Pharma Mar, S.A., Sociedad Unipersonal
Colmenar Viejo, Madrid, Spain

CLINICAL STUDY REPORT

APL-B-016-05

PHASE I-II MULTICENTER, RANDOMIZED, OPEN-LABEL, CLINICAL AND PHARMACOKINETIC STUDY OF PLITIDEPSIN, ADMINISTERED ALONE OR IN COMBINATION WITH DACARBAZINE, AS FRONT-LINE THERAPY TO SUBJECTS WITH UNRESECTABLE ADVANCED MELANOMA

Investigational Medicinal Product:	Plitidepsin
Name of Test Drug:	Aplidin®
Study Design:	Dose-finding, open-label, multicenter, phase I-II clinical trial
Protocol Number:	APL-B-016-05
Study Start Date:	6 January 2006 (First consent signed)
Study Completion Date:	19 October 2010 (Date reported to the Competent Authorities)
Principal Investigator Name and Affiliation:	**Ruth Plummer, M.D.** Newcastle General Hospital Newcastle upon Tyne, United Kingdom
Responsible Medical Officer:	**Arturo Soto Matos-Pita, M.D.** Clinical Research and Development Director Pharma Mar, S.A., Sociedad Unipersonal (abbreviated as PharmaMar S.A. in this report) Avenida de los Reyes, 1; Polígono Industrial La Mina-Norte 28770 Colmenar Viejo, Madrid, Spain Phone: +34 91 846 6053 Fax: +34 91 823 4504 E-mail: asoto@pharmamar.com
Earlier Approved Reports:	None
Version:	Final version
Approval Date:	12 August 2011

This study was conducted in compliance with Good Clinical Practice (GCP)

NOTE:

This report has been written according to the International Conference of Harmonization (ICH) HARMONISED TRIPARTITE GUIDELINE E3: "STRUCTURE AND CONTENT OF CLINICAL STUDY REPORTS":

- EU: adopted by CPMP, December 1995, issued as CPMP/ICH/137/95.
- MHLW: adopted May 1996, PAB/PCD Notification No.335.
- FDA: published in the Federal Register, Vol. 61, July 17, 1996, page 37320.

The primary objective of the first (phase I) stage of this study was to determine the safety and to identify the maximum tolerated dose (MTD) and the recommended dose (RD) of plitidepsin combined with dacarbazine (DTIC). Both the MTD and the RD were part of the safety results. Therefore, in order to show first safety parameters, including the MTD and the RD, the content of Sections 11 (Efficacy evaluation) and 12 (Safety evaluation) has been exchanged.

Additionally, a summary of results regarding dose-limiting toxicities (DLTs), MTD and RD is described in Section 11.2.

2. SYNOPSIS

<table>
<tr><td>Name of Sponsor(s)/Company(ies):
PharmaMar, S.A.</td><td colspan="2" rowspan="3">Individual Study Table Referring to Part of the Dossier

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<tr><td>Name of finished product:
Aplidin®</td></tr>
<tr><td>Name of active ingredient(s):
Plitidepsin</td></tr>
<tr><td>Protocol number</td><td colspan="3">APL-B-016-05</td></tr>
<tr><td>Title of the study</td><td colspan="3">Phase I-II Multicenter, Randomized, Open-label, Clinical and Pharmacokinetic Study of Plitidepsin, Administered Alone or in Combination with Dacarbazine, as Front-line Therapy to Subjects with Unresectable Advanced Melanoma.</td></tr>
<tr><td>Investigators / Study centers</td><td colspan="3">Raquel Andrés, M.D. Hospital Clinico Lozano Blesa, Zaragoza, Spain.
Ewa Chmielowska, M.D. Centrum Onkologii Prof. F. Lukaszczyka, Bydgoszcz, Poland.
Carmen Crespo, M.D. Hospital Ramón y Cajal, Madrid, Spain.
Lev Demidov, M.D. Russian Cancer Research Center, Moscow, Russia.
Larry Hayward, M.D. Western General Hospital, Edinburgh, United Kingdom.
Jacek Jassem, M.D. Samodzielny Publiczny Szpital Kliniczny 1, Gdansk, Poland.
Galina Kudryavtseva, M.D. Medical Radiological Research Center, Obninsk, Russia.
Paul Lorigan, M.D. Christie Hospital, NHS Trust, Manchester, United Kingdom.
Vladimir Moiseyenko, M.D. Petrov Research Institute of Oncology, St. Petersburg, Russia.
Ruth Plummer, M.D. Newcastle General Hospital, Newcastle upon Tyne, United Kingdom.
Laslo Roman, M.D. Leningrad Regional Oncology Dispensary, St. Petersburg, Russia.
Virtudes Soriano, M.D. Instituto Valenciano de Oncología, Valencia, Spain.</td></tr>
<tr><td>Publication (references)</td><td colspan="3">At the time of this report no articles have been published on the study described herein.
Preliminary results of this study were presented at:
American Society of Clinical Oncology (ASCO) 2009 Meeting. “Plummer R, Hayward L, Lorigan P, Soriano V, Jassem J, Moiseyenko V, Szyldergemajn S, Prados R, Smyth J, Calvert H. Plitidepsin alone or with dacarbazine (DTIC) as first-line treatment for advanced unresectable melanoma (AUM). J Clin Oncol 2009, 27(15 Suppl): Abstract 9059”.
American Society of Clinical Oncology (ASCO) 2010 Meeting. “Plummer ER, Lorigan P, Hayward L, Jassem J, Demidov L, Moiseyenko V, Soriano V, Chmielowska E, Prados R, Szyldergemajn S. Plitidepsin (APL) alone or with dacarbazine (DTIC) as first-line treatment for advanced unresectable melanoma (AUM). J Clin Oncol 2010, 28(15 Suppl): Abstract 8537”.</td></tr>
<tr><td>Study period:
. First consent signed
. Last consent signed
. First infusion administered
. Last infusion administered
. Last follow-up
. Date of completion reported to authorities</td><td colspan="2">6 January 2006
23 June 2009

