL

4.1 Duration of the Trial Individually per Patient

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

- **Pre-treatment:** from signature of the IC to the first trial drug infusion.
- **Treatment:** from first infusion of trial 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 trial termination (clinical cut-off), whichever occurs first.

Patients will be considered to be on-trial 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 trial, in which case the EOT visit should be performed immediately before the start of the new therapy, whenever possible.

4.2 Duration of the Trial (for the Whole Trial)

- **Planned start date:** 1Q2016
- **Planned enrolment period:** approximately 24 months.
- **Total duration of the trial:** approximately 30 months.
- **Planned end-of-trial date (clinical cut-off):** six months after the last patient's treatment discontinuation (last patient-last visit), or nine months after accrual of the last evaluable patient, whichever occurs first. If there are patients still being treated at the planned cut-off date, the actual cut-off date will be the date when those patients have have completed the ongoing treatment cycle and the corresponding EOT visit.

4.3 Subject Participation

Only after giving IC, may patients be enrolled in the trial by completing the electronic screening form. The electronic screening form will be checked and approved by the Medical Responsible at PharmaMar. No patient can be treated until PharmaMar has given treatment authorization. A patient enrollment number will be provided and registration will be confirmed and checked by PharmaMar.

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 trial periods: pre-treatment, treatment and follow-up. Patients will receive trial medication while it is considered to be in their best interest (see Section 4.4). In responding patients, treatment may continue upon Investigators' discretion.

Patients will be considered to be on-trial 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 trial drug administration.

4.4 Discontinuations

A discontinuation occurs when an enrolled patient ceases to participate in the trial, regardless of the circumstances, prior to completion of the protocol. The Investigator must determine the primary reason for discontinuation, which will be recorded on the electronic Case Report Form (e-CRF). The final evaluation required by the protocol will be performed 30 ± 5 days after the last dose of trial therapy. A trial discontinuation must be reported immediately to the clinical monitor or his/her designated representative if it is due to a SAE.

If the patient is discontinued from the trial 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. Trial 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 trial.
- Patient refusal and/or non-compliance with trial 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.

Trial discontinuation could also occur due to a Sponsor's decision or unforeseen reasons. Patients withdrawn from the trial must not re-enter this trial at any time. Trial withdrawal due to an AE (see Section 6.2.1) should be distinguished from withdrawal due to insufficient response.

4.5 Replacement of Patients

Patients must be replaced if they are not evaluable for efficacy, the primary objective of the trial. Patients must have received at least one complete treatment cycle (two plitidepsin infusions, four bortezomib injections and four doses of dexamethasone), or the equivalent doses over two cycles and must have had at least one tumor assessment.

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 ICs (general and PGx sub-study) signed by the patient.	Prior to any trial-specific procedure.
	• 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. • Transfusion requirements.	Within 14 days prior to inclusion.
	• Clinical assessment of signs and symptoms (tumor-related or not). • Complete physical examination, including weight, height and vital signs assessment (HR, ABP and temperature). • Clinical neurological assessment.	Within 14 days prior to inclusion. Repeat prior to the first infusion[§†].
	• Baseline ECOG PS (Appendix 2).	Within 14 days prior to inclusion. Repeat prior to the first infusion.
2. Hematology	• Differential WBC count. • Hematocrit, hemoglobin. • Platelet count.	Within 7 days prior to inclusion. Repeat prior to the first infusion[†].
3. Coagulation panel	• PT, INR, APTT.	Within 14 days prior to inclusion.
4. Biochemistry	• Biochemistry A: AP, AST, ALT, LDH, GGT, bilirubin, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}), glucose, CPK, CPK-MB fraction (if applicable).	Within 7 days prior to inclusion. Repeat prior to the first infusion[§†].
	• Biochemistry B: Total proteins and albumin.	Within 7 days prior to inclusion[§].
5. Creatinine and Cr Cl	• Calculated or measured (Appendix 4).	Within 7 days prior to inclusion.
6. Urinalysis	• Dipstick, sediment.	Within 14 days prior to inclusion.
7. Viral serology	• HBV and HCV. • CMV in patients who have undergone allogeneic BM transplantation.	Within 14 days prior to inclusion.
8. Pregnancy test	• Serum or urine in women of childbearing potential.	Within 7 days prior to enrolment.

Screening and baseline	Investigations	Time
9. 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. Repeat prior to the first infusion[§].
10. AEs	• Evaluation of AEs (NCI-CTCAE v4.0).	SAEs will be collected from the time of signature of the ICF.
Disease assessment[¥]		
11. Serum protein	• Protein electrophoresis. • Serum Ig determination and M-protein measurement and IF • SFLC.	Within 14 days prior to inclusion. Repeat prior to the first infusion[§].
12. Urine protein	• 24-h urine protein electrophoresis • UFLC. • Urine M-protein measurement and IF.	
13. Serum beta-2-microglobulin and C-reactive protein		Within 14 days prior to inclusion.
14. Bone marrow	• BM morphology. • BM cytometry (if available).	Within 21 days prior to treatment.
	• BM FISH (Appendix 6) • BM cytogenetics.	At diagnosis and prior to entering the trial.
	• PGx sub-study (available stored BM samples) (only if written informed consent given).	Within 21 days prior to treatment.
15. Clinical and radiological tumor assessment in the presence of soft tissue plasmacytoma	• CT-scan or MRI of all measurable/evaluable involved sites.	Within 14 days prior to inclusion.
16. Skeletal evaluation	• X-ray of skull, vertebral column pelvis and proximal long bones or MRI.	Within 4 weeks prior to inclusion.
17. Other relevant tests	• Where indicated, according to the clinical context.	Within 14 days prior to inclusion.

