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CLINICAL TRIAL PROTOCOL

Randomized, Multicenter, Open-label, Phase III Study of Plitidepsin in Combination with Dexamethasone vs. Dexamethasone Alone in Patients with Relapsed/Refractory Multiple Myeloma

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

Protocol No.: APL-C-001-09

EudraCT No.: 2009-016138-29

NCT Code: 01102426

Protocol version 4.0: 10 July 2017

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CLINICAL TRIAL PROTOCOL

APL-C-001-09
(ADMYRE: Aplidin – Dexamethasone in RElapsed/Refractory MYeloma)

Randomized, Multicenter, Open-label, Phase III Study of Plitidepsin in Combination with Dexamethasone *vs.* Dexamethasone Alone in Patients with Relapsed/Refractory Multiple Myeloma

INVESTIGATIONAL MEDICINAL PRODUCT: Aplidin® (plitidepsin)

Combination with: Dexamethasone

Protocol Code: **APL-C-001-09**

EudraCT: 2009-016138-29

Protocol Version 4.0
Substantial Amendment #3 (edition 10 July 2017) is included

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

Confidentiality statement

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QT-Related Polymorphisms Assessments Laboratory	Transgenomic, Inc. Five Science Park. New Haven, CT06511. USA.	Phone: +1 (877) 274 9432 Fax: +1 (855) 263 8668

A list of additional study contacts will be supplied as an additional document.

PRINCIPAL INVESTIGATORS

A complete list of investigators will be supplied as an additional document.

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SYNOPSIS

TITLE	Randomized, Multicenter, Open-label, Phase III Study of Plitidepsin in Combination with Dexamethasone *vs.* Dexamethasone Alone in Patients with Relapsed/Refractory Multiple Myeloma
CODE	APL-C-001-09
LOCATION OF INVESTIGATORS AND SITES	The complete list of investigators is available in a separate document.
NUMBER OF SITES	Approximately 40-80 centers worldwide.
CLINICAL TRIAL OBJECTIVES **Primary**	▪ To compare the efficacy of plitidepsin in combination with dexamethasone *vs.* dexamethasone alone as measured by progression-free survival (PFS) in patients with relapsed/refractory multiple myeloma (MM).
Secondary	▪ To evaluate tumor response according to the International Myeloma Working Group (IMWG) criteria. ▪ To assess duration of response (DR) and overall survival (OS). ▪ To assess efficacy in patients who undergo crossover from dexamethasone alone to plitidepsin and dexamethasone combination. ▪ To characterize and compare the safety profile on both arms in this population. ▪ To characterize the pharmacokinetics (PK) and pharmacokinetic /pharmacodynamic (PK/PD) relationship.
PATIENT ELIGIBILITY **Inclusion Criteria**	1. Age ≥ 18 years. 2. Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) ≤ 2 (see Appendix 2). 3. Life expectancy ≥ 3 months. 4. Patients previously diagnosed with multiple myeloma based on IMWG diagnostic criteria (see Appendix 4). 5. Patients must have relapsed or relapsed and refractory multiple myeloma (MM) (Appendix 5) after at least three but not more than six prior therapeutic regimens for MM, including induction therapy and stem cell transplant in candidate patients, which will be considered as only one regimen.

	6. Patients must have received previous bortezomib-containing and lenalidomide-containing regimens (or thalidomide where lenalidomide is not available), unless unable to tolerate either of them. 7. Patients must have measurable disease defined as: a) For secretory MM: any quantifiable serum monoclonal protein value and, where applicable, urine light-chain excretion ≥ 200 mg/24 hours. b) For oligo- or non-secretory MM: presence of soft tissue (not bone) plasmacytomas, as determined by clinical examination or applicable radiographs [i.e., magnetic resonance imaging (MRI), computed tomography (CT)-scan], and/or by the presence of abnormal serum free light chains (sFLC): involved FLC level ≥ 10 mg/dl provided the serum FLC ratio is abnormal. 8. At least two-week washout period since the end of last therapy (six weeks if previous nitrosoureas-containing regimen), given recovery to grade ≤ 1 from any non-hematological related adverse event (AE) derived from previous treatment (excluding alopecia). 9. Adequate bone marrow (BM), renal, hepatic, and metabolic function (assessed ≤ 7 days before inclusion in the study): a) Absolute neutrophil count (ANC) ≥ 1.0 x 10^9/l (≥ 0.5 x 10^9/l if due to extensive and documented 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. b) Platelet count ≥ 50 x 10^9/l (≥ 25 x 10^9/l if due to extensive and documented BM disease involvement). c) Hemoglobin ≥ 8.5 g/dl. • Patients may receive red blood cells (RBC) and/or erythropoietin (EPO), and/or platelets transfusions in accordance with institutional guidelines. d) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 3.0 x the upper limit of normal (ULN). e) Total bilirubin ≤ 1.0 x ULN or direct bilirubin ≤ 1.0 x ULN when total bilirubin is above the upper limit of normal. f) Calculated creatinine clearance (CrCl) ≥ 30 ml/minute (by means of Cockcroft and Gault´s formula) (see Appendix 3). g) Creatine phosphokinase (CPK) ≤ 2.5 x ULN. h) Albumin ≥ 2.5 g/dl. 10. Left ventricular ejection fraction (LVEF) by echocardiogram (ECHO) or multiple-gated acquisition scan (MUGA) above the lower limit of normal (LLN).

	11. Women of childbearing potential must have a negative serum pregnancy test before study entry. Both women and men must agree to use a medically acceptable method of contraception throughout the treatment period and for six months after discontinuation of treatment. 12. Voluntarily signed and dated written informed consent prior to any specific study procedure.
PATIENT ELIGIBILITY **Exclusion Criteria**	1. Concomitant diseases/conditions: a) History or presence of angina, myocardial infarction, clinically relevant valvular heart disease, cardiac amyloidosis or congestive heart failure within the last 12 months. b) Symptomatic arrhythmia (excluding anemia-related sinusal tachycardia grade ≤ 2) or any arrhythmia requiring ongoing treatment, and/or prolonged QT-QTc grade ≥ 2; or presence of unstable atrial fibrilation. Patients with stable atrial fibrillation on treatment are allowed provided they do not meet any other cardiac or prohibited drug exclusion criterion. c) Active uncontrolled infection. d) Morphological or cytological features of myelodysplasia and/or post-chemotherapy aplasia on BM assessment. e) Myopathy > grade 2 or any clinical situation that causes significant and persistent elevation of CPK (>2.5 x ULN in two different determinations performed one week apart). f) Known human immunodeficiency virus (HIV) infection (HIV testing is not required unless infection is clinically suspected). g) Known active hepatitis B or C virus (HBV or HCV) infection. h) Limitation of the patient's ability to comply with the treatment or follow-up requirements. i) Any other major illness that, in the Investigator's judgment, will substantially increase the risk associated with the patient's participation in this study. j) Peripheral neuropathy > grade 2. 2. Women who are pregnant or breast feeding. 3. Concomitant medications that include corticosteroids, chemotherapy, or other therapy that is or may be active against MM, within two weeks prior to Cycle 1 Day 1. Concurrent corticosteroids are allowed, provided they are administered at an equivalent prednisone dose of ≤ 10 mg daily, as premedication for blood products only. 4. Known history of peptic ulcer and/or major upper gastrointestinal bleeding episode occurring during last year before study entry and/or related to prior steroid-based therapy. 5. Relevant history of mood-disturbances changes associated with previous steroid-based therapy.

	6. Disease-related symptomatic hypercalcemia despite optimal medical therapy. 7. Known hypersensitivity to any involved study drug or any of its formulation components.
PATIENT ELIGIBILITY **Number of patients**	Approximately, 210 progression or death events will be needed for the evaluation of the primary endpoint (PFS). A total of approximately 250 patients will be randomized in a 2:1 ratio to: 1. Arm A (plitidepsin + dexamethasone combination): approximately 167 patients. 2. Arm B (dexamethasone single agent): approximately 83 patients. An early futility analysis will be performed with the data collected when 40 patients in Arm A are evaluable for response. Patients without valid disease evaluation during treatment will not be replaced for the main analysis, and will be analyzed as per ITT according to randomization.
CLINICAL TRIAL DESIGN	Prospective, open-label, two-arm, 2:1 randomized phase III study. The efficacy of plitidepsin in combination with dexamethasone *vs.* dexamethasone alone will be studied by means of PFS calculated using the IMWG uniform response criteria, and the evaluation of secondary efficacy endpoints. Patients in the control arm (dexamethasone alone, Arm B) who have documented disease progression according to Investigator's criteria, after a minimum of eight weeks from randomization, should be offered crossover to the combination arm (plitidepsin + dexamethasone, Arm A) upon Sponsor agreement. An Independent Review Committee (IRC) consisting of medical specialists directly involved in the care of patients with MM but not taking part in this trial as investigators or sub investigators, will review all efficacy data and will assign the date of progression/censoring and objective response according to their independent evaluation. This IRC will be blinded regarding to treatment arm allocation and identity of the cases reviewed. An Independent Data Monitoring Committee (IDMC), including specialists in MM and in medical statistics, will review the results of the protocol-specified analyses performed by an independent statistician, including investigators and IRC efficacy assessments and safety information. Then, the IDMC will provide advice on the conduct of the study. Operational details for the IRC and IDMC will be detailed in the corresponding charters.

Duration of study period (per patient)	Patients will be evaluated at scheduled visits in up to three study periods: ▪ **Pre-treatment**: from signature of informed consent to first administration of study drugs. ▪ **Treatment**: up to 30 days after the day of the last dose of study drug administration, unless the patient starts any new antitumor therapy outside this clinical trial or dies, in which case the date of administration of this new therapy or the date of death will be considered the date of treatment discontinuation. An end-of-treatment visit will be performed within 30 days (± two days) after last dose administration, unless the patient starts any new antitumor therapy outside this clinical trial, in which case the end-of-treatment visit should be performed immediately before the start of the new therapy (ideally the day before or the same day). ▪ **Follow-up**: after treatment discontinuation, patients will be followed every four weeks until resolution of toxicities if any. Patients who discontinued treatment without progression will be followed every four weeks until disease progression or other antitumor therapy, whichever occurs first. After progression, all patients will be followed every three months for survival until death, or until the date of study termination, whichever occurs first. Patients will be considered to be **on-study** from the signature of the informed consent to the end of the follow-up period. Patients will be considered to be **on-treatment** for the duration of their treatment and 30 days following the last treatment dose. Those patients in the control arm (Arm B) who **crossed over** to the combination arm (Arm A) after disease progression will be considered on-treatment for the duration of their whole treatment (dexamethasone alone + dexamethasone in combination with plitidepsin) and during 30 days following the last treatment dose. Patients will receive the study medications while it is considered to be in their best interest. Specifically, treatment will continue until: ▪ Disease progression (except for those patients treated with dexamethasone alone and eligible to be offered crossover to combination therapy). ▪ Unacceptable toxicity. ▪ Intercurrent illness of sufficient magnitude to preclude safe continuation of the study. ▪ Patient refusal and/or non compliance with study requirements. ▪ Protocol deviation with an effect on the risk/benefit ratio of the clinical trial. ▪ Treatment delay > 2 weeks from the theoretical treatment date (except in case of clear clinical benefit, with the Sponsor's approval). ▪ Requirement of > 2 dose reductions of either drug.

<table>
<tr><td>INVESTIGATIONAL DRUG

Formulation</td><td>▪ Plitidepsin will be supplied as a powder and solvent for concentrate for solution for infusion. The 2-mg vial should be reconstituted with a 4-ml ampoule of reconstitution solution. The composition of reconstitution solution is Cremophor EL/ethanol/water for injection (15/15/70% v/v/v).
▪ Commercially available dexamethasone formulations will be used.</td></tr>
<tr><td>Mode of administration, dose and schedule</td><td>1. Arm A:
a) Dexamethasone: 40 mg orally on Day 1, 8, 15 and 22 every four weeks (q4wk) at least one hour before plitidepsin infusion.
b) Plitidepsin: 5 mg/m^2 intravenously (i.v.) diluted to a total volume of 250 ml in 0.9% saline (or 5% glucose) via a central venous catheter (suggested) or diluted to a total volume of 500 ml in 0.9% saline (or 5% glucose) via a peripheral line. Infusion will be performed through a pump device over three hours (fixed rate) on Day 1 and 15 q4wk.
2. Arm B:
a) Dexamethasone: 40 mg orally on Day 1, 8, 15 and 22 q4wk.
A cycle is defined as a four-week period.</td></tr>
<tr><td>Criteria for treatment continuation</td><td>Before the administration of each dose (re-treatment), patients must fulfill the baseline criteria defined in the following table:
<table>
<tr><th></th><th>Plitidepsin (Arm A)</th><th>Dexamethasone (Arm A and B)</th></tr>
<tr><th></th><th>Day 1 [a] & 15 [b]</th><th>Day 1 [a] & 15</th></tr>
<tr><td>ANC</td><td>≥ 1.0 x 10^9/l or return to baseline values if extensive BM involvement</td><td>-</td></tr>
<tr><td>Platelets</td><td>≥ 50 x 10^9/l or return to baseline values if extensive BM involvement</td><td>-</td></tr>
<tr><td>Hemoglobin</td><td>≥ 8.5 g/dl</td><td>≥ 8.5 g/dl</td></tr>
<tr><td>Direct bilirubin if total bilirubin is above the upper normal limit</td><td>≤ 1.0 x ULN</td><td>-</td></tr>
<tr><td>AST/ALT</td><td>≤ Grade 2</td><td>-</td></tr>
<tr><td>Muscular toxicity (myalgia, muscular weakness, CPK increase)</td><td>≤ Grade 2</td><td>≤ Grade 2</td></tr>
<tr><td>Other non-hematological drug-related AEs (except for increased GGT and/or AP, not optimally treated nausea and vomiting or hypertension, alopecia)[c]</td><td>≤ Grade 1</td><td>≤ Grade 1</td></tr>
<tr><td>ECG, ECHO/MUGA[d]</td><td>Same as baseline</td><td>Same as baseline</td></tr>
<tr><td colspan="3">a. If a patient does not meet the requirements for treatment continuation on Day 1 of the following cycle, the infusion of study drugs will be withheld until recovery or for a maximum of 14 days. After this period, if delay is due to toxicity assessed as related to study</td></tr>
</table></td></tr>
</table>

	drug, a dose decrease of 20% is mandatory; up to a maximum of two individual dose reductions are allowed. Patients needing additional dose reductions must be withdrawn from the trial. b. If a patient does not meet the requirements for treatment continuation on Day 15, the administration of plitidepsin will be omitted. Patients requiring frequent dose omissions may have a dose reduction of 20% upon Investigator and Sponsor agreement. In no case more than two dose reductions are allowed. c. Any grade accepted for increased GGT and/or AP, and up to grade 2 peripheral neuropathy. d. To be performed every three months unless more frequent assessments are clinically indicated. AEs: adverse event(s); ANC: absolute neutrophil count; AP: alkaline phosphatase; BM: bone marrow; AST/ALT: aspartate aminotransferase/alanine aminotransferase; CPK: creatine phosphokinase; GGT: γ-glutamyltranspeptidase; ECG: electrocardiogram; ECHO/MUGA: echocardiogram/multiple-gated acquisition scan.
Dose reduction criteria	Patients experiencing frequent dose omissions and/or unacceptable toxicity, defined as • less than 50% compliance with 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 (in patients with extensive BM involvement), and/or • any grade ≥ 3 clinically relevant non-hematological toxicity other than non-optimally treated nausea and vomiting, diarrhea lasting < 48 hours and/or grade ≥ 3 asthenia/fatigue lasting < 5 days, may continue treatment after reducing 20% of the dose of plitidepsin (first reduction to 4 mg/m^2, and second reduction to 3.2 mg/m^2), upon Sponsor agreement, if patient benefit is perceived. Dexamethasone doses are to be reduced by 50%, up to a maximum of two consecutive dose reductions (first one, 20 mg Days 1,8,15 and 22, and second, 20 mg Days 1 and 15 of each 28-day cycle), if a patient experiences • muscular toxicity of grade ≥ 3 (weakness, myalgia and/or CPK elevations), or • drug-related grade ≥ 3 fatigue, or • mood disturbances or • agitation of grade ≥ 2 or • grade ≥ 3 fluid retention or • grade 4 clinically documented infection these dose reductions are to be implemented independently from plitidepsin dose reductions, if required. Once a dose reduction has been implemented, dose will not be re-escalated thereafter. No more than two dose reductions (of either compound, if required) are allowed. Patients needing additional dose reductions must be withdrawn from the trial.

Dose reduction criteria for both study drugs, plitidepsin and dexamethasone, are summarized in the following table:

Toxicity	Worst grade	Plitidepsin	Dexamethasone
Less than 50% compliance with treatment schedule		Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Febrile neutropenia	≥ 3	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Neutropenia lasting > 7 days (except for patients with extensive BM involvement)	4	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Thrombocytopenia (except for patients with extensive BM involvement)	4	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Toxicity	**Worst grade**	**Plitidepsin**	**Dexamethasone**
Thrombocytopenia with grade ≥ 3 bleeding (in patients with extensive bone marrow involvement)	4	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Any clinically relevant and/or non- hematological toxicity (except non-optimally treated nausea and vomiting, diarrhea < 48 hours and/or asthenia/fatigue lasting < 5 days)	≥ 3	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Muscular toxicity (weakness, myalgia and/or CPK elevations)	≥ 3	First episode: decrease to 4mg/m^2, then reduce dexamethasone; if toxicity recurs, reduce to 3.2 mg/m^2	First reduce plitidepsin; if persistence, decrease dexamethasone to 20 mg Days 1,8,15,22, of each cycle; if toxicity recurs, decrease again first plitidepsin and, if persistence, then decrease dexamethasone dose to 20 mg Days 1 and 15 of each cycle. Two dose reductions are allowed for either agent (only for muscular toxicity).
Mood disturbances/agitation	≥ 2	No reduction	First reduction to 20 mg Days 1,8,15,22 of each cycle, then to 20 mg Days 1 and 15 of each cycle.
Fluid retention	≥ 3	No reduction	First reduction to 20 mg Days 1,8,15,22 of each cycle, then to 20 mg Days 1 and 15 of each cycle.
Clinically documented infection	4	No reduction	First reduction to 20 mg Days 1,8,15,22 of each cycle, then to 20 mg Days 1 and 15 of each cycle.

Prophylactic medication	**1. Arm A:** All patients must receive the following prophylactic medication 20-30 minutes before infusion of plitidepsin: ▪ Ondansetron 8 mg i.v. or equivalent (granisetron 3 mg i.v. preferred when available). ▪ Diphenhydramine hydrochloride 25 mg i.v. or equivalent, and ▪ Ranitidine 50 mg i.v. or equivalent. Oral metoclopramide and/or extended oral ondansetron (or their equivalents) may be used as per Investigator's criteria/institutional guidelines. **2. Arm B:** No prophylactic medication specified.
Allowed medications/therapies	1. Platelet and red cells transfusions. 2. Erythropoietin. 3. Bisphosphonates according to the American Society of Clinical Oncology (ASCO) guidelines. 4. Therapies for the treatment of preexisting and/or emergent medical conditions not specifically forbidden as per protocol elsewhere. 5. Antiemetics (excluding steroids) according to institutional or ASCO guidelines. 6. Granulocyte colony stimulating factor (G-CSF)/granulocyte-macrophage colony stimulating factor (GM-CSF) according to institutional or ASCO guidelines. 7. Palliative local radiation of a plasmacytoma. The irradiated lesion will then not be considered an area of measurable/evaluable disease. 8. Systemic and/or local therapies for symptomatic relief, particularly in the case of diarrhea or skin toxicity. 9. Patients in the plitidepsin + dexamethasone arm who develop grade ≥ 2 muscular toxicity may be empirically treated with oral L-carnitine at a total daily amount of up to 3 g, divided into three doses, until it decreases to grade ≤ 1. 10. Adequate analgesic medication, including opioids for symptomatic pain relief if indicated. 11. Drugs known to prolong QT interval and/or induce Torsades de Pointes should be avoided whenever possible (see Appendix 7, drugs labeled as 1).
Prohibited medications/therapies	1. Concomitant administration of any other antineoplastic therapy. 2. Other investigational agents. 3. Immunosuppressive therapies, except single bolus hydrocortisone used eventually as treatment for hypersensitivity reactions, if required. 4. Primary prophylaxis with colony-stimulating factors such as G-CSF

	and GM-CSF.
EVALUATION CRITERIA	**Primary endpoint:** ▪ Efficacy: • PFS, according to IRC assessment, as per intention-to-treat (ITT) analysis. **Secondary endpoints:** ▪ Efficacy: objective response rate (RR); best overall response including rate of minor response (MR) or better (according to the IMWG response criteria), RR to combination treatment in patients who crossed over after progression on dexamethasone alone; time-to-event endpoints: DR, and OS. Intrapatient response and PFS comparison of patients who crossed over from Arm B to Arm A. Both IRC and Investigator's assessment will be used for the determination of RR, DR and PFS. ▪ Safety: patients are evaluable for general safety if they received at least one dose of study treatment. Safety will be evaluated in each arm separately. AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4. The primary study analysis will be based on externally assessed PFS data in the **ITT efficacy population**, defined as ***all patients randomized to either treatment arm.*** PFS will be calculated from randomization to the first evidence of progressive disease (PD) (IMWG criteria) or death due to any cause. If the patient receives further antitumor therapy before PD and within the timeframe expected for first follow-up, PFS will be censored on the date of the last disease assessment prior to the administration of this antitumor therapy. If the patient is lost to follow-up for the assessment of progression, or has more than one missing follow-up between the date of last tumor assessment and the date of progression, death or further antitumor therapy, the PFS will be censored at the date of last valid tumor assessment before the missing evaluations. DR will be calculated from the date of first documentation of response to the date of disease progression or death. The same censoring rules described above for PFS calculation will be also considered for DR. OS is defined as the time from the date of randomization to the date of death or last contact. An external IRC, blinded to treatment arm, will assign the objective response and a progression or censoring date for each patient based on laboratory data, radiologic and bone marrow assessments when required, and evaluation of all relevant clinical information, according to a predefined algorithm provided in a separate charter. By design, disease response will be assessed every four weeks symmetrically across treatment arms irrespectively of treatment delays or omissions. Disease assessments (e.g., serum or urine M protein, sFLC) and evaluation of extent of disease will be done within two weeks

	before ***randomization*** and every four weeks thereafter in the absence of PD while on treatment. If disease progression has not occurred at treatment termination, then disease assessments should continue every four weeks until evidence of disease progression, start of subsequent anticancer treatment, or death, whichever occurs first. PFS and objective tumor response will be assessed according to IMWG criteria. Centralized laboratory reports and copies of CT scans, MRI (in case of soft tissue plasmacytoma) and any other documented means to evaluate tumor response or progression should be available for IRC review.
PHARMACO-KINETIC EVALUATIONS	Plitidepsin pharmacokinetics (PK) will be evaluated in a minimum of 100 patients included in the plitidepsin/dexamethasone arm (Arm A). A total of 13 blood samples will be collected during the first three infusions [infusions 1 and 2 of Cycle 1 (Days 1 and 15), and infusion 1 of cycle 2 (Day 1)] using a sparse sampling schedule. Last sample will be collected immediately previous to infusion 2 of cycle 2 (Day 15) (see details in Table 5 of this protocol). Pharmacokinetic parameters will be calculated with population methods, using the population PK model previously developed with PK data from 411 patients treated with single-agent plitidepsin.
QT SUBSTUDY	The primary objective of the QT substudy is to assess the potential effects of plitidepsin administered at a therapeutic dose on the duration of the QT/QTc interval, measured by ECGs, in patients with relapsed/refractory multiple myeloma. Secondary objectives are: to evaluate QTc changes over the treatment period; to characterize the plitidepsin (real and estimated) whole blood concentration/QTc relationship (or PK/PD relationship); and to explore related ECG parameters. This substudy will be conducted on patients included in study APL-C-001-09 and randomized to the experimental arm (plitidepsin + dexamethasone), who voluntarily sign and date the informed consent form to participate in the QT substudy, and who fulfill the following at screening for the main study: • A 12-lead ECG consistent with normal cardiac conduction and function, showing sinus rhythm, pulse rate between 45 and 100 bpm, QRS interval <120 ms, and PR interval <200 ms. • Blood pressure between 90 and 150 mmHg systolic, inclusive, and not higher than 90 mmHg diastolic. • Serum electrolyte levels ≤ grade 1 (i.e. Ionic Ca^{++}: 1.0 – 1.5 mmol/L; K^{+}: 3 – 5.5 mmol/L; Mg^{++}: 0.5 – 1.23 mmol/L). • Prior exposure to anthracyclines at a cumulative dose of doxorubicin or equivalent, ≤ 450 mg/m². • Patients who at screening are not on medication that is known to

	prolong the QT interval. Patients must have been off these medications for a minimum of 48 hours prior to plitidepsin administration on Day 1 and Day 15. This substudy will be conducted at some of the sites participating in study APL-C-001-09. **Evaluations of the effect of plitidepsin on QTc:** ECGs will be obtained digitally using an ECG continuous 12-lead digital recorder on Day 1 and Day 15. The change in QTc from baseline to each postdose time point on Day 1 and Day 15 (ΔQTc) will be evaluated. The QTc measurements at each of the scheduled time points along with their corresponding real and estimated whole blood plitidepsin concentration will be compared to explore any concentration-effect relationship for plitidepsin.
STATISTICAL CONSIDERATIONS	**Randomization** Eligible patients will be stratified according to their ECOG-PS score (0 and 1 *vs.* 2) and Durie-Salmon stage (I/II *vs.* III) and then randomized using a 2:1 randomization procedure to Arm A (plitidepsin in combination with dexamethasone) or Arm B (dexamethasone alone). Patients will be assigned to each group by strata random lists, so that a patient will have a two-thirds chance of getting Arm A (plitidepsin in combination with dexamethasone) and a one-third chance of getting Arm B (dexamethasone alone). The random permuted blocks method will be used; the size of the blocks in the randomization list will be fixed and not accessible to the investigators. To select the blocks, a uniform (0, 1) variable with a random seed will be used. **Sample size** Approximately 210 progression or death events would be needed in this trial to detect a HR of 0.625 in favor of the combination arm (equivalent to an increase of 60% in PFS, i.e., from 10 to 16 weeks, from 12 to 19.2 weeks, from 16 to 25.6 weeks, etc.) with 90% power and 1-sided 2.5% significance level. As a preliminary hypothesis, it is estimated that up to 250 randomized patients will be needed to achieve the 210 events in 24-30 months. **Statistical Analyses** All randomized patients during the trial will be included in the final efficacy analyses, while the first 40 patients evaluable for response in Arm A will be included in an early futility analysis. A response rate (IMWG criteria) of at least 30% (twelve or more responses by IRC review) will be taken as threshold for continuation of the study. A minimum response rate of 30% has been considered as clinically significant in this setting. This result will ensure that the lower limit of the exact binomial 95% Confidence Interval for the response rate will be greater than 15% (95% CI in case of 12 responses would be 16.6% - 46.5%). No claim for superiority in efficacy will be formulated in this interim analysis and no alpha-spending for the analysis of PFS is foreseen.

