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

FINAL STUDY REPORT

APL-B-012-02

A PHASE II MULTICENTER, OPEN-LABEL, CLINICAL AND PHARMACOKINETIC STUDY OF APLIDIN® AS A 1-HOUR WEEKLY I.V. INFUSION, IN PATIENTS WITH RELAPSED OR REFRACTORY INDOLENT NON-HODGKIN'S LYMPHOID NEOPLASMS

Compound name (INN):	Plitidepsin
Investigational Medicinal Product:	Aplidin®
Study design:	Non-randomized, open-label, single-arm, multicenter, exploratory, phase II study
Protocol number:	APL-B-012-02
Study start date:	7 July 2005 (First consent signed)
Study completion date:	30 April 2007 (Last follow-up)
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Earlier approved reports:	None
Version:	Final version
Approval date:	11 April 2008

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

2. SYNOPSIS

Name of Sponsor/ Company: Pharma Mar, S.A. **Name of finished product:** Aplidin® **Name of active ingredient(s):** Plitidepsin	**Individual Study Table Referring to Part of the Dossier** **Volume:** **Page:**	(*For National Authority Use only*)
Protocol number	APL-B-012-02	
Study title	A Phase II Multicenter, Open-label, Clinical and Pharmacokinetic Study of Aplidin® as a 1-Hour Weekly i.v. Infusion, in Patients with Relapsed or Refractory Indolent Non-Hodgkin's Lymphoid Neoplasms.	
Principal investigator	**Dolores Caballero Barrigón, M.D.**, Hospital Universitario de Salamanca	
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Study centers	**Hospital Universitario de Salamanca** Departamento de Hematología Paseo de San Vicente, 58-182 37007 Salamanca, Spain **Clínica Universitaria de Navarra** Servicio de Hematología Av. Pío XII, 36 31008 Pamplona, Spain **Hospital General Gregorio Marañón** Departamento de Oncología C/ Dr. Esquerdo, 46 28007 Madrid, Spain **Hospital de la Princesa** Servicio de Hematología C/ Diego de León 62 28006 Madrid, Spain **Hospital Clínico Universitario de Valencia** Servicio de Hematología y Oncología C/ Blasco Ibañez17 46010 Valencia, Spain	**Hospital Morales Meseguer** Departamento de Hematología. C/ Marqués de los Vélez, s/n 30008 Murcia, Spain **Hospital Universitario Marqués de Valdecilla** Servicio de Hematología Av. de Valdecilla s/n 39008 Santander, Spain **Hospital de la Santa Creu i Sant Pau** Departamento de Hematología C/ Sant Antoni Mª Claret 167. 08025 Barcelona, Spain **Institut Gustave Roussy** Département Médecin 39-53, rue Camille Desmoulins 94805 Villejuif Cedex, France **Oncology Institute of Southern Switzerland** Division of Medical Oncology Ospedale San Giovanni Via Vincenzo Vela 6 CH - 6500 Bellinzona, Switzerland
Publications (references)	At the time of this report no articles have been published on the study described herein.	
Study period: **- First consent signed** **- Last consent signed** **- First infusion administered** **- Last infusion administered** **- Last follow-up**	 7 July 2005 2 August 2006 8 July 2005 4 August 2006 30 April 2007	**Phase of Development:** Phase II
Objectives	**Primary Objective:**	• To assess the antitumor activity of plitidepsin given as a 1-hour weekly i.v. infusion in patients with relapsed or refractory indolent non-Hodgkin's

