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CLINICAL STUDY REPORT

APL-B-020-10

OPEN-LABEL, PHASE II CLINICAL TRIAL OF APLIDIN® (PLITIDEPSIN) IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF) AND POST POLYCYTHEMIA VERA/ESSENTIAL THROMBOCYTHEMIA (POST-PV/ET) MYELOFIBROSIS

Investigational Medicinal Product/Study Drug:	Aplidin® (Plitidepsin)
Study Design:	Open-label, non-randomized, phase II trial.
Protocol Number:	APL-B-020-10
Study Start Date:	8 July 2010 (Date of first registration)
Study Completion Date:	Competent Authorities were notified of study termination on 4 March 2011 6 April 2011 (Date of last follow-up)
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Earlier Approved Reports:	None
Version:	Final version
Approval Date (planned):	7 February 2012

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

2. SYNOPSIS

Name of Sponsor(s)/Company(ies): Pharma Mar S.A. Pharma Mar USA, Inc **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-020-10	
Title of the study	Open-label, Phase II Clinical Trial of Aplidin® (Plitidepsin) in Patients with Primary Myelofibrosis (PMF) and Post Polycythemia Vera/Essential Thrombocythemia (Post-PV/ET) Myelofibrosis	
Coordinating Investigator	**Animesh D. Pardanani, M.D, PhD.** Mayo Clinic Cancer Center Rochester, Minnesota (USA)	
Co-investigators / Study centers	**Alessandro M. Vannucchi, MD** University of Florence AOU Careggi Florence, Firenze (Italy)	
Publication (references)	At the time of this report no articles have been published on the study described herein.	
Study period: **. First registration** **. Last registration** **. First infusion** **. Last infusion** **. Last follow-up**	 8 July 2010 11 November 2010 12 July 2010 5 January 2011 6 April 2011	**Phase of Development:** Phase II
Study objectives	**Primary:** • To assess the objective response rate (ORR) of plitidepsin according to the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) in patients with: ▪ primary myelofibrosis (PMF), ▪ post-polycythemia vera myelofibrosis (post-PV MF), or ▪ post-essential thrombocythemia myelofibrosis (post-ET MF). **Secondary:** • To evaluate the safety and tolerability of plitidepsin in this study population. • To determine the effect of plitidepsin on: ▪ bone marrow (BM) or peripheral blood histologic and cytogenetic findings, and ▪ peripheral blood granulocyte JAK2V617F allele burden. • To determine the quality of life (QoL) and symptoms assessment according to the Myelofibrosis Symptom Assessment Form (MFSAF), after treatment with plitidepsin. **Exploratory:** • To determine other time-related parameters, according to available follow-up data [i.e., time to response, duration of response (DR), progression-free survival (PFS), or overall survival (OS)].	
Methodology	This was an open-label, non-randomized, phase II trial. The primary endpoint of this trial was the treatment success rate, with success defined as confirmed disease objective response according to the IWG-MRT consensus criteria. Thus, a confirmed response was deemed to be an objective status of partial or complete remission, or clinical improvement (CI), according to the IWG-MRT consensus criteria (i.e., on two consecutive evaluations performed at least eight weeks apart). The design chosen was the optimum among the family of Simon two-stage designs, with a first stage between ten and 12 patients, alpha ≤ 0.1 and beta ≤ 0.1. In a first stage, a minimum of ten evaluable patients had to be accrued to test the null hypothesis, Ho: response rate (RR) $\leq$15% vs. Ha: RR $\geq$35%. At this first stage, the largest success rate in the sample that would cause the proposed treatment regimen to	