	The main analysis will be conducted after 210 progression and/or death events are observed. The PFS assessed by the IRC will be used for the primary analysis, while the Investigator assessment will be used for the secondary analysis of PFS. An interim analysis of OS will be performed concomitantly with the final PFS analysis. A final analysis of OS will be performed when 80% of death events (approximately 200 death events) have occurred, or 24 months after the inclusion of the last patient, whichever occurs first. **Note:** Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A (plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death events are not achieved on 19 November 2017, the study might be further extended by three to six months. The unstratified log-rank test will be used to compare the PFS of Arm A and B. A stratified log-rank test for the main endpoint (PFS) will be performed as supportive analysis. Secondary time-to-event endpoints (DR, OS) will be analyzed according to the Kaplan-Meier method and compared between treatment groups using the log-rank test. Counts and percentages, with their corresponding exact 95% confidence intervals, will be calculated for the response rate (IMWG criteria and, separately, minor response). The Fisher's exact test will be used to compare the RRs of Arm A and B. Both the IRC and Investigator's assessments will be used for RR, PFS and DR. Efficacy parameters vs. baseline covariates will be analyzed and appropriate tests will be used (i.e., Fisher's exact test and logistic regression for categorical variables; the log-rank test or Cox regression for time-to-event variables, etc.). Exploratory intrapatient comparison of response and PFS (before and after crossover) will be performed for patients who switch from Arm B to Arm A after disease progression.
ANTICIPATED STUDY DATES	▪ Protocol submission: 1Q2010. ▪ Planned start date (first patient on study): 2Q2010. ▪ Complete enrollment: 60 months (2Q2010-2Q2015). ▪ Planned study termination date: it will be set after the occurrence of 80% of death events, 24 months after the accrual of the last randomized patient, or IDMC recommendation (whichever occurs first). **Note:** Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A (plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the

	duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death events are not achieved on 19 November 2017, the study might be further extended by three to six months. The futility analysis is foreseen after 40 patients evaluable for response are recruited in Arm A, approximately 12 months after the recruitment start. All patients on active treatment at the date of study termination will be offered to continue to receive plitidepsin-based treatment off-study according to the Investigator's criteria and upon Sponsor agreement.

SCHEDULE OF ASSESSMENTS AND PROCEDURES

PROCEDURE	Pre-treatment	Treatment First cycle				Treatment Subsequent cycles				End of treatment	Follow-up [16]
		1	8	15	22	29=1	8	15	22		
Written informed consent	Before any procedures	-	-	-	-	-	-	-	-	-	-
Demographic data	-14 to 0	-	-	-	-	-	-	-	-	-	-
Medical history/ Baseline condition	-14 to 0	-	-	-	-	-	-	-	-	-	-
Primary diagnosis / Prior treatment(s)	-14 to 0	-	-	-	-	-	-	-	-	-	-
Assessment of signs and symptoms[1]	-14 to 0	-	-	-	-	-	-	-	-	-	-
Complete physical examination, including weight and height[1] and BSA.	-14 to 0	-	-	-	-	●	-	-	-	●	-
Performance status (ECOG)	-14 to 0	●	-	●	-	●	-	●	-	●	●
Vital signs (heart rate, blood pressure, temperature)	-14 to 0		-	●	-	●	-	●	-	●	-
Hematology[2,3]	-7 to 0	-	-	●	-	●	-	●	-	●	-
Coagulation tests[2,4]	-7 to 0	-	-	-	-	●	-	-	-	●	-
Biochemistry A[2]	-7 to 0	-	-	●	-	●	-	●	-	●	-
Biochemistry B[2]	-7 to 0	-	-	-	-	●	-	-	-	●	-
Pregnancy test (if applicable)[5]	-7 to 0	Repeat if applicable								-	
ECG (before treatment on both arms) [6]	-7 to 0	●	-	●	-	●	-	●	-	●	-
LVEF by ECHO or MUGA [7]	-14 to 0	Every 12 weeks (± 2 week tolerance)								●	-
Pharmacokinetics [8]	-	●		●		●	-	-	-	-	-
Intercurrent events, concomitant diseases and treatments	-14 to 0	Throughout study									-
Adverse events	NA	Throughout study									● [14]
Local serum and Urine proteins[9]	-28 to 0	-	-	-	-	-	-	-	-	-	-
Central Lab Serum protein [10]	NA	●	-	-	-	●	-	-	-	●	● [15]
Central Lab Urine protein [11]	NA	●	-	-	-	●	-	-	-	●	● [15]
Serum β-2 microglobulin	-14 to 0	Every 8 weeks								● [15]	-
Bone marrow [12]	-28 to 0	To confirm a presumed CR								If clinically indicated	If clinically indicated
Radiological tumor assessment only if soft tissue plasmacytoma is present at baseline [13]	-28 to 0	Every 8 weeks or if response is observed or if clinically indicated								●	● [13]
Skeletal evaluation [17]	-28 to 0 (+1 week tolerance)	If clinical symptoms suggest new bone lesions								●	If clinically indicated
Plitidepsin treatment (Arm A only)	-	●	-	●	-	●	-	●	-	-	-
Dexamethasone treatment (both arms)	-	●	●	●	●	●	●	●	●	-	-
QT substudy [18]	●	See Appendix 8								-	

A 2-day window is allowed for Hematology, Biochemistry A, Serum Protein, Urine Protein and Serum β-2 Microglobulin.

A 5-day window is allowed for Biochemistry B and Coagulation Tests.

A 2-week window is allowed for radiological tumor assessment.

A 2-week window is allowed for LVEF assessment by ECHO or MUGA.

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

1. Repeat prior to first infusion, if treatment will be administered more than 2 weeks after the screening tests.
2. Repeat prior to first infusion, if treatment will be administered more than 1 week (2 days tolerance) after the screening tests. If CTCAE Grade ≥3 occurs, the abnormal test(s) should be assessed at least every 2-3 days until recovery.
3. At least every 2-3 days in the case of grade 4 non-febrile neutropenia and every day in case of grade 4 febrile neutropenia.
4. Close monitoring of patients taking oral anticoagulants is required.
5. Serum human chorionic gonadotropin (HCG). Repeat during treatment if applicable, always prior to study drug administration.
6. It should allow rhythm definition (at least 30 seconds of duration). Before each study drug administration, symmetrically on both arms. After the first two cycles, ECG will be performed only on Day 1 of each cycle.
7. During treatment, LVEF (when due) will be performed within 7 days of plitidepsin administration, except in non-primarily cardiac origin acutely ill hospitalized patients, until resolution.
8. Plitidepsin pharmacokinetics (PK) will be evaluated in at least 100 patients receiving both plitidepsin and dexamethasone. A total of 13 blood samples will be collected during the first three infusions [infusions 1 and 2 of Cycle 1 (Days 1 and 15), and infusion 1 of Cycle 2 (Day 1)] using a sparse sampling schedule. Last sample will be collected immediately previous to infusion 2 of Cycle 2 (Day 15) (see details in Table 5).
9. Serum and urine protein analysis performed at the institution to assess/document disease relapse after last treatment will be used for screening (tests performed within 4 weeks prior to registration will be allowed).
10. Serum protein electrophoresis, immunoglobulins quantification, M-protein identification and quantification, serum free light chains (sFLC) ratio and immunofixation will be performed in the Central Lab starting on Day 1 of Cycle 1 prior to treatment (complete protein panel). Patients with secretory MM will be followed with serum electrophoresis, immunoglobulins quantification and M protein quantification; patients with oligo or non-secretory MM will be followed with sFLC ratio, in case it has been abnormal at study entry; in case of M protein disappearance, immunofixation will be done to confirm CR, and if CR is confirmed, sFLC ratio will be performed, to determine stringent complete response (sCR). All protein assays will be done symmetrically every four weeks (i.e., Day 1 of each 4-week cycle, with a window of ± 2 days) in both treatment arms, irrespectively of treatment delays or omissions.
11. Urine immunoelectrophoresis and immunofixation in Central Lab: on Day 1 of Cycle 1, and afterwards, only if Bence-Jones proteinuria was present at entering the study. To be done symmetrically every 4 weeks (with a window of ± 2 days) in both treatment arms, irrespectively of treatment delays or omissions.
12. At screening (within 4 weeks prior to registration). In all patients, BM evaluation is mandatory at screening and while on treatment in case of CR (including flow cytometry or immunohistochemistry, to demonstrate the presence of clonal plasma cells, only in those patients with presumed CR). In patients with non-secretory MM, it must be repeated 6 weeks later to confirm response or as clinically indicated.
13. In case of measurable soft tissue plasmacytoma, involvement assessments should be done to evaluate all known affected sites every 8 weeks to confirm response or as clinically indicated. In truly non-secretory patients who discontinued treatment without progression, and only evaluable by measurable plasmacytomas, radiological assessments should continue every 8 weeks until progression.
14. Patients withdrawn with a drug-related AE should be followed until recovery. Beyond 30 days after the last administration of study drug, only those procedures that are relevant to any persisting toxicities need to be performed.
15. Only in patients who discontinued treatment without documented disease progression.
16. Bone marrow evaluation, radiological tumor assessment and skeletal evaluation will be performed only on pre-treatment and treatment evaluations. These evaluations will not be performed after the end of treatment. After the end of treatment, all patients will be followed every three months for survival until death, or until the date of study termination. The follow-up visits will be performed by phone only if the patient is unable to go to the study center owing to the seriousness of his/her disease. This will only apply to patients who are followed up after discontinuing treatment due to disease progression.
17. May be evaluated using CT-scan or X-ray, provided the same procedure is used throughout the study.
18. Optional substudy (a separate ICF needs to be signed).

Note: One patient was still undergoing treatment on 19 May 2017. However, as the primary endpoint of the study (progression-free survival) was fulfilled, and this extended follow-up is focused only on survival, no information such as central laboratory data or images will be sent for independent review. Nevertheless, laboratory determinations or images will be done locally, if clinically indicated, in order to perform tumor assessment.

Hematology: Differential white blood cells (WBC), hemoglobin, hematocrit and platelets.

Biochemistry A: AST, ALT, total bilirubin, direct bilirubin in case total bilirubin is above ULN, alkaline phosphatase (AP), creatinine, calculated creatinine clearance (Cockcroft and Gault´s formula; see Appendix 3), glucose, serum electrolytes (Na^{+}, K^{+}, Mg^{++}, Ca^{++}), creatine phosphokinase (CPK), CPK-MB fraction (should be measured only if CPK is abnormally high) and troponin I.

Biochemistry B: Uric acid, lactate dehydrogenase (LDH), total proteins and albumin.

Coagulation tests: PT, PTT and INR.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ABP	Arterial Blood Pressure
AE(s)	Adverse Event(s)
ALL	Acute Lymphoblastic Leukemia
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ANOVA	Analysis Of Variance
AP	Alkaline Phosphatase
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
AST	Aspartate Aminotransferase
AUC	Area Under The Curve
βhCGs	Beta Human Chorionic Gonadotrophins
BM	Bone Marrow
B_{max}	Maximal Plitidepsin Concentration into Red Blood Cells
BP	Blood Pressure
BPM	Beats Per Minute
BSA	Body Surface Area
CBP	CREB binding protein
CDK	Cyclin-dependent Protein Kinase
C	Cycle
CI	Confidence Interval
CL	Clearance
C_{max}	Maximum Plasma Concentration
CPK	Creatine Phosphokinase
CPK-MB	Serum CPK Isoenzymes (Found In Cardiac Muscle)
CR	Complete Response
CrCl	Creatinine Clearance
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
CT-scan	Computed Tomography Scan
CV	Cardiovascular
d/D	Day(s)
DI	Dose Intensity
DLT	Dose-Limiting Toxicity
DNA	Deoxyribonucleic Acid
DR	Duration of Response
ECG	Electrocardiogram

ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EOI	End of Infusion
EPO	Erythropoietin
ER	Endoplasmic Reticulum
EU	European Union
GC	Glucocorticoids
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GGT	γ-Glutamyltranspeptidase
GI	Gastrointestinal
GM-CSF	Granulocyte/Macrophage Colony Stimulating Factor
GMT	Greenwich Mean Time
GR	GC Receptor
GSH	Gluthatione Reduced
h	Hour(s)
HAT	Histone Acetyltransferase
Hb	Hemoglobin
HBV	Hepatitis B Virus
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HDAC	Histone Deacetylase
HIV	Human Immunodeficiency Virus
HBR	Heart Beating Rate
HR	Hazard Ratio; Heart Rate
IB	Investigator Brochure
IC_{50}	Half Maximal Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committees
Ig	Immunoglobulin
IMWG	International Myeloma Working Group
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
IRB	Institutional Review Board
IRC	Independent Review Committee
ISS	International Staging System
i.v.	Intravenous

IVRS	Interactive Voice Response System
ITT	Intention-To-Treat
K_d	Plitidepsin Plasma Concentration at which the Plitidepsin Bound to Red Blood Cells is Half-maximal
LDA	Low Density Array
LDH	Lactate Dehydrogenase
LLN	Lower Limit of Normal
LVEF	Left Ventricular Ejection Fraction
MEF	Mouse Embryonic Fibroblast
MM	Multiple Myeloma
MP	Melphalan plus Prednisone
MR	Minor Response
MRI	Magnetic Resonance Imaging
ms	Milliseconds
MTD	Maximum Tolerated Dose
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUGA	Multiple-gated Acquisition Scan
NCI	National Cancer Institute
NCI-CTC	National Cancer Institute Common Toxicity Criteria
NHL	Non-Hodgkin Lymphoma
NSCLC	Non-Small Cell Lung Cancer
ORR	Overall Response Rate
OS	Overall Survival
PCR	Polymerase Chain Reaction
PD	Progressive Disease; Pharmacodynamics
PFS	Progression-free Survival
PK	Pharmacokinetics
p.o.	Per os, Oral(ly)
PR	Partial Response
PS	Performance Status
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PTCL	Peripheral T-Cell Lymphoma
Q_2	Intercompartmental Exchange Flow for the Shallow Compartment
Q_3	Intercompartmental Exchange Flow for the Deep Compartment
q4w	Every Four Weeks
qPCR	Quantitative Polymerase Chain Reaction
RBC	Red Blood Cell
RD	Recommended Dose
RNA	Ribonucleic Acid

RP-HPLC	Reversed-Phase High Performance Liquid Chromatography
RR	Response Rate
RT	Plasma-to-blood Ratio; Reverse Transcription, Room Temperature
SAE(s)	Serious Adverse Event(s)
SCLC	Small Cell Lung Carcinoma
sCR	Stringent Complete Response
SCT	Stem Cell Transplantation
SD	Stable Disease
sFLC	Serum Free Light Chains
SOI	Start Of Plitidepsin Infusion
SPC	Summary of Product Characteristics
$t_{1/2}$	Half-life
TdP	Torsades de Pointes
TSA	Trichostatin A
TTP	Time to Tumor Progression
ULN	Upper Limit of Normal
UV light	Ultraviolet Light
US	Ultrasound (Cardiac)
V_1	Central Volume of Distribution
V_2	Volume of Distribution of the Shallow Compartment
V_3	Volume of Distribution of the Deep Compartment
VEGF	Vascular Endothelial Growth Factor
VEGFR-1	Vascular Endothelial Growth Factor Receptor Type-1
VGPR	Very Good Partial Response
V_{ss}	Volume of Distribution in Steady State
WBC	White Blood Cells

1 INTRODUCTION

Despite recent advances in the treatment of multiple myeloma (MM), most patients ultimately relapse. The disease remains incurable and there is an urgent need for developing new therapeutic options including investigational drugs. The addition of corticosteroids to active new investigational drugs in MM is a major way to improve the outcome, particularly because this agent has non-cross-resistance and a different toxicity profile when compared to other agents commonly used for MM treatment.

1.1 OVERVIEW OF THE DISEASE

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 (NCI) indicates that, in the USA, 19,920 new cases of MM will be diagnosed in 2009 and 10,690 patients will die from their disease (1). Similar figures occur in Europe, with an incidence of 6.0/100,000 habitants per year and a mortality rate of 4.1/100,000 habitants per year (2). In the Western hemisphere, about 1% of cancer-related deaths are due to myeloma. Traditionally, MM has been staged according to the Durie-Salmon system, based on the amount of abnormal monoclonal immunoglobulin in the blood or urine; blood calcium levels; the amount of bone damage shown by X-rays, blood hemoglobin levels, and renal function (3). More recently, the International Staging System (ISS), which relies on the levels of albumin and beta-2-microglobulin, has been validated (4). Nevertheless, though both systems are based on easily obtained clinical and laboratory parameters, and predict for survival, it has recently been suggested that newer parameters should be included in the prognostic initial evaluation of MM patients (5, 6). The present recommendation is to include both classifications in clinical trials, as they provide complementary information (6).

Recent advances have identified new prognostic markers, such as the complete deletion of chromosome 13 or its long arm as detected by karyotyping, the t(4;14) or t(14;16) translocations, and the increased bone marrow microvascular density.

The disease primarily affects elderly individuals, with a median age of 60 years at diagnosis. From the time of diagnosis, the survival without treatment is between 6 to 12 months and extends to three years with chemotherapy. MM is treatable but rarely curable. Most patients receive multiple treatments over the course of their disease, and the precise sequence of therapy and regimens used can be quite variable. With standard-dose chemotherapy, patients have a median survival of 24–30 months. Twenty-five percent of patients survive five years or longer, and the 10-year survival is approximately 3% (1).

1.2 CURRENT TREATMENT FOR MULTIPLE MYELOMA

Failure of standard-dose chemotherapy to cure this disease has led to study the use of dose intensified chemotherapy regimens. Conditionings may involve ablative or reduced intensity, non-myeloablative regimens, and may be followed by autologous or allogeneic stem-cell rescue. Despite recent advances in supportive care measures, the morbidity and mortality related to intensified chemotherapy regimens are still of concern, particularly in the aged and frail population; therefore, this treatment option has been reserved only for younger patients with adequate performance status (7).

Currently the standard treatment for suitable MM patients aged <65 years is induction chemotherapy followed by autologous stem-cell transplantation (SCT), which has been associated with increased response rates (complete response of 30-40%), overall survival (54-62 months) and event-free survival (25-43 months) when compared to traditional chemotherapy (7), although these results are currently being challenged by the high response rates achieved with the introduction of the so called "novel agents", which are increasingly being used as part of first-line combinations.

In elderly patients, namely those over 65 years of age, SCT has been associated with unacceptably higher morbidity and mortality. Therefore, more conservative approaches, such as the use of standard-dose melphalan plus prednisone (MP) or prednisone alone (but at higher doses) have become a widely-accepted standard of care (8). However, MP is now challenged, as new therapeutic options like thalidomide, lenalidomide and bortezomib, initially approved in the relapsed/refractory setting, are now moving upfront as part of new combination regimens. Two recently finished randomized trials have shown the superiority of adding thalidomide to MP compared to MP alone in elderly patients (9, 10). Likewise, the VISTA trial has shown superiority when bortezomib was added to MP in a similar patient population (11).

In patients with relapsed or refractory MM, thalidomide, lenalidomide and bortezomib have been used as single agents, with responses of 30%, 38% and 27%, respectively (12-14). However, in these and other studies evaluating therapies in relapsed/refractory MM patients (15-18), progression-free survival (PFS) and time to progression (TTP) were in the range of 4-9 months, thus demonstrating a step forward, but also the limitations and the current need for new therapeutic options in this truly palliative disease setting.

Despite all these therapeutic advances, MM remains an incurable disease. No standard treatment is available and therapeutic options are limited, thus representing a clear unmet medical need. Options may include palliative care, steroid treatment, oral alkylating agents, or participation in clinical trials. Of note, a previous exploratory phase II trial in which plitidepsin was administered with or without the addition of dexamethasone has provided evidence of activity in this patient population (see details in Section 1.3.3.3).

In conclusion, all these innovative approaches have significantly changed the available therapeutic options, especially in elderly patients and in patients with relapsed/refractory MM, as they are not likely to show cross-resistance or the toxicities commonly seen with traditional chemotherapy regimens based on alkylators and anthracyclines. Nonetheless, newer toxicity patterns are also emerging, such as an increased thrombotic risk and neurotoxicity, which may be cumulative. These new drugs are being combined with classic agents, such as melphalan, prednisone and anthracyclines, as an upfront treatment; however, their exact role and sequence in the changing landscape of MM therapy remains to be defined.

1.2.1 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 mechanism of action by which plitidepsin inhibits cell growth and/or induces cell death remains to be fully characterized, the major effects of plitidepsin can be at least partially attributed to a cell cycle block in the G0/G1 phases and the induction of apoptosis (19-21) 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 caspases-dependant 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 (22). It seems that the majority of the pharmacologic 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 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 bone marrow reserve capacity. Consequently, plitidepsin may display a positive profile for combination with other agents in chemotherapy regimens, avoiding overlapping toxicity.

1.3 INFORMATION ON STUDY DRUG: PLITIDEPSIN

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 (23), and a rapid and sustained activation of JNK, p38 MAPK and ERK in human breast cancer cells (MDA-MB-231) (24). 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 (21). 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 (24). Plitidepsin effects on the Rac1, a pleiotropic regulatory protein, would contribute to the sustained activation of JNK (19, 25). 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 (26). 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 (24, 25), thus demonstrating a common mechanism in cells of different tumor origins. Latest studies have shown that plitidepsin is able to increase levels of cell membrane phospholipid oxidation and deoxyribonucleic acid (DNA) oxidation in vitro (19).

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 (22), suggesting that the block of cell growth might be mediated by dual inhibition of VEGF autocrine loop. Supporting these findings, plitidepsin has been shown highly cytotoxic on acute myelogenous leukemia cells, both on regular cultures (K-562, HEL and HL-60) and on blasts obtained from patients (27). 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 (28).

In summary, it appears that the pharmacologic 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 *In vitro and in vivo Data as Single Agent*

In vitro studies demonstrated antiproliferative activity against a broad spectrum of tumor types, namely bladder cancer, breast cancer, stomach cancer, lung cancer, melanoma, neuroblastoma, prostate cancer, thyroid cancer (with IC_{50} values ranging from 10^{-7} to 10^{-9} M), and leukemia, myeloma and lymphoma (with IC_{50} values ranging from 10^{-8} to 10^{-9} M) (29).