11 January 2006

16 March 2010
22 September 2010
19 October 2010</td><td>Phase of Development:

Phase I-II</td></tr>
<tr><td>Study objectives</td><td colspan="3">Primary:
To determine the efficacy of plitidepsin, alone or in combination with dacarbazine (DTIC), in the front-line treatment of patients with unresectable advanced melanoma.
To determine the maximum tolerated dose (MTD) and recommended dose (RD) of plitidepsin administered in combination with DTIC to patients with advanced unresectable melanoma.</td></tr>
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<tr><td></td><td colspan="2">Secondary:
• To further study the pharmacokinetics (PK) of plitidepsin, including drug-drug PK interactions between plitidepsin and DTIC.
• To evaluate the safety and tolerability of plitidepsin, administered alone or in combination with DTIC, as front-line therapy of patients with unresectable advanced melanoma.</td></tr>
<tr><td>Methodology</td><td colspan="2">This was a dose-finding, open-label, multicenter, phase I-II clinical trial conducted in adult men and women with histologically confirmed unresectable advanced malignant melanoma that was either metastatic or relapsing/progressing after primary treatment for locoregional disease.
• The phase I (dose-finding) stage was an open-label, non-randomized, multicenter, dose-escalating study, with the primary objective of determining the MTD and RD of plitidepsin administered as 1-hour infusion on Days 1, 8, and 15 in combination with DTIC as 1-hour infusion on Day 1 every four weeks (q4wk). The starting doses were plitidepsin 1.8 mg/m^2 and DTIC 800 mg/m^2.
• The phase II (therapeutic/exploratory) stage was an open-label, randomized, multicenter study designed to assess the efficacy of plitidepsin alone (3.2 mg/m^2 as 1-hour infusion on Days 1, 8, and 15 q4wk) and of the plitidepsin and DTIC combination at the RD determined in the phase I stage.</td></tr>
<tr><td>Number of patients (planned and analyzed)</td><td colspan="2">Planned number of patients:
• <u>Phase I stage.</u>
A total of 8-18 patients were to be treated with plitidepsin combined with DTIC during the phase I stage.
• <u>Phase II stage.</u>
Patients in this stage were randomized to receive plitidepsin alone (Arm A) or combined with DTIC (Arm B). A Simon two-stage design was used to test the null hypothesis that $P \leq 0.060$ versus the alternative hypothesis that $P \geq 0.250$; this design had an expected sample size of 20.53 and a probability of early termination of 0.728. If the treatment was actually not effective, there was a 0.086 probability of concluding that it was. If the treatment was actually effective, there was a 0.069 probability of concluding that it was not.
In accordance with this design, 17 evaluable patients were to be recruited into each arm. If at least two patients in one arm showed response, additional patients were to be recruited so as to bring patient accrual in that arm to 30. If four or more responses were observed in that arm, the corresponding treatment would be considered for further development.
Patients analyzed:
• <u>Phase I stage.</u>
Twenty-eight patients were enrolled during the phase I stage. All were distributed among four dose levels and were treated with plitidepsin as a 1-hour infusion on Days 1, 8 and 15 followed by DTIC as a 1-hour infusion on Day 1 q4wk:

<table>
<tr><th>Dose level</th><th>Plitidepsin / DTIC dose (mg/m^2)</th><th>No. of patients</th></tr>
<tr><td>I</td><td>1.8 / 800</td><td>7</td></tr>
<tr><td>II</td><td>2.4 / 800</td><td>8</td></tr>
<tr><td>III</td><td>3.0 / 800</td><td>8</td></tr>
<tr><td>IV</td><td>2.4 / 1000</td><td>5</td></tr>
</table>
• <u>Phase II stage.</u>
At the go/no-go decision in the first part of the phase II stage, responses had been found in two of 17 patients evaluable for efficacy in Arm B, and in none of 16 evaluable patients in Arm A. As a result, patient accrual into Arm A was discontinued and new patients were recruited into Arm B only during the second part of this stage.</td></tr>
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	Overall, 58 patients were enrolled during the phase II stage: 20 in Arm A and 38 in Arm B. All 20 patients in Arm A were treated with plitidepsin 3.2 mg/m^2 as a 1-hour infusion on Days 1, 8 and 15 q4wk. In Arm B, 36 patients were treated with plitidepsin 2.4 mg/m^2 as a 1-hour infusion on Days 1, 8 and 15 followed by DTIC 800 mg/m^2 as a 1-hour infusion on Day 1 q4wk (i.e., the RD established during the phase I stage of this study).	
Diagnosis and main selection criteria	**Inclusion Criteria** 1. Voluntary written informed consent of the patient, obtained before any study-specific procedure. 2. Malignant melanoma, from cutaneous or non-cutaneous primary site, histologically proven. 3. Advanced melanoma. a. Metastatic melanoma (M1a, M1b, M1c). b. Melanoma relapsing /progressing after primary treatment for locoregional disease. 4. Age ≥ 18 years. 5. Patient with measurable disease using the Response Evaluation Criteria In Solid Tumors (RECIST, version 1.0) (only applicable for the second, randomized, therapeutic-exploratory phase of the trial). 6. Recovery from any non-hematological drug-related adverse event derived from previous treatment, excluding alopecia and National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade < 2 symptomatic peripheral neuropathy. 7. Laboratory values within 14 days prior to first infusion: - Platelet count ≥ 100 x 10^9/l, hemoglobin (Hb) ≥ 9.5 g/dl, and absolute neutrophil count (ANC) ≥ 1.5 x 10^9/l. - Alkaline phosphatase (AP) ≤ 2.5 x the upper limit of normal (ULN) (≤ 5 x ULN in case of extensive bone metastases). - Aspartate aminotransferase (AST) ≤ 2.5 x ULN. - Alanine aminotransferase (ALT) ≤ 2.5 x ULN. - Total bilirubin ≤ 1.5 x ULN (unless due to indirect hyperbilirubinemia). - Creatinine clearance ≥ 40 ml/minute or creatinine ≤ 1.4 mg/dl (calculated from Cockcroft and Gault's formula). - Creatine phosphokinase (CPK) ≤ 2.5 x ULN. - Albumin ≥ 2.5 g/dl. 8. Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2. 9. Life expectancy ≥ 3 months. 10. Left ventricular ejection fraction (LVEF) within normal limits. 11. Childbearing potential women had to have a negative serum pregnancy test before study entry. Both women and men had to agree to use a medically acceptable method of contraception throughout the treatment period and for three months after discontinuation of treatment. Acceptable methods of contraception included complete abstinence, intrauterine device (IUD), oral contraceptive, subdermal implant and double barrier (condom with a contraceptive sponge or contraceptive suppository). **Exclusion Criteria** 1. Patients who received any prior systemic therapy for the treatment of metastatic melanoma. 2. Patients relapsing/progressing within six months after the end of adjuvant or neo-adjuvant systemic drug therapy for non-metastatic melanoma. 3. Patients with loco-regional melanoma previously treated with loco-regional drug therapy that only had disease relapse or progression within the treated area. 4. Patients for whom surgery would be expected to result in cure or significant palliation. 5. Less than four weeks from radiation therapy (eight weeks in case of extensive prior radiotherapy).	

