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CLINICAL STUDY REPORT

PM104-B-003-10

PHASE II MULTICENTER, OPEN-LABEL, CLINICAL AND PHARMACOKINETIC STUDY OF ZALYPSIS® (PM00104) IN PATIENTS WITH UNRESECTABLE LOCALLY ADVANCED AND/OR METASTATIC EWING FAMILY OF TUMORS (EFT) PROGRESSING AFTER AT LEAST ONE PRIOR LINE OF CHEMOTHERAPY

Compound Number:	PM00104
Investigational Medicinal Product:	Zalypsis®
Study Design:	Multicenter, open-label, exploratory phase II clinical and pharmacokinetic trial
Protocol Number:	PM104-B-003-10
Eudra CT:	2010-022221-15
Study Start Date:	22 December 2010 (First consent signed)
Study Completion Date:	24 April 2012 (Date of the end of the study according to the study protocol) 21 May 2012 (Date of last follow-up)
Principal/Coordinating Investigator Name and Affiliation:	**Sant P. Chawla, M.D.** Sarcoma Oncology Center, 2811 Wilshire Blvd, Suite 414, Santa Monica, CA 90403, USA. Phone: +1 310 552 9999 Fax: +1 310 201 66 85 E-mail: santchawla@aol.com
Responsible Medical Officer:	**Arturo Soto Matos-Pita, M.D.** Clinical Research and Development Director PharmaMar S.A., Sociedad Unipersonal (abbreviated as PharmaMar in this report) Avenida de los Reyes, 1 Polígono Industrial La Mina-Norte 28770 Colmenar Viejo, Madrid, Spain Phone: +34 918 466 053 Fax: +34 918 234 504 E-mail: asoto@pharmamar.com
Earlier Approved Reports:	None
Version:	Final version
Approval Date:	15 May 2013

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

2. SYNOPSIS

Name of Sponsor(s)/Company(ies): Pharma Mar S.A., Sociedad Unipersonal Pharma Mar USA, Inc. **Name of finished product:** Zalypsis® **Name of active ingredient(s):** PM00104	**Individual Study Table Referring to Part of the Dossier** **Volume:** **Page:**	(***For National Authority Use only***)
Protocol number	PM104-B-003-10	
Eudra CT	2010-022221-15	
Title of the study	Phase II multicenter, open-label, clinical and pharmacokinetic study of Zalypsis® (PM00104) in patients with unresectable locally advanced and/or metastatic Ewing Family of Tumors (EFT) progressing after at least one prior line of chemotherapy.	
Coordinating investigator	**Sant P. Chawla M.D.** Sarcoma Oncology Center, Santa Monica, CA, USA.	
Study centers	Sarcoma Oncology Center, Santa Monica, CA, USA. Istituto Ortopedico Rizzoli, Bologna, Italy. Seattle Cancer Care Alliance, Seattle, WA, USA. Centre Léon Bérard, Lyon, France. St. Jude Children's Research Hospital, Memphis, TN, USA.	
Publication (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 dose first cycle** **. First dose last cycle** **. Last follow-up**	 22 December 2010 1 November 2011 4 January 2011 24 January 2012 21 May 2012	**Phase of Development:** Phase II
Study objectives	**Primary:** • To determine the antitumor activity of PM00104 administered as a 1-hour intravenous (i.v.) infusion on Day 1, 8 and 15 every four weeks (d1, d8 and d15, q4wk) to patients with advanced and/or metastatic EFT. **Secondary:** • To determine the time-to-event efficacy parameters. • To characterize the safety profile and tolerability of PM00104 in patients with unresectable advanced and/or metastatic EFT. • To characterize the pharmacokinetics (PK) of PM00104 when administered as a single-agent to patients with EFT. • To determine the pharmacodynamic profile by measuring the effect of PM00104 on the number of Ewing's sarcoma circulating tumor cells (CTCs) at different times of treatment and its correlation with the clinical outcome. • To determine the pharmacogenomic (PGx) profile. Hypothesis-generating exploratory PGx analyses were to be conducted to correlate the molecular parameters found in the tumor and blood samples of the patients with the clinical results achieved with PM00104. Pharmacodynamic analysis of CTCs and PGx analyses were eventually not performed due to the lack of clinical benefit reported in this phase II clinical trial.	
Methodology	Exploratory, open-label, two-stage, single-arm phase II clinical trial evaluating PM00104 in patients with advanced and/or metastatic EFT who failed standard chemotherapy. The schedule (1-hour i.v. infusion d1, d8 and d15 q4wk) and dose (2 mg/m^2) evaluated in this phase II clinical trial were those recommended in a previous phase I study conducted in patients with advanced solid tumors (PM104-A-004-05).	
Number of patients (planned and analyzed)	**Planned number of patients:** The study protocol established that, in the first stage, at least 12 evaluable patients had to be recruited. After testing the drug on 12 evaluable patients in this first stage, the trial was to be closed if there was not any responder. In case to proceed to the second stage, 17 additional patients (for a total of 29 evaluable patients) were to be recruited. If the total number responding was less than or equal to two patients, the treatment was to be considered not interesting in this disease setting and with this schedule	

