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CLINICAL TRIAL PROTOCOL

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

INVESTIGATIONAL MEDICINAL PRODUCTS: PM00104 (Zalypsis®)
Protocol No.: PM104-B-003-10
EudraCT: 2010-022221-15
NCT Code: 01222767

FINAL version 1.0: 30 July 2010

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INVESTIGATIONAL MEDICINAL PRODUCT/STUDY DRUG: Zalypsis® (PM00104)

Protocol Code: PM104-B-003-10

EudraCT: 2010-022221-15

FINAL version 1.0: 30 July 2010

This study will be conducted in compliance with the protocol, GCP and applicable regulatory requirements.

Confidentiality Statement

STUDY CONTACTS	NAME AND ADDRESS	PHONE AND FAX NUMBER, AND E-MAIL ADDRESS
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PRINCIPAL INVESTIGATORS

A complete list of Investigators, including the Principal Investigators, will be available as a separate document.

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SYNOPSIS

TITLE	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.
PROTOCOL CODE	PM104-B-003-10
INVESTIGATORS / TRIAL LOCATION	A minimum of six centers in Europe and the USA are expected to participate. A full list of investigators will be available as a separate document.
STUDY OBJECTIVES **Primary**	• To determine the antitumor activity of Zalypsis® 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 time-to-event efficacy parameters. • To characterize the safety profile and tolerability of Zalypsis® in patients with unresectable advanced and/or metastatic EFT. • To characterize the pharmacokinetics (PK) of Zalypsis® when administered as a single-agent to patients with EFT. • To determine the pharmacodynamic profile by measuring the effect of Zalypsis® 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 will be conducted to correlate the molecular parameters found in the tumor and blood samples of the patients with the clinical results achieved with Zalypsis®.
STUDY DESIGN	Multicenter, open label, phase II clinical trial with single-agent Zalypsis® given as a 1-hour i.v. infusion on d1, d8 and d15, q4wk, to patients with advanced and/or metastatic EFT who failed to standard chemotherapy. The primary endpoint of the study is the overall response rate (ORR), defined as the percentage of patients with objective response (OR), either complete response (CR) or partial response (PR), as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) v. 1.1. The study will consist of two stages. If in the first stage there are no responders after testing the drug on 12 evaluable patients, the trial will be terminated. If the trial goes on to the second stage,

	17 additional evaluable patients will be accrued. A total of 29 evaluable patients will be studied. If the total number of responders is ≤ 2, the drug will be considered as not interesting in the setting of patients treated for this disease with this schedule. Treatment will be administered in the absence of disease progression and/or unacceptable toxicity. In case of obtaining a CR, two additional cycles will be administered and then the treatment will be stopped.
STUDY POPULATION	Patients with EFT progressing after standard treatment with systemic chemotherapy are eligible for this trial. To be included in the study, patients have to fulfill all inclusion criteria and none of the exclusion criteria.
INCLUSION CRITERIA	1. Voluntary written informed consent, obtained from the patient or his/her representative before the beginning of any specific study procedures. 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) score ≤ 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 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 will be designated as "non-target" lesions unless progression is 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 or normality (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 ≥ 25 g/l. 13. Left ventricular ejection fraction (LVEF) within normal limits (LVEF of at least 50%). 14. Women of childbearing potential must have a negative serum pregnancy test before study entry. Both women and men must agree to use a medically acceptable method of contraception throughout the treatment period and for three months after discontinuation of treatment. Acceptable methods of contraception include complete abstinence, intrauterine device (IUD), oral contraceptive, subdermal implant and double barrier (condom with a contraceptive sponge or contraceptive suppository).
Pharmacodynamic and pharmacogenomic substudy inclusion criteria	• All patients included in trial PM104-B-003-10 will be eligible. • Only those patients who have themselves or their representatives voluntarily signed the Informed Consent Form for the PGx study will participate.
EXCLUSION CRITERIA	1. Prior therapy with Zalypsis®. 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 spine, provided that acute effects of radiation treatment have resolved. Hormonal therapy and palliative radiation therapy (i.e., for control of pain from bone metastases) must be discontinued before study entry. 5. Patients with a prior invasive malignancy (except non-melanoma skin cancer and *in situ* cervix carcinoma) who have 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 (CNS) 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 are 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) Known hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. f) Immunocompromised patients, including those known to be infected with the human immunodeficiency virus (HIV). 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, will 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) in case of 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 must 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®.
Pharmacodynamic and pharmacogenomic substudy exclusion criteria	• Patients who do not consent to participate in this substudy (by themselves or through their representatives). Refusal to participate in the PGx substudy will not affect participation in the main trial PM104-B-003-10.
No. of patients	A total of 29 evaluable patients are expected to participate in this trial.