D0=C1D1

[¥]The day of inclusion is the day the patient is formally registered in the study by the Sponsor(s).

[§]A 2-day window is allowed for hematology, biochemistry A and B, coagulation panel, creatinine, CrCl, serum protein, urine protein tests, physical examination, assessment of patients' signs and symptoms and neurological assessments. A 2-week window is allowed for radiological tumor (to confirm response) and LVEF (by ECHO or MUGA) assessments.

[†]To be repeated prior to the first infusion if more than seven days have elapsed from the last measurement.

ABP, arterial blood pressure; AE, adverse event; ALT, alanine aminotransferase; AP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BM, bone marrow; C, cycle; CMV, cytomegalovirus; CPK, creatine

Screening and baseline	Investigations	Time

phosphokinase, CPK-MB fraction, CPK isoenzymes found in cardiac muscle (it will be performed only if CPK is elevated); 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; FISH, fluorescence in situ hybridization; FLC, free light chains; GGT, ɣ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HR, heart rate; ICF, informed consent form; 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; PGx, pharmacogenomics; PT, prothrombin time; SAE, serious adverse event; SFLC, Serum FLC; TTP, time to progression; UFLC, urine FLC;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 (Appendix 2). • Physical examination, including weight, BSA and vital signs (HR, ABP, temperature). • Assessment of patient's signs and symptoms (disease related or not). • Clinical neurological assessment.	D1 of each cycle.
	• Concomitant treatments (especially transfusion requirements)[a]. • Intercurrent adverse events.	Throughout the trial.
2. Hematology	• Differential WBC count. • Hematocrit, hemoglobin. • Platelet count.	D1, D15 of each cycle[b§].
3. Coagulation panel	• PT, INR, APTT.	D1 of each cycle from C2[§].
4. Biochemistry	• Biochemistry A: AP, AST, ALT, LDH, GGT, bilirubin, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}), glucose, CPK, CPK-MB fraction (if applicable).	D1, D15 of each cycle[c§].
	• Biochemistry B: Total proteins and albumin.	D1 of each cycle from C2[§].
5. Creatinine and CrCl	• Calculated or measured (Appendix 4).	D1 of each cycle from C2[§].
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.	D1 of each cycle from C2 (an early 2-day window is allowed)[¥].
8. 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.	D1 of each cycle.
	• LVEF by ECHO or MUGA scan.	Every 12 weeks[§].
9. AEs	• Evaluation of AEs (NCI-CTCAE v4.0).	Throughout the trial.

Treatment period	Investigations	Time
10. PK	• As in Section 6.3.	C1 and C2.
Disease assessment[d]		
11. Serum protein	• Protein electrophoresis • Serum Ig determination and M-protein quantitation and IF. • SFLC.	D1 of each cycle[§].
12. Urine protein	• 24-h urine protein electrophoresis. • Urine M-protein quantitation and IF. • UFLC.	D1 of each cycle[§].
13. C-reactive protein		Every eight weeks.
14. Bone marrow[e]	• BM morphology. • BM cytometry (if available).	When all parameters indicate CR. When clinically indicated.
	• PGx sub-study.	If response is observed.
15. 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.
16. 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.
17. Other relevant tests	• Where indicated, according to the clinical context.	When clinically indicated.

D0=C1D1

[§]A 2-day window is allowed for hematology, biochemistry A and B, coagulation panel, creatinine, CrCl, serum protein, urine protein tests, physical examination, assessment of patients' signs and symptoms and neurological assessments. A 2-week window is allowed for radiological tumor (to confirm response) and LVEF (by ECHO or MUGA) assessments.

Note: windows for laboratory assessments only apply prior to the scheduled infusion (e.g. 2-day window = within 48 hours before the following scheduled infusion, etc.). Windows for radiological tumor and LVEF assessments (by MUGA or ECHO) apply either before or after the corresponding scheduled infusion.

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.

[¥]For women of childbearing potential a serum or urine β-HCG analysis should be done. During the on-treatment period, testing should be repeated every cycle, or at least, every four weeks.