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		lymphoid neoplasms.
	Secondary Objective:	• To further investigate the safety profile of plitidepsin given as a 1-hour weekly i.v. infusion in this patient population. • To evaluate the pharmacokinetics (PK) of this schedule of plitidepsin in this patient population.
Methodology	Non-randomized, open-label, single-arm, multicenter, exploratory, phase II study.	
Number of subjects/patients	**Study design:** A Simon's two-stage MiniMax design was applied to test the null hypothesis that $p<0.1$ value *versus* the alternative hypothesis that it was $p\geq0.25$. With this study design, the expected sample size of this model was 31.20 patients and the probability of early termination was 0.648. If plitidepsin was not effective, there was a 0.098 probability of concluding that it was. Otherwise, if plitidepsin was actually effective, there was a 0.099 probability of concluding that it was not. **Planned number of patients:** Plitidepsin was to be tested on 21 evaluable patients in the first stage, and the trial had to be discontinued if ≤2 responses (complete response [CR]/unconfirmed complete response [CRu] and partial [PR]/nodular partial response [nPR]) were observed. Otherwise, the trial had to go onto the second stage and 29 additional evaluable patients had to be treated in the second stage of this study to reach the total of 50 patients. The Simon's design stated that if the total number of patients with an objective tumor response was ≤7, this schedule was not to be considered for further evaluation. **Patients analyzed:** Finally, the study was closed early after the first phase with eight included and treated patients, as the efficacy reported in the six evaluable patients (five SD according to the guidelines of the National Cancer Institute [NCI]-sponsored Working Group guidelines for non-Hodgkin's Lymphoma [NHL] and chronic lymphocytic leukemia) was considered too low to merit further interest for plitidepsin as single-agent in indolent NHL.	
Diagnosis and main criteria for inclusion	**Inclusion Criteria:** 1. Signed informed consent by the patient before starting any specific study procedures. 2. Histologically confirmed indolent lymphoid neoplasms, including the following: 2.1. Mature (peripheral) B-cell neoplasms: • B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma; • B-cell prolymphocytic leukemia; • Lymphoplasmacytic lymphoma; • Splenic marginal zone B-cell lymphoma (with or without villous lymphocytes); • Hairy cell leukemia; • Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type; • Nodal marginal zone B-cell lymphoma (with or without monocytoid B cells); • Follicular lymphoma (except large cell); • Mantle-cell lymphoma (except diffuse pattern or blastoid variant). 2.2. Mature (peripheral) T-cell neoplasms • T-cell prolymphocytic leukemia; • T-cell granular lymphocytic leukemia; • Mycosis fungoides/Sezary syndrome. 3. Lymphoproliferative malignancy either after relapse, following a response to standard or high-dose chemotherapy, or after refractory disease to chemotherapy with a clinical	

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need to start a new chemotherapy, or malignancies with no other alternative therapy that could demonstrate an improvement in survival or quality of life.

3.1. Relapsed disease was defined as development of any of the following symptoms after a prior response and with duration of at least six months:
- Lymphadenopathy;
- Splenomegaly;
- Malignant lymphocytosis greater than 5000 x 10^9/l;
- Infiltration of the bone marrow with malignant lymphocytes.

3.2. Refractory disease:
- No PR to prior therapy;
- CR or PR lasting for less than six months.

4. Patients with follicular NHL had to be previously treated with anti-CD20 monoclonal antibody.
5. Presence of at least one bidimensional measurable lesion greater than 2 cm in its longer diameter or malignant lymphocytosis greater than 5 x 10^9/l. Any other procedure for measurable disease in particular cases had to be approved by Pharma Mar.
6. Recovery from any non-hematological toxicity derived from previous treatments. The presence of alopecia and National Cancer Institute Common Toxicity Criteria (NCI-CTC) grade <2 symptomatic peripheral neuropathy was allowed.
7. Age ≥18 years.
8. Performance status (Eastern Cooperative Oncology Group, ECOG) ≤2.
9. Adequate renal, hepatic, and bone marrow function (to be documented ≤14 days before inclusion in the study):
   - Neutrophil count ≥1.5 x 10^9/l;
   - Platelet count ≥100 x 10^9/l;
   - Hemoglobin ≥8 g/dl;
   - Creatinine clearance ≥40 ml/min (calculated according to the Cockcroft and Gault formula);
   - Serum bilirubin ≤1.5 mg/dl and alkaline phosphatase (AP) ≤2.5 x upper limit of normal (ULN) (≤5 x ULN in case of extensive bone metastases);
   - Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤2.5 x ULN (≤5 x ULN in case of liver metastases);
   - Albumin ≥25 g/l.

Lower hematological values due to bone marrow infiltration had to be accepted by Pharma Mar, which had previously been agreed between the Sponsor and the investigator.