In addition, a model in MM has been explored (30). Plitidepsin exhibited, at clinically achievable concentrations, 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 tumors cell lines such as lung, breast, thyroid, prostate, stomach, bladder, and kidney, as well as human malignant cell lines of hematological origin.

In the hollow fiber *in vivo* model, in which athymic rats were treated with i.v. plitidepsin, tumors of the bladder, stomach, and prostate were shown to be susceptible

to the drug. In xenograft models, activity was noted against human renal and pancreatic tumors when injected to athymic mice.

Additionally, the antitumor and antiangiogenic effects of plitidepsin were evaluated in the 5T33MM syngeneic orthotopic model of MM (31). 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 (32).

1.3.2.3 *In vitro and in vivo Data in Combination*

Clinical experience in the management of MM patients supports the concept that drug combinations induce higher response rates 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 combination of plitidepsin with dexamethasone that was selected for further development in solid tumors and preclinical data is summarized below.

1.3.2.3.1 Non-clinical Data of the Plitidepsin-Dexamethasone Combination

The combination of plitidepsin with dexamethasone was explored regarding the viability of MM1.S and U266-LR7 cells. Both non-constant ratio (for suboptimal doses of drugs) and constant ratio experimental designs were used. The effects of single and combined treatments after 72 hours were evaluated by MTT assays. The results clearly show that plitidepsin increased the anti-MM effect of dexamethasone. The combination was additive but tended to be synergistic at higher doses (30) (Figure 1).

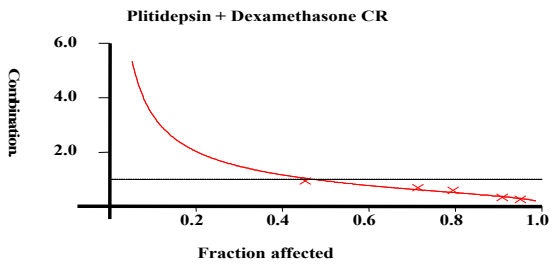

Figure 1. MM1.S cell line, 48 h incubation

Similar results were obtained in a study by Medina *et al.* (Internal Report, March 2008), where the combination of plitidepsin and dexamethasone (at a fixed plitidepsin to dexamethasone ratio of 1:50) was tested in four MM cell lines (MM1.S, ARP, RPMI-

8226 and MM1.R (dexamethasone-resistant). As shown in Figure 2, the combination (Apl+Dex) resulted in a significant increase in cell toxicity when added to the MM cell lines MM1.S, ARP, and RPMI-8226 compared to either drug alone (Apl/Dex). In contrast, only plitidepsin had an effect on the dexamethasone-resistant cell line, MM1.R.

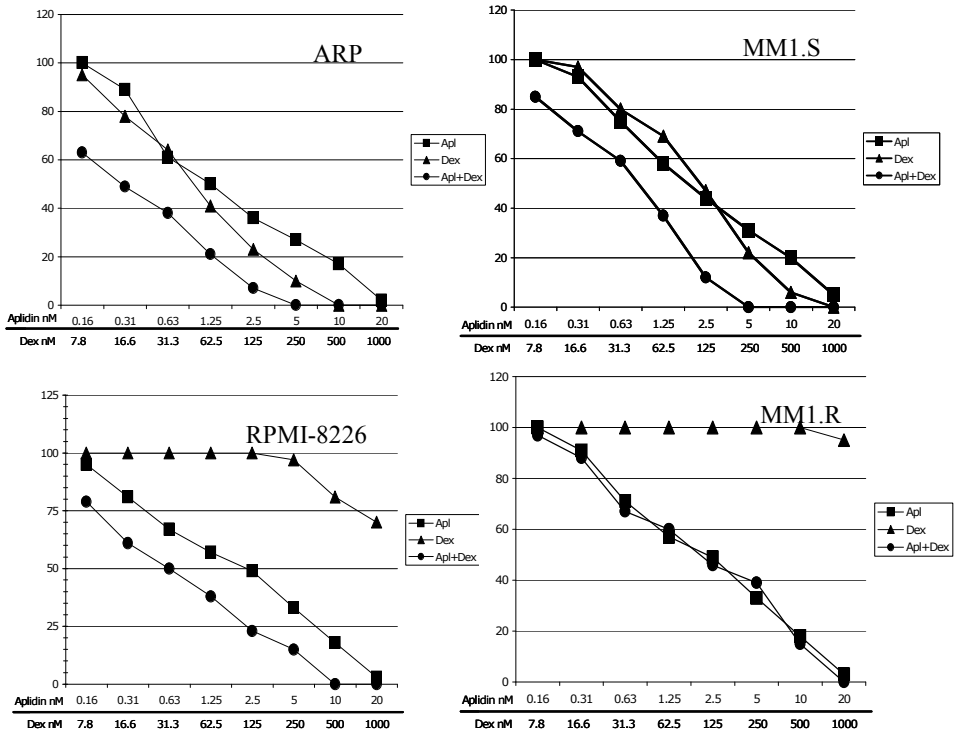

Figure 2. Activity of the combination plitidepsin + dexamethasone in MM cell lines

This combination was synergistic for the cell lines MM1.S, ARP, and RPMI-8226 at all tested (nanomolar) concentrations of the drugs, while it was additive for MM1.R at high concentrations.

1.3.2.3.2 Molecular Rationale Based on Dexamethasone Mechanism of Action and Similarities with Plitidepsin Activity.

The pleiotropic molecular effects elicited by the plitidepsin treatment do not allow concluding which pathways are the most important for its antitumor activity; however, they offer a potentially unique mechanism of action 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 results 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 (33-35). 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 (35-38).

Thus, dexamethasone acts over genes responsible for the induction of apoptosis in lymphoid cells, in which seems a plitidepsin complementary pathway, therefore enhancing plitidepsin cytotoxicity (39-42).

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 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) (36, 43).

On the other hand, both agents have been found to disrupt 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 caspases cascade.

Moreover, plitidepsin-induced suppression of caspases inhibitors (FLIP, survivin) may contribute to the increased sensitivity of plitidepsin-treated MM cells to caspases-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 (44). Likewise, both had a synergistic effect on NF-kappaB (survival transcription factor) and its inhibitor (45, 46).

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 (35).

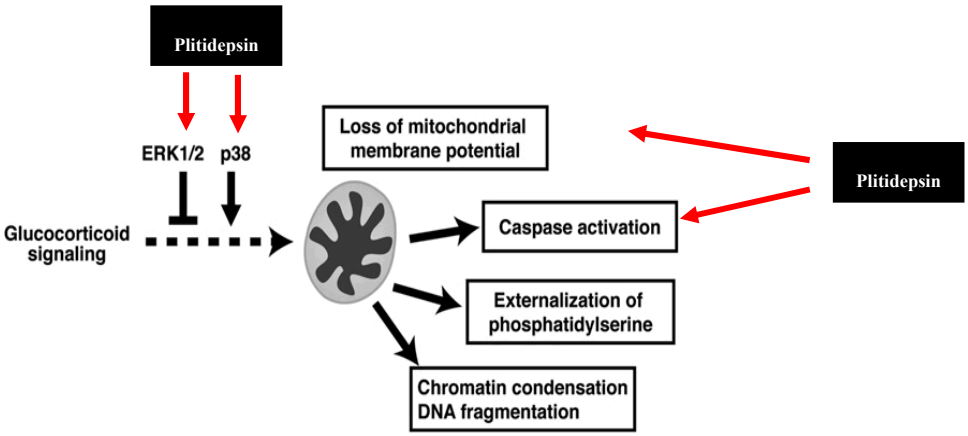

Figure 3. Plitidepsin-dexamethasone molecular model of apoptosis

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

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 (47, 48). Both complexes are closely related to chromatin remodeling and consequently, to the modulation of gene expression induced by dexamethasone.

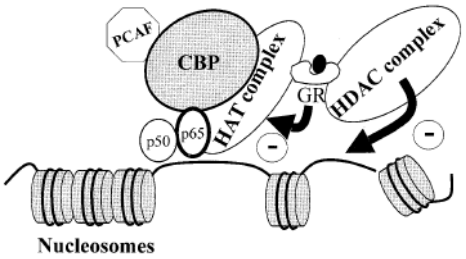

Figure 4. Glucorticoids and the chromatin complex

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

Therefore, histone H4 K5 acetylation can be considered as a marker of dexamethasone transactivation. Dexamethasone predominantly targeted acetylation on histone H4 K5 and K16 in all subjects. However, 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 same complex in the presence of p65 and GR and that they can each act independently. This mechanism for glucocorticoid repression is novel and 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 its use in vivo at the clinical setting. HDACs were also pointed out to 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 at random.

1.3.2.4 *Toxicology*

Plitidepsin, given by i.v. injection in daily 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. The main organs

additionally affected 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) (49). These and other data (50) indicate that plitidepsin might be a potential compound in multidrug chemotherapy regimen, assuming that it does not increase hematotoxicity significantly, which is often the dose-limiting toxicity of these regimens.

1.3.2.5 *Safety Pharmacology*

1.3.2.5.1 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. Infusion of plitidepsin at doses up to 0.03 mg/kg (0.6 mg/m^2) did not affect ABP. HBR increased from 2 to 48 hours 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 hours after discontinuation of the plitidepsin infusion.

1.3.2.5.2 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 hours post-dosing with 1.50 mg/kg (9 mg/m^2).

1.3.2.5.3 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

To date, plitidepsin has been evaluated in adult patients as single-agent or in combination with other antineoplastic drugs, and as single-agent in pediatric patients.

As of March 2009, more than 700 patients had been recruited into clinical trials with plitidepsin (PharmaMar internal data):

- A phase I program using single-agent plitidepsin in adult patients with solid malignancies or non-Hodgkin lymphoma (NHL) has been completed.
- There are several finished or ongoing studies of plitidepsin in combination with other agents:
  - Phase I trial of plitidepsin with carboplatin in patients with advanced solid malignancies or lymphoma: completed.
  - Phase Ib trial of plitidepsin in combination with bortezomib and dexamethasone in relapsed/refractory multiple myeloma: closed recruitment.
  - Phase Ib trial of plitidepsin in combination with sorafenib or gemcitabine in patients with solid tumors or lymphomas: open recruitment.
  - Phase Ib trial of plitidepsin in combination with bevacizumab or docetaxel in patients with solid tumors: open recruitment.
  - Phase I-II trial of plitidepsin in combination with dacarbazine as first-line treatment in patients with unresectable advanced melanoma: the phase I part has been already completed.
- Phase II studies with single-agent plitidepsin have been conducted to evaluate its activity against different solid tumors and hematological malignancies.

Particularly of interest is a phase II trial of plitidepsin alone and/or with the addition of dexamethasone in patients with suboptimal response in relapsed/refractory multiple myeloma that has been recently completed. The final results of this trial were presented during the American Society of Hematology (ASH) meeting in December 2008 in San Francisco, USA (Abstract # 3700).

1.3.3.1 *Single-Agent Phase I Trials*

Results obtained in single-agent phase I trials in adult patients are summarized in Table 1.

Table 1. Plitidepsin: single-agent 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. patients	35	27	49	47	20	37
Highest dose (mg/m^2)	4.50	6	3.60	7	8	1.50
DLTs	muscular hepatic	muscular hepatic	muscular	muscular	muscular flu-like	muscular emesis skin rash diarrhea
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

24 h i.v. d 1,8,15 q4w: 24-hour intravenous infusion for three weeks every four weeks; c.i.v.i.: continuous intravenous infusion; DI: Dose intensity; DLT, dose-limiting toxicity; NCI-CTC, National Cancer Institute Common Toxicity Criteria; RD: recommended dose.
Hepatic = Increased values of serum enzymes (grade 3-4 CTCAE)
Muscular = myalgia, muscular weakness or elevated CPK (grade 3-4 NCI-CTC)

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 DLTs, without unexpected toxicities independently of schedule.

1.3.3.2 *Single-Agent Phase II Trials*

As single-agent in phase II clinical trials and up to March 2009, plitidepsin has been administered to patients with:

- Unresectable advanced renal cancer or advanced colorectal cancer in objective progression (81 patients).
- Unresectable advanced medullar thyroid carcinoma (16 patients).
- Unresectable advanced carcinoma of the exocrine pancreas (19 patients).
- Advanced non-small cell lung cancer (NSCLC) progressing after first-line chemotherapy (21 patients).
- Advanced or metastatic transitional cell carcinoma of the urothelium relapsing or progressing after first-line chemotherapy (21 patients).
- Small-cell lung carcinoma (SCLC) relapsing or progressing after first-line chemotherapy (19 patients).
- Advanced or metastatic melanoma relapsing or progressing after first-line systemic therapy (37 patients).
- Locally advanced or metastatic squamous cell carcinoma of the head and neck (10 patients).
- Relapsing or refractory androgen-independent prostate adenocarcinoma (8 patients).
- Relapsed or refractory indolent NHL (8 patients).
- Relapsed or refractory aggressive NHL (51 patients).
- Relapsed or refractory multiple myeloma (51 patients).

- Relapsed or refractory acute lymphoblastic leukemia (ALL) (17 patients).

The results from completed and ongoing phase II trials confirmed the preliminary safety profile described from the subset of patients treated at the proposed RD in phase I trials. For the 255 patients treated with plitidepsin in the dose-escalating phase I studies, most of the AEs and laboratory abnormalities which constituted DLTs consisted of transient liver biochemical disorders without clinical manifestations and muscular events, mainly myalgia, muscular weakness and creatine phosphokinase (CPK) elevations, without myoglobin increase or signs/symptoms of rhabdomyolysis. These events were in most cases mild, and reverted upon dose modification or drug discontinuation. Other common events were mild emesis and diarrhea and cutaneous events (erythema, pruritus, rash). Mild hypersensitivity reactions have been reported to be probably related to the Cremophor oil contained in the formulation. Of these, less than 3% had been serious (i.e., anaphylactic shock) and all reactions have been reversible after stopping the infusion and implementation of routine symptomatic treatment. No fatal events have occurred. Yet, allergic prophylaxis (Appendix 1) has been made mandatory in all subsequent trials since 2005. Of note, hematological toxicity was almost absent from plitidepsin single agent trials.

Non-serious cardiac events (rhythm alterations, ECG abnormalities) have been occasionally reported in association with plitidepsin treatment; however, severe and life-threatening cardiovascular events were rare and causality was usually confounded by underlying disease or pre-existing conditions. No chronic or dose-cumulative cardiac toxicity has been observed so far. Regular assessment of cardiac function and cardiac parameters (i.e., troponin I) and mandatory ECG monitoring have been implemented in all ongoing and planned trials in order to closely evaluate the cardiac safety profile of plitidepsin.

Regarding efficacy, plitidepsin alone has shown consistent antitumor activity in pretreated patients with renal cancer, melanoma, neuroblastoma, non-cutaneous mature aggressive peripheral T-cell lymphoma (PTCL) and multiple myeloma. In addition clinically meaningful stabilizations (≥ 3 months) have been achieved in patients with several others solid tumor types (i.e., colorectal cancer, neuroendocrine tumors, NSCLC, hepatocellular carcinoma, etc.).

Based on the aforementioned results and its relative good tolerance, the clinical development of plitidepsin as a single agent in multiple myeloma, PTCL and melanoma, as well as combined with several agents (including dacarbazine for advanced malignant melanoma, bortezomib or lenalidomide for relapsed/refractory multiple myeloma, and sorafenib or gemcitabine for advanced solid tumors or lymphomas), is currently underway.

1.3.3.3 *Phase II Trial of Plitidepsin in Combination with Dexamethasone*

The safety and efficacy of plitidepsin in patients with relapsed or refractory MM have been investigated in a phase II study and final results have been reported (51). This was an open-label study of plitidepsin given as a 3-h infusion every two weeks, with the addition of dexamethasone allowed in patients with suboptimal response to plitidepsin alone (defined as PD after 3 cycles or SD after 4 cycles of plitidepsin). Primary endpoint was the objective response rate (ORR = CR+ PR [partial response] + MR

[minimum response]) according to strict Bladé criteria (52). Secondary endpoints were time to progression (TTP) and safety.

Between June 2004 and March 2008, 53 relapsed/refractory MM patients were included and 51 received plitidepsin-based therapy. The results showed a promising activity in this heavily treated subset of patients, resulting in an ORR of 12%, and up to 21% when dexamethasone was added (primary efficacy endpoint: ORR in the intent-to-treat population according to Myeloma Response Criteria). This benefit was also translated in terms of TTP.

Patients received a median number of four prior lines of therapy (range: 1-18) and 47 of them were evaluable for response. During the first phase of the study, 21 patients received plitidepsin as monotherapy. The overall response rate (ORR) for the first stage was 9%. During the second phase, 26 additional patients were evaluable for response. ORR to plitidepsin as monotherapy in this second cohort was 11%.

Dexamethasone was subsequently added to plitidepsin treatment in 18 patients evaluable for efficacy, being the response in this subgroup of 21%.

Overall, pooled data of all evaluable patients (n=47) receiving plitidepsin as monotherapy in the two stages resulted in an ORR of 12% (1 PR, 2 MR). Median time to progression (TTP) was 3 months (95% CI: 0.9-2.8 months) and median overall survival (OS) for the first 21 treated patients was 17 months. The addition of dexamethasone also increased median TTP to 4.2 months (95% CI: 3.4-7.7 months), which represented a 40% increase compared to plitidepsin alone (TTP of 3 months). The safety profile was acceptable and was not significantly changed after dexamethasone addition. Particularly the incidence of grade 3-4 drug-related hematological toxicity was very low, with only 11% and 21% of the patients experiencing grade 3-4 neutropenia and thrombocytopenia respectively with the combination (similar figures for plitidepsin alone)

1.3.4 Safety Issues

1.3.4.1 *Relevant Safety Information from Phase II Trials*

The results from completed and ongoing phase II trials confirmed the preliminary safety profile described from the subset of patients treated at the proposed recommended dose in phase I trials. The majority of AEs and laboratory abnormalities which constituted dose-limiting toxicities consisted of transient liver biochemical disorders without clinical manifestations and muscular events, mainly myalgia, muscular weakness and creatine phosphokinase (CPK) elevations without myoglobin increase, or signs/symptoms of rhabdomyolysis. These events were in most cases mild, and reverted upon dose modification or drug discontinuation. Other common events were mild emesis and diarrhea and cutaneous events (erythema, pruritus, and rash) or hypersensitivity reactions. Serious hematological toxicity was negligible.

Non-serious cardiac events (rhythm alterations, ECG abnormalities) have been occasionally reported in association with plitidepsin treatment, however, severe and life-threatening cardiovascular events were rare and causality was usually confounded by underlying disease or pre-existing conditions. A close monitoring of cardiac function is being implemented in ongoing trials to reassess the cardiac safety profile of plitidepsin.

1.3.4.2 *Clinical Safety*

For each AE, figures represent the frequency of its worst grade by patient and regardless of causality.

1.3.4.2.1 Muscular Adverse Events

Muscular AEs, together with CPK increases, constituted the main DLTs reported with plitidepsin when administered at the doses recommended for further development.

Most muscular AEs found in patients treated with the 1-hour infusion in the phase I trial were mild or moderate and consisted of myalgia (50%) and muscle weakness (17%), and only one grade 3-4 event was reported (muscle weakness, 7%). As for the other schedules, most muscular adverse events reported in patients in the three 1-hour infusion phase II trials, were mild or moderated myalgia (25%) and muscle weaknesses (15%). Grade $\geq$ 3 events were reported in eight occasions: two cases of myalgia and four of muscle weakness, one of muscle cramp and one of back pain (n=87).

1.3.4.2.2 Biochemical Laboratory Abnormalities

Grade $\geq$ 3 elevations of ALT and AST (aspartate aminotransferase) were reported in 27.5% in the 1-hour Day 1, 8, 15 q4w schedule in phase II trials. However these elevations were transient and reversible being asymptomatic in the vast majority of cases. Other severe biochemical disorders were rare and no remarkable differences in incidence of biochemical or metabolic abnormalities were observed as compared to other schedules.

1.3.4.2.3 Gastrointestinal Symptoms

As for phase I and phase II trials, severe drug-related gastrointestinal AEs were uncommon, and none of them reached grade 4 severity. Nausea was the most frequent gastrointestinal event. Its incidence was higher with the 24-hour infusion schedule (64%) as compared to the 3-hour (47%) and the 1-hour infusion (35%) schedules. Grade 3-4 emesis was rare in patients treated with 1-hour infusion.

1.3.4.2.4 Neurological Adverse Events

Specific neurological testing was performed only in the phase I trial evaluating the daily x 5 dosing. Test results showed a decrease in dynamometer readings in most patients without other abnormalities. There was no apparent correlation between dose level of plitidepsin and strength testing abnormalities in this trial. No grade 3 events were reported. Overall, the incidence of neurological AEs was generally low.

1.3.4.2.5 Hematological Abnormalities

As for other schedules, the most common hematological abnormality in the 1-hour Day 1, 8, 15 q4w schedule in phase II trials was hemoglobin decrease (90%). Lymphocyte decrease occurred in 66% of the patients, and thrombocytopenia was observed in 60% (26% of them were grade $\geq$ 3). However, taking into account the prior clinical experience with plitidepsin and its preclinical toxicology profile, it was concluded that most of these episodes of hematological abnormalities were not related to plitidepsin but

to the underlying disease (acute leukemia, lymphoma and multiple myeloma). In fact, several patients already suffered from leukopenia and/or thrombocytopenia at baseline. In general, these events did not worsen after plitidepsin treatment, but rather their values improved significantly in patients showing objective response to treatment.

1.3.4.2.6 Constitutional Symptoms

Asthenia was the constitutional AE most frequently found in patients in phase II studies with single-agent plitidepsin. A total of 31% of patients treated with the 1-hour infusion had asthenia, with 8% of cases being grade 3-4. However, its significance may be confounded, since the underlying malignancy is frequently a cause of asthenia in cancer patients.

Other severe AEs reported within this schedule were grade 3 edema in one patient (1%) and pyrexia also in one (1%) patient. Mild grade 1-2 pyrexia was reported in other schedules, particularly in the 3-hour i.v. infusion every two weeks (69% of patients). It was usually associated with shivering and developed within 24 hours after the start of the infusion.

One patient had multi-organ failure and died, most likely as a consequence of the underlying disease.

1.3.4.2.7 Infusion Site Events

When central venous access was not used, local problems related to drug administration were frequent, including erythema, pain during infusion and phlebitis.

Injection site reactions were more common in patients receiving plitidepsin as a 24-hour infusion than with any other schedule. Shortening the infusion time was associated with a marked reduction in the incidence of injection site reactions. A total of 2% of patients reported infusion site reactions with the 3-hour infusion and 5 mg/m^2 dose (1/3 of which were grade ≥ 3), and 1% of patients with mild-moderate events were found with the 1-hour infusion schedule. In all cases, plitidepsin should be administered through infusion pumps with special filter-containing infusion lines, via central venous access.

1.3.4.2.8 Cardiac Safety

An update of the safety information on cardiac events occurred during clinical trials evaluating plitidepsin as single-agent treatment in adult patients with advanced solid tumors or hematological malignancies was done using a cutoff for data analysis of 20 November 2008 (see Investigator Brochure). Forty-six of the 578 patients (8.0%) treated with plitidepsin as a single agent up to that cutoff date had at least one cardiac AE. Eleven of these 46 patients (1.9% of the total of 578 patients) had cardiac AEs related to plitidepsin and eight among them were reported as serious adverse events (SAEs). The majority of cardiac events occurred in phase II studies (37 of 46 for overall events), and particularly in two studies, APL-B-003-02 (pancreatic cancer) and APL-B-011-02 (prostate cancer) with a higher than expected incidence.

Cardiac AEs observed to date in plitidepsin trials are clinically heterogeneous; the most frequent type is harmless rhythm alterations, as no life-threatening ventricular arrhythmias occurred, while the rest of events are relatively infrequent. No fatal

outcome was reported as a consequence of all cardiac AEs analyzed. Relevant predisposing factors identified in univariate and multivariate analyses are mostly related with patient's baseline characteristics and disease-related characteristics rather than with drug exposure or treatment-related characteristics, the analyzed cardiac events do not follow a dose cumulative pattern of occurrence, and none among all PK parameters explored did show a correlation, although other potential relevant factors remain yet to be identified and further characterized. As of 20 November 2008, with data available on 578 adult advanced cancer patients treated with singe-agent plitidepsin, cardiac safety does not seem to be of special concern.

In order to fully monitor cardiac safety, additional cardiovascular explorations have become mandatory in all current and future plitidepsin protocols, including serial ECG and troponin I determination and periodic left ventricular ejection fraction (LVEF) measurement by echocardiogram (ECHO) or multiple-gated acquisition scan (MUGA) testing. In addition, patients with a cardiovascular background or any prior interventions predisposing to cardiac toxicity are excluded from these protocols.