ABP, arterial blood pressure; AE, adverse event; ALT, alanine aminotransferase; AP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HCG, human chorionic gonadotropin; 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 elevated); 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; GGT, ɣ-glutamyl transpeptidase; 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; PGx, pharmacogenomics; PT, prothrombin time; SFLC, Serum FLC; UFLC, urine FLC;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 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 evaluable patients 30 ± 5 days after the last dose of trial 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 (Appendix 2). • Complete physical examination, including weight and vital signs (HR, ABP, temperature) • Concomitant treatments (including transfusion requirements). • Clinical neurological assessment.	Thirty (±5) days after the last dose of trial therapy.
2. Hematology	• Differential WBC count. • Hematocrit, hemoglobin. • Platelet count.	Thirty (±5) days after the last dose of trial therapy.
3. Coagulation panel	• PT, INR, APTT.	Thirty (±5) days after the last dose of trial therapy.
4. Biochemistry	• Biochemistry A: AP, AST, ALT, LDH, GGT, bilirubin, electrolytes (Na^{+}, K^{+}, Mg^{++}, Ca^{++}), glucose, CPK, CPK-MB fraction (if applicable). • Biochemistry B: total proteins, albumin.	Thirty (±5) days after the last dose of trial therapy.
5. Creatinine and CrCl	• Calculated or measured.	Thirty (±5) days after the last dose of trial therapy.
6. Urinalysis	• Dipstick, sediment.	Thirty (±5) days after the last dose of trial therapy.
7. Pregnancy test	• Serum or urine.	For women of childbearing potential, 30 (±5) days after the last dose of trial therapy.
8. AEs	• Evaluation of AEs (NCI-CTCAE v4).	Thirty (+5) days after the last dose of trial 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 trial 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 trial therapy.
11. Urine protein	• 24-h urine protein electrophoresis. • Urine M-protein quantitation and IF. • UFLC	Thirty (±5) days after the last dose of trial therapy.
12. C-reactive protein		Thirty (±5) days after the last dose of trial therapy.

End of treatment visit	Investigations	Time
13. BM	• BM morphology. • BM cytometry (if available). • BM cytogenetic (if available).	If clinically indicated, thirty (±5) days after the last dose of trial therapy.
14. Clinical and radiological tumor assessment in the presence of soft tissue plasmacytoma	• CT-scan or MRI of all measurable/evaluable sites.	If indicated, thirty (±5) days after the last dose of trial 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 trial therapy.
16. Other relevant tests	• Where indicated, according to the clinical and laboratory context.	Thirty (±5) days after the last dose of trial therapy.

ABP, arterial 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 elevated); 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; GGT, ɣ-glutamyl transpeptidase; 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; UFLC, urine FLC;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, trial 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. TRIAL 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 and contains plitidepsin as the active ingredient ad 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.

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 reconstituted solution should be determined based on the dose established for each individual patient:

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

For detailed instructions on reconstitution and dilution refer 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	Administration time
Ondansetron or equivalent	8.0 mg	intravenous	30-60 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 is available for use as i.v. infusion and s.c. injection. For further information on bortezomib, please refer to the EU SmPC (Summary of Product Characteristics) and/or USP (US Product Information).

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. For further information on the drug product, please refer to the EU SmPC or the USP.

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 be treated until PD. If the patient responds to treatment or achieves SD and bortezomib toxicity precludes any further treatment, treatment may continue with plitidepsin and dexamethasone at the same dose upon Investigator's decision and agreement with the Sponsor. Once plitidepsin treatment is stopped, patients must be discontinued from the trial.

Table 6. Trial drug administration schedule.

Agent	Dose	Route	Day
Dexamethasone	40 mg	Oral	D1, 8, 15 and 22*
Plitidepsin	5 mg/m^2	Intravenous	D1 and 15 q4wk*
Bortezomib	1.3 mg/m^2	Subcutaneous	D1, 4, 8 and 11 q4wk*

* Patients will be treated until PD. If the patient responds to treatment or achieves SD and bortezomib toxicity precludes any further treatment, treatment may continue with plitidepsin and dexamethasone at the same dose upon Investigator's decision and agreement with the Sponsor. Once plitidepsin treatment is stopped, patients must be discontinued from the trial.
D, day; PD, disease progression; q4wk, every four weeks; SD, stable disease.

5.5 Criteria for Treatment Continuation

Re-treatment criteria for plitidepsin and dexamethasone will be monitored on D1 and D15 and re-treatment criteria for bortezomib will be monitored on D1, as defined in Table 7:

Table 7. Criteria for plitidepsin and bortezomib treatment continuation.

	Plitidepsin/Dexamethasone	Bortezomib
	1[a] and 15[b]	**Day 1[a]**
ANC	1.0 x 10^9/L (≥ 0.5 x10^9/L if due to extensive BM involvement)	0.75 x 10^9/L (≥ 0.5 x10^9/L if due to extensive BM involvement)
Platelet count	≥ 50.0 x 10^9/L (≥ 25.0 x 10^9/L if ≥ 50% of BM nucleated cells are plasma cells)	≥ 25.0 x 10^9/L
Hemoglobin	≥ 8.0 g/dL	≥ 8.0 g/dL
Serum total bilirubin	≤ 1.5 x ULN[e]	≤ 3.0 x ULN[e]
AST/ALT	≤ 3.0 x ULN	≤ 5.0 x ULN
AP	≤ 2.5 x ULN	≤ 5.0 x ULN
Muscular toxicity (myalgia, muscular weakness, CPK increase)	< Grade 2	< Grade 3
Other non-hematological drug-related AEs (except for increased GGT, non-optimally treated nausea and vomiting, hypertension, alopecia)[c]	< Grade 2	< Grade 3[f]
ECG	Baseline values	Baseline values
ECHO/MUGA[d]	Baseline values	Baseline values