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	6. Evidence of progressive central nervous system (CNS) metastases or any symptomatic brain or leptomeningeal metastases. 7. Other relevant diseases or adverse clinical conditions: - History or presence of unstable angina, myocardial infarction, valvular heart disease or congestive heart failure. - Any grade of cardiac arrhythmia according to NCI-CTCAE v.3.0. - Previous mediastinal radiotherapy. - Uncontrolled arterial hypertension despite optimal medical therapy. - History of significant neurological or psychiatric disorders. - Active infection. - Myopathy. - Significant non-neoplastic liver disease (e.g., cirrhosis, active chronic hepatitis). - Uncontrolled endocrine diseases (e.g., diabetes mellitus, hypothyroidism or hyperthyroidism) (i.e., requiring relevant changes in medication within the last month, or hospital admission within the last three months). - Immunocompromised patients, including patients known to be infected by human immunodeficiency virus (HIV). - Any other major illness that, in the investigator's judgment, would substantially increase the risk associated with the patient's participation in this study. 8. Pregnant or lactating women. 9. Limitation of the patient's ability to comply with the treatment or follow-up procedures and visits defined in the study protocol. 10. Known hypersensitivity to any component of the study drug products, including plitidepsin, DTIC, Cremophor, mannitol, citrate, or ethanol. 11. Treatment with any investigational product in the 30-day period before inclusion in the study.	
Test product, dose and mode of administration	**Plitidepsin** Plitidepsin was supplied by PharmaMar (Colmenar Viejo, Madrid, Spain) as a lyophilized powder for concentrate for solution for infusion. It was administered **as a 1-hour intravenous (i.v.) infusion on Days 1, 8 and 15 in 4-week cycles (q4wk)**. The following **2-mg vial batche**s were used: #04H27, #05E25, #05C10, #06B15, #06K08, #07I27 and #08I16. **Dacarbazine** DTIC was supplied as a powder for injection that also contained anhydrous citric acid and mannitol. It was administered **as a 1-hour infusion on Day 1 (immediately after the plitidepsin infusion) q4wk.** Commercially available vials were provided, with the following strengths and batch numbers: • **500-mg vial batches:** #M80219AAE and #M50124AAGB. • **1000-mg vial batches:** #M50329AAE, #M80117AAE, #M60418AAE, #M80609ACE, #M90106AAE, #M80513ABE and #23011908E.	
Duration of treatment	Treatment was administered until progressive disease; life-threatening, unmanageable or unacceptable toxicity; withdrawal of patient's consent; treatment delay for more than two weeks (except in case of obvious patient's benefit); or intercurrent serious illness. After the end of study treatment (regardless of the reason for discontinuation), patients underwent toxicity, hematology and biochemistry assessments within 30 days after the administration of the last study drug dose and until the recovery/stabilization of all toxicities that occurred during protocol treatment. Beyond 30 days after the last study drug administration, only those procedures that were relevant to response assessment or any remaining toxicity needed to be performed. All serious adverse events (SAEs) occurring within 30 days of the last study drug administration were reported. Beyond this time limit, only drug-related SAEs were reported.	

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Criteria for evaluation	
Efficacy	The primary efficacy endpoint was the objective tumor response, defined as the combined rate of complete response (CR) and partial response (PR) according to RECIST (version 1.0). All patients who received at least two doses of plitidepsin and had at least one disease assessment (performed at least eight weeks after the start of treatment) were considered evaluable for efficacy. Secondary efficacy endpoints were duration of tumor response, duration of stable disease, time to progression (TTP), progression-free survival (PFS) and overall survival (OS).
Safety	All patients who had received one infusion of plitidepsin were considered evaluable for safety. Safety parameters included the description of toxic deaths, premature withdrawals from treatment due to toxicity, description of adverse events (AEs), description of SAEs, and evaluation of toxicity according to the NCI-CTCAE grading (version 3.0).
DLTs, MTD and RD	Dose-limiting toxicities (DLTs) were evaluated during Cycle 1 in patients enrolled into the phase I stage and evaluable for DLTs, and were defined as follows: • Hematological drug-related adverse events: o Grade 4 neutropenia (ANC < 0.5 x 10^9/l) lasting more than five days. o Grade 4 neutropenia with concomitant fever (i.e., body temperature ≥ 38.5°C). Fever could not be disease-related. o Grade 4 neutropenia and sepsis or other severe infection. o Thrombocytopenia < 25 x 10^9/l. • Any other grade 3/4 non-hematological AE suspected to be related to study drug(s), except nausea/vomiting (unless the patient was receiving an optimal anti-emetic regimen), hypersensitivity reactions, or non-clinically relevant biochemical abnormalities [e.g., isolated gamma-glutamyltransferase (GGT) increase]. o Grade 3/4 increases in AST/ALT were individually discussed, and might not be considered DLT if: - They were disease-related. - They were not associated with clinically relevant drug-related clinical symptoms. - They were the only abnormality in liver function tests besides eventual GGT increases (including drug-related direct hyperbilirubinemia of any grade, drug-related grade > 1 AP increase, drug-related grade > 1 decreases in albumin or prothrombin), and there were no other relevant drug-related biochemistry abnormalities. - They were rapidly reversible (i.e., recovery to ≤ 2.5 x ULN by Day 29) and did not compromise the administration of plitidepsin on Days 8 and 15. • Delay in the administration of a subsequent dose of plitidepsin exceeding two weeks, due to AEs suspected to be related to study drug. • Omission of infusions on Days 8 and 15, due to AEs suspected to be related to the study drug(s). The **MTD** was defined as the highest dose level of the plitidepsin and DTIC combination at which at least two of 3-6 patients experienced DLTs in Cycle 1. The **RD** was defined as the highest dose level of the plitidepsin and DTIC combination at which less than two of six patients showed a DLT during Cycle 1.
Pharmacokinetics	All patients included in this study were to be sampled for pharmacokinetics (PK). Blood samples were to be taken at specific times before, during and after administration of the first plitidepsin infusion.
Pharmacogenomics	Paraffin-embedded or fresh frozen tumor tissue samples were obtained from patients consenting for the pharmacogenomic (PGx) testing.
Statistical methodology	Summary tables, data listings and statistical analyses were generated using the SAS statistical package (version 8.2). Statistical tests had an exploratory purpose only and had a threshold of $\alpha = 5$ %. All tables were stratified by initial dose level of plitidepsin and DTIC in the phase I stage and by randomization group in the phase II stage. All