1.3.4.2.9 Conclusions on Safety Results

Plitidepsin alone or in combination with carboplatin, dacarbazine or dexamethasone has a tolerable safety profile with most AEs mild to moderate and usually reversible upon drug reduction or discontinuation. The negligible hematological toxicity makes this compound a good candidate for combination with standard chemotherapy.

Overall, the currently available clinical data provide reassurance that the toxicity of plitidepsin appears to be acceptable in the clinical setting.

1.3.4.3 *Summary of Pharmacokinetic Results*

Pharmacokinetic data have been collected from patients in phase I and II studies following i.v. administration of plitidepsin using the following schedules: 24-hour infusion every week (Day 1 and 15 q4wk), 3-hour infusion every other week (Day 1 and 15 q4wk), 1-hour infusion every week (Day 1, 8, 15 q4wk), 24-hour infusion every other week (Day 1 and 15 q4wk) and 1-hour infusion for five consecutive days every three weeks (Day 1-5 q3wk).

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, thus 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, with samples collected at later time points than in the phase I trials. Furthermore, patients included in phase II trials had samples taken for pharmacokinetic (PK) evaluation during both the first and third treatment cycle, allowing for a more extensive analysis. Population methodology was used, as the phase II studies had sparse sampling.

Thus, the population PK analysis of plitidepsin was performed based on data from 13 clinical studies, including all four adult patients, single-agent phase I studies (APL-A-001-98, APL-A-002-98, APL-A-003-98 and APL-A-004-98) and nine phase II studies

(APL-B-001-01, APL-B-002-02, APL-B-005-02, APL-B-006-02, APL-B-007-02, APL-B-011-02, APL-B-013-02, APL-B-014-03, APL-B-015-04), using the NONMEM software. Blood and plasma concentration-time data from phase I studies with extensive sampling were pooled with sparse PK sampling from patients with advanced cancer included in phase II studies. In these studies, patients received i.v. plitidepsin as monotherapy at doses ranging from 0.13 to 8.0 mg/m^2 and given as 1-hour or 24-hour infusions weekly; 3-hour or 24-hour q2wk, or 1-hour infusion daily for five consecutive days every 21 days. In total, 411 patients and 5585 plitidepsin concentration measurements, including 2900 blood and 2685 plasma concentrations, were pooled in the population PK analysis.

An open, three-compartment disposition model with linear elimination and linear distribution from the central compartment to peripheral compartments was used to describe the PK of plitidepsin in plasma. The model was parameterized in terms of clearance (CL), central (V_1) and peripheral volumes of distribution for the shallow (V_2) and the deep (V_3) compartments, intercompartmental exchange flows for shallow (Q_2) and the deep (Q_3) compartments. The concentration of plitidepsin into red blood cells was modeled as a non-linear function, where B_{max} corresponds to the maximal plitidepsin concentration into red blood cells and k_d is the plitidepsin plasma concentration at which the plitidepsin bound to red blood cells is half-maximal. Between and within subject variabilities were assumed to be log-normally distributed.

Final parameter estimates describing the PK of plitidepsin in cancer patients are presented in Table 2. The plitidepsin initial fast distribution half-life was about 21 minutes followed by a dominant elimination half-life of about 8.9 hours and a terminal half-life of about 88 hours that constituted on average 27.2% of the overall area under the plasma concentration *vs.* time curve.

Table 2. Final pharmacokinetic parameter estimates for plitidepsin.

Pharmacokinetic parameter	**Typical value***	**Variability**	
		Between patients (%)	**Within patients (%)**
V_1 (l)	103 (8.87)	74.2 (30.5)	--
CL (l/h)[a,b]	28.7 (4.77)	55.0 (16.6)	38.3 (25.6)
V_2 (l)[b]	417 (5.68)	55.1 (16.1)	--
Q_2 (l/h)	136 (6.46)	64.0 (20.4)	--
V_3 (l)	1320 (7.17)	88.7 (32.8)	--
Q_3 (l/h) [a]	18.1 (6.13)	--	53.4 (19.1)
Bmax (µg/l)	309 (10.2)	60.8 (12.9)	21.8 (27.5)
k_d (µg /l)	23.5 (11.8)	--	--

*Results expressed as parameter (RSE: relative standard error of parameter estimate, %).
a. Correlation between IIV of Cl and Q_3 set to 1. Expansion factor of Q_3 is 0.757 (RSE=17.2%).
b. Correlation between IIV of Cl and V_2 is 0.521 (RSE= 19.1%).
Residual variability, expressed as percentage:
-Blood: 24.5 (RSE = 7.35 %).
-Plasma: 38.1 (RSE =9.86 %).
Bmax: maximal plitidepsin concentration into red blood cells; k_d: plitidepsin plasma concentration at which the plitidepsin bound to red blood cells is half-maximal; V_1: central volume of distribution; V_2: volume of distribution of the shallow compartment; V_3: volume of distribution of the deep compartment; CL: clearance, Q_2: intercompartmental exchange flow for the shallow compartment, Q_3: intercompartmental exchange flow for the deep compartment.

As a summary, the main conclusions of the population PK report are:

- A three-compartment disposition model with linear elimination is suitable to describe the plasma PK profiles of plitidepsin after i.v. administration to cancer patients.
- Non-linear binding to red blood cells is evident from blood concentrations. However, its clinical relevance is limited since the plasma concentrations achieved with the dosing regimens used in phase II program are below the estimated k_d value and the effect of the hematocrit on blood concentrations is limited.
- No evidence of time-dependent kinetics was observed. Actually, plitidepsin systemic exposure following repeat i.v. administration q2wk evidenced minimal accumulation in plasma.

The subject-related covariates, including age, body weight, sex, laboratory liver function parameters (AST, ALT, ALP and total bilirubin) and renal function (creatinine clearance), total protein, serum albumin, hemoglobin levels and leukocytes counts had no discernable impact on the PK parameters of plitidepsin within the range of values evaluated and, consequently, plitidepsin dose adjustment on the basis of these covariates is not warranted.

Between and within patients variability in PK is moderate to large, but similar to other anticancer drugs.

The following Section details the individual PK results from the relevant phase I clinical trial in which the 3-hour infusion every two weeks was used.

1.3.4.3.1 Three-hour Every Two Weeks Intravenous Infusion Regimen of Plitidepsin (Study APL-A-001b-98)

Median half-life values were 35.3, 31.7, 31.5 and 38.5 hours following administration of plitidepsin at 3, 4, 5 and 6 mg/m^2, respectively. Corresponding clearance values were 5.3, 4.1, 5.9 and 1.7 l/h, respectively and corresponding V_{ss} values were 171, 148, 199 and 130 l, respectively. Median C_{max} at the same dose levels were 78.3, 97.5, 82.6 and 294.9 ng/ml respectively, and median AUC_{inf} were 967, 1772, 1392 and 6057 h*ng/ml respectively.

Plitidepsin AUC_{inf} and C_{max} values increased with dose, although dose-proportionality of plitidepsin in blood was not proven, most probably due to the limited sample size for all dose levels except for 5 mg/m^2 and a high interpatient variability.

The effect of age, weight and body surface area (BSA) on the pharmacokinetics of plitidepsin in blood were inconclusive. There were no statistically significant gender effects.

1.3.4.3.2 Excretion of Plitidepsin

Urinary excretion of unchanged compound is a minor elimination route, with average recoveries of 2-5% over 48 hours and maximum values below 15%. The major routes of elimination have not as yet been determined.

There were no correlations between kinetic parameters and variations in hepatic and renal function in the population analysis of adult phase I results.

1.4 INFORMATION ON STUDY DRUG: DEXAMETHASONE

Please refer to the Dexamethasone Drug Information, provided as a separate document, for further information on dexamethasone.

1.5 STUDY RATIONALE

1.5.1 Rationale for the Proposed Drug Combination

Clinical development of anticancer treatments usually requires combination of more than one active drug for improving efficacy, with each agent having different targets or mechanisms of action in order to prevent or delay the development of tumor resistance. Ideally, from the preclinical point of view, drugs may have shown either additive or synergistic antitumor activity. It is equally important to avoid agents with overlapping toxicity profiles for the development of successful novel combinations.

1.5.1.1 *Plitidepsin*

Plitidepsin (Aplidin®) is a novel anticancer compound that has shown activity as single agent in a recently completed phase II study in relapsed/refractory MM patients (51). In a first stage, this trial explored the objective response to plitidepsin at 5 mg/m^2 as a 3-hour i.v. infusion administered every two weeks (q2wk), with 10% of 21 evaluable patients having a PR and, additionally, 14% of patients having clinically meaningful disease stabilization (SD $\geq$ 3 months), with a median TTP of 2.3 months. Based on preclinical results that showed additive to synergistic effects of dexamethasone/plitidepsin combination (30), in a second stage of this phase II trial, patients who experienced suboptimal response (SD or PD) after three to four plitidepsin infusions were allowed to receive oral dexamethasone 20 mg daily on Days 1 through 4 q2wk (160 mg total monthly dose) added to plitidepsin. In this cohort of patients, 28% of the 18 evaluable patients had an objective response and TTP was significantly prolonged to 4.2 months. A similar overall safety profile was found, although with a slight increase in muscular events (mostly myalgia and reversible CPK increase) and a mild decrease in grade 3-4 transaminases increase. Of note, as much as two thirds of these patients had previously received bortezomib and thalidomide or lenalidomide and HDT and PBSC-T, whereas all patients had steroids as part of a prior therapy, with a median of four prior lines of systemic treatments. Although the trial design had limitations that precluded comparison between cohorts, both regimens were well tolerated and showed clinical activity in this heavily pretreated population for which limited clinical options were available. The addition of dexamethasone after three to four cycles of plitidepsin did not change dramatically the response (some disease stabilizations were found in patients who were progressing on plitidepsin alone, and one patient who was stable responded after dexamethasone addition), but responses appeared to be more durable and steeped. Therefore, it seems logical to add dexamethasone to plitidepsin upfront in order to optimize any synergistic effect that may occur clinically to achieve a better and longer disease control.

This approach has been extensively used for most active agents in MM. In fact, corticosteroids have long been a central component of the treatment for MM, with the most commonly used regimens being high-dose dexamethasone alone (53), melphalan and prednisone (8, 54), vincristine, doxorubicin, and dexamethasone (VAD) (55, 56), thalidomide, lenalidomide plus dexamethasone (57-59), and bortezomib plus dexamethasone. Moreover, dexamethasone can be used as monotherapy as well, the dose and regimen being typically around 20-40 mg on Days 1-4, 9-12, and 17-20 of a 28- to 35-day cycle for a total of 240-480 mg per cycle (53), although adverse reactions may be observed including principally endocrine axis suppression, serious infections derived from clinically relevant immunosuppression, and confusion or mood changes including acute psychosis. In fact, dose of dexamethasone has recently raised some concerns particularly after the ECOG 4A03 study. The Eastern Cooperative Oncology Group, compared the administration of lower doses of dexamethasone (40 mg d 1, 8, 15, and 22 p.o. q4wk, for a total monthly dose of 160 mg) plus lenalidomide with high-dose dexamethasone at the usual dose (40 mg d 1-4, 9-12, and 17-20 p.o. q4wk, for a total monthly dose of 480 mg) plus the same lenalidomide regimen, and reported a significant improvement in overall survival with a better safety profile for the lower dose arm (60). A second study with lenalidomide plus dexamethasone in the relapsed/refractory setting showed that patients who had dose reductions of dexamethasone have shown a significantly higher overall response rate, including a higher complete response (CR), PFS and improved median overall survival, when compared to those who received dexamethasone at the assigned dose, with adverse events rates comparable between both groups (61). Finally, and based on data from last available publications on dexamethasone and bortezomib combinations (62), dexamethasone administered at reduced doses (20 mg) the same day and the day after bortezomib infusion up to a total dose of 160 mg/cycle, has been associated with a better safety profile while maintaining the same level of activity.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To compare the efficacy of plitidepsin with dexamethasone *vs.* dexamethasone alone, as measured by PFS, in patients with relapsed/refractory MM.

2.2 SECONDARY OBJECTIVES

- To evaluate tumor response according to the IMWG criteria (Appendix 5).
- To assess duration of response (DR) and overall survival (OS).
- To assess efficacy in patients who undergo crossover from dexamethasone alone to plitidepsin and dexamethasone combination.
- To characterize and compare the safety profile on both arms in this population.
- To characterize the PK and pharmacokinetic/pharmacodynamic (PK/PD) relationship.

3 OVERALL STUDY DESIGN

This is a prospective, open-label, two-arm, 2:1 randomized phase III study. The efficacy of plitidepsin in combination with dexamethasone (Arm A) *vs.* dexamethasone alone

(Arm B) will be studied by means of PFS calculated using the IMWG uniform response criteria (Appendix 5), and the evaluation of secondary efficacy endpoints.

Patients in the control arm (dexamethasone alone, Arm B) who have documented disease progression according to the Investigator's criteria after a minimum of eight weeks from randomization should be offered crossover to combination arm (plitidepsin + dexamethasone, Arm A) upon Sponsor agreement.

An Independent Review Committee (IRC), consisting of medical specialists directly involved in the care of MM patients, but not being investigators or subinvestigators in this trial, will review all efficacy data and will assign the date of progression/censoring and objective response according to their independent evaluation. This IRC will be blinded to treatment arm allocation and identity of the cases reviewed (Section 5.6).

An Independent Data Monitoring Committee (IDMC), including specialists in MM and in medical statistics, will review the results of the analysis performed by an independent statistician. Then, the IDMC will advise the Sponsor about the study conduct.

Operational details for the above mentioned IRC and IDMC will be detailed in the corresponding charters.

4 PATIENT DEFINITION

4.1 STUDY POPULATION

A total of approximately 250 patients will be randomized in a 2:1 ratio (combination: dexamethasone alone).

An early futility analysis will be performed with data from 40 patients evaluable for response in Arm A.

Patients without valid disease evaluation during treatment will not be replaced for the main analysis, and will be analyzed as per ITT according to randomization.

4.2 PATIENT ELIGIBILITY

4.2.1 Inclusion criteria

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

1. Age ≥ 18 years.
2. ECOG PS ≤ 2 (see Appendix 2).
3. Life expectancy ≥ 3 months.
4. Patients previously diagnosed with multiple myeloma based on IMWG diagnostic criteria (see Appendix 4).
5. Patients must have relapsed or relapsed and refractory multiple myeloma (MM) (Appendix 5) after at least three, but not more than six, prior therapeutic regimens for MM, including induction therapy and stem cell transplant in candidate patients, which will be considered as only one regimen.

6. Patients must have received previous bortezomib-containing and lenalidomide-containing regimens (or thalidomide where lenalidomide is not available), unless unable to tolerate either of them.

7. Patients must have measurable disease defined as:

    a) For secretory MM: any quantifiable serum monoclonal protein value and, where applicable, urine light-chain excretion ≥ 200 mg/24 hours.

    b) For oligo or non-secretory MM: presence of soft tissue (not bone) plasmacytomas, as determined by clinical examination or applicable radiographs [i.e., magnetic resonance imaging (MRI), computed tomography (CT)-scan], and/or by the presence of abnormal serum free light chains (sFLC): involved FLC level ≥ 10 mg/dl provided the serum FLC ratio is abnormal.

8. At least two-week washout period since the end of last therapy (six weeks if previous nitrosoureas-containing regimen), given recovery to grade ≤ 1 from any non-hematological related adverse event (AE) derived from previous treatment (excluding alopecia).

9. Adequate BM, renal, hepatic, and metabolic function (assessed ≤ 7 days before inclusion in the study):

    a) Absolute neutrophil count (ANC) ≥ 1.0 x 10^9/l (≥ 0.5 x 10^9/l if due to extensive and documented BM disease involvement by ≥ 50% of plasma cells in BM biopsy).

        - Screening of ANC should be independent of granulocyte- and granulocyte/macrophage-colony stimulating factors (G-CSF and GM-CSF) support for at least one week and of pegylated G-CSF for at least two weeks.

    b) Platelet count ≥ 50 x 10^9/l (≥ 25 x 10^9/l if due to extensive and documented BM disease involvement).

    c) Hemoglobin ≥ 8.5 g/dl.

        - Patients may receive red blood cells (RBC) and/or erythropoietin (EPO) and/or platelets transfusions in accordance with institutional guidelines.

    d) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 3.0 x the upper limit of normal (ULN).

    e) Total bilirubin ≤ 1.0 x ULN or direct bilirubin ≤ 1.0 x ULN when total bilirubin is above the upper limit of normal.

    f) Calculated creatinine clearance (CrCl) ≥ 30 ml/minute (by means of Cockcroft and Gault´s formula) (Appendix 3).

    g) Creatine phosphokinase (CPK) ≤ 2.5 x ULN.

    h) Albumin ≥ 2.5 g/dl.

10. Left ventricular ejection fraction (LVEF) by echocardiogram (ECHO) or multiple-gated acquisition scan (MUGA) above the lower limit of normal (LLN).

11. Women of childbearing potential must have a negative serum pregnancy test before study entry. Both women and men must agree to use a medically acceptable method of contraception throughout the treatment period and for six months after discontinuation of treatment.
12. Voluntarily signed and dated written informed consent prior to any specific study procedure.

4.2.2 Exclusion criteria

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

1. Concomitant diseases/conditions:
   a) History or presence of angina, myocardial infarction, clinically relevant valvular heart disease, cardiac amyloidosis or congestive heart failure within the last 12 months.
   b) Symptomatic arrhythmia (excluding anemia-related sinusal tachycardia grade ≤ 2) or any arrhythmia requiring ongoing treatment, and/or prolonged QT-QTc grade ≥ 2; or presence of unstable atrial fibrilation. Patients with stable atrial fibrillation on treatment are allowed provided they do not meet any other cardiac or prohibited drug exclusion criterion.
   c) Active uncontrolled infection.
   d) Morphological or cytological features of myelodysplasia and/or post-chemotherapy aplasia on BM assessment.
   e) Myopathy > grade 2 or any clinical situation that causes significant and persistent elevation of CPK (> 2.5 x ULN in two different determinations performed one week apart).
   f) Known human immunodeficiency virus (HIV) infection (HIV testing is not required unless infection is clinically suspected).
   g) Known active hepatitis B or C virus (HBV or HCV) infection.
   h) Limitation of the patient's ability to comply with the treatment or follow-up requirements.
   i) Any other major illness that, in the Investigator's judgment, will substantially increase the risk associated with the patient's participation in this study.
   j) Peripheral neuropathy > grade 2.
2. Women who are pregnant or breast feeding.
3. Concomitant medications that include corticosteroids, chemotherapy, or other therapy that is or may be active against MM, within two weeks prior to Cycle 1 Day 1. Concurrent corticosteroids are allowed, provided they are administered at an equivalent prednisone dose of ≤ 10 mg daily, as premedication for blood products only.
4. Known history of peptic ulcer and/or major upper gastrointestinal bleeding episode occurring during last year before study entry and/or related to prior steroid-based therapy.

5. Relevant history of mood-disturbances changes associated with previous steroid-based therapy.
6. Disease-related symptomatic hypercalcemia despite optimal medical therapy.
7. Known hypersensitivity to any involved study drug or any of its formulation components.

5 PLAN OF STUDY

5.1 PATIENT PARTICIPATION

Patients will receive the study treatment for as long as it is considered beneficial for the patient (see Section 5.2). Patients will be evaluated at scheduled visits in up to three study periods:

Pre-treatment: from the signature of the informed consent to the first administration of study drugs.

Treatment: up to 30 days after the day of the last dose of study drug administration, unless the patient starts any new antitumor therapy outside this clinical trial or dies, in which case the date of administration of this new therapy or the date of death will be considered the date of last treatment discontinuation. An end-of-treatment visit will be performed within 30 days (± two days) after last dose administration, unless the patient starts any new antitumor therapy outside this clinical trial, in which case the end-of-treatment visit should be performed immediately before the start of the new therapy (ideally the day before or the same day).

Follow-up: After treatment discontinuation, patients will be followed every four weeks until resolution of toxicities if any. Patients who discontinued treatment without progression will be followed every four weeks until disease progression or other antitumor therapy whichever occurs first. After progression, all patients will be followed every three months for survival until death, or until the date of study termination, whichever occurs first.

Study termination date is defined as the date in which 80% of death events occurred, 24 months after the accrual of the last randomized patient, or IDMC recommendation (whichever occurs first).

Note: Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A (plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death events are not achieved on 19 November 2017, the study might be further extended by three to six months.

Patients will be considered to be **on-study** from the signature of the informed consent to the end of the follow-up period. Patients will be considered to be **on-treatment** for the duration of their treatment and 30 days following the last treatment dose. Those patients in the control arm (Arm B) who **crossed over** to the combination arm (Arm A) after disease progression will be considered on-treatment for the duration of their whole

treatment (dexamethasone alone + dexamethasone in combination with plitidepsin) and during 30 days following the last treatment dose.

Patients may withdraw consent at any time.

5.2 TREATMENT DISCONTINUATION

Patients will receive the study drugs while it is considered to be in their best interest. Specifically, treatment will continue until:

- Disease progression (except for those patients treated with dexamethasone alone and eligible to be offered crossover to combination therapy).
- Unacceptable toxicity.
- Intercurrent illness of sufficient magnitude to preclude safety continuation of the study.
- Patient refusal, and/or non compliance with study requirements.
- Protocol deviation with an effect on the risk/benefit ratio of the clinical trial (Section 5.3).
- Treatment delay > 2 weeks from the theoretical treatment date (except in case of clear clinical benefit, with the Sponsors' approval).
- Requirement of > 2 dose reductions of either drug.

Patients who are withdrawn from Arm A for any reasons or from Arm B for any reason other than progression must not be re-treated in the context of this study at any time. For follow-up activities, please refer to Section 5.13.

5.3 PROTOCOL DEVIATIONS

A protocol deviation is defined as any departure from what is described in the protocol of a clinical trial approved by an Independent Ethics Committee/Institutional Review Board (IEC/IRB) and Competent Authorities. Therefore, it 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 the Good Clinical Practice (GCP) guidelines or ethical issues (e.g., issues related to obtaining the patients' Informed Consent, data reporting, the responsibilities of the Investigator, etc.).

Deviations with no effects 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 investigational medicinal product (plitidepsin) due to not following dose adjustment specifications or an incorrect preparation of the medication.

- Deviations related to the following of GCP guidelines as described in the protocol and regulations in force, such as deviations when obtaining the Informed Consent or not following the terms established for reporting Serious Adverse Events, etc.

The Investigators may suggest to the Sponsor the authorization of certain protocol deviations, especially if they are related to the inclusion/exclusion criteria or if they may have an effect on the evaluability of the patients. 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, which are related to ethical issues, fulfillment of GCP guidelines and trial procedures, will be notified to the pertinent IEC/IRB and, if pertinent, to the relevant authorities as established by local regulations.

5.4 REPLACEMENT OF PATIENTS

The study analyses will be performed in the study population of all randomized patients. As detailed above (see Section 4.1), those patients not evaluable for response will not be replaced and they will be included in the final analyses.

Patients not evaluable for response:

- A patient evaluable for response should have measurable disease at baseline and should have completed at least one full cycle of treatment or have received two incomplete cycles followed by at least one response assessment, unless the patient had been withdrawn from the study due to early disease progression or drug-related toxicity, in which case the patient will be considered as "early progression" or "treatment failure", respectively.
- Patients will be non-evaluable if there is a protocol deviation resulting in an impossibility of drawing conclusions about the efficacy of the study therapy.

These non-evaluable patients will be included in the general safety analysis (if appropriate) and in planned efficacy analyses.

Patients without valid disease evaluation during treatment will not be replaced for the main analysis and will be analyzed as ITT in either randomized arm. All randomized patients will be evaluable for the primary endpoint (PFS) in an ITT analysis.

The early futility analysis will be mainly based on the efficacy data of the first 40 patients in Arm A evaluable for response. However, the IDMC will be provided with data from all randomized patients at that point in order to evaluate the safety profile and advice on the further conduct of the study.

5.5 DURATION OF THE CLINICAL TRIAL

- Protocol submission: 1Q2010.
- Planned start date (first patient on study): 2Q2010.
- Complete enrollment: 60 months (2Q2010 - 2Q2015).
- Planned study termination date: it will be set after the occurrence of 80% of death events, 24 months after accrual of the last randomized patient, or IDMC recommendation (whichever occurs first).

  Note: Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A

(plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death events are not achieved on 19 November 2017, the study might be further extended by three to six months.

The futility analysis is foreseen after 40 patients evaluable for response are recruited in Arm A, approximately 12 months after the recruitment start.