	Plitidepsin/Dexamethasone	Bortezomib

[a]If a patient does not meet the requirements for plitidepsin/dexamethasone treatment continuation while meeting the requirements for bortezomib treatment continuation on D1 of the following cycle, the plitidepsin/dexamethasone dose will be omitted and bortezomib will be administered.
If a patient does not meet the requirements for plitidepsin/dexamethasone and bortezomib treatment continuation on D1 of the following cycle, plitidepsin/dexamethasone and bortezomib will be delayed until recovery or for a maximum of 14 days. After this period, if the delay is due to toxicity assessed as related to a trial drug, a dose reduction (according to the "Criteria for Dose Reduction" section) is mandatory.
[b]If a patient does not meet the requirements for plitidepsin/dexamethasone treatment continuation on D15 of the following cycle, the plitidepsin/dexamethasone dose will be omitted.
[c]Any grade accepted for increased GGT.
[d]To be performed every three months unless more frequent assessments are clinically indicated.
[e]Except if Gilbert syndrome is clearly documented and other liver function tests are normal.
[f]If peripheral neuropathy (PN) occurs, follow the guidelines for bortezomib dose reduction available in the "Criteria for Dose Reduction" section.
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-glutamyl transpeptidase; L, liter; ULN, upper limit of normality; PN, peripheral neuropathy.

5.6 Criteria for Dose Reduction

5.6.1 Plitidepsin

Under the following circumstances, patients may continue plitidepsin treatment after a 20-25% dose reduction, upon Investigator's decision and agreement with the Sponsor, if patient benefit is perceived:

- Less than 50% compliance with the treatment schedule, and/or
- Grade ≥ 3 febrile neutropenia, or
- Grade 4 neutropenia and infection, or grade 4 neutropenia lasting > 7 days (except for patients with extensive BM involvement), and/or
- Grade 4 thrombocytopenia (except for patients with extensive BM involvement), and/or
- Grade 4 thrombocytopenia with grade ≥ 3 bleeding (except for patients with extensive BM involvement), and/or
- Grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations). In the presence of muscular toxicity, plitidepsin will be reduced first; if toxicity persists, dexamethasone will then be reduced.
- Any grade ≥ 3 clinically relevant non-hematological toxicity other than non-optimally treated nausea and vomiting, diarrhea lasting < 48 h and/or grade ≥ 3 asthenia/fatigue lasting < 5 days.

Up to a maximum of two plitidepsin dose reductions will be allowed (from 5.0 mg/m^2 to 4 mg/m^2 and from 4 mg/m^2 to 3.0 mg/m^2) upon Sponsor's agreement, if patient benefit is perceived. After two dose reductions, trial treatment will be discontinued.

5.6.2 Dexamethasone

Under the following circumstances, and after recovery from toxicity, patients may continue dexamethasone treatment after a 50% dose reduction:

- Grade ≥ 3 muscular toxicity (weakness, myalgia and/or CPK elevations) (in the presence of muscular toxicity, plitidepsin will be reduced first; if toxicity persists, dexamethasone will then be reduced), or
- Drug-related grade ≥ 3 fatigue, or
- Grade ≥ 2 mood disturbances or agitation, or
- Grade ≥ 3 fluid retention, or
- Grade 4 clinically documented infection.
- Grade ≥ 3 gastrointestinal disorders, despite optimal treatment.
- Acute pancreatitis (discontinue dexamethasone without previous dose reduction).
- Grade ≥ 3 hyperglycemia, despite optimal treatment.

Up to a maximum of two consecutive dose reductions (i.e., 20 mg D1, 8, 15 and 22, and 20 mg D1 and 15 of each 28-day cycle) will be allowed upon Sponsor's agreement, if patient benefit is perceived. After two dose reductions, dexamethasone will be discontinued.

5.6.3 Bortezomib

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

Table 8. Dose modifications for bortezomib-related hematological and non-hematological toxicity.

Severity of toxicity	Recommended modification of bortezomib dose and regimen
≥ Grade 3 febrile neutropenia, or Grade 4 neutropenia, or Grade 4 thrombocytopenia, or Grade 3 thrombocytopenia with bleeding	If two or more doses are omitted consecutively or within the same cycle, then reduce bortezomib one dose level
Herpes Zoster reactivation (any grade)	Hold therapy until lesions are dry
≥ Grade 3 bortezomib-related non-hematological toxicity (as judged by the Investigator)	If two or more doses are omitted consecutively or within the same cycle, then reduce bortezomib one dose level

Table 9. Dose modifications for bortezomib-related peripheral neuropathy.

Severity of PN signs and symptoms[a]	Recommended modification of bortezomib dose and regimen [76]
Grade 1 (paresthesia; weakness and/or loss of reflexes) without pain or loss of function	For patients receiving twice-weekly bortezomib, change to once-per-week schedule (D1, 8, 15, 22, q4wk), then Reduce current dose by one DL (from 1.3 to 1.0 mg/m^2) (from 1.0 to 0.7 mg/m^2), then Discontinue bortezomib

Severity of PN signs and symptoms[a]	Recommended modification of bortezomib dose and regimen [76]
Grade 1 with pain or grade 2 (with no pain but limiting instrumental ADLs[b])	For patients receiving twice-weekly bortezomib, change to a once-per-week (D1, 8, 15, 22, q4wk) schedule, then Reduce current dose by one DL, (from 1.3 to 1.0 mg/m^2) (from 1.0 to 0.7 mg/m^2) or consider temporary discontinuation; upon resolution to grade ≤ 1, re-start once-per-week (D1, 8, 15, 22, q4wk) dosing at the same DL, then Discontinue bortezomib
Grade 2 with pain or grade 3 (limiting self-care and ADL[c]) or grade 4	Discontinue bortezomib

[a]Based on posology modifications in phase II and III MM studies and post-marketing experience. Grading based on NCI-CTCAE v 4.0.
[b]Instrumental ADL: refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[c]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.