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	be considered ineffective was 10% (one confirmed response or no response), and the smallest success rate in the sample that would be considered worthy of further study is 20% (at least two confirmed responses). If the latter occurred, 35 additional evaluable patients had to be recruited in a second stage. A response rate of at least 22.2% (this is, at least ten confirmed responses) in the total of 45 patients would have allowed to conclude that the drug was effective. If the drug was actually not effective, there was a 0.0978 probability of concluding that it were (the probability of type I error is < 0.1). If the drug was actually effective, there was a 0.0992 probability of concluding that it was not (the probability of type II is error < 0.1). Patients were to receive a maximum of six cycles of treatment with plitidepsin. Patients with stable disease (SD), clinical improvement (CI), or partial or complete remission (per IWG-MRT criteria) who tolerated the drug well were allowed to continue receiving plitidepsin at the discretion of the Investigator and with approval from the Sponsor. Toxicity, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific discoveries and changes in standard care, were taken into account in any decision to terminate the study.	
Number of patients (planned and analyzed)	**Planned number of patients:** In a first stage, a minimum of ten evaluable patients were to be accrued to test the null hypothesis. It was anticipated the recruitment of additional patients to account for ineligibility, cancellation, major treatment violation, or other reasons. If success rate in the sample would cause the proposed treatment regimen to be considered worthy of further study, 35 additional evaluable patients had to be recruited in a second stage. **Number of patients analyzed:** Finally, a total of 12 patients were included in the first stage. The trial did not proceed into the second stage.	
Diagnosis and main selection criteria	**<u>Inclusion Criteria</u>** Patients who met all following criteria were to participate in the study: 1) Diagnosis of PMF or post-ET/PV MF as per revised World Health Organization (WHO) criteria. 2) High-risk or intermediate-2 risk myelofibrosis (MF) as defined by the International Prognostic Scoring System (IPSS); or intermediate-I risk MF associated with symptomatic splenomegaly/hepatomegaly and/or unresponsive to available therapy. 3) At least 18 years of age, with life expectancy ≥12 weeks. 4) Able to provide informed consent and being willing to sign an informed consent form (ICF). 5) Eastern Cooperative Oncology Group (ECOG) performance status ≤2. 6) Acceptable organ function within seven days of initiating study drug, as evidenced by the following: a) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 3.0 x upper limit of normal (ULN). b) Direct bilirubin ≤ 1.0 x ULN. c) Absolute neutrophil count (ANC) ≥ 1.0 x 10^9/L. d) Platelet count ≥ 25 x 10^9/L. e) Left ventricular ejection fraction (LVEF) within normal limits. f) Creatine phosphokinase (CPK) ≤ 2.5 x ULN. g) Creatinine clearance ≥ 40 mL/min. **<u>Exclusion Criteria</u>** Patients who met any of the following criteria were to be excluded from participating in the study:	

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1) Previous treatment with plitidepsin.
2) Any of the following therapies within two weeks prior to initiation of study drug:
   a) chemotherapy (e.g., hydroxyurea),
   b) immunomodulatory drug therapy (e.g., thalidomide),
   c) immunosuppressive therapy,
   d) corticosteroids >10 mg/day prednisone or equivalent, or
   e) erythropoietin.
3) Incomplete recovery from major surgery within four weeks of study entry.
4) Radiation therapy within four weeks of study entry.
5) Women of childbearing potential, unless surgically sterile for at least three months (i.e., hysterectomy), or postmenopausal for at least 12 months (follicle-stimulating hormone, FSH > 30 U/mL), or unless they agreed to take appropriate precautions to avoid pregnancy (with at least 99% certainty) throughout the treatment period and for six months after discontinuation of the treatment. Permitted methods for preventing pregnancy had to be communicated to the study patients and their understanding had to be confirmed.
6) Men who partner with a woman of childbearing potential, unless they agreed to take appropriate precautions to avoid pregnancy (with at least 99% certainty) throughout the treatment period and for six months after discontinuation of the treatment. Permitted methods for preventing pregnancy had to be communicated to the study subjects and their understanding had to be confirmed.
7) Women who were pregnant or breastfeeding.
8) Myopathy grade > 2 or any clinical situation that caused significant and persistent elevation of CPK (> 2.5 x ULN in two different determinations performed one week apart).
9) Known positive status for human immunodeficiency virus (HIV).
10) Active hepatitis B or C virus (HBV or HCV) infection and/or significant non-neoplastic liver disease (i.e., cirrhosis).
11) Diagnosis of another invasive malignancy unless free of disease for at least three years following therapy with curative intent. Patients with early-stage basal cell or squamous cell skin cancer, cervical intraepithelial neoplasia, cervical carcinoma *in situ*, or superficial bladder cancer, may be eligible to participate at the Investigator's discretion.
12) Any acute active infection.
13) Serious concomitant systemic disorders that would compromise the safety of the patient or limit the patient's ability to complete or comply with the study, including:
   a) Uncontrolled medical illness that the Investigator feels could compromise the patient's tolerance to the study medication.
   b) Uncontrolled or unstable angina, myocardial infarction, cerebrovascular accident, valvular heart disease or congestive heart failure (New York Heart Association Classification 3 or 4), within 12 months prior to initiation of study drug.
   c) Pulmonary embolism within three months prior to initiation of study drug.
   d) Uncontrolled arterial hypertension (≥ 160/110 mmHg) despite optimal medical therapy.
   e) Previous treatment with doxorubicin at cumulative doses ≥ 450 mg/m².
   f) Symptomatic arrhythmia (excluding anemia-related sinusal tachycardia grade ≤ 2), or prolongation of the QTc (Bazzett's, QTcB) interval to >450 msec for men or >470 msec for women at pre-study screening, unless attributable to pre-existing bundle branch block.