All patients on active treatment at the date of study termination will be offered to continue receiving plitidepsin-based treatment off-study according to the Investigator's criteria and upon Sponsor agreement.

5.6 INDEPENDENT REVIEW AND DATA MONITORING COMMITTEES

An Independent Review Committee (IRC), consisting of medical specialists directly involved in the care of MM patients, but not being investigators or subinvestigators in this trial, will review all efficacy data and will assign the date of progression/censoring and objective response according to their independent evaluation. This IRC will be blinded to treatment arm allocation and identity of the cases reviewed.

Pertinent clinical or laboratory records [protein M measurements in blood and urine and/or serum free light chains (sFLC) measurements in patient samples will be centralized for adequate monitoring and auditing purposes] and original copies of all tumor images, whenever appropriate, are to be made available by the Investigator on request from the Committee.

An Independent Data Monitoring Committee (IDMC), including specialists in MM and in medical statistics, will review the results of the analysis performed by an independent statistician. Then, the IDMC will advise the Sponsor about the study conduct.

A futility analysis will be performed when data from the 40 patients evaluable for efficacy in Arm A are available. The IDMC will advise on the further conduct of the study using the protocol-specified efficacy criteria for study continuation in addition to the evaluation of safety profile in both treatment arms.

Operational details for the above mentioned IRC and IDMC will be detailed in the corresponding charters.

5.7 PRE-TREATMENT ASSESSMENTS

During the pre-treatment period, and after signing the informed consent, patients should undergo a series of investigations to determine their eligibility for the study (Table 3).

Table 3. Pre-treatment period

PRE-TREATMENT	INVESTIGATIONS	TIME
History and clinical examination	Written informed consent signed by the patient.	Prior to any study-specific procedure.
	Demographic data.	Within two weeks prior to registration.

PRE-TREATMENT	INVESTIGATIONS	TIME
	Medical history includes: Date and stage at first diagnosis. Previous specific treatments (radiotherapy, chemotherapy, immunotherapy and investigational agents), with dates; best response and date of progression or relapse. Complete physical examination, including weight, height and BSA. Assessment of signs and symptoms. Intercurrent events, concomitant diseases and treatments. Baseline PS (ECOG), vital signs (heart rate, BP, temperature).	Repeat vital signs and PS (ECOG) on first infusion. Repeat complete physical examination and signs and symptoms assessment prior to first infusion, if treatment will be administered > 2 weeks after the screening test.
Hematology	Differential WBC, hemoglobin, hematocrit and platelets.	Within one week prior to registration. Repeat prior to first infusion, if treatment will be administered more than 1 week after the screening test.
Biochemistry	**Biochemistry A:** AST, ALT, total bilirubin, direct bilirubin (in case total bilirubin is above ULN), AP, creatinine, calculated creatinine clearance (Cockcroft and Gault´s formula; see Appendix 3), glucose, serum electrolytes (Na+, K+, Mg++, Ca++), CPK (CPK-MB fraction should be measured only if CPK is abnormally high) and troponin I. **Biochemistry B**: Uric acid, LDH, total proteins, albumin **Coagulation tests:** PT, PTT and INR.	Within one week prior to registration. Repeat prior to first infusion, if treatment will be administered more than 1 week after the screening test.
Pregnancy test, if applicable	Serum HCG	Within one week prior to registration.
Cardiac assessment	ECG: It should allow rhythm definition (at least 30 seconds of duration). Troponin I. LVEF assessment by ECHO or MUGA.	Within one week prior to registration. Within one week prior to registration. Within two weeks prior to registration.
Local Serum protein	Serum protein electrophoresis, immunoglobulins quantification, M-protein identification and quantification, serum free light chains (sFLC), sFLC ratio and immunofixation (complete protein panel).	Within four weeks prior to registration. Repeat on first infusion prior to treatment in the Central Lab on Day 1 of Cycle 1.
Local Urine protein	Urine immunoelectrophoresis and immunofixation.	Within four weeks prior to registration (up to two additional days of tolerance window). Repeat on first infusion prior to treatment in the Central Lab on Day 1 of Cycle 1.
Serum β-2 microglobulin		Within two weeks prior to registration.
Bone marrow		Within four weeks prior to registration.

PRE-TREATMENT	INVESTIGATIONS	TIME
Radiological tumor assessment only in case of soft tissue plasmacytoma(s) present at baseline		Within four weeks prior to registration.
Skeletal evaluation	May be done using CT-scan or X-ray, provided the same procedure is used throughout the study.	Within four weeks prior to registration (+1 week tolerance).

BSA: body surface area; PS-ECOG: performance status according to Eastern Cooperative Oncology Group; BP: blood pressure; WBC: white blood cells; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ULN: upper limit of normal; AP: alkaline phosphatase CPK: creatine phosphokinase; CPK-MB: serum CPK isoenzymes (found In cardiac muscle); CT: computed tomography; LDH: lactate dehydrogenase; PT: prothrombin time, PTT: partial thromboplastin time, INR: international normalized ratio; ECG: electrocardiogram; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; MUGA: multiple-gated acquisition scan; HCG: human chorionic gonadotropin; FLC: free light chain; D, day; C, cycle.

5.8 PATIENT REGISTRATION

After ensuring that the patient meets all eligibility criteria and has given written informed consent, he/she can be registered in the study by means of the Interactive Voice Response System (IVRS, ICOPhone™). Authorized site staff will be provided with a personal user ID and PIN Code to access the system. To start the randomization process, the site responsible should dial the country-specific phone number supplied in the IVRS Manual (given as a separate document). The system will require the following information to be provided: patient number (six digits: four digits for site code, pre-assigned, plus two numbers assigned consecutively for every site's patients), date of birth, ECOG-PS score, Durie-Salmon stage at first diagnosis and planned treatment date (the planned treatment date must be seven days or less from the Randomization date). A confirmation report will be sent via e-mail once the randomization call will end, listing the above information plus the treatment arm the patient has been assigned to. The aforementioned patient number should be used on all future documentation and correspondence referring this patient.

A patient who has been treated prior to registration will not be accepted for the study at a later date.

5.9 PATIENT ASSIGNMENT TO TREATMENT GROUP

See Section 5.10 Patient Randomization.

5.10 PATIENT RANDOMIZATION

Eligible patients will be stratified according to their ECOG-PS score (0 and 1 *vs.* 2) and Durie-Salmon stage (I/II *vs.* III) and then randomized using a 2:1 randomization procedure to Arm A (plitidepsin in combination with dexamethasone) or Arm B (dexamethasone alone). Randomization will be used to avoid bias in the assignment of subjects to treatment, and to increase the likelihood that known and unknown subject attributes (e.g., demographics and baseline characteristics) are evenly balanced across treatment groups.

Randomization of patients should occur as close in time as possible before administration of the first dose of study drug(s) and must occur within 7 days of the patient receiving the first dose of study treatment. Patients will be assigned to each

group by strata random lists, so that a patient will have a two-thirds chance of getting Arm A (plitidepsin in combination with dexamethasone) and a one-third chance of getting Arm B (dexamethasone alone). The random permuted blocks method will be used; the size of the blocks in the randomization list will be fixed and not accessible to the investigators. To select the blocks, a uniform (0, 1) variable with a random seed will be used.

5.11 EVALUATION DURING TREATMENT

Table 4. Evaluation during treatment

TREATMENT	INVESTIGATIONS	TIME
Clinical examination	Physical exam including weight, height and BSA. PS (ECOG), vital signs (heart rate, BP, body temperature). Concomitant treatments.	On D1 of each cycle before study drug administration. On D1 and D15 of every cycle before study drug administration. Throughout the study.
Hematology	Differential WBC, hemoglobin, hematocrit and platelets.	On D1 and D15 of every cycle before study drug administration, with a window of -2 days. If CTCAE grade ≥ 3 occurs, the hematological counts should be assessed at least every 2-3 days in the case of grade 4 non-febrile neutropenia and everyday in case of grade 4 febrile neutropenia.
Biochemistry	**Biochemistry A:** AST, ALT, total bilirubin, direct bilirubin (in case total bilirubin is above ULN), AP, creatinine (calculated creatinine clearance by Cockcroft and Gault´s formula; see Appendix 3), glucose, serum electrolytes (Na^{+}, K^{+}, Mg^{++}, Ca^{++}), CPK, (CPK-MB fraction should be measured only if CPK is abnormally high) and troponin I*.	On D1 and D15 of every cycle before study drug administration, with a window of -2 days. If CTCAE grade ≥ 3 occurs, the abnormal test(s) should be assessed at least every 2-3 days until recovery.
	Biochemistry B: Uric acid, LDH, total proteins, albumin. **Coagulation tests**: PT, PTT and INR.	On D1 of every cycle, except on D1 of Cycle 1, and with a window of -5 days.
Pregnancy test	Serum HCG	Repeat if applicable; always prior to study drug administration.
Adverse Events		Throughout the study.
Cardiac assessment	ECG: It should allow rhythm definition (at least 30 seconds of duration). Troponin I*. LVEF assessment by ECHO or MUGA.	Before each study drug administration, symmetrically in both arms. After the first two cycles, ECG will be done only on D1 of each cycle. Every 12 weeks, with a window of ± 2 weeks. When due, LVEF will be performed within 7 days of plitidepsin administration, except in non-primarily cardiac origin acutely ill

TREATMENT	INVESTIGATIONS	TIME
		hospitalized patients, until resolution.
Pharmacokinetics	Plitidepsin PK will be evaluated in at least 100 patients receiving both plitidepsin and dexamethasone (Arm A).	A total of 13 blood samples will be collected during the first three infusions [infusions 1 and 2 of Cycle 1 (Days 1 and 15), and infusion 1 of cycle 2 (Day 1)] using a sparse sampling schedule (see details in Table 5)
Central Lab Serum protein	Patients with secretory MM will be followed with serum electrophoresis, immunoglobulins quantification and M protein quantification in the Central Lab starting on Day 1 of Cycle 1; patients with oligo or non-secretory MM, will be followed with sFLC ratio, in case it has been abnormal at study entry; in case of M protein disappearance, immunofixation will be done to confirm CR, and if CR is confirmed, sFLC ratio will be performed, to determine sCR.	All protein assays will be done symmetrically every four weeks (i.e., Day 1 of every 4-week cycle, with a window of ± 2 days) in both treatment arms, irrespectively of treatment delays or omissions.
Central Lab Urine protein	Only if Bence-Jones proteinuria is present at entering the study, in the Central Lab starting on Day 1 of Cycle 1.	Every four weeks (with a window of ± 2 days) symmetrically in both treatment arms, irrespectively of treatment delays or omissions.
Serum β-2 microglobulin		Every 8 weeks (with a window of ±2 days).
Bone marrow	In all patients while in treatment, BM evaluation is mandatory at screening and in case of CR (including flow cytometry or immunohistochemistry, to demonstrate the presence of clonal plasma cells, only in those patients with presumed CR). In patients with non-secretory MM it must be repeated 6 weeks later to confirm response or as clinically indicated.	When serum protein indicates CR.
Radiological tumor assessment only in case of measurable soft tissue plasmacytoma		Every 8 weeks (with a window of 2 weeks) or if response is observed or if clinically indicated.
Skeletal evaluation	May be done using CT-scan or X-ray, provided the same procedure is used throughout the study.	If clinical symptoms suggest new bone lytic lesion.

* In case of serum troponin I increase, an ECG and ECHO will be performed as soon as possible, as well as further studies if judged necessary by clinical investigator. Any increase of troponin I ≥ ULN together with evidence of cardiac damage by ECG or ECHO/MUGA will be followed accordingly by a cardiologist and the patient will immediately and definitively discontinue study treatment.

PS-ECOG: Eastern Cooperative Oncology Group; BSA: body surface area; BP: blood pressure; WBC: white blood cells; CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events; AST: aspartate aminotransferase; ALT: alanine

TREATMENT	INVESTIGATIONS	TIME

aminotransferase; ULN: upper limit of normal; AP: alkaline phosphatase; CPK: creatine phosphokinase; CPK-MB: serum CPK isoenzymes (found in cardiac muscle); CT: computed tomography; LDH: lactate dehydrogenase; PT: prothrombin time, PTT: partial thromboplastin time, INR: international normalized ratio; ECG: electrocardiogram; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; MUGA: multiple-gated acquisition scan; PK: pharmacokinetics; HCG: human chorionic gonadotropin; BM: bone marrow; FLC: free light chain; CR: complete response; MM: multiple myeloma; sCR, stringent complete response; D, day.

Note: One patient was still undergoing treatment on 19 May 2017. However, as the primary endpoint of the study (progression-free survival) was fulfilled, and this extended follow-up is focused only on survival, no information such as central laboratory data or images will be sent for independent review. Nevertheless, laboratory determinations or images will be done locally, if clinically indicated, in order to perform tumor assessment.

5.12 EVALUATION AT THE END OF TREATMENT

The end-of-treatment visit will be scheduled 30 days (± two days) after the administration of the last dose of the study therapy, unless another anticancer treatment is started before. In that case, the end-of-treatment visit should be performed immediately before the start of the new therapy (ideally the day before or the same day).

At the time of patient discontinuation (regardless of the reason), the same complete work-up done before study entry will be repeated.

- Physical examination, weight and height.
- ECOG performance status.
- Vital signs.
- Hematology and coagulation tests.
- Biochemistry (A and B).
- ECG: It should allow rhythm definition (at least 30 seconds of duration)
- LVEF assessment by ECHO or MUGA.
- Safety assessment (AEs).
- Serum protein: serum protein electrophoresis, immunoglobulins quantification, M-protein identification and quantification, and serum sFLC if indicated.
- Urine protein: Only if Bence-Jones proteinuria is present at entering the study.
- Serum β-2 microglobulin: only if the patient discontinues treatment without documented disease progression.
- Bone marrow (if clinically indicated).
- Radiological tumor assessment only in case of soft tissue plasmacytoma present at entering the study.
- Skeletal evaluation.
- Concomitant treatments.

Adverse events must be assessed for 30 days after the administration of the last study treatment. All SAEs occurring within 30 days of the last study drug administration will be reported. Beyond this period of time, only those suspected to be treatment-related SAEs will be reported (Section 9.2.2).

The Sponsor will evaluate any safety information that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

5.13 FOLLOW-UP AFTER END-OF-TREATMENT VISIT

Bone marrow evaluation, radiological tumor assessment and skeletal evaluation will be performed only on pre-treatment and treatment evaluations. These evaluations will not be performed after the end of treatment.

Patients who discontinued treatment without progression will be followed every four weeks until disease progression or other antitumor therapy, whichever occurs first. After progression, all patients will be followed every three months for survival until death, or until the date of study termination, whichever occurs first. Study termination date is defined as the date in which 80% of death events occurred, 24 months after the accrual of the last randomized patient, or IDMC recommendation (whichever occurs first).

Note: Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A (plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death events are not achieved on 19 November 2017, the study might be further extended by three to six months.

All AEs suspected to be related to study drug must be followed after the time of therapy discontinuation until the event or its sequel resolve or stabilize at a level acceptable to the Investigator and the clinical monitor or his/her designated representative. After treatment discontinuation, patients will be followed every four weeks until resolution of toxicities, if any.

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

The follow-up visits will be performed by phone only if the patient is unable to go to the study center owing to the seriousness of his/her disease. This will only apply to patients who are followed up after discontinuing treatment due to disease progression.

6 PHARMACOKINETICS

Plitidepsin pharmacokinetics (PK) will be evaluated in at least 100 patients receiving both plitidepsin and dexamethasone (Arm A) at selected sites. Patients will be sampled during Days 1 and 15 of the Cycle 1 and on Day 1 of the Cycle 2 using a sparse sampling schedule.

In the treatment infusions with blood sampling for pharmacokinetics, the infusion rate will be established so that it ensures that the total plitidepsin dose is infused over 3 hours. Drug will be infused at a constant rate throughout the 3-hour period. In order to obtain reliable pharmacokinetic information, the infusion rate should not be modified once the infusion begins. If a variation in the infusion time eventually occurs, it is very important to reflect it in the CRF, writing clearly the time of the beginning and the end

of the infusion. The infusion rate should not be changed to maintain the scheduled duration of infusion. It would be enough just to record the actual duration in the CRF and in the pharmacokinetic sampling sheet in the cycles in which PK sampling is performed.

Blood samples will be obtained through a peripheral vein located in the contralateral side to that of the infusion. In any case, the sampling vein has to be different to that in which study drug is infused. Even the last sample must never be collected from the infusion catheter. If the blood sample is obtained from a catheter, the first ml of blood will be discarded to avoid the dilution of the sample with the solution used to keep it permeable.

Four-ml blood samples will be collected in sodium heparin tubes. Sample tubes will be gently inverted several times to assure adequate mixing with the heparin and the blood (without centrifugation) should be transferred to the provided polypropylene tubes and stored at -20ºC until the shipping to the analysis laboratory. The tubes will be provided by PharmaMar.

A total of 13 blood samples will be collected during the first three infusions (infusions 1 and 2 of Cycle 1, and infusion 1 of Cycle 2) at the time points detailed in Table 5.

Table 5. Sampling points for pharmacokinetic evaluations.

Sample number	Cycle	Infusion	Time (from start of each infusion)	Time* (relative to EOI)
1	1	1	0 h	Preinfusion
2	1	1	3 h	Just before EOI
3	1	1	4 h	One hour after EOI
4	1	1	6 h	Three hours after EOI
5	1	2	0 h	Preinfusion
6	1	2	3 h	Just before EOI
7	1	2	4 h	One hour after EOI
8	1	2	6 h	Three hours after EOI
9	2	1	0 h	Preinfusion
10	2	1	3 h	Just before EOI
11	2	1	4 h	One hour after EOI
12	2	1	6 h	Three hours after EOI
13	2	1	~336	Two weeks after EOI**

*The length of the infusion is three hours.
**Previous to second infusion of the second cycle.
EOI: end of infusion.

The accurate recording of actual dosing and sampling times is much more important than the strict adherence to the scheduled times.

Once all samples from a patient have been collected they should be shipped to the central laboratory for pharmacokinetics as soon as possible, ideally the next shipping day. The time span between the moment the last PK sample for a patient has been collected and the shipment of all the samples from this patient to the central lab should not exceed two months.

Samples will be identified with the following data: study reference, patient number, sample number, date and time of collection. At all time the confidentiality of patient's data will be maintained.

A manual of instructions for sample collection, labeling, storage, and shipment will be provided as a separate document.

7 TREATMENT

7.1 STUDY MEDICATIONS

For information regarding dexamethasone please refer to Dexamethasone Drug Information, provided as a separate document. For detailed instructions regarding plitidepsin inventory, handling, reconstitution, dilution, storage, accountability and disposal, please refer to the updated Aplidin® Preparation Guide for Infusion, also provided as separate document.

Commercially available oral dexamethasone formulation will be provided by the Sponsor.

7.1.1 Quantitative and Qualitative Composition

7.1.1.1 *Plitidepsin*

Plitidepsin will be supplied as a powder and solvent for concentrate for solution for infusion. The 2-mg vial should be reconstituted with a 4-ml ampoule of reconstitution solution. The composition of reconstitution solution is Cremophor EL/Ethanol/Water for injection, 15%/15%/70% (v/v/v).

7.2 ADMINISTRATION OF STUDY MEDICATION

The administration of each study medication will be as follows:

- **Arm A**:
  a) Dexamethasone: 40 mg orally on Day 1, 8, 15 and 22 every four weeks (q4wk) at least one hour before plitidepsin infusion.
  b) Plitidepsin: 5 mg/m^2 intravenously (i.v.) diluted to a total volume of 250 ml in 0.9% saline (or 5% glucose) via a central venous catheter (suggested) or diluted to a total volume of 500 ml in 0.9% saline (or 5% glucose) via a peripheral line. Infusion will be performed through a pump device over three hours (fixed rate) on Day 1 and 15 q4wk.
- **Arm B:**
  a) Dexamethasone: 40 mg orally on Day 1, 8, 15 and 22 q4wk.

A cycle is defined as a four-week period.

7.3 CRITERIA FOR TREATMENT CONTINUATION AND RETREATMENT

Patients may be treated with additional cycles of plitidepsin and dexamethasone or dexamethasone alone, as long as no unacceptable toxicity and/or disease progression is documented (see Section 5.2).

Complete blood counts, blood chemistries and other tests will be done prior to starting the next treatment cycle (please refer to Section 5.7 and Section **5.11** for the full list of tests to be done before each drug administration and/or initiation of a new treatment cycle).

Table 6 lists the minimum requirements needed to re-expose the patient to the study drug(s) in each group. Other factors might be considered critical by the Investigator. In this case, these factors should be appropriately documented in the patient's record and on the CRF, and discussed with the Sponsor.

If the criteria listed in Table 6 are not met on the day of the next planned dose, administration of the study drug(s) should be delayed. The criteria for treatment administration should be reevaluated weekly. The new cycle will start upon recovery to the values listed in those tables, for Day 1.

A maximum cycle delay of two weeks is allowed for recovery from drug-related AEs. If no recovery was found after the two-week delay, the patient should discontinue the treatment, except —upon Sponsor's agreement— in cases of obvious patient benefit in continuing the treatment according to the investigator's criteria.

Before the administration of each dose (retreatment), patients must fulfill the baseline criteria defined in Table 6.

Table 6. Criteria for continuation of treatment

Criteria for continuation of treatment	**Plitidepsin (Arm A)**	**Dexamethasone (Arm A and B)**
	Day 1 [a] and 15 [b]	**Day 1 [a] and 15**
ANC	≥ 1.0 x 10^9/l or return to baseline values if extensive BM involvement	-
Platelets	≥ 50 x 10^9/l or return to baseline values if extensive BM involvement	-
Hemoglobin	≥ 8.5 g/dl	≥ 8.5 g/dl
Direct bilirubin if total bilirubin is above the upper normal limit	≤ 1.0 x ULN	-
AST/ALT	≤ Grade 2	-
Muscular toxicity (myalgia, muscular weakness, CPK increase)	≤ Grade 2	≤ Grade 2
Other non-hematological drug-related AEs (except for increased GGT and/or AP, not optimally treated nausea and vomiting or hypertension, alopecia)[c]	≤ Grade 1	≤ Grade 1

Criteria for continuation of treatment	Plitidepsin (Arm A)	Dexamethasone (Arm A and B)
	Day 1 [a] and 15 [b]	Day 1 [a] and 15
ECG, ECHO/MUGA[d]	Same as baseline	Same as baseline

a. If a patient does not meet the requirements for treatment continuation on Day 1 of the following cycle, the infusion of study drugs will be withheld until recovery or for a maximum of 14 days. After this period, if delay is due to toxicity assessed as related to study drug, a dose decrease of 20% is mandatory; up to a maximum of two individual dose reductions are allowed. Patients needing additional dose reductions must be withdrawn from the trial.
b. If a patient does not meet the requirements for treatment continuation on Day 15, the administration of plitidepsin will be omitted. Patients requiring frequent dose omissions may have a dose reduction of 20% upon Investigator and Sponsor agreement. In no case more than two dose reductions are allowed
c. Any grade accepted for increased GGT and/or AP, and up to grade 2 peripheral neuropathy.
d. To be performed every three months unless more frequent assessments are clinically indicated.

AEs: adverse event(s); ANC: absolute neutrophil count; AP: alkaline phosphatase; BM: bone marrow; AST/ALT: aspartate aminotransferase/alanine aminotransferase; CPK: creatine phosphokinase; GGT: γ-glutamyltranspeptidase; ECG: electrocardiogram; ECHO/MUGA: echocardiogram/multiple-gated acquisition scan.

7.4 DOSE REDUCTION CRITERIA

Patients experiencing frequent dose omissions and/or unacceptable toxicity, defined as

- less than 50% compliance with 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 (in patients with extensive BM involvement), and/or
- any grade ≥ 3 clinically relevant non-hematological toxicity other than non-optimally treated nausea and vomiting, diarrhea lasting < 48 hours and/or grade ≥ 3 asthenia/fatigue lasting < 5 days

may continue treatment after reducing 20% of the dose of plitidepsin (first reduction to 4 mg/m^2, and second reduction to 3.2 mg/m^2), upon Sponsor agreement, if patient benefit is perceived.

Dexamethasone doses are to be reduced by 50%, up to a maximum of two consecutive dose reductions (first one, 20 mg Days 1,8,15 and 22, and second, 20 mg Days 1 and 15 of each 28-day cycle), if a patient experiences muscular toxicity of grade ≥ 3 (weakness, myalgia and/or CPK elevations), or drug-related grade ≥ 3 fatigue, or mood disturbances or agitation of grade ≥ 2 or grade ≥ 3 fluid retention or grade 4 clinically documented infection. These dose reductions are to be implemented independently from plitidepsin dose reductions, if required.