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	analyses were based on the actual treatment each patient received, rather than on the intended randomized treatment. Any departures from the planned treatment according to the randomization schedule were listed and documented in the clinical study report. Categorical variables were summarized in frequency tables; percentages in the summary tables were rounded and could therefore not always add up to exactly 100%. Continuous variables were summarized and presented with summary statistics (i.e., mean, standard deviation, median and range). The main characteristics concerning inclusion in the study, population definitions, overall reasons for discontinuations by cycle and dose level, discontinuations due to AEs, deaths and other reasons of withdrawal and protocol deviations were presented. The number of patients enrolled per investigator, the patient disposition and the reasons for treatment discontinuation were summarized. The demographics and baseline characteristics of all recruited patients were summarized. Age, sex, race, baseline weight, height, body mass index (BMI), performance status (PS) and body surface area (BSA) were summarized descriptively. Time from initial diagnosis to the first plitidepsin infusion was calculated in months and summarized descriptively. A frequency tabulation of the number of patients with and the different types of previous cancer surgery, radiotherapy or medical therapy was given. The number of lines and the number of agents of prior therapy were summarized. A summary of prior relevant history and signs and symptoms was presented per patient. Concomitant therapies were categorized per Anatomical Therapeutic Chemical Classification System (ATC) class and coded term, and the number of patients receiving each type of therapy at study entry was given. For hematology and serum biochemistry abnormalities at baseline, the NCI-CTCAE grades of toxicity were displayed by frequency tabulation and baseline values were to be summarized. The last value before the first plitidepsin infusion was considered the baseline value. The DLTs, the MTD and the RD were defined for the phase I stage of the study. The total number of cycles received by each patient was calculated. Descriptive statistics of treatment duration, cumulative dose, dose intensity, and relative dose intensity were described for all patients who received at least one infusion of plitidepsin and DTIC, and for all patients who received at least one infusion of single-agent plitidepsin during the phase II stage. The number of patients with a cycle delay, the duration of each delay, the number of patients with a dose reduction and the number of patients with both a cycle delay and a dose reduction while on treatment were summarized. Listings of reasons for delay and reasons for reduction were provided by patient and by cycle. In addition, a listing of patients with skipped infusions during any cycle was prepared per product. A frequency table with the objective response rate was prepared and the exact binomial 95% confidence interval (CI) for the response rate was calculated. Median time-dependent parameters and their fix-time estimations were analyzed according to the Kaplan-Meier method. All analysis of safety endpoints was descriptive. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 5.1). Toxicities and laboratory results were coded using the NCI-CTCAE, version 3.0. All AEs (drug-related and others) were summarized by severity (worst toxicity grade by patient and by cycle). All deaths occurring during treatment or within 30 days from the last treatment administration were tabulated, together with the primary cause of death. Additional safety analyses could be determined at any time in order to most clearly enumerate the rates of toxicities and to further define the safety profile of plitidepsin. The PK parameters were tabulated, and selected parameters were graphically displayed per dose level. The potential influence on selected PK parameters of selected demographic and clinical dichotomous variables (e.g., gender, laboratory test results above/below selected cut-off values) were evaluated by Student`s t test or Mann-Whitney's U test, as appropriate. For multinomial variables, analysis of variance was used. For selected continuous demographic and clinical variables (e.g., age, laboratory	