Up to a maximum of two bortezomib consecutive dose reductions will be allowed (from 1.3 mg/m^2 to 1.0 mg/m^2 and from 1.0 mg/m^2 to 0.7 mg/m^2) upon Sponsor's agreement, if patient benefit is perceived. After two dose reductions, bortezomib will be discontinued and treatment with plitidepsin/dexamethasone will be continued.

Once a dose reduction of any trial drug has been implemented, the dose will not be re-escalated thereafter.

5.7 Permitted Treatment and Medication

5.7.1 Transfusions

5.7.1.1 Guidelines for Platelet Transfusions

Thrombocytopenia can occur as a consequence of BM infiltration by myeloma cells or may be related to trial 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.

5.7.1.2 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.

5.7.2 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 American Society of Clinical Oncology [ASCO] guidelines) [77].
- Use of G-CSF/GM-CSF according to institutional or ASCO guidelines [78] 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.
- Patients receiving aminobisphosphonates before trial entry may continue to receive them during the trial according to ASCO guidelines.

5.8 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 products.
- Other disease-modifying treatment (e.g., alpha interferon) is strictly prohibited.
- Primary prophylaxis with colony-stimulating factors such as G-CSF (i.e., within the first cycle).

5.9 Drug Accountability

Proper drug accountability will be done by the trial 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. TRIAL ASSESSMENTS

6.1 Efficacy Assessment

Response or disease progression will be assessed on D1 of each therapy cycle according to the International Myeloma Working Group (IMWG) criteria [79] 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), DOR, TTP, PFS, EFS, OS and OS rate at 6 (OS6) and 12 (OS12) months.

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 plasmacytomas 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 Clinical 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 do not need 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.
- OS6 and OS12, defined as the Kaplan-Meier estimate of the percentage of patients who are alive at six and 12 twelve months from treatment start, respectively.
- OS, defined as the time from treatment start to the date of death (of any cause) or last patient contact.

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% Confidence Interval (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, fluorescence *in situ* hybridization (FISH), pharmacogenomics (PGx) 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 trial therapy.

6.2 Safety Assessment

Individual patients will be evaluated from treatment start until 30 days after the last IMP administration or until the patient starts a new antitumor therapy, or until the date of death, whichever occurs first.

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. Treatment delays, dose reduction requirements and reasons for discontinuation will be monitored throughout the study.

Any treatment-related AE will be followed until recovery to at least grade 1 or stabilization of symptoms, whichever occurs first.

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 trial treatment. An AE can therefore be any unfavorable and unintended sign, (e.g., an abnormal laboratory finding), or a disease temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product. Illnesses with onset during the study or exacerbation of pre-existing illnesses, including but not limited to

clinically significant changes in physical examination findings and abnormal objective tests/procedures findings (e.g., X-ray, ECG) should be recorded.

The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- The test result is associated with clinically significant symptoms and/or,
- The test result leads to a change in the study dosing or discontinuation from the clinical trial, significant additional concomitant drug treatment or other therapy, and/or,
- The test result leads to any of the outcomes included in the definition of a SAE, and/or,
- The test result is considered to be clinically relevant by the Investigator.

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 an event or an AE is medically significant or not, in particular those events 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.

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 trial 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 trial entry). Any surgery or procedure planned before trial 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 Reference Safety Information. The Reference Safety Information for the evaluation of expectedness will be:

- The IB in force for plitidepsin.
- The SmPC for bortezomib and dexamethasone.

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 trial or exacerbations of pre-existing illnesses should be recorded.

6.2.1.8 Adverse Events Related to the Trial Treatment

An AE is considered related to the IMPs if the Investigator's assessment of the causal relationship to the IMPs is "yes". The Investigator will assess the causal relationship of the IMPs to all AEs. The Sponsor will also consider related to the IMPs those events for which the Investigator assesses the causal relationship with the IMPs as "Unknown" when it cannot rule out a role of the IMP(s) with the events.

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 trial drug(s) [IMP(s)] to the Investigators and to the Independent Ethics Committee (IEC)/Institutional Review Boards (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 IMPs (including combination and comparator products) according to the following criteria:

- Related to trial drug(s): There is a reasonable possibility that the IMP(s) caused the SAE.
- Not related to trial drug(s): there is no reasonable possibility that the IMP(s) caused the SAE and other causes (as listed below) are more probable.
  - ✓ Disease under study.
  - ✓ Other illness (must be specified).
  - ✓ Previous/concomitant treatment/therapy.
  - ✓ Unknown but not related to trial drug.
- Unknown: only to be used in some situations when the Investigator has insufficient information.