Dose reduction criteria for both study drugs are summarized in Table 7.

Table 7. Dose reduction criteria for plitidepsin and dexamethasone.

Toxicity	Worst grade	Plitidepsin	Dexamethasone
Less than 50% compliance with treatment schedule		Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Febrile neutropenia	≥ 3	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Neutropenia lasting > 7 days (except for patients with extensive BM involvement)	4	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Thrombocytopenia (except for patients with extensive BM involvement)	4	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Thrombocytopenia with grade ≥ 3 bleeding (in patients with extensive bone marrow involvement)	4	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Any clinically relevant and/or non-hematological toxicity (except non-optimally treated nausea and vomiting, diarrhea < 48 hours and/or asthenia/fatigue lasting < 5 days)	≥ 3	Decrease to 4 mg/m^2, then to 3.2 mg/m^2	No reduction
Muscular toxicity (weakness, myalgia and/or CPK elevations)	≥ 3	First episode: decrease to 4 mg/m^2, then reduce dexamethasone; if toxicity recurs, reduce to 3.2 mg/m^2	First reduce plitidepsin; if persistence, decrease dexamethasone to 20 mg Days 1,8,15,22 of each cycle; if toxicity recurs, decrease again plitidepsin and, if persistence, then decrease dexamethasone dose to 20 mg Days 1 and 15 of each cycle. Two dose reductions are allowed for either agent (only for muscular toxicity).
Mood disturbances/agitation	≥ 2	No reduction	First reduction to 20 mg Days 1,8,15,22 of each cycle, then to 20 mg Days 1 and 15 of each cycle.
Fluid retention	≥ 3	No reduction	First reduction to 20 mg Days 1,8,15,22 of each cycle, then to 20 mg Days 1 and 15 of each cycle.

Toxicity	Worst grade	Plitidepsin	Dexamethasone
Clinically documented infection	4	No reduction	First reduction to 20 mg Days 1,8,15,22 of each cycle, then to 20 mg Days 1 and 15 of each cycle.

Once a dose reduction has been implemented, dose will not be re-escalated thereafter. No more than two dose reductions (of either compound, if required) are allowed. Patients needing additional dose reductions must be withdrawn from the trial.

7.5 CONCOMITANT MEDICATION

All tumor-specific prior chemotherapy, radiation therapy and all relevant information must be recorded on the patient's CRF.

In addition, reasonable efforts will be made to determine all treatments received by the patient during administration of the study drugs. This information must be documented in the concomitant therapy Section of the CRF.

Cardiac safety of plitidepsin will be monitored with ECG, LVEF and troponin I measurement during the trial. Some drugs may prolong QT_C interval duration. These drugs are allowed as concomitant medication, and the investigators should be aware of this potential event.

Close monitoring of patients taking oral anticoagulants is required.

7.5.1 Prophylactic Medication

1. Arm A:

All patients must receive the following prophylactic medication 20-30 minutes before infusion of plitidepsin:

- Ondansetron 8 mg i.v. or equivalent (granisetron 3 mg i.v. preferred when available).
- Diphenhydramine hydrochloride 25 mg i.v. or equivalent, and
- Ranitidine 50 mg i.v. or equivalent.

Oral metoclopramide and/or extended oral ondansetron (or their equivalents) may be used as per Investigator's criteria/institutional guidelines (63).

2. Arm B: No prophylactic medication specified.

7.5.2 Allowed Medications/Therapies

1. Platelet and red cells transfusions.
2. Erythropoietin.
3. Bisphosphonates during the study according to the ASCO guidelines (64).
4. Therapies for the treatment of preexisting and/or emergent medical conditions not specifically forbidden as per protocol elsewhere.

5. Antiemetics (excluding steroids) according to institutional or ASCO guidelines (65).
6. Granulocyte colony stimulating factor (G-CSF)/granulocyte-macrophage colony stimulating factor (GM-CSF) and/or blood derived products transfusions, according to institutional or ASCO guidelines (66, 67).
7. Palliative local radiation of a plasmacytoma. The irradiated lesion will then not be considered an area of measurable/evaluable disease.
8. Systemic and/or local therapies for symptomatic relief, particularly in the case of diarrhea or skin toxicity.
9. Patients in the plitidepsin + dexamethasone arm who develop grade ≥ 2 muscular toxicity may be empirically treated with oral L-carnitine at a total daily amount of up to 3 g, divided into three doses, until it decreases to grade ≤ 1.
10. Adequate analgesic medication, including opioids for symptomatic pain relief if indicated.
11. Drugs known to prolong QT interval and/or induce Torsades de Pointes should be avoided whenever possible (see Appendix 7, drugs labeled as 1).

7.5.3 Prohibited Medications/Therapies

1. Concomitant administration of any other antineoplastic therapy.
2. Other investigational agents.
3. Immunosuppressive therapies, except single bolus hydrocortisone used eventually as treatment for hypersensitivity reactions, if required.
4. Primary prophylaxis with colony-stimulating factors, such as G-CSF and GM-CSF.

7.5.4 Drug Accountability

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

All unused drug supplied by the Sponsor will be properly destroyed at the investigational site. Documentation of this procedure must be provided, or with the agreement of Sponsor returned to the drug repository.

7.6 VERIFICATION OF COMPLIANCE WITH TREATMENT REGIMEN

The compliance of the patient is under direct supervision of the Investigator.

8 STUDY EVALUATIONS

8.1 EFFICACY

Primary

- PFS, according to IRC assessment, as per intention-to-treat (ITT) analysis.

Secondary

- Objective RR.
- Best overall response including rate of minor response (MR) or better (according to the IMWG criteria) (Appendix 5).
- RR to combination treatment in patients who crossed over after progression on dexamethasone alone.
- Time-to-event endpoints: DR and OS.
- Intrapatient response and PFS comparison of patients who crossed over from dexamethasone alone (Arm B) to plitidepsin plus dexamethasone combination (Arm A).

The primary study analysis will be based on externally assessed PFS data in the **ITT efficacy population**, defined as ***all patients randomized to either treatment arm.***

PFS will be calculated from randomization to the first evidence of PD (IMWG criteria) or death due to any cause. If the patient receives further antitumor therapy before PD and within the timeframe expected for first follow-up, PFS will be censored on the date of the last disease assessment prior to the administration of this antitumor therapy. If the patient is lost to follow-up for the assessment of progression, or has more than one missing follow-up between the date of last tumor assessment and the date of progression, death or further antitumor therapy, the PFS will be censored at the date of last valid tumor assessment before the missing evaluations.

DR will be calculated from the date of first documentation of response to the date of disease progression or death. The same censoring rules described above for PFS calculation will be also considered for DR.

OS is defined as the time from the date of randomization to the date of death or last contact.
An external IRC, blinded to treatment arm, will assign a progression or censoring date for each patient based on laboratory data and radiologic and bone marrow assessments when required and evaluation of all relevant clinical information, according to a predefined algorithm provided in a separate charter. Both IRC and Investigator's assessment will be used for the determination of RR, DR and PFS.

By design, disease response will be assessed every four weeks symmetrically across treatment arms irrespectively of treatment delays or omissions. Disease assessments (e.g., serum or urine M protein, serum FLC) and evaluation of extent of disease will be done within two weeks before ***randomization*** and every four weeks thereafter in the absence of PD while on treatment. If disease progression has not occurred at treatment termination, then disease assessments should continue every four weeks until evidence of disease progression, start of subsequent anticancer treatment, or death, whichever occurs first.

PFS and objective tumor response will be assessed symmetrically across treatment arms every four weeks while on treatment, irrespectively of dose omissions or delays, according to IMWG criteria. Centralized laboratory reports and copies of CT scans, MRI (in case of soft tissue plasmacytoma) and any other documented means to evaluate tumor response or progression should be available for IRC review.

8.2 SAFETY

Patients are evaluable for general safety if they received at least one dose of the study treatment. Safety will be evaluated in each arm separately according to the actual treatment received.

AEs will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.

8.3 EVALUATION OF PHARMACOKINETICS

At least 100 patients will be evaluated for PK in patients receiving both plitidepsin and dexamethasone during the first three infusions. The effect of the concomitant administration of dexamethasone on the plitidepsin PK and the relationship between the plitidepsin PK exposure and toxicity/efficacy parameters will be evaluated.

9 ADVERSE EVENTS REPORTING

9.1 DEFINITIONS

9.1.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient or a clinical investigation patient administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), or a disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Any event involving adverse drug reactions, illnesses with onset during the study or exacerbations of pre-existing illnesses should be recorded, including but not limited to clinically significant changes in physical examination findings and abnormal objective test findings (e.g., x-ray, ECG). 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.

9.1.2 Serious Adverse Event (SAE)

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.
- Is any suspected transmission of an infectious agent via a medicinal product.

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

9.1.2.1 *Death*

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

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

9.1.2.2 *Life Threatening Event*

Any event in which the patient was 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.

9.1.2.3 *Hospitalization or Prolongation of Hospitalization*

Any AE 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 unless exempted from SAE reporting (see Section 9.2.2). 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:

a) Reasons described in protocol (e.g., study drug administration, protocol-required intervention/investigations, etc). However, events requiring hospitalizations or prolongation of hospitalization as a result of a complication of therapy administration or clinical trial procedures will be reported as SAEs.

b) Hospitalization or prolonged hospitalization for technical, practical or social reasons, in absence of an AE.

c) Pre-planned hospitalizations: any pre-planned surgery or procedure must be documented in the source documentation. 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 the following:

d) An emergency visit due to an accident where the patient is treated and discharged.

e) When the subject is held 24 hours for observation and finally is not admitted.

f) Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (i.e., laser eye surgery, arthroscopy, etc).

9.1.3 Unlisted /Unexpected Adverse Event

An AE, the nature or severity of which is not consistent with the applicable reference safety information.

The Sponsor will use as the reference safety information for the evaluation of listedness/expectedness the following documents:

- The current Investigator's Brochure (IB) for any PharmaMar study drug without a marketing authorization or used outside the conditions of its marketing authorization.
- The summary of product characteristics (SPC) or its national equivalent [i.e., U.S. package inserts (USPI)] for non-PharmaMar study drugs used within the conditions of its marketing authorization. For dexamethasone the expectedness will be assessed against the safety sections in the label maintained at http://www.drugs.com/pro/dexamethasone.html.

9.1.4 Adverse Reactions

All untoward and unintended responses to an investigational medicinal product related to any dose administered. This definition covers also medication errors and uses outside what is foreseen in the protocol, including overdose, lack of efficacy, misuse and abuse of the product.

9.1.5 Adverse Events Related to Study Drugs

An AE is considered related to a study drug/ investigational medicinal product (IMP) if the Investigator assessment of causal relationship to the study drug is "Y" (see below).

The Investigator will assess the causal relationship of each of the study drugs to the SAE.

The Sponsor may also consider related to the study drug those events for which the Investigator assesses the causal relationship with the study drug as "Uk" when it cannot rule out a role of the study drug in the event. Refer to Section 10.1.6 for causality scale.

9.1.6 Expedited Reporting

The Sponsor is responsible for the appropriate expedited reporting of suspected unlisted / unexpected and related serious adverse reactions (SUSARs), including the following events of special interest: misuse, overdose, medication error and abuse, to the Competent Authorities. The Sponsor will also report all SAEs, including those which are unlisted / unexpected and related to the study drug(s), to the Investigators and to the Independent Ethics Committees/Institutional Review Boards (IECs/IRBs) according to the current legislation unless otherwise required and documented by the IECs/IRBs.

9.1.7 Assessment of Causal Relationship to the Study Drug

The Investigator must provide an assessment of causal relationship of each of the clinical trial study drugs (including combination and comparator products) to the SAE according to the following scale:

Y There is a reasonable possibility that the study drug(s) caused the SAE

N There is no reasonable possibility that the study drug(s) caused the SAE and other causes are more probable.

Uk Only to be used in special situations where the Investigator has insufficient information (i.e. the patient was not seen at his/her centre) if none of the above can be used.

9.2 PROCEDURES

9.2.1 Reporting of Adverse Events

The Sponsor will collect AEs until 30 days after administration of the last dose of study drug(s) or until the start of a new antitumor therapy, whichever occurs first. All AEs suspected to be related to study drug(s) must be followed after the time of therapy discontinuation until the event or its sequelae resolve or stabilize at an acceptable level to the Investigator and the Sponsor.

All AEs, including misuse, overdose and abuse must be recorded using medical terminology in the source document and the CRF. Whenever possible the Investigator will record the main diagnosis instead of the signs and symptoms normally included in the diagnoses.

Investigators must assess severity (grade) of the event following NCI-CTCAE v. 4 and assign relationship to each study drug; pursue and obtain information adequate both 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 relevant information as requested by the Sponsor in addition to that on the CRF.

Abnormal laboratory tests occurring during the study are AEs, but they should be collected only in the AE section of the CRF in some cases:

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

Otherwise laboratory results should be reported in the corresponding section of the CRF (e.g. biochemistry, hematology).

9.2.2 Reporting Serious Adverse Events

The Sponsor will collect SAEs from the signing of the Informed Consent Form onwards. If the patient is definitively included in the study, this information will also be recorded in the AE section of the CRF, if applicable.

SAEs will be collected until 30 days after administration of the last dose of study drug(s) or until the start of a new antitumor therapy, whichever occurs first. Beyond this period of time, only those SAEs suspected to be related need to be reported. Nonetheless, the Sponsor will evaluate any safety information that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

Exemptions from SAE REPORTING: Events of "disease progression" even if they fulfill any seriousness criterion (i.e., fatal, requiring hospitalization, etc) are exempted from SAE reporting and will only be reported in the applicable CRF page(s).

All SAEs (as defined above) regardless of treatment group or relationship to study drug/IMP must be reported immediately and always within 24 hours to the PharmaMar Pharmacovigilance Department by fax (+34 91 846 6004) or telephone (+34 91 846 6054). Out of office hours (GMT), assistance on SAE reporting can be obtained by calling the Pharmacovigilance Service +34 91 823 47 49.

The preferred reporting method is by faxing the completed "Serious Adverse Event Form" provided by Pharmamar. An initial report by telephone must be followed by a completed SAE form the investigational staff within one working day.

All SAEs suspected to be related to the study drug(s) must be followed until the event or its sequelae resolves or stabilizes at an acceptable level by the Investigator and the clinical monitor or his/her designated representative.

9.2.3 Reporting Pregnancy Cases Occurred within the Clinical Trial

National regulations require that clinical trial Sponsors collect information on pregnancies occurring during clinical trials, in which exposure to the study drugs at any moment of the pregnancy, via either maternal or paternal exposure is suspected.

Therefore, pregnancy and suspected pregnancy (including a positive pregnancy test regardless of age or disease state) of a female patient or the female partner of a male patient occurring while the patient is on study drug, or within 30 days of the patient's discontinuation visit, is considered an immediately reportable event.

The Investigator will report the following events immediately and always within 24 hours from first knowledge:

- Any occurrence of a pregnancy where any kind of exposure to the drug(s) is suspected
- 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 study drug(s)]
- All reports of elevated/ questionable or indeterminate beta human chorionic gonadotrophins (βhCGs)

Immediately after detecting a case of suspected pregnancy in a female clinical trial patient, the decision on her continued participation in the clinical trial will be jointly taken by the trial patient, the Investigator and the Sponsor, with the patient's best interest in mind. A decision to continue the pregnancy will require immediate withdrawal from the trial. If the trial is blinded, the Investigator will open the blind whenever the treatment information is needed for the management of the patient.

Any pregnancy, suspected pregnancy, or positive pregnancy test must be reported to PharmaMar Pharmacovigilance immediately by facsimile using the Pregnancy Report form. In the case of pregnancy of the female partner of a trial Patient the Investigator will obtain her informed consent to provide the information by using the applicable form provided by the Sponsor who will also advise the Investigator in these situations.

The Investigator will follow the pregnancy until its outcome, and must notify PharmaMar Pharmacovigilance the outcome of the pregnancy within 24 hours of first knowledge as a follow-up to the initial report.

For any event during the pregnancy, which meets a seriousness criterion (including fetal or neonatal death or congenital anomaly), the Investigator will also follow the procedures for reporting SAEs (complete and send the SAE form to PharmaMar Pharmacovigilance by facsimile within 24 hours of the Investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death at any time thereafter that the Investigator suspects is related to the exposure to the study drug(s) should also be reported to PharmaMar Pharmacovigilance by facsimile within 24 hours of the Investigators' knowledge of the event.

10 STATISTICAL METHODS

10.1 SAMPLE SIZE

1. **Arm A** (plitidepsin plus dexamethasone combination): approximately 167 patients.
2. **Arm B** (dexamethasone single agent): approximately 83 patients.

The number of patients randomized in the trial has been calculated based on PFS estimates obtained from the previous phase II study (APL-B-014-03).

Approximately 210 progression or death events would be needed in this trial to detect a HR of 0.625 in favor of the combination arm (equivalent to an increase of 60% in PFS, i.e., from 10 to 16 weeks, from 12 to 19.2 weeks, from 16 to 25.6 weeks, etc.) with 90% power and 1-sided 2.5% significance level. As a preliminary hypothesis, it is estimated that up to 250 randomized patients will be needed to achieve the 210 events in 24-30 months.

10.2 STATISTICAL ANALYSIS

10.2.1 Demographics

Descriptive statistics (mean, median, standard deviation and 95% confidence interval, range of value, frequencies and percentages) will be used. Tables by randomization group will be displayed.

Comparative study with descriptive methods will be done to check that patients' baseline characteristics are similar between treatment groups

10.2.2 Safety

Patients are evaluable for general safety if they received any study treatment. Safety will be evaluated in each arm separately according to the actual treatment received.

AEs will be graded according to CTCAE, version 4.0.

Descriptive statistics will be employed to characterize the profiles of drug-related adverse events, drug-related deaths, SAE and drug-related treatment discontinuation. Tables by randomization group will be displayed.

10.2.3 Efficacy Analysis

10.2.3.1 *Early Futility Analysis*

An early futility analysis will be performed when information from 40 patients in Arm A are evaluable for response. A response rate (IMWG criteria) of at least 30% (12 or more responses by IRC review) will be taken as threshold for continuation of the study. A minimum response rate of 30% has been considered as clinically significant in this setting. This result will ensure that the lower limit of the exact binomial 95% Confidence Interval for the response rate will be greater than 15% (95% CI in case of 12 responses would be 16.6% - 46.5%).

However, the information from all randomized patients in both arms at that time will be used by the IDMC to evaluate the safety profile and to provide the Sponsor with a recommendation for the further study conduct. No claim for superiority in efficacy will be formulated in this interim analysis and no alpha-spending for the analysis of PFS is foreseen.

10.2.3.2 *Patients' Evaluability for Response*

All patients who have completed at least one evaluable cycle or who have received two uncompleted cycles followed by at least one response assessment not less than eight weeks (± one week) after treatment onset, will be considered evaluable for response. In addition, any eligible patients who receive at least one treatment cycle or have received two uncompleted cycles and experience disease progression or die due to progressive disease prior to response evaluation will be considered evaluable for response and will be categorized as an "early progression".

Patients withdrawn due to drug-related toxicity without any tumor assessments after the start of study treatment will be considered as “treatment failures”.

Patients withdrawn due to significant clinical deterioration of unknown reason, hypersensitivity reactions, or refusal to continue on study for any reason or unrelated AEs without any disease assessments after the start of study treatment will be considered not evaluable for efficacy and their response will be categorized as “non evaluable”.

Binomial estimates with exact 95% CIs will be calculated for the analysis of response rate. Randomized patients not evaluable for response will be excluded from the denominator exclusively for the futility analysis but will be included in all final efficacy analyses.

10.2.3.3 *Final PFS Analysis and Interim OS analysis*

The final PFS analysis will be performed when at least 210 progression or death events are observed.

The PFS assessed by the Independent Review Committee (IRC) will be used for the primary analysis, while the Investigator assessment will be used for the secondary analysis of PFS.

The unstratified log rank test will be used to compare the PFS of Arm A and Arm B.

Secondary time-to-event endpoints (DR, OS) will be analyzed according to the Kaplan-Meier method and compared between treatment groups using the log-rank test. A stratified log-rank test for the main endpoint (PFS) will be performed as supportive analysis. Both the IRC and Investigator’s assessments will be used.

Counts and percentages, with their corresponding exact 95% confidence intervals, will be calculated for the RR (IMWG criteria and, separately, minor response). The Fisher’s exact test will be used to compare the response rates of the Arm A and B.

10.2.3.4 *Evaluation of Overall Survival*

Although the study is powered for the evaluation of the main endpoint, PFS, two analyses of OS will be performed to ascertain if a trend in OS is observed in favor of the experimental arm. An interim analysis of OS will be performed concomitantly with the final PFS analysis (see Section 10.2.3.3). A final analysis of OS will be performed when 80% of death events (approximately 200 death events) have occurred or 24 months after the inclusion of the last patient, whichever occurs first. At the interim OS analyses, the significance level determined by the O’Brien-Fleming boundary with overall 2.5% 1-sided significance level will be used.

Note: Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A (plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death

events are not achieved on 19 November 2017, the study might be further extended by three to six months.

It is anticipated that an indeterminate number of patients in the control arm will switch treatment after progression to plitidepsin plus dexamethasone. This could cause the size of the effect on OS to be difficult to interpret. Consequently, if the OS results show to be substantially influenced by crossover, the Sponsor will study the estimated effect of crossover by means of rank preserving structural failure time models for correcting for treatment changes (68).

10.2.3.5 *Other Analyses of Efficacy*

Efficacy parameters versus baseline covariates will be analyzed and appropriate tests will be used (i.e., the Fisher exact test and logistic regression for categorical variables, the log-rank test or Cox regression for time-to-event variables, etc.).

Exploratory intrapatient comparison of response and PFS (before and after crossover) will be performed for patients who switch from Arm B to Arm A after disease progression.

10.2.4 Pharmacokinetics

Pharmacokinetic parameters will be calculated using population methods, after pooling data from this study with data obtained in other plitidepsin single-agent studies. The effect of dexamethasone on the plitidepsin pharmacokinetics will be evaluated by testing the improvement in the "Objective Function" of the population model after the inclusion of a shift variable on every relevant PK parameter. Bayesian estimation of the individual PK parameters will be correlated with toxicity and response outcomes. Logistic regression will be used for dichotomic or categorical outcomes, such as response/no response or toxicity grade. For continuous outcomes, such as transaminase values, linear regression will be applied. The influence of the plitidepsin PK on time to event variables, such as PFS, will be evaluated using Cox regression.

10.3 INTERIM ANALYSIS

Protocol-specified analyses are foreseen at the time of the futility evaluation (approximately 40 evaluable patients for response in Arm A), final PFS analysis (main endpoint, approximately 210 progression or death events) and follow-up evaluation of OS when 80% of death events (approximately 200 death events) have occurred or 24 months after the inclusion of the last patient, whichever occurs first. Accrual will be on-hold while data for the futility analysis is being assessed.

Note: Two years after the last patient accrual, on 19 May 2017, the final OS analysis was done based on a total of 195 death events (i.e., 76.5% of the 255 randomized patients): 123 events in Arm A (plitidepsin plus dexamethasone) and 72 events in Arm B (dexamethasone). Then, the duration of this study is prolonged for six additional months, until 19 November 2017, in order to continue follow-up in the alive patients, and then to be able to reach the pre-specified total of 80% of death events (approximately 204 death events) or even more. If the required death events are not achieved on 19 November 2017, the study might be further extended by three to six months.

11 ADMINISTRATIVE SECTION

11.1 ETHICS

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

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

The study will be conducted in compliance with the protocol. The protocol, any amendments and the patient informed consent will receive Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval/favorable opinion prior to initiation.

The decision of the IEC/IRB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of the study.

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

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

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

11.2 MONITORING, AUDITING AND INSPECTING

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

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

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

Systems with procedures will be implemented to assure the quality of every aspect of the study.

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

Participation in this study implies acceptance of potential inspection by national or foreign health authorities.

11.3 PATIENT INFORMED CONSENT

The rights, safety and well being of the trial patients are the most important considerations and should prevail over interests of science and society.

The Patient Information sheet will include all elements required by ICH, GCP and applicable Regulatory requirements.

The Investigator, or a person designated by the Investigator, must provide the patient with a copy of the consent form and written fully information about the study in language that is non-technical and easily understood. The Investigator should allow time necessary for the patient or the patient's legally acceptable representative to inquire about the details of the study; then, the informed consent must be freely signed and personally dated by the patient and by the person who conducted the informed consent discussion before commencement of the study. The patient should receive a copy of the signed informed consent and any other written information provided to study patients prior to patient's participation in the trial.