10. Left ventricular ejection fraction (LVEF) within normal limits.

Exclusion Criteria:

1. Prior therapy with plitidepsin.
2. Concomitant therapy with any anti-lymphoproliferative agent, including glucocorticoids at a daily dose greater than 10 mg (prednisone or equivalent), unless they were indicated for symptom control and documented disease progression.
3. Aggressive histological conversion.
4. Human immunodeficiency virus (HIV)-associated lymphoma.
5. Central nervous system (CNS) lymphoma.
6. More than five prior lines of systemic biological agents or systemic chemotherapies.
7. Prior gene therapy with viral vectors.
8. Washout periods since the end of the prior therapy less than:
   - Six weeks for nitroso-urea or high dose chemotherapy;
   - Four weeks for other chemotherapies or biological agents;

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- Four weeks for radiation or radioimmunotherapy (six weeks in case of prior extensive external beam radiation; i.e. more than 25% of bone marrow distribution);
- Four weeks for major prior surgery;
- Thirty days for any investigational product.

9. Pregnant or lactating women.
10. Men and women of reproductive potential who were not using effective contraceptive methods (one or more of the following):
    10.1. Complete abstinence from intercourse from two weeks prior to administration of plitidepsin, throughout the study, and for at least six months after completion or premature discontinuation from the study to account for elimination of the investigational drug; or,
    10.2 Patient or patient's partner physical sterilization; or,
    10.3. One of the following, for female patients or female partners of male patients:
    - Implants of levonorgestrel; or,
    - Injectable progestogen; or,
    - Oral contraceptive (combined or progestogen only; subject taking oral contraceptives should have been on a stable regimen for at least two months prior to screening); or,
    - Any intrauterine device (IUD) with published data showing that the lowest expected failure rate is less than 1% per year; or,
    - Double barrier method (two physical barriers or one physical barrier plus spermicide); or,
    - Any other method with published data showing that the lowest expected failure rate for that method is less than 1% per year.
11. History of another neoplastic disease (except for non-melanoma skin cancer, carcinoma *in situ* of any site or any other cancer curatively treated with no evidence of disease for at least 10 years).
12. Known symptomatic cerebral or leptomeningeal involvement.
13. Other relevant diseases or adverse clinical conditions:
    - History or presence of unstable angina pectoris, myocardial infarction, valvular heart disease or congestive heart failure;
    - Previous mediastinal radiotherapy;
    - Uncontrolled arterial hypertension despite optimal medical therapy;
    - Previous treatment with doxorubicin at cumulative doses that exceed 400 mg/m²;
    - Symptomatic arrhythmia or any arrhythmia requiring treatment;
    - Abnormal electrocardiogram (ECG);
    - History of significant neurological or psychiatric disorders;
    - Active infection; infection by HIV, hepatitis B virus (HBV) or hepatitis C virus (HCV);
    - Myopathy or any clinical situation that causes significant and persistent elevation of creatine phosphokinase (CPK) (>2.5 x ULN in two different assessments one week apart);
    - Significant non-neoplastic liver disease (e.g., cirrhosis, active chronic hepatitis);
    - Uncontrolled endocrine diseases (e.g., diabetes mellitus, hypothyroidism or hyperthyroidism) (i.e., requiring a change in medication within the last month or a hospital admission within the last three months).
14. Treatment with any investigational product in the 30-day period before inclusion in the study.
15. Known hypersensitivity to plitidepsin, mannitol, cremophor EL, or ethanol.
16. Limitation of the patient's ability to comply with the treatment or follow-up protocol.