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	Patients analyzed: Data from 16 treated patients were evaluable for efficacy analysis. No objective responses were observed. Four patients had stable disease as best response to PM00104 treatment. Therefore, in accordance to the study protocol, a "no go decision" was taken and recruitment was closed without proceeding to the second stage.	
Diagnosis and main selection criteria	**Inclusion Criteria** Patients who met all following criteria participated in the study: **1.** Voluntary written informed consent, obtained from the patient or his/her representative before the beginning of any specific study procedure. **2.** Age ≥ 16 years. **3.** Histologically or cytologically confirmed EFT, with recurrent disease. **4.** Documented failure to at least one prior chemotherapy regimen for their disease. **5.** Radiographic documentation of disease progression at study entry. **6.** Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) ≤ 2. **7.** Life expectancy ≥ 3 months. **8.** Complete recovery from the effects of drug-related adverse events (AEs) derived from previous treatments, excluding alopecia and grade 1 peripheral neuropathy, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, v.4.0). **9.** At least one measurable lesion ("target lesion" according to the Response Evaluation Criteria in Solid Tumors, RECIST v.1.1), located in a non-irradiated area and adequately measured less than four weeks before study entry. Tumors within a previously irradiated field were designated as "non-target" lesions unless progression was clearly documented or biopsy proven. **10.** Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/l; platelet count ≥ 100 x 10^9/l, and hemoglobin ≥ 9 g/dl. **11.** Adequate renal function: calculated creatinine clearance (using Cockcroft and Gault´s formula) ≥ 30 ml/min. **12.** Adequate hepatic function: a) Total bilirubin ≤ 1.5 x upper limit of normal (ULN), unless due to Gilbert's syndrome. b) Alanine aminotransferase (ALT), aspartate aminotransferase (AST) ≤ 3 x ULN (≤ 5 x ULN in case of hepatic metastases), and alkaline phosphatase (AP) ≤ 2.5 x ULN (≤ 5 x ULN in case of extensive bone involvement). c) Albumin ≥ 2.5 g/dl. **13.** Left ventricular ejection fraction (LVEF) within normal limits (LVEF ≥ 50%). **14.** Women of childbearing potential 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 contraceptive device, oral contraceptive, subdermal implant and double barrier (condom with a contraceptive sponge or contraceptive suppository). **Exclusion Criteria** Patients who met any of the following criteria were to be excluded from participating in the study: **1.** Prior therapy with PM00104. **2.** Pregnant or lactating women or women of childbearing potential not using an appropriate contraceptive method. **3.** Less than three weeks from prior radiation therapy, biological therapy or chemotherapy. **4.** Less than six weeks from prior nitrosourea, mitomycin C, high-dose chemotherapy or radiotherapy involving the whole pelvis or over 50% of the	

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spine, provided that acute effects of radiation treatment had resolved. Hormonal therapy and palliative radiation therapy (i.e., for control of pain from bone metastases) had to be discontinued before study entry.

5. Patients with a prior invasive malignancy (except non-melanoma skin cancer and *in situ* cervix carcinoma) who had had any evidence of disease within the last five years or whose prior malignancy treatment contraindicates the current protocol therapy.

6. Evidence of progressive or symptomatic central nervous system metastases or leptomeningeal metastases.

7. Other diseases or serious conditions:

a) Increased cardiac risk as defined by:
- Unstable angina or myocardial infarction within 12 months before inclusion in the study.
- New York Heart Association (NYHA) grade II or greater congestive heart failure.
- Symptomatic arrhythmia or any arrhythmia requiring ongoing treatment.
- Abnormal electrocardiogram (ECG), i.e., patients with the following were excluded: QT prolongation - QTc > 480 msec; signs of cardiac enlargement or hypertrophy, bundle branch block; partial blocks; signs of ischemia or necrosis, and Wolff Parkinson White patterns.
- History or presence of valvular heart disease.
- Uncontrolled arterial hypertension despite optimal medical therapy.
- Previous mediastinal radiotherapy.
- Previous treatment with doxorubicin at cumulative doses exceeding 400 mg/m^2.

b) History of significant neurological or psychiatric disorders.

c) Active infection requiring systemic treatment.

d) Significant non-neoplastic liver disease (e.g., cirrhosis).

e) Hepatitis B or C virus infection.

f) Immunocompromised patients, including those infected with the human immunodeficiency virus.

g) Uncontrolled (i.e., requiring relevant changes in medication within the last month or hospital admission within the last three months) endocrine diseases (e.g., diabetes mellitus, hypo- or hyperthyroidism, adrenal disorder).