During a patient's participation in the trial, any updates to the consent 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 a person designated by the Investigator, should inform the patient of any new information relevant to the patient's willingness to continue participation in the study, before obtaining the written consent.

11.4 CONFIDENTIALITY/ PATIENTS IDENTIFICATION

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational medicinal product used in this study. It is the Investigator's responsibility that sufficient information appertaining to the identity of the patients will be retained.

The trial monitor, Sponsor's auditor, IRB/IEC, or Competent Authorities should have direct access to all requested trial related records and agree to keep the identity of study patients confidential.

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

11.5 CASE REPORT FORMS

Case report forms (CRFs) will be used for recording all data for each patient. It is the responsibility of the Investigator to ensure that the CRFs are properly and completely

filled in. CRFs must be completed for all patients who have given informed consent and have been admitted into the study.

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

The data collected in the CRF will be entered into the Sponsor databases that comply with the Spanish Act which implements the 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.

11.6 INSURANCE

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

11.7 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 of time, if required by the applicable regulations.

11.8 USE OF INFORMATION AND PUBLICATION

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

If the study is part of a multicenter study, the first publication of the study shall be made in conjunction with the presentation of a joint, multicenter publication of the study results with the Investigators and the Institutions from all appropriate sites contributing data, analysis and comments. However, if such a multicenter publication is not submitted within 12 months after conclusion, abandonment or termination of the study at all sites, the present study may be published individually in accordance with the procedure established before.

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

12 REFERENCES

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13 APPENDICES

APPENDIX 1: CLINICAL MANAGEMENT OF HYPERSENSITIVITY REACTIONS TO PLITIDEPSIN®

• **If Severe/life threatening reactions** *(NCI CTCAE v4 ≥ grade 3)*

Patients must have at least one of the following:

-Cardiovascular symptoms: *hypertension ≥ 160/100 mmHg or tachycardia ≥ 120bpm or hypotension (systolic pressure < 90mmHg) requiring vasopressor therapy, or anaphylactic shock or*

-Respiratory symptoms: *Angioedema, generalized wheezing and/or respiratory distress requiring oxygen therapy (pulse gasometry with < 92% O_2 saturation at ambient air).*

Then:

1) Immediately stop infusion. A trained physician must clinically assess the patient.

2) If pulse gasometry < 92% O_2 saturation at ambient air, consider immediately O_2 mask ± bronchodilators (i.e., generalized wheezing).

3) Administer diphenhydramine 50 mg i.v. or equivalent, and hydrocortisone 100 mg up to a maximum of 300 mg i.v.

4) Add epinephrine (adrenaline) if clinically indicated.

Do not re-start infusion. Withdraw the patient from the study.

Event should be reported as a serious adverse event. Send an SAE form by fax within 24 hrs.

Carry out final disease assessments, post-treatment evaluations and follow-up as required by protocol.

CLINICAL MANAGEMENT OF HYPERSENSITIVITY REACTIONS TO PLITIDEPSIN® (CONT.)

If Mild to Moderate reactions/not life threatening *(NCI CTCAE v4.0 ≤ grade 2)*

Patients may have any of the following:

-Mild to moderate*: facial and/or trunk flushing, rash and/or pruritus and/or mild dyspnea (shortness of breath) and/or coughing and/or chest discomfort.*

BUT NONE of the previous severe/life threatening criteria.

Then:

1) Immediately stop infusion and assess vital signs and pulse gasometry. Ideally a trained physician must clinically assess the patient.

2) Check if premedication was administered properly (if not, administer it as per protocol and re-start infusion similarly as before, withholding it a minimum of 30 minutes after premedication was given).

3) If symptoms persist after stopping infusion, give additional diphenhydramine, 50 mg i.v. or equivalent, and hydrocortisone 100 mg bolus i.v. or equivalent.

4) Reassess symptoms and vital signs after 30 minutes.

4.A- If normal or improving, then infusion could be re-started at 1/3 (one third) of the initial drip rate during the first hour, and then, infusion rate could be increased according to tolerance. The decision about retreating the patient or not should be taken according to Investigator's judgment on benefit/risk balance. Any further infusions should be initiated at this reduced rate and should be given with prophylactic premedication as per protocol. If no hypersensitivity reactions are observed, the rate of infusion to be applied could be set back to the initial one.

4.B- If there is no sign of improvement of symptoms after 30 minutes, a decision must be taken by the investigator in agreement with the patient in order to:

- Eventually repeat medication (anti-H_1 and/or corticoids) if necessary and/or wait up to one hour for symptoms to resolve.

- Omit infusion until resolution (< two weeks) (in this case further infusions might start at a reduced rate, according to 4.A

-Withdraw the patient from study.

- Report the event as a serious adverse only if it fulfills any of the seriousness criteria indicated in the SAE section of the protocol.

APPENDIX 2: PERFORMANCE STATUS (ECOG)

Grade	ECOG*
0	**Fully active, able to carry on all pre-disease performance without restriction**
1	**Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work**
2	**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	**Capable of only limited self-care, confined to bed or chair more than 50% of waking hours**
4	**Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair**
5	**Dead**

*As published in Am. J. Clin. Oncol *5:649-655, 1982*: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group.*

APPENDIX 3: COCKCROFT AND GAULT'S FORMULA

For calculating creatinine clearance:

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

$$\text{Creatinine clearance (ml/min)} = \frac{[(140\text{-age (years)}) \times \text{weight (Kg)}]}{72 \times \text{serum creatinine (}\mu\text{mol/l)} \times 0.0113} \times G^{1}$$

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

Reference:

Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

APPENDIX 4: DIAGNOSTIC CRITERIA

Multiple myeloma:

All three criteria must be met, except as noted:

1) Clonal bone marrow plasma cells ≥ 10%.
2) Presence of serum and/or urinary monoclonal protein (except in patients with true non-secretory multiple myeloma).
3) Evidence of end-organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically
   - Hypercalcemia: serum calcium > 11.5 mg/100 ml or
   - Renal insufficiency: serum creatinine > 1.73 mmol/l
   - Anemia: normochromic, normocytic with a hemoglobin value of > 2 g/100 ml below the lower limit of normal or a hemoglobin value of 10 g/100 ml
   - Bone lesions: lytic lesions, severe osteopenia or pathologic fractures.

References:

The International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Haematol 2003; 121: 749–757.

Rajkumar SV, Kyle RA. Multiple myeloma: diagnosis and treatment. Mayo Clinic Proc 2005; 80: 1371–1382.

APPENDIX 5: INTERNATIONAL MYELOMA WORKING GROUP UNIFORM RESPONSE CRITERIA FOR MULTIPLE MYELOMA

(Anderson KC, Kyle RA, Rajkumar SV, Stewart AK, Weber D, Richardson P. Clinically relevant end points and new drug approvals for myeloma. Leukemia 2008; 22: 231–239).

- **Complete response (CR)**

- Negative immunofixation of serum and urine, and
- Disappearance of any soft tissue plasmacytomas, and
- <5% plasma cells in bone marrow.

- **Stringent complete response (sCR)**

CR as defined above plus

- Normal FLC ratio, and
- Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence.

- **Very good partial response (VGPR)**

- Serum and urine M-component detectable by immunofixation but not on electrophoresis or > 90% or greater reduction in serum M-component plus urine M-component of 100mg per 24 h.

- **Partial response (PR)**

- ≥ 50% reduction of serum M protein and reduction in 24-h urinary M protein by ≥ 90% or to < 200mg per 24 h.
- If the serum and urine M protein are unmeasurable, a > 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M protein criteria.
- If serum and urine M protein are unmeasurable, and serum free light assay is also unmeasurable, ≥ 50% reduction in bone marrow plasma cells is required in place of M protein, provided baseline percentage was > 30%.
- In addition to the above criteria, if present at baseline, ≥ 50% reduction in the size of soft tissue plasmacytomas is also required.

- **Stable disease (SD)**

- Not meeting criteria for CR, VGPR, PR or progressive disease

- **Progressive disease (PD)**

Increase of 25% from lowest response value in any one or more of the following:

- Serum M-component (absolute increase must be ≥ 0.5 g/100 ml) and/or
- Urine M-component (absolute increase must be ≥ 200mg per 24 h) and/or
- Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be > 100 mg/l)
- Bone marrow plasma cell percentage (absolute % must be ≥ 10%)
- Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas
- Development of hypercalcemia (corrected serum calcium > 11.5 mg/100 ml) that can be attributed solely to the plasma cell proliferative disorder

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 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; complete, PR and SD categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed.

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

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

<u>Additional response criteria for specific disease stages</u>

(Anderson KC, Kyle RA, Rajkumar SV, Stewart AK, Weber D, Richardson P. Clinically relevant end points and new drug approvals for myeloma. *Leukemia* 2008; 22: 231–239).

Definition of relapsed myeloma and relapsed and refractory myeloma

- **Relapsed myeloma**: at least one prior regimen, and not meeting criteria for relapsed and refractory myeloma
- **Relapsed and refractory myeloma**: relapse of disease while on salvage therapy, or progression within 60 days of most recent therapy

Minor response (MR) in patients with relapsed refractory myeloma

- ≥ 25% but < 49% reduction of serum M protein and reduction in 24 h urine M protein by 50–89%, which still exceeds 200 mg per 24 h

- In addition to the above criteria, if present at baseline, 25–49% reduction in the size of soft tissue plasmacytomas is also required
- No increase in size or number of lytic bone lesions (development of compression fracture does not exclude response)

APPENDIX 6: STAGING SYSTEMS

Durie-Salmon Staging Criteria

Stage	Durie-Salmon Criteria	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	ß2-M <3.5 and Albumin ≥3.5
II	Neither stage I nor stage III	Neither stage I nor stage III
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	ß2-M >5.5
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		

New International Staging System

Stage	Criteria	Median Survival (months)
I	Serum ß2-microglobulin < 3.5 mg/L	62
	Serum albumin ≥3.5 g/dl	
II	Not stage I or III*	44
III	Serum ß2-microglobulin ≥5.5 mg/L	29

* 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 7: LISTS OF DRUGS THAT PROLONG THE QT INTERVAL AND/OR INDUCE TORSADES DE POINTES VENTRICULAR ARRHYTHMIA

Source: www.azcert.org

Because the evidence for risk of TdP is often imperfect, AZCERT, inc. has divided the drugs into four groups based on their analysis of the evidence:

1. Risk of TdP: Substantial evidence supports the conclusion that these drugs prolong QT intervals and have a risk of TdP when used as directed in labeling.

2. Possible risk of TdP: Substantial evidence supports the conclusion that these drugs can cause QT prolongation but there is insufficient evidence that the drugs, when used as directed in labeling, have a risk of causing TdP.

3. Conditional risk of TdP: Substantial evidence supports the conclusion that these drugs prolong QT and have a risk of developing TdP but only under certain known conditions.

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

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Alfuzosin (Uroxatral®)	Alpha1-blocker/benign prostatic hyperplasia		2
Amantadine (Symmetrel®)	Dopaminergic/anti-viral/anti-infective/Parkinson's disease		2
Amiodarone (Cordarone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	1
Amiodarone (Pacerone®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males,TdP risk regarded as low	1
Amisulpride (Solian® and others)	Antipsychotic, atypical	Risk of TdP with overdose - not available in U.S.	3
Amitriptyline (Elavil®)	Tricyclic antidepressant/depression	Risk of TdP with overdosage	3
Arsenic trioxide (Trisenox®)	Anti-cancer/leukemia		1
Artenimol + piperaquine (Eurartesim®)	Anti-malarial/	Not available in U.S.	2
Astemizole (Hismanal®)	Antihistamine/allergic rhinitis	No longer available in U.S.	1
Atazanavir (Reyataz®)	Protease inhibitor/HIV		2
Azithromycin (Zithromax®)	Antibiotic/bacterial infection		1
Bedaquiline (Sirturo®)	Anti-infective/drug-resistant Tuberculosis	Black Box for QT	2
Bepridil (Vascor®)	Anti-anginal/heart pain	Females>Males	1
Chloral hydrate (Noctec®)	Sedative/sedation/insomnia		2
Chloroquine (Aralen®)	Anti-malarial/malaria infection		1

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Chlorpromazine (Thorazine®)	Anti-psychotic/anti-emetic/schizophrenia/nausea		1
Ciprofloxacin (Cipro®)	Antibiotic/bacterial infection	Drug interaction risk - metabolic inhibitor	3
Cisapride (Propulsid®)	GI stimulant/heartburn	No longer available in U.S.	1
Citalopram (Celexa®)	Anti-depressant/depression		1
Clarithromycin (Biaxin®)	Antibiotic/bacterial infection		1
Clomipramine (Anafranil®)	Tricyclic Antidepressant/depression		3
Clozapine (Clozaril®)	Anti-psychotic/schizophrenia		2
Desipramine (Pertofrane®)	Tricyclic Antidepressant/depression	Risk of TdP with overdosage	3
Diphenhydramine (Benadryl®)	Antihistamine/allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	3
Diphenhydramine (Nytol®)	Antihistamine/allergic rhinitis, insomnia	Risk of QT increase/TdP in overdosages	3
Disopyramide (Norpace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	1
Dofetilide (Tikosyn®)	Anti-arrhythmic/abnormal heart rhythm	Females > Males	1
Dolasetron (Anzemet®)	Anti-nausea/nausea, vomiting		2
Domperidone (Motilium®)	Anti-nausea/nausea	Not available in U.S.	1
Doxepin (Sinequan®)	Tricyclic Antidepressant/depression		3
Dronedarone (Multaq®)	Anti-arrhythmic/atrial fibrillation		2
Droperidol (Inapsine®)	Sedative;anti-nausea/anesthesia adjunct, nausea		1
Eribulin (Halaven®)	Anti-cancer/metastatic breast neoplasias		2
Erythromycin (E.E.S.®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	1
Erythromycin (Erythrocin®)	Antibiotic;GI stimulant/bacterial infection; increase GI motility	Females>Males	1
Escitalopram (Cipralex®)	Anti-depressant/major depression/ anxiety disorders		1
Escitalopram (Lexapro®)	Anti-depressant/major depression/ anxiety disorders		1
Famotidine (Pepcid®)	H2-receptor antagonist/peptic ulcer/ GERD		2
Felbamate (Felbatrol®)	Anti-convulsant/seizure		2
Fingolimod (Gilenya®)	Immunosuppressant/multiple sclerosis		2
Flecainide (Tambocor®)	Anti-arrhythmic/abnormal heart rhythm		1
Fluconazole (Diflucan®)	Anti-fungal/fungal infection	Drug interaction risk- metabolic inhibitor. Can also increase QT at high doses - 800 mg/day	3
Fluoxetine (Sarafem®)	Anti-depressant/depression		3
Fluoxetine (Prozac®)	Anti-depressant/depression		3
Foscarnet (Foscavir®)	Anti-viral/HIV infection		2
Fosphenytoin (Cerebyx®)	Anti-convulsant/seizure		2
Galantamine (Reminyl®)	Cholinesterase inhibitor/ dementia, Alzheimer's		3

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Gatifloxacin (Tequin®)	Antibiotic/bacterial infection	Oral/I.V. forms no longer available in U.S. and Canada, only ophthalmic	2
Gemifloxacin (Factive®)	Antibiotic/bacterial infection		2
Granisetron (Kytril®)	Anti-nausea/nausea and vomiting		2
Halofantrine (Halfan®)	Anti-malarial/malaria infection	Females>Males	1
Haloperidol (Haldol®)	Anti-psychotic/schizophrenia, agitation	TdP risk with I.V. or excess dosage	1
Ibutilide (Corvert®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	1
Iloperidone (Fanapt®)	Antipsychotic, atypical/schizophrenia		2
Imipramine (Norfranil®)	Tricyclic antidepressant/depression	TdP risk with excess dosage	3
Indapamide (Lozol®)	Diuretic/stimulate urine & salt loss		2
Isradipine (Dynacirc®)	Anti-hypertensive/high blood pressure		2
Itraconazole (Sporanox®)	Anti-fungal/fungal infection	Drug interaction risk - metabolic inhibitor	3
Ketoconazole (Nizoral®)	Anti-fungal/fungal infection	Prolongs QT & Drug interaction risk - metabolic inhibitor.	3
Lapatinib (Tykerb®)	Anti-cancer/breast cancer, metastatic		2
Lapatinib (Tyverb®)	Anti-cancer/breast cancer, metastatic		2
Levofloxacin (Levaquin®)	Antibiotic/bacterial infection		2
Levomethadyl (Orlaam®)	Opiate agonist/pain control, narcotic dependence	Not available in U.S.	1
Lithium (Eskalith®)	Anti-mania/bipolar disorder		2
Lithium (Lithobid®)	Anti-mania/bipolar disorder		2
Mesoridazine (Serentil®)	Anti-psychotic/schizophrenia		1
Methadone (Dolophine®)	Opiate agonist/pain control, narcotic dependence	Females>Males	1
Methadone (Methadose®)	Opiate agonist/pain control, narcotic dependence	Females>Males	1
Mirtazapine (Remeron®)	Anti-depressant/		2
Moexipril/HCTZ (Uniretic®)	Anti-hypertensive/high blood pressure		2
Moxifloxacin (Avelox®)	Antibiotic/bacterial infection		1
Nicardipine (Cardene®)	Anti-hypertensive/high blood pressure		2
Nilotinib (Tasigna®)	Anti-cancer/leukemia		2
Nortriptyline (Pamelor®)	Tricyclic Antidepressant/depression		3
Octreotide (Sandostatin®)	Endocrine/acromegaly, carcinoid diarrhea		2
Ofloxacin (Floxin®)	Antibiotic/bacterial infection		2
Olanzapine (Zyprexa®)	Antipsychotic, atypical/schizophrenia, bipolar	Combo c fluoxetine: Symbyax	2
Ondansetron (Zofran®)	Anti-emetic/nausea and vomiting		2
Oxytocin (Pitocin®)	Oxytocic/labor stimulation		2
Paliperidone (Invega®)	Antipsychotic, atypical/schizophrenia		2

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Paroxetine (Paxil®)	Anti-depressant/depression		3
Pentamidine (NebuPent®)	Anti-infective/pneumocystis pneumonia	Females>Males	1
Pentamidine (Pentam®)	Anti-infective/pneumocystis pneumonia	Females>Males	1
Perflutren lipid microspheres (Definity®)	Imaging contrast agent/echocardiography		2
Pimozide (Orap®)	Anti-psychotic/Tourette's tics	Females>Males	1
Probucol (Lorelco®)	Antilipemic/hypercholesterolemia	No longer available in U.S.	1
Procainamide (Pronestyl®)	Anti-arrhythmic/abnormal heart rhythm		1
Procainamide (Procan®)	Anti-arrhythmic/abnormal heart rhythm		1
Protriptyline (Vivactil®)	Tricyclic antidepressant/depression		3
Quetiapine (Seroquel®)	Anti-psychotic/schizophrenia		2
Quinidine (Quinaglute®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	1
Quinidine (Cardioquin®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	1
Quinine sulfate (Qualaquin®)	Anti-malarial/malaria or leg cramps	TdP with overdose or drug-drug or drug food interaction	3
Ranolazine (Ranexa®)	Anti-anginal/chronic angina		2
Risperidone (Risperdal®)	Anti-psychotic/schizophrenia		2
Ritonavir (Norvir®)	Protease inhibitor/HIV		3
Roxithromycin* (Rulide®)	Antibiotic/bacterial infection	*Not available in U.S.	2
Sertindole (Serdolect®)	Antipsychotic, atypical/anxiety, schizophrenia	Not available in U.S.	2
Sertindole (Serlect®)	Antipsychotic, atypical/anxiety, schizophrenia	Not available in U.S.	2
Sertraline (Zoloft®)	Anti-depressant/depression		3
Sevoflurane (Ulane®)	Anesthetic, general/anesthesia	Label warning for patients with congenital long QT or patients taking QT prolonging drugs	1
Sevoflurane (Sojourn®)	Anesthetic, general/anesthesia	Label warning for patients with congenital long QT or patients taking QT prolonging drugs	1
Solifenacin (VESIcare®)	muscarinic receptor anatagonist/treatment of overactive bladder		3
Sotalol (Betapace®)	Anti-arrhythmic/abnormal heart rhythm	Females>Males	1
Sparfloxacin (Zagam®)	Antibiotic/bacterial infection	No longer available in U.S.	1
Sunitinib (Sutent®)	Anti-cancer/RCC, GIST		2
Tacrolimus (Prograf®)	Immunosuppressant/Immune suppression		2
Tamoxifen (Nolvadex®)	Anti-cancer/breast cancer		2
Telithromycin (Ketek®)	Antibiotic/bacterial infection		2
Terfenadine (Seldane®)	Antihistamine/allergic rhinitis	No longer available in U.S.	1
Thioridazine (Mellaril®)	Anti-psychotic/schizophrenia		1
Tizanidine (Zanaflex®)	Muscle relaxant/		2
Trazodone (Desyrel®)	Anti-depressant/depression, insomnia		3

Generic Name (Brand Name)	Drug Class / Clinical Usage	Comments	Risk List
Trimethoprim-Sulfa (Septra® or Bactrim®)	Antibiotic/bacterial infection	Also available in DS (double strength)	3
Trimipramine (Surmontil®)	Tricyclic Antidepressant/depression		3
Vandetanib (Caprelsa®)	Anti-cancer/thyroid cancer		1
Vardenafil (Levitra®)	Phosphodiesterase inhibitor/vasodilator		2
Venlafaxine (Effexor®)	Anti-depressant/depression		2
Voriconazole (VFend®)	Anti-fungal/anti-fungal		2
Ziprasidone (Geodon®)	Anti-psychotic/schizophrenia		2

APPENDIX 8: QTC SUBSTUDY PROTOCOL

An Uncontrolled, Open-label, Substudy within APL-C-001-09 Clinical Study (ADMYRE) in Patients with Relapsed/Refractory Multiple Myeloma, Evaluating the Potential Effects of Plitidepsin on the QT Intervals of the Electrocardiogram.

1. INTRODUCTION

Plitidepsin showed no relevant cardiac toxicity during preclinical/toxicology studies. However, other cyclic depsipeptides have been linked to increased cardiac toxicity, and thus a retrospective study of the cardiac adverse events [e.g., rhythm abnormalities, myocardial injury events, electrocardiogram (ECG) alterations] that occurred in all patients treated with single-agent plitidepsin was conducted. This study showed that the frequency of these events was not different to that reported in the age-matched healthy population, thereby suggesting that plitidepsin has a safe cardiac risk profile in cancer patients.[1]

1.1. SUBSTUDY RATIONALE

An undesirable property of some drugs is their ability to delay cardiac repolarization, an effect that can be measured as prolongation of the QT interval on the surface electrocardiogram (ECG). The QT interval represents the duration of ventricular depolarization and subsequent repolarization, and is measured from the beginning of the QRS complex to the end of the T wave. A delay in cardiac repolarization creates an electrophysiological environment that favors the development of cardiac arrhythmias, most clearly torsades de pointes (TdP), but possibly other ventricular tachyarrhythmias as well. TdP is a polymorphic ventricular tachyarrhythmia that appears on the ECG as continuous twisting of the vector of the QRS complex around the isoelectric baseline. A feature of TdP is pronounced prolongation of the QT interval in the supraventricular beat preceding the arrhythmia. TdP can degenerate into ventricular fibrillation, leading to sudden death. While the degree of QT prolongation is recognized as an imperfect biomarker for proarrhythmic risk, in general there is a qualitative relationship between QT prolongation and the risk of TdP, especially for drugs that cause substantial prolongation of the QT interval. Because of its inverse relationship to heart rate, the measured QT interval is routinely corrected by means of various formulae to a less heart rate-dependent value known as the QTc interval. It is not clear, however, whether arrhythmia development is more closely related to an increase in the absolute QT interval or QTc. Most drugs that have caused TdP clearly increase both the absolute QT and the QTc (hereafter called QT/QTc). Documented cases of TdP (fatal and nonfatal) associated with the use of a drug have resulted in the withdrawal from the market of several drugs and relegation of other drugs to second-line status. Because prolongation of the QT/QTc interval is the ECG finding associated with the increased susceptibility to these arrhythmias, an adequate premarketing investigation of the safety of a new pharmaceutical agent should include rigorous characterization of its effects on the QT/QTc interval.[2]

1.2. SUBSTUDY DESIGN RATIONALE

Plitidepsin is an anticancer agent that should not be administered to healthy subjects. Plitidepsin is administered at maximum tolerated dose, so it cannot be tested safely at supratherapeutic concentrations, even in cancer patients.