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	test results), the relationship with selected PK parameters was graphically explored and assessed using correlation and regression methods. The potential influence of the selected PK parameters on efficacy and safety was graphically explored.	
Results (1): Patient characteristics	Phase I stage Of the 28 patients enrolled in this stage, 16 (57.1%) were male, all were Caucasians and their median age was 48 years (range, 20-77 years). Twelve patients (42.9%) had ECOG PS scores of 1-2, and eight (28.6%) had LDH values > 1.1 x ULN. Disease at baseline was metastatic in 26 patients (96.3%) and locally advanced in one (3.7%). The median time from first diagnosis to first infusion was 40.1 months (range, 2.7-199.6 months). The median number of sites of disease per patient at baseline was 3.0 (range, 1-7 sites). The most common sites were the lung (n=19, 70.4%) and lymph nodes (n=18, 66.7%). Visceral sites were involved in 10 patients (37.0%). All 28 enrolled patients underwent surgery prior to entering the study. The most common surgical procedures were local excision of lesions (n=21, 75.0%) and lymph nodes (n=13, 46.4%). Ten patients (35.7%) received prior radiotherapy. Two patients (7.1%) were given one line each of prior biological therapy (interferon alfa) in the adjuvant setting. Phase II stage *Arm A (Plitidepsin 3.2 mg/m^2)* Ten of 20 (50.0%) patients enrolled into this arm were male. All patients were Caucasians and their median age was 51.5 years (range, 36-78 years). More patients in this arm had ECOG PS scores of 1-2 [n=16 (80.0%)] and LDH > 1.1 x ULN [n=16 (80.0%)] than in Arm B. All had metastatic disease at baseline. The median time from first diagnosis to first infusion was 32.0 months (range, 2.2-136.6 months). The median number of sites of disease per patient at baseline was 3.5 (range, 1-8 sites). Sixteen patients (80.0%) had three or more sites of disease at baseline. The most common sites were the lymph nodes (n=17, 85.0%), the lung (n=11, 55.0%), the liver (n=11, 55.0%) and the bone (n=7, 35.0%). Visceral sites were involved in 12 patients (60.0%). All 20 patients underwent surgery prior to entering the study. The most common procedures were local wide excision of lesions (n=11, 55.0%), local excision of lesions (n=10, 50.0%) and lymph nodes (n=9, 45.0%). Ten patients (50.0%) were given prior radiotherapy. In addition, one patient (5.0%) was given biological therapy (interferons) in the adjuvant setting. *Arm B (Plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2)* Twenty-one of 38 (55.3%) patients enrolled into this arm were male. All were Caucasians and their median age was 55.5 years (range, 21-76 years). In this arm, 17 patients (44.7%) had ECOG PS scores of 1-2 and 15 (39.5%) had LDH > 1.1 x ULN. All had metastatic disease at baseline. The median time from first diagnosis to first infusion was 24.2 months (range, 1.3-159.5 months). The median number of sites of disease per patient at baseline was 3.0 (range, 1-7 sites). Twenty-four patients (64.9%) had three or more sites of disease at baseline. The most common sites were the lymph nodes (n=27, 73.0%), the lung (n=25, 67.6%), the liver (n=17, 45.9%) and the bone (n=6, 16.2%). Visceral sites were involved in 24 patients (64.9%). Thirty-six (94.7%) patients underwent surgery prior to entering the study. The most common procedures were local excision of lesions (n=26, 68.4%) and lymph nodes (n=15, 39.5%). Seven (18.4%) patients were given prior radiotherapy. In addition, three (7.9%) were given prior systemic therapy, which consisted of biological therapy (interferons) in the adjuvant setting in two patients, and chemotherapy (mitomycin and 5-fluorouracil) in the neoadjuvant setting in one.	

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Results (2): Extent of exposure to investigational product	**Drug Exposure:** Phase I stage Seventy-six cycles of plitidepsin and DTIC were administered, with a median of 2 cycles (range, 1-8 cycles) per patient. A total of 190 plitidepsin infusions were administered during this stage. At the RD of plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2, the median plitidepsin cumulative dose per patient was 12.7 mg/m^2 (range, 2.4-33.6 mg/m^2), the median dose intensity was 1.2 mg/m^2/week (range, 0.6-2.0 mg/m^2/week) and the median relative dose intensity was 66.8% (range, 33.1-99.2%). For DTIC, the median cumulative dose per patient at the RD was 1,583.7 mg/m^2 (range, 795.7-4,000.0 mg/m^2), the median dose intensity was 189.5 mg/m^2/week (range, 151.0-198.9 mg/m^2/week) and the median relative dose intensity was 94.8% (range, 75.5%-99.5%). Phase II stage *Arm A (Plitidepsin 3.2 mg/m^2)* Thirty-two cycles and 75 infusions of single-agent plitidepsin were administered, with a median of 1.5 cycles (range, 1-4 cycles) per patient. The median total plitidepsin dose per patient was 9.7 mg/m^2 (range, 3.0-27.1 mg/m^2), the median dose intensity was 1.6 mg/m^2/week (range, 0.8-2.4 mg/m^2/week) and the median relative dose intensity was 67.0% (range, 31.7%-100.9%). *Arm B (Plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2)* A total of 125 cycles of plitidepsin and DTIC were administered, with a median of 2 cycles (range, 1-11 cycles) per patient. Overall, 277 plitidepsin infusions were administered in this arm. The median total plitidepsin dose per patient was 9.6 mg/m^2 (range, 2.4-67.2 mg/m^2), the median dose intensity was 1.1 mg/m^2/week (range, 0.6-1.8 mg/m^2/week) and the median relative dose intensity was 61.8% (range, 33.0%-100.0%). For DTIC, the median total dose per patient was 1,600.0 mg/m^2/wk (range, 797.9-8,789.5 mg/m^2), the median dose intensity was 199.8 mg/m^2/wk (range, 99.7-203.1 mg/m^2/wk) and the median relative dose intensity was 99.9% (range, 49.9%-101.6%). **Dose Delays, Omissions and Reductions:** Phase I stage Seventeen cycles were delayed in 14 patients. Five delays were related to the study treatment: four were due to non-hematological toxicity [ALT increase (n=2); CPK increase (n=1); and precordial oppression (n=1)] and one was due to hematological toxicity (neutropenia). The other 12 delays were unrelated to treatment. A total of 34 plitidepsin dose omissions occurred in this stage, and most (n=19, 55.9%) involved the infusion scheduled on Day 15. Thirty-one were related to treatment, either due to non-hematological toxicity [transaminase increase alone or concomitant with other reasons (26 omissions); respiratory infection; cardiac AE (grade 4 atrial fibrillation); direct bilirubin increase; and a combination of nausea, vomiting, anemia and pancytopenia (one omission each)], or hematological toxicity (thrombocytopenia; one omission). The other three omissions were unrelated to treatment. Eleven dose reductions occurred in nine patients during this stage. Nine reductions affected plitidepsin only and were due to non-hematological toxicity [ALT increase (n=3); hepatic toxicity with asthenia and myalgia; nausea and vomiting; and direct bilirubin increase (n=1 each)] or to reasons unrelated to treatment (n=3). The other two dose reductions affected DTIC only and were the result of hematological toxicity related to DTIC [pancytopenia (n=1)], or of reasons unrelated to treatment (n=1). Phase II stage *Arm A (Plitidepsin 3.2 mg/m^2)* Three delays occurred in Arm A: two due to non-hematological toxicity (transaminase increases) and one due to reasons unrelated to plitidepsin.	

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Twelve dose omissions were found, and most (eight omissions, 66.7%) affected the infusion scheduled on Day 8. Six were due to non-hematological toxicity [ALT increase alone or concomitant with myalgia (three omissions); CPK increase; fatigue concomitant with muscular weakness; and extrasystoles (one omission each)]. The other six were due to reasons unrelated to plitidepsin.
A total of three dose reductions occurred in three patients. All were due to non-hematological toxicity: transaminase increase, CPK increase, and direct bilirubin increase (n=1 each).