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Test product, dose and mode of administration, batch numbers	Plitidepsin was supplied by Pharma Mar S.A. (Colmenar Viejo, Madrid, Spain) as a lyophilized powder for concentrate for solution for infusion. Two different sizes of vials containing 0.5 mg and 2 mg of the active ingredient plitidepsin, respectively, and mannitol as the inactive ingredient were provided. The reconstitution solution was supplied in ampoules containing 1 ml and 4 ml of Cremophor/ethanol/Water for Injection (WFI) (15%/15%/70% v/v/v). Both vials and reconstituted solution had to be stored in a locked area with limited access at 5 ± 3ºC, and protected from light. Upon reconstitution of the 0.5-mg and 2-mg plitidepsin vials with 1-ml and 4-ml ampoules of reconstitution solvent, respectively, the reconstituted solution was clear, colorless, free from visible particles, and contained 0.5 mg/ml of plitidepsin. If not immediately used, this solution was chemically and physically stable for 24 hours (more if reconstitution was performed at controlled and validated aseptic conditions) at 2-8ºC. To prepare the drug for i.v. infusion, the reconstituted solution had to be immediately diluted with 0.9% w/v sodium chloride solution for infusion. The allowed dilution range was 1:10 to 1:400 (v:v). The total volume of infusion was to be 250 ml for administration through the central venous catheter (recommended way). In cases where administration occurred through a peripheral line, the reconstituted drug was to be diluted to 500 ml of total volume. An in-line filter of 0.2 µm had to be used for infusion. The infusion rate was to be established in a manner that ensured that the total dose of infusion was infused within one hour, either through a peripheral or a central i.v. line. The batch numbers of plitidepsin used in this study were as follows: • **0.5-mg vials:** #01G02 and #04K25 • **2-mg vials:** #03I25, #04H27, #05C10, and #05E25.	
Duration of treatment	Plitidepsin was to be administered at a starting dose of 3.2 mg/m^2, as a 1-hour continuous i.v. infusion, on days 1, 8, and 15 every four weeks. A set of consecutive three-week treatment followed by one resting week was considered a cycle (one cycle = four weeks). Treatment cycles were repeated weekly for a maximum of six months (six cycles) until disease progression, significant increase in tumor-related symptoms, unmanageable toxicity, withdrawal of patient consent or treatment delay >2 weeks (except in case of obvious patient's benefit). Only exceptionally, treatment duration was to be exceed six months in patients with clinical benefit and good tolerance, according to physician's discretion, and with the written permission of the sponsor.	
Criteria for evaluation	**Primary Endpoint:** • Objective tumor response, defined as the sum of CR/CRu and PR/nPR. **Secondary Endpoints:** • Time to response and duration of response. • Time to progression (TTP) and time to subsequent chemotherapy. • Progression-free survival (PFS) and overall survival (OS; follow-up of survival was to be finished at two years after the last-treatment visit of the last patient). • Description of PK parameters (second stage only). • Characterization of toxicity, serious adverse events (SAEs) and treatment withdrawals.	
Statistical methodology	Objective response rates were analyzed in all evaluable patients (i.e., patients having received a minimum of two treatment cycles of plitidepsin and with at least one disease assessment). Additionally, eligible patients who had received plitidepsin and experienced early disease progression prior to a formal response evaluation or stopped treatment because of unmanageable toxicity were to be considered evaluable for response and categorized as "non-responders". Safety parameters had to be evaluated in all subjects who received at least one infusion (or part of it) of plitidepsin according to NCI-CTC (version 3.0).	