8. Any other major illness that, in the Investigator's judgment, could substantially increase the risk associated with the patient's participation in the study. The Investigator should feel free to consult the Study Coordinator or the Sponsor(s) for uncertainty in this regard.

9. Limitation of the patient's ability to comply with the treatment or to follow-up at a participating center. Patients enrolled into this trial had to be treated and followed at a participating center.

10. Treatment with any investigational product within 30 days prior to inclusion in the study.

11. Known hypersensitivity to any component of Zalypsis®.

Test product, dose and mode of administration	PM00104 was administered at a dose of 2 mg/m^2 as a 1-hour i.v. infusion on Day 1, 8 and 15 q4wk. Before PM00104 infusion, the patients received prophylactic treatment for emesis consisting of dexamethasone 8 mg i.v. and 5-HT3 antagonists (ondansetron 8 mg or equivalent), according to the American Society of Clinical Oncology (ASCO) guidelines for drugs with moderate emetic risk. If necessary, in addition to the above, 10 mg of metoclopramide orally every 8 hours could be administered, or the duration of treatment with 5-HT3 antagonists and/or dexamethasone could be extended. PM00104 was provided as a powder for concentrate for solution for infusion in only

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	one strength (2.5 mg/vial). The numbers of PM00104 batches were as follows: • **2.5-mg vial batches:** 00203 and 01015.	
Duration of treatment	Patients remained on treatment in the absence of confirmed or unacceptable toxicity that was not resolved after applying the appropriate dose delays, omissions or reductions.	
Criteria for evaluation **Efficacy** **Pharmacokinetics** **Pharmacogenomics** **Pharmacodynamics** **Safety**	 The primary efficacy endpoint was overall response rate (ORR), defined as the percentage of patients with confirmed objective response (OR), either complete response (CR) or partial response (PR) according to the RECIST v.1.1. Secondary endpoints of efficacy included duration of response (DR), progression-free survival (PFS) and overall survival (OS). PM00104 PK profile was assessed by non-compartmental methods. The study included hypothesis-generating exploratory PGx analyses to be conducted to correlate the molecular parameters found in the tumor and blood samples of the patients with the clinical results achieved with PM00104. Finally, these PGx analyses were not performed due to the lack of clinical benefit reported in this phase II clinical trial. A substudy to evaluate Ewing's sarcoma CTCs as a prognostic factor but also as a pharmacodynamic marker of response to PM00104 treatment was also planned. This analysis was eventually not performed due to the lack of clinical benefit reported in this phase II clinical trial. All patients who received at least one total or partial infusion of PM00104 were evaluable for safety.	
Statistical methodology	Continuous variables were tabulated and presented with summary statistics (i.e., mean, standard deviation, median and range). Categorical variables were summarized in frequency tables by means of counts and percentages. **Efficacy:** Binomial estimates with exact 95% confidence intervals were calculated for the analysis of the main endpoint (ORR). Time-to-event endpoints (DR, PFS and OS) were analyzed according to the Kaplan-Meier method. **Pharmacokinetics:** All individual PK parameters were tabulated and summarized for the evaluable PK population using count (n), arithmetic mean, median, maximum, minimum, standard deviation (SDev) and coefficient of variation (CV%). Linear regression of natural log-transformed plasma PK parameters predicted by natural log-transformed demographic and other log-transformed covariates (liver enzymes, creatine phosphokinase, bilirubin, creatinine and creatinine clearance, total proteins and hematological parameters) was carried out. Ninety-five percent confidence intervals (CIs) for the slope, and p-test with a level of significance of 0.05 were calculated. **Safety:** Safety was evaluated using clinical examinations, which comprised vital signs analysis, clinical assessment of AEs and serious adverse events (SAEs), changes in laboratory parameters (hematological and biochemical, including liver function tests), deaths, reason for study discontinuations, dose delays and reductions, and any other analyses that could be considered necessary to characterize the safety profile of PM00104 in advanced and/or metastatic EFT. Cardiac tests as troponin I, ECG and LVEF measurements were analyzed descriptively. All AEs were classified according to the NCI-CTCAE, v.4.0, and were coded using MedDRA, v.11.0.	
Results (1): Patient characteristics	Most patients (n=12; 70.6%) were males, their median age was 23 years (range, 15-53 years), and the majority (94.1%) had ECOG PS=0-1. The most common primary tumor type was osseous Ewing's sarcoma (n=13; 76.5%) and the most reported primary disease locations at diagnosis were lower extremity and trunk/abdominal wall (n=7; 41.2% each). Disease at study entry was metastatic in all patients. The median number of sites involved per patient was 2 (range, 1-4 sites). The most common disease locations were lung (n=13; 76.5%) and lymph nodes (n=4; 23.5%). Fourteen patients (82.4%) had previously received radiotherapy and undergone	