Arm B (Plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2)
Twenty-five dose delays occurred in this arm. Fifteen were due to treatment-related reasons, including non-hematological toxicity [ALT increase (n=9); CPK increase (n=2); and fatigue (n=1)] and hematological toxicity [neutropenia alone or concomitant with thrombocytopenia (n=3)]; the other ten delays were unrelated to treatment.
A total of 86 plitidepsin dose omissions were found, mostly (n=47, 54.7%) involving the infusion scheduled on Day 8. Seventy-six dose omissions were related to treatment. Of these, 72 were due to non-hematological toxicity: transaminase increases (56 omissions); increased troponin alone or concomitant with fatigue and muscular weakness (four omissions); nausea and vomiting alone or with fatigue (n=3); bilirubin increase (n=2); pericarditis (n=2); a combination of hypokalemia and prolonged QT interval; altered bowel habits; diarrhea and abdominal pain; fatigue; and CPK increase (one omission each). Four were due to hematological toxicity: thrombocytopenia alone or concomitant with anemia (three omissions); and pancytopenia (one omission). The other ten dose omissions were unrelated to treatment.
A total of nine dose reductions occurred in nine patients. Seven affected plitidepsin only and were due to non-hematological toxicity [ALT increase (n=4); bilirubin increase; and CPK increase (n=1 each)] or to reasons unrelated to treatment (n=1). The other two reductions affected both plitidepsin and DTIC, and were due to non-hematological toxicity (bilirubin increase) or a combination of hematological and non-hematological toxicity (neutropenia, thrombocytopenia and ALT increase).

Study Discontinuation:
Phase I stage
Most enrolled patients (n=20; 71.4%) discontinued due to disease progression. Six (21.4%) discontinued due to toxicity: hypersensitivity (n=4); pancytopenia (n=1); and atrial fibrillation (n=1). The other two (7.1%) discontinued due to other reasons: to undergo surgery for remaining lesions and due to target lesions being a hepatic hemangioma instead of metastatic melanoma (n=1 each).

Phase II stage
Arm A (Plitidepsin 3.2 mg/m^2)
Most enrolled patients (n=14; 70.0%) discontinued due to disease progression. Five (25.0%) discontinued due to toxicity: CPK and ALT increase lasting > 2 weeks (n=2); hypersensitivity reaction (n=2); and grade 2 extrasystoles (n=1). In addition, one (5.0%) discontinued owing to other reasons: surgery for debulking an eye lesion.

Arm B (Plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2)
Nineteen patients (50.0%) discontinued due to disease progression. Eight (21.1%) discontinued due to toxicity: hypersensitivity (n=5); pancytopenia; ALT increase; and a combination of mild pleural effusion and pericarditis (n=1 each). Finally, 11 (28.9%) discontinued due to other reasons: investigator's decision (n=2); fatigue alone or with hypotension; achievement of complete response; unstable angina; AP increase lasting > 2 weeks; amylase increase; immunosuppression and herpes zoster; finding of CNS metastases; and loss to follow-up (n=1 each).

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Results (3): Dose-limiting toxicities, maximum tolerated dose, and recommended dose (phase I stage only)	**Dose-limiting Toxicities (DLTs):** Twenty-one of 28 patients treated with plitidepsin and DTIC were evaluable for DLTs. Four had DLTs: one (grade 3 ALT increase) of six evaluable patients at plitidepsin 1.8 mg/m^2 and DTIC 800 mg/m^2, one (grade 3 ALT increase) of five evaluable patients at plitidepsin 2.4 and DTIC 800 mg/m^2, and two (one with grade 3 ALT increase and one with grade 4 pancytopenia and grade 4 febrile neutropenia) of four evaluable patients at plitidepsin 2.4 and DTIC 1000 mg/m^2. All DLTs were transient. No DLTs were found at plitidepsin 3.0 mg/m^2 and DTIC 800 mg/m^2, but this dose level was associated with a high number of skipped infusions (11 of 69 scheduled infusions in 23 cycles, 15.9%) due to ALT increases. **Maximum Tolerated Dose (MTD) and Recommended Dose (RD):** The MTD for the combination was plitidepsin 2.4 mg/m^2 and DTIC 1000 mg/m^2, and the RD was plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2.	
Results (4): Safety	Phase I stage All 28 treated patients were evaluable for safety. At the RD, the most common treatment-related AEs were fatigue (62.5% of patients/60.0% of cycles), nausea (62.5% of patients/35.0% of cycles), diarrhea (37.5% of patients/20.0% of cycles) and anorexia (37.5% of patients/15.0% of cycles). Most were mild or moderate. Severe treatment-related AEs comprised grade 3 fatigue (n=2); grade 3 vomiting; grade 3 diarrhea; grade 3 hypersensitivity; grade 3 respiratory tract infection; grade 3 weakness; grade 4 pancytopenia; and grade 4 neutropenic sepsis (n=1 each). Regardless of their relationship to treatment, most hematological abnormalities were mild or moderate. At the RD, severe hematological abnormalities consisted of grade 3/4 lymphopenia (25.0% of patients/25.0% of cycles), grade 3/4 leukopenia (25.0% of patients/15.0% of cycles), grade 4 neutropenia (25.0% of patients/10.0% of cycles), grade 4 thrombocytopenia (25.0% of patients/10.0% of cycles) and grade 3 anemia (25.0% of patients/10.0% of cycles). All severe hematological abnormalities at the RD were found in two patients who already had grade 2 lymphopenia and grade 1/2 anemia at baseline; these abnormalities were categorized as treatment-related grade 4 pancytopenia in one patient, and were concomitant with treatment-related grade 4 neutropenic sepsis in the other. Grade 4 neutropenia in all treated patients appeared at a median of 14 days (range, 13-15 days) after dosing and lasted a median of 11 days (range, 7-14 days), whereas grade 4 thrombocytopenia occurred at a median of 14 days (range, 13-14) after dosing and lasted a median of 12 days (range, 7-14 days). One case of grade 4 febrile neutropenia was found and was concomitant with other severe hematological abnormalities. Most biochemical abnormalities were mild or moderate regardless of their relationship to treatment. The only severe abnormality at the RD was grade 3 ALT increase, which was found in 25.0% of patients/10.0% of cycles. In all treated patients, it appeared at a median of 7.0 days (range, 6-21 days) after dosing, peaked on that same day and returned to grade $\leq$ 2 levels at a median of 17.0 days (range, 13-35 days). A total of 56 SAEs occurred at all dose levels in this stage. Fourteen of them were considered related to treatment: grade 3 hypersensitivity; grade 3 vomiting; grade 3/4 neutropenic sepsis; grade 4 pancytopenia (n=2 each); grade 4 febrile neutropenia; grade 4 thrombocytopenia; grade 3 fatigue; grade 3 diarrhea; grade 4 atrial fibrillation and grade 1 electrocardiogram (ECG) T wave abnormal (n=1 each). Both episodes of neutropenic sepsis and one of pancytopenia were considered suspected unexpected serious adverse reactions (SUSARs). Nine patients died up to the date of last follow-up. All deaths were due to progression of the malignant disease. Phase II stage *Arm A (Plitidepsin 3.2 mg/m^2)* Twenty patients in this arm were treated with single-agent plitidepsin and were thus evaluable for safety.	