The impact of the disease, together with age, comorbidities and comedications present in patients with cancer is such that they are unable to tolerate excessive testing. Therefore, the search for a precise characterization of the effects of the drug on the QTc must be carefully balanced against the feasibility of the study design. Furthermore, the duration of the QTc interval is a less-than-perfect marker of the risk of proarrhythmia.[3]

Patients with refractory malignancies and expected reduced survival could justifiably be excused from the burden of time-matched baseline ECG values and separate administration of a positive control.[4]

Therefore, this substudy will be conducted in patients who may benefit from the anticancer activity of plitidepsin at the recommended dose, such as those enrolled in clinical trial APL-C-001-09. The potential effects of a 5 mg/m^2 plitidepsin dose given as a 3-hour intravenous infusion on the QTc interval duration will be assessed when the patient is treated with plitidepsin for the first time (Day 1 of Cycle 1). In order to detect any delayed drug effect on the QTc interval, these investigations will be repeated at the second infusion of plitidepsin (Day 15 of Cycle 1), when the drug is expected to reach the steady state in spite of plitidepsin's long terminal half-life in blood, approximately 88 hours, although concentrations on Day 15 are negligible.

This substudy will be conducted at some of the sites participating in study APL-C-001-09.

Although the daily variability in QTc interval of cancer patients has not been well described, it may be even larger because enrolled patients are likely to have advanced age, concomitant medical problems and prior QT-prolonging chemotherapy.[5] For this reason, an intrapatient standard deviation of 18 milliseconds is assumed. To reduce intrapatient variability due to measurement error, it has been recommended that the baseline QTc be rigorously investigated and be expressed as the mean of multiple electrocardiogram assessments.[6] For this purpose, two sets of triplicate ECG tracings recorded before plitidepsin administration will be considered baseline assessments: one set before and one after prophylactic medication administration on Days 1 and Day 15.

Electrocardiogram assessments will be performed by means of continuous 12-lead ECG automated digital collection.

All digital ECG continuous recordings will be sent to a third-party central ECG laboratory for measurement of intervals, diagnostics of abnormalities and review of ECG waveform morphology review. This will prevent the potential introduction of bias during the analysis of ECG recordings.

1.3. DNA COLLECTION RATIONALE

Genetic variation can be an important contributory factor to interindividual differences in QT/QTc changes to drug exposure. The collection of an additional sample will allow for genetic evaluation of mutations associated with ion channelopathies in case marked drug-related changes of the QT/QTc interval are observed. Details are provided in Section 7.4 QT-Related Polymorphisms Assessment.

2. OBJECTIVES

Based on the aforementioned scientific background, the primary objective of the present substudy is to assess the potential effects of plitidepsin administered at a therapeutic dose on the duration of the QT/QTc interval, measured by ECGs, in patients with relapsed/refractory multiple myeloma.

Secondary objectives are: to evaluate QTc changes over the treatment period; to characterize the plitidepsin (real and estimated) whole blood concentration/QTc relationship (or PK/PD relationship); and to explore related ECG parameters.

3. OVERALL SUBSTUDY DESIGN

This is an uncontrolled, open-label, substudy within APL-C-001-09 clinical trial (ADMYRE) in patients with relapsed/refractory multiple myeloma, evaluating the potential effects of plitidepsin on the QT/QTc intervals of the ECG.

Patients will be enrolled as needed to ensure at least 36 evaluable patients with evaluable ECG data.

Patients will be admitted to the study site on Days 1 and 15 (at least 2 hours before plitidepsin administration) until completion of the pharmacokinetic (PK) blood sample collection scheduled at three hours after EOI. After completing all other baseline assessments on Days 1 and 15, a 12-lead continuous ECG recorder will be set in place and patients will be resting in a supine position for at least one hour prior to the start of the plitidepsin infusion (SOI).

Patients will be administered the prophylactic medication at 20-30 minutes before SOI.

Patients will be at the study site until completion of the PK blood sample collection at three hours after EOI and then will leave the study site. Patients will be asked to:

- keep calm and relaxed until the next day (i.e., at 21 hours after EOI), when the ECG recorder will be removed.

- remain in a supine position from 10 minutes before 6, 9 and 21 hours after EOI. Specific times will be reminded (see section 4.4 Patient's Card)

Meals on Day 1 and Day 15 should be standardized. Patients will have lunch at 1.5 hours after EOI, which must be completed at least 30 minutes before the next PK blood sample collection (i.e., 3 hours after EOI). The activities of patients at the study site will be modified to avoid strenuous effort or emotional excitement.

An additional blood sample (10 mL) may be collected on Day 1 prior to treatment to allow for genetic evaluation of mutations associated with ion channelopathies, in case marked drug-related changes of the QT/QTc interval are observed. Participation in the QT-related polymorphisms assessment is optional.

Details on the timing of the treatment and assessments are given in the substudy's Schedule of Assessments and Procedures (see below).

4. PATIENT DEFINITION

4.1. SUBSTUDY POPULATION

Patients will be enrolled in the substudy as needed to ensure that at least 36 patients have completed all required assessments (see Section 5.1).

The inclusion and exclusion criteria for enrolling patients in this substudy are described in the following sections.

4.2. PATIENT ELIGIBILITY

4.2.1. Inclusion Criteria

In order to be enrolled in the substudy, patients must satisfy the following criteria at screening for the main study:

1. Patient included in the clinical study APL-C-001-09 and randomized to the experimental arm (plitidepsin + dexamethasone).

2. Patient with voluntarily signed and dated informed consent to participate in the QT substudy.

3. A 12-lead ECG consistent with normal cardiac conduction and function, showing:

    - Sinus rhythm.

    - Pulse rate between 45 and 100 bpm.

    - QRS interval <120 ms.

    - PR interval <200 ms.

4. Blood pressure between 90 and 150 mmHg systolic, inclusive, and not higher than 90 mmHg diastolic.

5. Serum electrolyte levels within seven days prior to first infusion, ≤ grade 1:
    - Ionic Ca^{++}: 1.0 – 1.5 mmol/L.
    - K^{+}: 3 – 5.5 mmol/L.
    - Mg^{++}: 0.5 – 1.23 mmol/L.

4.2.2. Exclusion Criteria

Patients who meet any of the following criteria will be excluded from participating in the substudy:

1. Has heart rhythm disturbances (e.g. atrial fibrillation), unusual T wave and U wave (if present) morphology, personal or family history of Long QT Syndrome, ECG findings of complete left bundle branch block, permanent ventricular pacemaker, or Brugada Syndrome.
2. Has any skin condition likely to interfere with ECG electrode placement, or a history of breast implant or thoracic surgery likely to cause abnormality in electrical conduction.
3. Prior exposure to anthracyclines at a cumulative dose of doxorubicin or equivalent greater than 450 mg/m².
4. Patients who at screening are on medication that is known to prolong the QT interval (refer to Appendix 7 of the main protocol). Patients must have been off these medications for a minimum of 48 hours prior to plitidepsin administration on Day 1 and Day 15.
5. History of illegal drug use or alcohol abuse within six months before study drug administration.

4.3. ACTIVITIES AND SUBSTANCES THAT PATIENTS MUST AVOID DURING THE SUBSTUDY

Potential patients must be willing to adhere to the following prohibitions and restrictions during the course of the substudy to be eligible for participation.

1. Must refrain from jogging, strenuous exercises of all types, and sunbathing from 48 hours before Days 1 and 15 until after the end of the continuous ~24-hour ECG recording on Days 1 and 15.
2. May not consume beverages containing alcohol from 24 hours before Days 1 and 15 until after the end of the continuous ~24-hour ECG recording on Days 1 and 15.
3. Must refrain from using any methylxanthine-containing products, (e.g., chocolate bars or beverages, coffee, tea, or colas) from 48 hours before Days 1 and 15 until after the end of the continuous ~24-hour ECG recording on Days 1 and 15.

4. Patients may consume standard meals during their stay at the study site. Meals will not contain spicy foods (i.e., black pepper, hot peppers, wasabi, etc). Excessive food consumption will not be permitted.

4.4. PATIENT'S CARD

The Investigator will provide the patient with a card listing all prohibited activities and substances during the substudy. This card will contain blank spaces for the Investigator to write down the specific times of the ECG timepoints at 6, 9 and 21 hours after EOI. The Investigator must remember to tell the patient that he/she must remain in a supine position from 10 minutes before these times.

5. PLAN OF SUBSTUDY

5.1. COMPLETION

A patient will be considered to have completed the substudy if he/she has evaluable ECG data.

5.2. WITHDRAWAL/DISCONTINUATION FROM THE SUBSTUDY

A patient will be withdrawn from the substudy for any of the following reasons:

- Withdrawal of substudy ICF.

- The patient does not comply with the substudy requirements, including inclusion criteria, exclusion criteria, and prohibitions and restrictions.

Patients who withdraw from the substudy will be replaced as needed in order to ensure that at least 36 evaluable patients complete the substudy.

5.3. WITHDRAWAL FROM QT-RELATED POLYMORPHISMS ASSESSMENT ONLY

A patient may withdraw his/her consent for the QT-related polymorphisms assessment while being evaluable for the substudy. In such a case, collected blood samples will be destroyed. To initiate the sample destruction process, the Investigator must notify the Sponsor's center contact and request sample destruction. In turn, the Sponsor's center contact will ask the central lab contact to destroy the sample. If requested, the Investigator will receive written confirmation from the Sponsor/central lab that the sample has been destroyed.

6. TREATMENT

6.1. PROPHYLACTIC MEDICATION

During the substudy (Cycle 1 of main study), all patients must receive the following prophylactic medication at 20-30 minutes before infusion of plitidepsin:

- Palonosetron 0.25 mg i.v. (tropisetron 5 mg i.v. could be considered if palonosetron is not available).
- Dexchlorpheniramine 5 mg i.v. or chlorpheniramine 10 mg i.v., (dexchlorpheniramine 6 mg p.o. or chlorpheniramine 10-15 mg p.o. could be considered, if i.v. formulations are not available).
- Ranitidine 50 mg i.v. or cimetidine 300 mg i.v.

Oral aprepitant may be used as per Investigator's criteria/institutional guidelines. Metoclopramide should be avoided.

The use of alternative prophylactic medications should be discussed in advance with the Sponsor.

6.2. PROHIBITED MEDICATIONS/THERAPIES

Drugs known to prolong QT interval and/or induce torsades de pointes are prohibited (refer to Appendix 7 of the main protocol). Patients must have been off these medications for a minimum of 48 hours prior to plitidepsin administration on Days 1 and 15.

7. SUBSTUDY EVALUATIONS

7.1. SUBSTUDY PROCEDURES

The substudy's Schedule of Assessments and Procedures summarizes the frequency and timing of continuous ECG recorder placement for pharmacodynamic (PD) assessments and PK and QT-related polymorphisms sample collection.

A 10 mL blood sample will be collected on Day 1 from patients who have consented to participate in the QT-related polymorphisms assessment of the substudy.

7.2. PHARMACOKINETIC EVALUATIONS

Plitidepsin PK assessment in patients participating in the substudy will be the same as the one conducted on patients in Arm A of the main study. Please refer to the main study protocol, Section 6.2.

7.3. PHARMACODYNAMIC EVALUATIONS

Patients will wear a continuous ECG recorder on Days 1 and 15, from prior to prophylaxis administration to 21 hours after EOI. This ECG recorder will be provided by ERT after the study site has registered online at ERT's MYSTUDY PORTAL™. Registration details will be provided to the site coordinator at each participating site.

The ECG recorder will be set in place once all safety assessments scheduled for the main study (i.e., vital signs, blood sample extraction for laboratory tests, and ECG) have been conducted.

On Day 1 and Day 15, serial ECGs with stable resting heart rate will be extracted from the continuous recording in triplicate (three 10-second digital ECGs in close succession) within a 10 minute (±5min) window at each scheduled time point of the ECG assessments:

- Pre-prophylaxis (Predose 1), post-prophylaxis and prior to SOI (Predose 2)*, 1.5 after SOI, just before the end of infusion (EOI)*, 0.5, 1*, 3*, 6, 9, and 21 hours after EOI.

*Triplicate ECGs will be extracted prior to the corresponding PK blood sample collection.

Patients will remain in the same comfortable supine position for at least 10 minutes before and after the time points for ECG and PK collection. Physical activity, external stimulation, and meal ingestion should be regulated to keep activity and emotional excitement to a minimum. ECG recordings at time points 6, 9 and 21 hours after EOI will be ambulatory.

All digital continuous ECG recordings will be sent to a third-party, central ECG laboratory (for triplicate ECG extraction at the scheduled time window, measurement of intervals, diagnostics of abnormalities and review of ECG waveform morphology). Quality assurance procedures will be used to ensure high precision and consistency of QT interval measurements.

For the triplicate ECGs collected at each time point, the average of the three measurements for each ECG parameter will be considered for all listing and statistical analyses described below.

7.3.1. QT Correction Methods

QT will be corrected for heart rate using the following methods:

- Primary: Fridericia's correction method (QTcF).[7]
- Secondary: Bazett's correction method (QTcB).[8]

7.3.2. Pharmacodynamic Endpoints

In principle, for each patient and each postdose time point on Day 1 and Day 15, baseline QTc intervals will be the mean of the Predose 1 and Predose 2 QTc values for that patient on Day 1.

If the mean Predose 2 QTc value of all evaluable patients is ≥10 ms longer than of the mean Predose 1 QTc value, an effect of prophylactic medication on QTc cannot be ruled out; therefore, individual Predose 2 QTc values will be considered as the baseline values.

The change from baseline (ΔQTc) will be calculated using the corresponding baseline QTc values. Specifically:

- ΔQTc at time t: Difference in QTc between scheduled time point t and baseline.

The primary pharmacodynamic (PD) endpoint will be ΔQTc at each scheduled time point. ΔQTc will be summarized where QTc is derived using both the QTcF and QTcB correction methods whereby QTc denotes a general notation of corrected QT using either method.

7.4. QT-RELATED POLYMORPHISMS ASSESSMENT

A 10 mL blood sample will be collected on Day 1 from patients who consented to take part in the QT-related polymorphisms assessment of the substudy at the time point indicated in the substudy's Schedule of Assessments and Procedures (see below). Participation in the QT-related polymorphisms assessment is optional.

A manual of instructions for sample collection, labeling, storage, and shipment will be provided as a separate document (Instruction Manual for the Collection, Labeling, Storage and Shipment of Pharmacokinetic Samples).

If a patient shows marked drug-related changes of the QTc interval and a genetic cause is suspected, that individual sample will be analyzed.

Genes currently hypothesized to potentially be relevant to irregular QT/QTc intervals such as KCNE1, KCNE2, KCNH2, KCNQ1 and SCN5A will be considered for genotyping.

When the ECG database will be locked, if no marked drug-related changes are observed, all samples for QT-related polymorphisms assessment from the substudy will be destroyed.

7.4.1. Confidentiality of Samples for QT-related Polymorphisms Assessment

PharmaMar shall comply with the 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 and on the free movement of such data.

Appropriate technical and organizational measures to protect the genetic data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration will be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of substudy patients confidential.

Samples will be stored until the ECG database has been locked, when the decision on whether it is necessary to rule out mutations associated with ion channelopathies, is made.

7.5. 12-LEAD ECG ACQUISITION

ECGs will be obtained digitally using a Mortara Instrument (Milwaukee, WI, USA) H-12+ ECG continuous 12 lead digital recorder, which will obtain ECGs on Day 1 and Day 15. The ECGs are stored continuously on a flash card and will not be available for review until the card is received by ERT and analyzed. ECGs to be used in the analysis will be selected by predetermined time points as detailed below and will be read centrally using a high-resolution manual on-screen caliper semiautomatic method with annotations. In this trial using pre-treatment ECGs as the baseline, the H-12+ recording

will be started approximately 1 hour prior SOI through approximately 21 hours post EOI in order to obtain the baseline ECGs as detailed below.

ECGs for the treatment days will be obtained as triplicate (3) 12-lead ECGs at each time point which will be downloaded from the H-12+ flash card approximately one minute apart on Day 1 and Day 15. On Day 1 and Day 15, baseline ECGs will be extracted (in triplicate) pre-prophylaxis (Predose 1), post-prophylaxis and prior to SOI (Predose 2). On Day 1 and Day 15, triplicate ECGs will be extracted at the following time points: 1.5 after SOI, just before the EOI, 0.5, 1, 3, 6, 9 and 21 hours after EOI.

7.6. ECG ANALYSIS METHODS

ECGs will be sent to ERT for a treatment-blinded high-resolution measurement of the cardiac intervals, and morphological assessment by a central cardiologist.

The 12-lead digital continuous ECG signal for each session in each patient will be recorded on compact flash memory cards provided to the site. The patient's unique identification number and demographic information will be recorded for each card. ERT will generate a 10-second, 12-lead digital ECG at each time point specified in the protocol. If targeted ECG time points are artifactual and of poor quality, ERT will capture analyzable 10-second ECGs as close as possible to the targeted time points.

Digital ECGs will be transmitted to ERT's validated data management system, EXPERT. Interval duration measurements will be collected using computer assisted caliper placements on three consecutive beats. Trained analysts will then review all ECGs for correct lead and beat placement and will adjudicate the pre-placed algorithm calipers as necessary using the proprietary validated electronic caliper system applied on a computer screen. A cardiologist will then verify the interval durations and perform the morphology analysis, noting any T-U wave complex that is compatible with an effect on cardiac repolarization.

The ECG analysis will be conducted in Lead II, or in Lead V5 if Lead II is not analyzable. If Lead V5 is not analyzable then Lead V2 will be used, followed by the most appropriate lead if necessary. ECG readers will be blinded to patient identifiers, and visit. All ECGs for a given patient will be analyzed by the same reader. Quality Assurance reports for inter- and intra-observer variability will be produced by the central ECG laboratory and provided to the Sponsor.

On-screen measurements of the RR, PR, QRS, and QT interval durations will be performed and variables for QTcF, QTcB, and heart rate (HR) will be obtained by the following processes:

Interval measurements will be obtained by the following method:

- Three (3) RR — mean RR Interval is reported.
- Three (3) PR — mean PR Interval is reported.
- Three (3) QRS — mean QRS Width is reported.
- Three (3) QT — mean QT Interval is reported.

The following calculations will be made from the interval measurements:

Three (3) QTcF measurements- QTc correction by the Fridericia's formula

- QTcF1 = QT1/$\sqrt[3]{RR1}$
- QTcF2 = QT2/$\sqrt[3]{RR2}$
- QTcF3 = QT3/$\sqrt[3]{RR3}$
- Mean QTcF = (QTcF1 + QTcF2 + QTcF3)/3

Three (3) QTcB measurements- QTc correction by the Bazett's formula

- QTcB1 = QT1/$\sqrt{RR1}$
- QTcB2 = QT2/$\sqrt{RR2}$
- QTcB3 = QT3/$\sqrt{RR3}$
- Mean QTcB = (QTcB1 + QTcB2 + QTcB3)/3

Three (3) Heart Rate measurements

- HR1 = 60 / RR1
- HR2 = 60 / RR2
- HR3 = 60 / RR3
- Mean HR = (HR1 + HR2 + HR3)/3

Each fiducial point (onset of P wave, onset of Q wave, offset of S wave, and offset of T wave) will be electronically marked. The original ECG waveform and such annotations will be saved separately in XML format for independent review.

8. STATISTICAL METHODS

A non-inferiority limit of 20 ms will be used for the comparison of the effects of plitidepsin on the QT/QTc intervals.

8.1. SAMPLE SIZE

Using a standard deviation of 18 ms in a sample size of 36 evaluable patients, the probability that the upper limit of the one-sided 95% confidence interval (CI) for the difference in mean QTc between each time point and baseline (ΔQTc) will fall below 20 ms is 80%, when the true difference in means equals 5 ms.

8.2. PHARMACODYNAMIC ANALYSES

The primary objective of this substudy is to measure the potential effect of plitidepsin on QTc. The primary comparison of interest will be ΔQTc at each scheduled time point. A non-inferiority criterion of 20 ms will be used to establish that postdose QTc is not inferior to baseline. Evaluable patients who have evaluable ECG data will be included in the primary analysis.

A mixed effect analysis of variance (ANOVA) model will be fitted with the QTc data as the dependent variable and intervention (baseline *vs.* postdose), scheduled time point t of measurement (where t will be baseline, 1.5 after SOI, just before the EOI, 0.5, 1, 3, 6, 9 and 21 hours after EOI) and treatment period (Day 1 and Day 15) as fixed effects and patient as a random effect. Using the estimated least square means and intrapatient variance obtained from this model, a one-sided 95% CI will be calculated for the

difference in mean QTc between baseline and each scheduled time point t. Non-inferiority will be concluded if at each scheduled time point the upper-limit of the one-sided 95% CI falls below 20 ms.

Mean ECG parameters (QT, QTc, RR, heart rate, QRS, and PR) and corresponding changes from baseline will be listed and summarized descriptively by time point t. Plots of mean QTc changes from baseline (ΔQTc) *vs.* the scheduled time point t will be prepared.

The incidence count and percentage of patients with QTc increase from baseline (ΔQTc) >30-60 ms and >60 ms will be tabulated for each time point t, age and gender. The incidence count and percentage of patients with any postdose QTc values >450 ms and >480 ms, will be tabulated for each time point t, and gender. Patients with QTc values >500 ms will be listed.

The incidence count of patients with PR >200 ms, QRS >120 ms, heart rate >100 bpm and <50 bpm, and ST segment and T and U wave abnormalities will be reported. The incidence count of patients with abnormalities in the ST segment (e.g. flat, depressed, elevated) and T wave (i.e. present or absent) and U wave (e.g. flattened, inverted, biphasic and notched) will be reported.

8.3. PHARMACOKINETIC/PHARMACODYNAMIC ANALYSES

The PK/PD analyses will be based on data collected from the scheduled time points of ECG measurement and:

1. their corresponding whole blood plitidepsin concentration.

2. their estimated whole blood plitidepsin concentration at the corresponding ECG time point by means of population PK modeling.

Evaluable patients (i.e., those who have evaluable ECG data) who withdraw before completing both days (i.e., Days 1 and 15) will be included in these analyses. Plots of individual QTc changes from baseline (ΔQTc) vs. the corresponding plitidepsin blood concentrations will be prepared to explore any concentration-effect relationship for plitidepsin.

The concentration–QTc relationship will be assessed using a linear mixed effects analysis of the relationship between ΔQTc data and plitidepsin concentration according to the equation: $\Delta QTc = \alpha + \beta*[plitidepsin]$, where inter-individual variability will be estimated for both intercept (α) and slope (β). The effect of treatment period (i.e. Day 1 or Day 15) on slope and intercept will also be evaluated. If the intercept term is not significant, the model will be re-fit with a zero intercept term. The predicted value of ΔQTc (along with 90% CI) will be estimated at the mean maximum plasma (C_{max}) values of plitidepsin.

Additionally, in a complementary PK/PD analysis, the relationship between QTc and plitidepsin whole blood concentration will be assessed using linear mixed effect modeling to estimate the maximal change (mean and upper 95% confidence limit) in the QTc associated with therapeutic concentrations of plitidepsin.

9. REFERENCES

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SCHEDULE OF ASSESSMENTS AND PROCEDURES

PROCEDURE	Screening	Day 1 Cycle 1								Day 15 Cycle 1							
		-2 hours	-30 min	predose	0	+3 hours (just before EOI)	+4 hours (1-h after EOI)	+4.5 hours (1.5-h after EOI)	+6 hours (3-h after EOI)	-2 hours	-30 min	predose	0	+3 hours (just before EOI)	+4 hours (1-h after EOI)	+4.5 hours (1.5-h after EOI)	+6 hours (3-h after EOI)
Written substudy-specific informed consent	Before any procedures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Written IC for QT-related polymorphisms assessment *	-14 to 0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Review of substudy-specific inclusion/exclusion criteria	-14 to 0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Patient admission at the study site and Holter placement **	-	●	-	-	-	-	-	-	-	●	-	-	-	-	-	-	-
Intravenous prophylaxis medication	-	-	●	-	-	-	-	-	-	-	●	-	-	-	-	-	-
Blood sample (10 mL) for QT-related polymorphisms	-	-	-	●	-	-	-	-	-	-	-	●	-	-	-	-	-
PK blood sample (4 mL)	-	-	-	●	-	●	●	-	●	-	-	●	-	●	●	-	●
Plitidepsin treatment	-	-	-	-	●	-	-	-	-	-	-	-	●	-	-	-	-
Meal	-	-	-	-	-	-	-	●	-	-	-	-	-	-	-	●	-

* Patient participation in QT-related polymorphisms assessment is optional. Sample will be collected on Day 1.

** The ECG recorder will be set in place once all safety assessments scheduled for the main study (i.e., vital signs, blood sample extraction for laboratory tests, and ECG) have been conducted.

ECG, electrocardiogram; EOI, end of infusion; IC, Informed Consent; PK, pharmacokinetics.