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	previous curative or palliative surgery. All 17 patients had previously received systemic anticancer therapy. Median number of lines of systemic anticancer therapy was four (range, 1-11 lines). The most common prior anticancer agents were nitrogen mustard analogues, vinca alkaloids and anthracyclines (n=17; 100.0% each).	
Results (2): Efficacy	The study protocol established that, in the first stage, at least 12 evaluable patients had to be recruited. Data from 16 treated patients were evaluable for efficacy analysis. No objective responses were observed. Four patients had stable disease as best response to PM00104 treatment. Median PFS was 1.8 months (95% CI, 0.9-3.5 months) and median OS was not reached (56.2% censored data). Therefore, in accordance to the study protocol, as the primary objective (clinically relevant antitumor response) was unmet during the first stage, a "no go decision" was taken and recruitment was closed without proceeding to the second stage.	
Results (3): Pharmacokinetics	Reliable PK profiles were available in 14 patients in the first infusion and in 11 patients in the second infusion. Mean PK results for PM00104 in the first infusion (C_{max}=21.23 µg/l and AUC=87.06 h·µg/l) were similar than those observed in the phase I clinical trial PM104-A-004-05 at 2.025 mg/m² in the first infusion (C_{max}=26.07 µg/l and AUC=88.44 h·µg/l). Additionally, the mean CL found in the current phase II trial (first infusion 57.35 l/h, second infusion 57.86 l/h) is in accordance with the CL (43.7 l/h) reported in the PM00104 population PK publication. Therefore, the PK behavior found here is similar to that reported in previous studies evaluating PM00104. No PK differences were reported between the first and second infusions. The inter- and intra- patient variability was between 20% and 30% for C_{max}, AUC and CL. There were no trends in C_{max} or AUC between infusions; therefore, PM00104 accumulation was not reported between the first two infusions. Regarding the effect of patient demographic characteristics on PM00104 PK, several relationships were detected among demographic characteristics and biochemical tests with respect to PK parameters. However, the population PK analysis conducted on data from four phase I clinical trials did not detect any relationship among any demographic characteristic. Then, the limited number of patients included in this study may detect relationships, which are not found when larger sample sizes are used. The effect of PM00104 exposure was only assessed along the first infusion on the hematological parameters. No relationship was found between exposure and hematological toxicity.	
Results (4): Safety	Sixteen of 17 patients included in this study were treated with PM00104 and were therefore evaluable for safety. The median number of cycles administered per patient was 2 (range, 1-6 cycles). Most PM00104-related AEs were grade 1/2; the most common were fatigue (n=6; 37.5%) and nausea (n=4; 25.0%). Only one case of fatigue reached grade 3 in one cycle. No deaths were reported as outcome of PM00104-related AEs. Seven patients died while on study, all of them due to progression of malignant disease. SAEs related to PM00104 treatment were not observed during this study. The most common hematological abnormalities regardless of relationship were lymphopenia and anemia, which were mainly grade 1/2. One case of neutropenia reached grade 4. Grade 3 clinically relevant hematological abnormalities included neutropenia (n=3; 18.8%) and thrombocytopenia (n=2; 12.5%). One case of grade 3 neutropenia and one case of grade 3 thrombocytopenia led to treatment discontinuation. Skipped doses were required in one patient due to grade 3 thrombocytopenia, and in other patient due to grade 3/4 neutropenia. Two cycle delays occurred in one patient due to grade 2 neutropenia, and in other patient due to grade 2 anemia. Biochemical abnormalities regardless of relationship were mostly grade 1/2. Only one case of creatinine increase reached grade 3. No effects on PM00104 treatment (discontinuations, dose reductions or dose delays) were caused by any of these abnormalities.	

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Conclusions	The primary endpoint of the study was unmet and, therefore, no further evaluation of PM00104 2 mg/m^2 1-hour i.v. infusion d1, 8 and 15 q4wk as treatment of patients with advanced and/or metastatic EFT who failed standard chemotherapy is planned. Pharmacokinetic results were very similar to those obtained in previous studies and in accordance with the population PK analysis previously published. This PM00104 schedule has an acceptable tolerability, with mostly mild to moderate, reversible and predictable adverse reactions.	
Date of report (final version)	15 May 2013	