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Most treatment-related AEs were mild or moderate. The most common were nausea (45.0% of patients/31.3% of cycles), fatigue (40.0% of patients/34.4% of cycles), vomiting (30.0% of patients/18.8% of cycles) and myalgia (20.0% of patients/18.8% of cycles). Four patients (20.0%) had six severe plitidepsin-related AEs: grade 3 nausea (n=2); grade 3 vomiting; grade 3 hypersensitivity; grade 3 fatigue; and grade 3 muscle weakness (n=1 each).

Regardless of relationship, most hematological abnormalities were mild or moderate. Severe abnormalities occurred in one patient (5.0%) and one cycle (3.1%) each and comprised grade 4 anemia and grade 3 lymphopenia. Of note, both patients with severe anemia and lymphopenia already had these hematological abnormalities at baseline. No cases of febrile neutropenia were found.

Most biochemical abnormalities regardless of relationship were also mild or moderate. The most common severe abnormality was grade 3/4 CPK increase (15.0% of patients/12.5% of cycles), followed by grade 3 amylase increase (10.5% of patients/6.9% of cycles) and grade 3 ALT increase (10.0% of patients/6.3% of cycles). Grade 3 ALT increase appeared at a median of 24 days (range, 21-27 days) after dosing and returned to grade ≤ 2 at a median of 29.5 days (range, 27-32 days). No severe increases in AST, AP, creatinine or total bilirubin levels were found.

A total of 23 SAEs were reported in Arm A; two of these SAEs in two patients (10.0%) were considered related to treatment. Both consisted of grade 2/3 hypersensitivity, resulted in treatment discontinuation and resolved without sequelae.

Eight patients died during the study period, all due to disease progression.

Arm B (Plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2)

Thirty-six patients in this arm were treated with plitidepsin and DTIC and were evaluable for safety.

Most treatment-related AEs were mild or moderate. The most common were nausea (69.4% of patients/36.8% of cycles), fatigue (41.7% of patients/32.0% of cycles), vomiting (36.1% of patients/12.8% of cycles) and hypersensitivity (25.0% of patients/7.2% of cycles). Thirteen patients (36.1%) had 19 severe treatment-related AEs: grade 3 fatigue (n=4); grade 3 hypersensitivity (n=3); grade 3 muscle weakness; grade 3 vomiting (n=2 each); grade 4 pancytopenia; grade 3 diarrhea; grade 3 nausea; grade 3 syncope; grade 3 cardiac troponin increase; grade 3 ECG T wave abnormal; grade 3 back pain; and grade 3 muscle cramps (n=1 each).

Most hematological abnormalities were mild or moderate, regardless of their relationship to treatment. Severe abnormalities consisted of grade 3/4 neutropenia (5.6% of patients/2.4% of cycles), grade 4 thrombocytopenia (5.6% of patients/1.6% of cycles), grade 3/4 anemia (2.8% of patients/3.2% of cycles), grade 3 lymphopenia (2.8% of patients/0.8% of cycles) and grade 4 leukopenia (2.8% of patients/0.8% of cycles). Most severe abnormalities occurred simultaneously in one patient and were globally categorized as treatment-related grade 4 pancytopenia. In addition, the single patient with severe anemia in this arm already had grade 2 anemia at baseline. No cases of febrile neutropenia were found.

Regardless of relationship, most biochemical abnormalities were mild or moderate. Most severe biochemical abnormalities did not reach grade 4. The most common was transient grade 3/4 ALT increase (27.8% of patients/12.1% of cycles), followed by transient grade 3 AST increase (5.6% of patients/1.6% of cycles), grade 3 amylase increase (2.9% of patients/0.9% of cycles) and grade 4 CPK increase (2.8% of patients/0.8% of cycles). Grade 3/4 ALT increase appeared at a median of 13 days (range, 6-35 days) after dosing and returned to grade ≤ 2 at a median of 26 days (range, 12-42 days), while grade 3 AST increase was also found at a median of 13 days (range, 6-20 days) after dosing and returned to grade 2 at a median of 19.5 days (range, 12-27 days). No severe increases in AP, creatinine or total bilirubin levels were found.