If the causality assessment is unknown and the Investigator cannot rule out relationship to the trial drug, then "unknown" should be chosen. If the causality assessment is "unknown but not related to the trial drug", this should be clearly documented on the trial 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 trial drug or until the start of a new antitumor therapy, or until the date of death, whichever occurs first. All AEs suspected to be related to the trial 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, medication error and uses outside what is foreseen within the protocol, must be recorded in English using medical terminology on the source document and the e-CRF. Whenever possible, the Investigator will record the main diagnosis instead of the signs and symptoms normally included in the diagnosis. Investigators must assess the severity (grade) of the event following NCI-CTCAE v4.0 and assign a relationship to trial medication, 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 trial 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 suspected relationship to the trial drug, must be reported immediately, and always within 24h to Pharmacovigilance electronically by completing the applicable e-CRF section. SAEs occurring during the screening phase (from ICF signature), pregnancy, off-study period, or in case of electronic reporting system failure, will be reported immediately, and within 24 hours to the Pharmacovigilance Department using a paper SAE form, by fax (+34 91 846 6004), e-mail (phv@pharmamar.com) or phone (+34 91 823 4569). Out of hours (Greenwich Meridien Time [GMT]), assistance on SAE reporting can be obtained by calling the Pharmacovigilance Department on +34 91 823 4742. SAEs initially reported conventionally by paper (SAE form), must be followed by a complete electronic SAE report through the e-CRF from the Investigational staff within one working day. After the database lock, only SAEs that are suspected to be related to the trial drug will trigger the reporting to Pharmacovigilance Department using a paper SAE form.

The Sponsor will collect SAEs from the time of signing of the IC. If the patient is definitively included in the trial, 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 trial drug, or until the start of a new antitumor therapy, or until the date of death of the patient, whichever occurs first. 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 trial 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 trial, 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 Reporting Pregnancy Cases Occurred During this Clinical Trial

Food and Drug Administration (FDA) and European Medicines Agency (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 (beta-hCGs).

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 trial drug, or within 30 days of the patient's discontinuation visit, is considered an immediately reportable event. The trial drug must be discontinued immediately and the patient instructed to return any unused portion of the trial drug to the Investigator. The pregnancy, suspected pregnancy, or positive pregnancy test must be

reported to the Pharmacovigilance 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 Pharmacovigilance Department at PharmaMar as a follow-up to the initial SAE report in patients who receive trial 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 Pharmacovigilance 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 trial drug should also be reported to the Pharmacovigilance 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 trial will be determined by the Investigator and the PharmaMar responsible 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 trial. AEs will be monitored by the Investigators and by the trial team at Pharma Mar, S.A. The personnel in charge of this process are defined in the section "Trial 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 trial 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.

6.3 Pharmacokinetics (PK) Assessment

All patients included in the trial will be sampled for PK. All sample collection dates and times will be recorded on the e-CRF.

Samples will be obtained during C1 and C2 for the PK analysis of plitidepsin. Time points for blood sample collection for the determination of whole blood concentrations of plitidepsin are detailed on Table 10.

Table 10. Sampling schedule for the determination of plitidepsin.

Sample Number	Cycle	Day	Time points for plitidepsin samples	Sampling window
1	1	1	Pre-infusion	--
2	1	1	3 h (just before the EOI)	+/- 2 min
3	1	4	72 h	+/- 24 h
4	1	8	168 h	+/- 24 h
5	1	11	240 h	+/- 24 h
6	2	1	Pre-infusion	--
7	2	1	3 h (just before the EOI)	+/- 2 min
8	2	4	72 h	+/- 24 h
9	2	8	168 h	+/- 24 h
10	2	11	240 h	+/- 24 h

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 will have a volume of 2 mL.

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 and the whole blood (without centrifugation) should be transferred to the provided polypropylene tubes and stored frozen until the time of shipping for analysis to the laboratory. The tubes will be provided by PharmaMar. All the material for PK procedures will be provided by the Sponsor(s).

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 two 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).

6.3.1 Analytical Procedures

Whole blood samples will be analyzed to determine the concentration of plitidepsin using a validated, specific and sensitive ultra performance liquid chromatography/mass spectrometry/mass spectrometry (UPLC-MS/MS) method by or under the supervision of the Sponsor.

6.3.2 Pharmacokinetic Calculations

PK parameters will be calculated using population methods, after pooling data from this study with data obtained from other studies.

6.4 Pharmacogenomic (PGx) Assessment

For those patients who consent to participate in the PGx sub-study, the response to study treatment will be correlated with the expression levels of selected genes. BM samples will be obtained at the time of the baseline visit and after a patient has responded to treatment. The analysis of potential predictive factors of response to plitidepsin and bortezomib will include factors related to the MOA of plitidepsin and/or bortezomib and/or those related to the pathogenesis of the disease.

The following analyses will be carried out in plasma cells isolated from BM aspirates:

- Quantitation of mRNA expression of selected genes related to the MOA of plitidepsin and/or bortezomib or to the pathogenesis of the disease by real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).
- Quantitation of protein expression of selected genes related to the MOA of plitidepsin and/or bortezomib or to the pathogenesis of the disease by IHC.
- Analysis of polymorphisms and mutations of the above mentioned selected genes will be analyzed, if relevant, by RT-PCR and/or DNA sequencing.

Expression levels of the different markers will be correlated with the patient's clinical outcome.

A manual of instructions for sample extraction, labeling, storage, and shipment will be provided.