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	14) Known hypersensitivity to the study drug or any of its formulation components (e.g., Cremophor®). 15) Treatment with any investigational product in the 30 days before inclusion in the study.	
Test product, dose and mode of administration	Plitidepsin (Aplidin®) was provided as a lyophilized powder for concentrate for solution for infusion and contains plitidepsin as the active ingredient and mannitol as the inactive ingredient. Before use, the vials were reconstituted with the solvent for reconstitution supplied in ampoules, each containing 4 mL of Cremophor/ethanol/water for injection (WFI) (15/15/70% v/v/v). Aplidin vials and reconstitution ampoules were to be stored in a locked area with limited access at 2 to 8ºC (36ºF to 46ºF). Aplidin vials had to be protected from exposure to light and kept in the outer carton. Plitidepsin was administered at 5 mg/m^2 intravenously (i.v.) diluted to a total volume of 250 ml in 0.9% saline or 5% dextrose solution, via a central venous catheter through a pump device over three hours (fixed rate) on Day 1 and 15 every four weeks (q4wk). The number of the plitidepsin batch in all patients was as follows: • **2-mg vial batch:** 08K20**.**	
Duration of study and treatment	Patients were to receive study treatment up to a maximum of six cycles, in the absence of unacceptable toxicity and/or early disease progression. Patients with SD, CI, or partial or complete remission (per IWG-MRT criteria) who tolerated the drug well might be allowed to continue to receive plitidepsin at the discretion of the Investigator and with approval from the Sponsor. Patients were to be considered **on-study** from the signature of the informed consent to the end of the follow-up period, and **on-treatment** for the duration of their treatment and until treatment discontinuation. Treatment discontinuation was defined as 30 days after the last dose of plitidepsin administration, unless the patient died or started any new antitumor therapy, in which case the date of death or the date of administration of this new therapy was considered the date of treatment discontinuation.	
Criteria for evaluation **Efficacy** **Safety**	The primary efficacy criterion for plitidepsin was the assessment of the ORR according to the IWG-MRT in patients with PMF, post-PV MF, or post-ET MF. Patients were considered evaluable for the primary efficacy endpoint if they had received at least one complete 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. QoL according to MFSAF was to be also evaluated until disease progression. As an exploratory objective, other time-related parameters (time to progression, DR, PFS, or OS) were to be assessed according to available follow-up data. Patients were evaluable for safety if they received at least one dose (complete or incomplete) of plitidepsin. Safety had to be evaluated using clinical examinations, which comprised vital signs analysis, clinical assessment of adverse events (AEs), changes in laboratory parameters (hematological and biochemical, including liver function tests) and any other analyses considered necessary.	
Statistical methodology	**<u>Main efficacy and safety parameters</u>** Descriptive statistics (mean, median, standard deviation and, range of value, frequencies and percentages with 95% confidence intervals), were to be used to characterize the efficacy and safety profile. Response was to be measured by CR rate, PR rate and CI rate, according to the IWG-MRT consensus criteria. All AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.4.0, and were coded using the Medical Dictionary for Regulatory Activities (MedDRA), v.12.0. **<u>Exploratory efficacy parameters</u>** Kaplan-Meier analysis was to be used for time-dependent parameters (DR, PFS, and OS).	