Twenty-eight SAEs were reported in this arm; of these, eight events in seven patients were treatment-related. These comprised grade 2/3 hypersensitivity (n=5); grade 3

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	vomiting; grade 3 syncope (which was considered a SUSAR); and grade 4 pancytopenia (n=1 each). The episodes of pancytopenia and vomiting, and the two episodes of grade 2 hypersensitivity, were related to both plitidepsin and DTIC. In contrast, all three episodes of grade 3 hypersensitivity were related to plitidepsin but not to DTIC. Finally, the grade 3 syncope was considered to have an unknown relationship with both drugs. Six patients discontinued as a result of treatment-related SAEs in Arm B: five due to grade 2/3 hypersensitivity and one due to grade 4 pancytopenia. All treatment-related SAEs resolved without sequelae. Three patients died during the study period, all due to disease progression.	
Results (5): Efficacy	Phase I stage Nineteen of 28 patients included and treated were evaluable for efficacy. One had PR and six had SD (including two who had unconfirmed PR). Two SDs occurred at plitidepsin 1.8 mg/m^2 and DTIC 800 mg/m^2, the PR and one SD at the RD, and three SDs (including the two unconfirmed PRs) at plitidepsin 3.0 mg/m^2 and DTIC 800 mg/m^2. Five of the six disease stabilizations lasted more than three months. Phase II stage *Arm A (Plitidepsin 3.2 mg/m^2)* - Primary efficacy endpoint: objective tumor response None of the 16 patients evaluable for efficacy in this arm had CR or PR. Two patients showed disease stabilization. - Secondary efficacy endpoints No median duration of tumor response could be calculated for this arm owing to the lack or response. The median duration of stable disease was 2.9 months (range, 2.8-3.0 months). TTP and PFS values were matched for all patients. The median PFS was 1.5 months (95% CI, 0.9-1.9 months). The median OS was 4.1 months (95% CI, 1.5-7.7 months). *Arm B (Plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2)* - Primary efficacy endpoint: objective tumor response Six patients in this arm were responders: one had CR and five had PR. Additionally, nine patients had disease stabilizations (including one with an unconfirmed PR) that in all cases lasted for $\geq$ 3 months. Hence, the objective tumor response for the plitidepsin and DTIC combination was 21.4% (95% CI, 8.3%-41.0%) for the 28 patients evaluable for efficacy and 16.7% (95% CI, 6.4%-32.8%) for the 36 treated patients. - Secondary efficacy endpoints The median duration of tumor response in the six responders in this arm was 4.5 months (range, 1.4-16.5+ months). The median duration of stable disease among the nine patients with disease stabilization was 3.9 months (range, 3.1-13.2 months). TTP and PFS values were matched in all patients. The median PFS was 3.3 months (95% CI, 1.6-4.6 months). No median OS could be calculated for this arm due to the high number of censored events (25 of the 28 evaluable patients).	
Results (6): Pharmacokinetics	The PK profile of plitidepsin administered alone at a dose of 3.2 mg/m² on Days 1, 8 and 15 q4wk to patients with advanced melanoma was characterized by a mean clearance (CL) of 15.4 l/h, a mean volume of distribution at steady-state (V_{ss}) of 652 l and a mean terminal half-life (HL) of 45.1 h. In patients treated with plitidepsin 2.4 mg/m^2 on Days 1, 8 and 15 in combination with DTIC at 800 mg/m^2 on Day 1 q4wk, the PK profile of plitidepsin showed a mean CL of 12.0 l/h, a mean V_{ss} of 456 l and a mean HL of 43.0 h. As for DTIC, its mean CL, V_{ss} and HL in plasma when administered at doses of 800 and 1000 mg/m² in combination with plitidepsin were 43.2 l/h, 94.6 l and 2.32 h, respectively. Overall, the	

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	values of the PK parameters found for plitidepsin alone or combined with DTIC in this study were similar to those obtained in a phase II study conducted in patients with relapsing/refractory multiple myeloma. A univariate analysis found that hemoglobin, hematocrit, bilirubin, serum albumin, total protein and ECOG PS at baseline had an effect on whole blood clearance and volume of distribution of plitidepsin. In addition, lactate dehydrogenase (LDH) at baseline had an effect on the half-life of plitidepsin. No evidence of drug-drug interaction between plitidepsin and DTIC was found.	
Results (7): Pharmacogenomics	The results of the pharmacogenomic substudy will be described in a separate report.	
Conclusions	The primary objectives of this phase I-II clinical trial in adult patients with unresectable advanced melanoma were met. The first, dose-escalating stage established plitidepsin 2.4 mg/m^2 and DTIC 1000 mg/m^2 as the MTD for plitidepsin as 1-hour infusion on Days 1, 8, and 15 in combination with DTIC as 1-hour infusion on Day 1 q4wk, whereas a dose of plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2 was declared the RD for further phase II evaluation of this regimen. The second, exploratory stage primary assessed the efficacy of plitidepsin 3.2 mg/m^2 as a 1-hour infusion on Days 1, 8 and 15 q4wk, and of plitidepsin combined with DTIC at the RD determined in the dose-finding stage. No responses were found in the group of patients treated with single-agent plitidepsin. In contrast, the combination of plitidepsin 2.4 mg/m^2 and DTIC 800 mg/m^2 induced objective tumor response in 21.4% of patients. Response to the combination consisted of one CR and five PR, and was found in six patients who had normal serum LDH levels ($\leq$ 1.1 x ULN) and an ECOG PS score $\leq$ 1 at baseline. The absence of responses found with single-agent plitidepsin in this study may be explained at least in part by the lack of patient stratification at screening, which resulted in patients randomized into this arm having poor prognostic factors (i.e., LDH levels, ECOG PS score). In contrast, patients enrolled into the combination arm had a much better prognosis at baseline. Furthermore, the six responses (21.4%) that occurred among patients treated with the combination may not be attributed only to DTIC because the dose of 800 mg/m^2 given to these patients is lower than the optimal dose of 1000 mg/m^2 used when DTIC is combined with other chemotherapeutic agents in the treatment of metastatic melanoma. The PK profiles obtained for plitidepsin alone or combined with DTIC were similar and in line with that reported previously in patients with relapsed/refractory multiple myeloma. No relevant differences were found between the safety profiles of plitidepsin alone and combined with DTIC. Both regimens were generally well tolerated and showed manageable toxicity. The plitidepsin infusion scheduled on Day 8 of each cycle was frequently omitted due to toxicity in patients treated with plitidepsin and DTIC, therefore suggesting that a biweekly schedule might be more convenient for this combination. These results warrant the conduct of further clinical trials to evaluate a biweekly schedule of plitidepsin 2.4 mg/m^2 as a 1-hour infusion combined with DTIC 800 mg/m^2 as a 1-hour infusion in patients with unresectable advanced melanoma, normal LDH levels and an ECOG PS score $\leq$ 1.	
Date of report (final version)	12 August 2011.	