7. STATISTICAL METHODS

7.1 Sample Size Considerations

Patients will be treated to test the null hypothesis (H_0) that 20% or fewer patients achieve a response according to IMWG criteria ($p \leq 0.20$) *versus* the alternative hypothesis (H_1) that 40% or more patients achieve a response according to IMWG criteria ($p \geq 0.40$). The

variance of the standardized test is based on the empirical estimate. The type I error rate (alpha) associated with this one-sided test is 0.025 and the type II error rate (beta) is 0.1; hence, statistical power is 90%. In order to test these hypotheses, it is necessary to recruit 64 evaluable patients.

A futility analysis based on the primary endpoint (ORR) is planned for the time when the first 20 evaluable patients have been recruited. The futility analysis will commence once patient number 20 has completed two full treatment cycles. Patient recruitment will not be halted during the conduct of this futility analysis. A spending function defined by the Gamma family with parameter (-2) has been selected. If there are two or fewer responders according to boundaries and sample size assumptions, then the alternative hypothesis could be rejected and recruitment might be stopped at that time. Otherwise, patient accrual will continue to a total of 64 patients.

Overall, if $\geq$ 21 (i.e., 33%) patients achieve a response, then the null hypothesis can be rejected.

7.2 Assessment of Efficacy

For the evaluation of ORR and clinical benefit, rates will be calculated with binomial exact CI 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). OS will be calculated from the date of the first infusion to the date of death (or last patient contact). If any patient is lost to follow-up before PD or death or receives another antitumor 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, TTP, PFS, EFS, OS6, OS12 and estimated rates of patients free of progression or alive will be calculated by Kaplan-Meier estimates with 95% CI.

7.3 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 and clinical laboratory results. Safety variables will be tabulated and presented for all patients who receive any amount of plitidepsin, bortezomib and dexamethasone. Exposure to trial drugs and reasons for discontinuation of trial treatment will be tabulated. Analyses will be performed in a descriptive fashion.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All AEs occurring during the trial 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 trial drug and up to 30 days after the last dose of trial 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 trial discontinuation will be tabulated.

Changes from baseline in clinical laboratory parameters will be summarized across time throughout the trial, 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, white blood cell (WBC) count, neutrophils, lymphocytes, platelets, AST, ALT, bilirubin, creatinine, AP, CPK and electrolytes. These tables will summarize the number of patients with each baseline NCI-CTCAE grade and changes to the maximum NCI-CTCAE grade during treatment.

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 trial analysis of drug-related events. A second set of tables including all events will also be presented.

7.4 Other Analyses

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

7.5 Baseline and Demographic Data

Baseline data such as demographics, serum calcium, renal function (creatinine [Cr], creatinine clearance [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 trial, prior anticancer therapy, biological values, prior relevant history, signs and symptoms, ECG and concomitant medication (Anatomical Therapeutic Chemical Drug Classification by the World Health Organization [ATC-WHO] coded) will be described.

7.6 Treatment Administration

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

7.7 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 Good Clinical Practice (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 trial (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 trial objectives, such as those involving the inclusion/exclusion criteria (which could mean that the patient is not eligible for the trial) 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 trial will be authorized.

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

7.8 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 trial 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.

7.9 Pharmacogenomics

Analysis of RNA/protein expression, polymorphisms and mutations will be performed blind, and clinical data compiled only after all analyses are completed. A Fisher's exact test/logistic regression for categorical variables and a log rank test/Cox regression for time-to-event variables will be used to test whether a specific profile is associated with clinical outcome after treatment with plitidepsin in combination with bortezomib and dexamethasone. The prognosis value of markers will be explored for objective clinical response, PFS and OS. In each case, if applicable, a multivariate model will be developed by stepwise selection. All tests of statistical significance will be two-sided, and significance will be set at 0.05.

7.10 Procedures for Reporting Deviations to the Original Statistical Analysis Plan

All deviations from the original statistical analysis plan will be provided in the final Clinical Study Report.

8. ADMINISTRATIVE REQUERIMENTS

This protocol will be approved by a Competent Authority before the beginning of the trial.

8.1 Ethics

This trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki (Appendix 7) and will be consistent with GCP and other applicable regulatory requirements.

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

The trial 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 trial will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of the trial.

The Investigator and/or the Sponsor is/are responsible for keeping the IEC/IRB informed of significant new information about trial 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 trial is to be conducted.

8.2 Monitoring, Auditing and Inspecting

The trial will be monitored by regular site visits and telephone calls to the Investigator by the clinical trial monitor designated by PharmaMar.

During site visits, the trial monitor should review original patient records, drug accountability records and document retention (trial file). Additionally, the monitor should observe trial 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 International Conference on Harmonization Good Clinical Practice (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 trial.

At any time during the course or at the end of the trial, 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 trial implies acceptance of potential inspections by national or foreign Competent Authorities.

8.3 Patient Informed Consent

Before agreeing to participate in this trial, all patients will be provided with full written information about the trial, as well as about other sub-studies (PGx sub-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 trial.

The Investigator, or designated person, must provide the patient with a copy of the Patient Information Sheet and consent forms and should allow the necessary time for the patient to inquire about the details of the trial and the PGx sub-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 trial. The patient should receive a copy of the signed IC and any other written information provided to patients prior to participation in the trial and the PGx sub-study.

During a patient's participation in the trial, 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 trial, before obtaining the written consent.

8.4 Confidentiality/Patients Identification

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

The trial monitor, auditor at PharmaMar, IRB/IEC, or Competent Authorities should have direct access to all requested trial related records and agree to keep the identity of trial 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.