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	<u>Other efficacy parameters</u> Change from baseline at each time point and treatment termination was to be analyzed for all MFSAF items and correlated with other objective efficacy parameters.	
Results (1): <u>Patient characteristics</u>	A total of 12 patients were included in the first stage of the trial. Their median age was 69.5 years (range, 59-78 years), more than half (58.3%) were female, and all but one (91.7%) had ECOG PS 0 or 1. Five patients (41.7%) had PMF, while four (33.3%) have post-ET and three (25.0%) post-PV at study entry. Most (75.0%) showed high-risk prognosis according to International Prognostic Scoring System (IPPS). Hepatosplenomegaly was palpable in all patients, with marked enlargement of the spleen ($\geq$ 10 cm) in eight of them (66.7%). All patients had anemia, mostly grade 2/3 (83.3%), that was associated with leukocytosis in five patients (41.7%), with thrombocytosis in one patient (8.3%) and with both abnormalities in one patient (8.3%). Other abnormalities at baseline were thrombocytopenia (50.0%), lymphopenia (41.7%), leukopenia (33.3%) and neutropenia (33.3%). The decrease of platelet, lymphocyte and WBC counts reached grade 3 severity in one patient (8.3%) each. Eleven patients (91.7%) had elevated lactate dehydrogenase (LDH) levels. Eleven patients (91.7%) had received prior immunomodulating and/or antineoplastic agents, being the most frequent hydroxycarbamide (50.0%) and thalidomide (41.7%). Four patients (33.3%) required antianemic preparations. At baseline, one patient (8.3%) had received one transfusion of platelets, and ten patients (83.3%) had received a median of 2 (range, 1-4) units of packed RBCs. One patient (8.3%) had undergone splenic radiation therapy.	
Results (2): <u>Efficacy</u>	Eleven patients were considered evaluable for the primary endpoint, objective response rate (ORR) according to IWG-MRT criteria, of whom 72.7% showed high-risk prognosis by IPSS. One patient (9.1%) had CI, nine patients (81.8%) had SD and one patient (9.1%) had PD as best response. No partial or complete remissions were recorded, and patients with SD showed no clinical benefit. Thus, ORR was 9.1% (95% CI, 0.2-41.3%). The duration of response was 2.3 months in the patient with CI, and ranged between 0.9 and 3.6 months (all data censored) the nine patients with SD. Seven of the 12 patients receiving plitidepsin refused further treatment after a median of 2 cycles (range, 1-4 cycles). With respect to the secondary endpoint of efficacy, the effects of plitidepsin on BM or peripheral blood histological and cytogenetic findings, as well as on blood granulocyte JAK2V617F allele burden, were not analyzed because observations were scant**.** Statistical analyses of patients' QoL by the MFSAF were inconclusive due to the low amount of data collected. Exploratory assessment of time-related characteristics showed a median progression-free survival of 4.6 months (95% CI, 1.4-4.6 months), with 88.9% of patients (95% CI, 68.4%-100%) being progression-free, and all patients being alive at three months. Median OS had not been reached at cutoff date (not enough survival data were available).	
Results (3): <u>Safety</u>	All 12 patients included in this study were treated with plitidepsin and, therefore, were evaluable for safety. The median number of cycles per patient was 2 (range, 1-4 cycles). The median time on treatment was 11.6 weeks (range, 6.1-20.0 weeks). The median cumulative dose was 20.1 mg/m^2 (range, 5.3-39.9 mg/m^2) and the median dose intensity was 2.2 mg/m^2/week (range, 1.3-2.5 mg/m^2/week). This resulted in a median relative dose intensity of 86.8% (range, 52.6-100.7%). The most common AEs related to the study treatment (all grades) were fatigue (50.0%), nausea (33.3%), vomiting (25.0%), and muscular weakness (25.0%). Three cases (25.0%) of grade 1/2 prolonged QT ECG of unknown relationship with plitidepsin were also recorded (two of these three patients had cardiac risk factors at baseline). Three patients (25.0%) had grade 3 AEs, which comprised fatigue in two patients (16.7%), and upper abdominal and chest pain in one patient (8.3%). No grade 4 drug-related AEs occurred.	

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	The most common hematological abnormality (all grades) was anemia, which occurred in all patients at all cycles, followed by lymphopenia (nine patients/19 cycles) and thrombocytopenia (eight patients/16 cycles). These abnormalities reached grade 3/4 severity in the following cases: anemia in nine patients/17 cycles, leukopenia in four patients/nine cycles, lymphopenia in four patients/six cycles, thrombocytopenia in four patients/six cycles and neutropenia in three patients/seven cycles. Additionally, five patients/11 cycles had leukopenia and five patients/10 cycles showed grade ≥2 neutropenia. A total of 47 units of RBCs and one unit of platelets were required by nine patients/11 cycles. WBC counts > ULN were documented in seven patients and platelet counts > ULN in four patients. Biochemical abnormalities were less common, and the only with effect on treatment was one case of grade 2 creatinine increase, which caused a dose delay. Five patients (41.7%) had serious AEs (SAEs). Upper abdominal and chest pain, reported in one patient, were the only plitidepsin-related SAEs occurred in this study. One patient died due to MF complications. Two patients discontinued plitidepsin administration: one due to grade 4 thrombocytopenia related to the disease, and other due to non drug-related SAEs (grade 3 pulmonary edema, bronchopneumonia and acute myocardial infarction). Four patients had delays associated to the following events: left ankle fracture, grade 2 blood creatinine increase, grade 4 neutropenia, grade 3 esophageal varices hemorrhage (SAE), and grade 2 bronchitis (none related to the study drug). Two patients had two dose omissions due to causes unrelated to plitidepsin: grade 2 rash macular and grade 3 gastrointestinal bleeding (SAE).	
Conclusions	Plitidepsin given at a dose of 5 mg/m^2 i.v. over three hours on Day 1 and 15 q4wk to patients with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (post-PV MF), or post-essential thrombocythemia myelofibrosis (post-ET MF) showed modest antitumor activity and was considered not worthy of consideration for further development in this indication. The toxicity profile of the study drug at this schedule of administration was consistent with that observed in other solid tumor and hematological trials conducted in patients with solid tumors and hematological diseases treated with plitidepsin.	
Date of report (final version)	7 February 2012.	