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	Descriptive statistics (mean, median, standard deviation and 95% confidence interval, range of value) were used in the analysis of demographic factors, response rate, safety profile and laboratory observations. Time-to-event efficacy endpoints (tumor response duration, time to objective tumor progression, TTP, PFS and OS) were analyzed according to the Kaplan-Meier method. PFS and OS rates at fixed time points (three and six months and one year) were estimated using Kaplan-Meier analysis. Whole blood samples (5 ml) for PK analysis had to be collected during the second stage of the study. However, as the study was closed early during the first phase, no PK analysis was performed.	
Results (1): Patient characteristics	A total of eight patients with indolent NHL were enrolled and treated at four medical institutions. All patients were Caucasians; six of them (75%) were male and had a median age of 70.5 years (range, 58-74 years). All patients had mature (peripheral) B-cell NHL according to the World Health Organization (WHO) classification, which either relapsed (n=7) following a response to chemotherapy or was refractory (n=1) to previous chemotherapy. Stage III/IV NHL according to the Ann Arbor lymphoma staging was found in most patients (n=7; 87.5%). Three patients (37.5%) had documented extranodal NHL. Only one patient showed well-defined and generalized "B" symptoms at diagnosis. All patients had received between one and five prior lines of chemotherapy, with a median of 4 lines and a median of 7.5 agents per patient (range, 4-11 agents). Five patients (62.5%) had previously undergone surgery in a diagnostic/exploratory setting, while therapeutic radiotherapy had been given to four patients (50.0%). Four patients (50.0%) entered the study showing grade 1/2 signs and symptoms of disease at baseline, with a median of 0.5 symptoms per patient (range, 0-3). Fatigue was the most frequent symptom. At baseline, four patients enrolled in this study received a median of 0.5 (range 0-4) concomitant drugs, which were administered for a median of 0.5 (range 0-4) indications and involved a median of 0.5 (range 0-2) body systems. Anemia (n=6; 75.0%), thrombocytopenia (n=4; 50.0%), and lymphopenia (n=2; 25.0%) were the only hematological abnormalities at baseline. All were grade 1/2 save for one patient (12.5%) with grade 3 lymphopenia and another one with grade 3 thrombocytopenia. No patients had leukopenia or neutropenia at baseline. Grade 1 increases in AP (n=3; 37.5%), AST (n=2; 25.0%), and ALT (n=1; 12.5%) levels were the only biochemical abnormalities at baseline. Additionally, one patient had grade 2 hyperglycemia.	
Results (2): Extent of exposure to investigational product	**Drug exposure:** Overall, 27 treatment cycles were administered to eight patients during the study, with a median of 3.5 cycles per patient (range, 1-6). Three patients (37.5%) received one cycle of treatment. The median relative calculated dose intensity was 97.3%. **Dose delays and reductions:** Four of the 19 cycles (21.1%) susceptible of delay were delayed in three of five patients (60.0%) who received more than one cycle. All cycles were delayed due to non-treatment-related reasons and delays ranged from 1-7 days. Only one patient (20.0%) had a dose reduction (-15.6%) during the study, due to treatment-related grade 2 ALT increase. **Treatment discontinuation:** Half of the patients discontinued the study because of disease progression (n=4, 50.0%). Other reasons for treatment discontinuation were toxicity (n=2; 25.0%: one patient with grade 2 myalgia and one with grade 4 CPK increase), and other reasons (n=2; 25.0%).	
Results (3): Efficacy	Six of eight patients were evaluable for the analysis of efficacy endpoints. No objective responses per the NCI-sponsored Working Group guidelines for NHL and chronic lymphocytic leukemia were found during the first stage of the study. Disease stabilization	

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	was observed in five of six evaluable patients (83.3%). With a median follow-up of 15.1 months (95% CI, 11.0-21.7), the median TTP and PFS were 4.0 months (95% CI, 3.5-5.1 months) and 4.7 months (95% CI, 3.8-5.1 months), respectively and the median OS was 12.4 months (95% CI, 8.1-16.4 months).	
Results (4): Safety	All eight patients were evaluated for extent of drug exposure and safety. Most plitidepsin-related adverse events (AEs) that occurred during the study were mild or moderate (grade 1/2) in severity and allowed the patients to remain on treatment. Only one patient (12.5%) had grade 3 plitidepsin-related diarrhea (which required no further action) during one cycle (3.7%). Only one SAE, which was considered unrelated to treatment, was observed in one patient who died due to grade 4 respiratory failure and septic shock before receiving the first plitidepsin dose. Two deaths occurred during follow-up, and both were due to progression of the underlying disease. No patients died during the treatment. The laboratory toxicities most frequently found after plitidepsin administration were anemia (100% of patients/100.0% of cycles), thrombocytopenia (75.0% of patients/59.3% of cycles), and increases in the levels of blood transaminases (ALT: 100.0% of patients/84.6 of cycles; AST: 71.4% of patients/53.8% of cycles) and CPK (57.1% of patients/21.7% of cycles). Anemia, thrombocytopenia and lymphopenia were the only hematological toxicities that reached grade 3 severity. Only one patient had grade 3/4 biochemical toxicities during the study (grade 3 AST and ALT increases and grade 4 CPK increase after cycle 1).	
Conclusions	Plitidepsin 3.2 mg/m^2 given as a 1-hour weekly i.v. infusion on day 1, 8, and 15 every four weeks to patients with relapsed or refractory indolent NHL was found to be a chemotherapy regimen with predictable and manageable toxicity. Plitidepsin treatment showed poor antitumor activity, which resulted in low accrual rate and in the premature closure of this exploratory clinical trial before the figure of 21 patients, scheduled by the protocol, had been reached in the first stage of the study design. No recommendation for further evaluation of plitidepsin as single-agent chemotherapy is considered in the treatment of patients with relapsed or refractory indolent NHL.	
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