8.5 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 trial.

The patient's source documentation, including but not limited to physician's patient records, nurses notes, pharmacy records, etc. should be maintained at the trial 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.

8.6 Insurance

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

8.7 Records Retention

The Investigator/Institution should maintain trial 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.

8.8 Use of Information and Publication

Before the Investigators of this trial submit a paper or abstract for publication or otherwise publicly disclose information concerning the trial 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 trial is part of a multicenter study, the first publication of the trial 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 trial at all sites, the present trial 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 trial 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 trial must be considered for co-authorship of the publication.

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10. APPENDICES

Appendix 1. 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 2. Performance Status

Performance Status Criteria

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

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. Cockcroft and Gault's Formula for Calculating Creatinine Clearance

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

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

[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 5. Contraception and pregnancy testing

This document is based on the Heads of Medicines Agencies' Recommendations Related to Contraception and Pregnancy Testing in Clinical Trials, published by the Clinical Trial Facilitation Group (CTFG) on 15 September 2014 and available at http://www.hma.eu/ctfg.html (accessed on 17 August 2015).

A woman is considered of childbearing potential (WOCBP) following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. Immediately after detecting a case of suspected pregnancy in a patient, the decision on her continued participation in the clinical trial will be jointly taken by the patient, the Investigator and the Sponsor, with the patient's best interest in mind. A decision to continue the pregnancy will require immediate discontinuation of any IMP.

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy. Fertile male patients included in this trial should refrain from fathering a child or donating sperm during the trial and for six months following the last IMP dose. If they have WOCBP partners the male subject should use condom during treatment and for four months following the last IMP dose.

Highly effective birth control methods are:

1. Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]
    a. oral
    b. intravaginal
    c. transdermal
2. Progestogen-only hormonal contraception associated with inhibition of ovulation [1]:
    a. oral
    b. injectable
    c. implantable[2]
3. Intrauterine device (IUD)[2]
4. Intrauterine hormone-releasing system (IUS)[2]
5. Bilateral tubal occlusion[2]
6. Vasectomized partner[2,3]
7. Sexual abstinence[4]

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method (see below).
[2] Contraception methods that are considered to have low user dependency.
[3] Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success.
[4] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together.

8. A combination of a male condom with either a cervical cap, a diaphragm or a sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods.

Contraception methods with low user dependency should preferably be used, in particular when contraception is introduced as a result of participation in this trial. It cannot be excluded that the IMP may reduce exposure to substrates of CYP3A through enzyme induction; the efficacy of hormonal contraceptives may be reduced if co-administered with this drug.

Appendix 6. Prognostic factors and risk-stratification in myeloma

Prognostic determinant	Standard-risk	High-risk	Therapeutic implication
Host factors	ECOG PS 0-2	ECOG PS 3-4	High-risk patients typically require a decrease in treatment intensity
	Normal renal function	Renal failure (serum creatinine ≥ 2.0)	
		Advanced age	
Tumor burden	Durie-Salmon stage I, II	Durie-Salmon stage III	Limited; some stage I patients require no therapy (smoldering myeloma, and some require radiation only (if solitary bone lesion)
Tumor biology (disease aggressiveness)	Hyperdiploidy	t(4;14)*	Treatment of high-risk patients remains unsatisfactory, but bortezomib appears to overcome some high-risk features (t4;14)
	t(11;14)	t(14;16)	
	t(6;14)	t(14;20)	
		del17p	
		High LDH	
		High plasma cell proliferative rate	
		High-risk signature on GEP	

Modified from Rajkumar et al with permission.
ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase.
*t(4;14) is considered "intermediate-risk" based on improved results seen now with bortezomib-based initial therapy.

Full details available in the full publication: Bergsagel PL, Mateos MV, Gutierrez, NC, Rajkumar SV and San Miguel JF. Improving overall survival and overcoming adverse prognosis in the treatment of cytogenetically high-risk multiple myeloma. Blood 2013; 121: 884-892

Appendix 7. 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
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

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 be applied with consideration of all other relevant paragraphs.

2) Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3) 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.”
4) It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
5) Medical progress is based on research that ultimately must include studies involving human subjects.
6) 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 proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
7) Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.
8) While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9) It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10) Physicians must 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.
11) Medical research should be conducted in a manner that minimizes possible harm to the environment.
12) Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13) Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14) Physicians who combine medical research with medical care should involve their patients in research only to the extent that this 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.
15) Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16) In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17) All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18) Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19) Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20) Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21) 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.

22) The design and performance of each research study involving human subjects must be clearly described and justified 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, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23) The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. 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 amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24) Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25) Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26) In medical research involving human subjects capable of giving informed consent, 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, post-study provisions 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.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27) When seeking informed consent for participation in a research study the physician must 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 must be sought by an appropriately qualified individual who is completely independent of this relationship.
28) For a potential research subject who is incapable of giving informed consent, 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 group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.
29) When a potential research subject who is deemed incapable of giving informed consent 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.
30) 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 group. In such circumstances the physician must 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 must be obtained as soon as possible from the subject or a legally authorized representative.
31) The physician must fully inform the patient which aspects of their 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 adversely affect the patient-physician relationship.
32) For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such

research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33) The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34) In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35) Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36) Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers 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. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37) In the treatment of an individual patient, where proven interventions do not exist or other known interventions 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 judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.