No. of sites	A minimum of six centers are expected to participate.
STUDY DRUG **Formulation**	Zalypsis® Zalypsis® is provided as a lyophilized powder for concentrate for solution for infusion in strength of 2.5 mg/vial.
Route of administration	Intravenous, as a 1-hour infusion by a central catheter.
Administered dose	Zalypsis® will be administered at a dose of 2 mg/m^2.
Treatment schedule	A treatment cycle consists of the drug administration on Day 1, 8 and 15 (study evaluations should be completed during each cycle prior to subsequent Zalypsis® infusion). Treatment cycles will be repeated every four weeks.
Prophylactic medication	Antiemetic prophylaxis: patients will receive prophylactic treatment for emesis for adult patients consisting of dexamethasone 8 mg i.v. and 5-HT3 antagonists (ondansetron 8 mg i.v., or granisetron 1 mg i.v., or tropisetron 5 mg i.v.) before the infusion of Zalypsis®, according to the American Society for Clinical Oncology (ASCO) guidelines for drugs with moderate emetic risk. If necessary, one or both of the following may also apply: • Adding 10 mg of metoclopramide orally every eight hours. • Extending the duration of treatment with 5-HT3 antagonists and/or dexamethasone. Secondary prophylaxis with colony-stimulating factors such as granulocyte or granulocyte-macrophage colony-stimulating factors (G-CSF or GM-CSF), or with erythropoietin is allowed according to the ASCO guidelines.
Criteria for continuation of treatment and for re-treatment	In order to be re-treated on Day 8 and Day 15 of the first cycle and on subsequent infusions, the patients will have to fulfill the following criteria: a) Platelet count ≥ 75 x 10^9/l, hemoglobin ≥ 9 g/dl and ANC ≥ 1.0 x 10^9/l. b) AP ≤ 2.5 x ULN (≤ 5 x ULN in case of extensive bone involvement). c) ALT, AST ≤ 3 x ULN (≤ 5 x ULN in case of hepatic metastases). d) Renal function: patients with calculated creatinine clearance (using Cockcroft and Gault's formula) ≥ 30 ml/min. e) Total bilirubin ≤ 1.5 x upper limit or normality (ULN), unless due to Gilbert's syndrome. f) Albumin ≥ 2.5 g/dl.

<table>
<tr><td></td><td>g) Other non-hematological toxicities grade ≤ 1 (grade 2 in case of asthenia).
If these criteria are not met on Day 8, 15 or on subsequent infusions, study drug administration should be skipped and criteria reevaluated weekly. Treatment administration will be resumed upon recovery of these parameters, according to these same criteria.
If a patient has to skip two doses (at Day 8 and Day 15) or has a delay > 2 weeks from the theorical day of re-treatment, the patient should discontinue from treatment. In the event of obvious clinical benefit, the patient will remain on treatment only after having discussed and agreed upon the case with the Sponsor(s), and upon recovery of all parameters according to the aforementioned criteria.</td></tr>
<tr><td>Dose reduction</td><td>Zalypsis® dose reductions will take place based on the worst treatment-related toxicity found since the last dose administration. The criteria for dose reduction are:
• Grade 4 neutropenia that lasts > 5 days.
• Febrile neutropenia.
• Grade 4 thrombocytopenia.
• Grade ≥ 3 transaminase increase that lasts > 7 days.
• Any other grade 3-4 adverse non-hematologic toxicity except nausea, vomiting and diarrhea (unless grade 3 or 4 nausea, vomiting and diarrhea persists despite use of adequate medication).
If the patient requires dose reduction, the new Zalypsis® dose level will be one of those described in the table below:
<table><tr><th>Dose level</th><th>Zalypsis® (mg/m^2)</th></tr><tr><td>1</td><td>2.0</td></tr><tr><td>-1</td><td>1.6</td></tr><tr><td>-2</td><td>1.3</td></tr></table>
No more than two dose reductions per patient (from 2 mg/m^2 to 1.6 mg/m^2 and then to 1.3 mg/m^2) will be allowed during the study. Patients requiring more than two dose reductions should discontinue the treatment, except in the event of obvious clinical benefit, in which case the patient could be allowed to remain on treatment after having discussed and agreed upon the case with the Sponsor(s).
No dose escalations will be allowed in this study.</td></tr>
<tr><td>EFFICACY EVALUATIONS</td><td>To be evaluable for efficacy:
• patients must have received at least four of the six infusions in the first two cycles (e.g., two infusions in each cycle, or three infusions in Cycle 1 and one infusion in Cycle 2), AND</td></tr>
</table>

	• patients must have at least one disease measurement recorded not less than six weeks after treatment onset. In addition, any eligible patient who receives at least two of the three infusions in one treatment cycle and experience disease progression or dies due to progressive disease (PD) prior to response evaluation will be considered evaluable for the main endpoint (ORR) and will be categorized as an "early progression". Patients withdrawn due to toxicity without any tumor assessment after the start of study treatment will be considered as "treatment failures" and will not be replaced. Patients withdrawn due to significant clinical deterioration of unknown reason, or due to hypersensitivity reactions or unrelated AEs, and patients who refuse to continue on study for any reason without any tumor assessment after the start of study treatment will be considered not evaluable for efficacy and will be replaced. Assessment of efficacy will be done using the RECIST v. 1.1 and will be essentially based on a set of measurable lesions identified at baseline as target lesions and followed until disease progression. A disease evaluation will be performed at baseline and every other cycle (± 1 week) until evidence of PD. The same procedure will be used to evaluate each identified lesion both at baseline and throughout the treatment period. In case of detection of an OR, either a CR or a PR, a confirmation assessment has to be performed after a minimum of four weeks from the first documentation of the response. Additionally, detection of the presence of CTCs by retrotranscriptase polymerase chain reaction (RT-PCR) analysis of EWS-FLI1 and EWS-ERG translocations in the blood of patients will be performed.
SAFETY EVALUATIONS	Patients will be evaluable for safety if they have received at least one total or partial infusion of Zalypsis®. Safety will be evaluated using clinical examinations, which will comprise vital signs analysis, clinical assessment of AEs, changes in laboratory parameters (hematological and biochemical, including liver and cardiac function tests) and any other analyses that may be considered necessary. All AEs will be classified according to the NCI-CTCAE v.4.0 and will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) v. 11.0.
PHARMACOKINETIC EVALUATIONS	The PK of plasma Zalypsis® will be evaluated during the first two infusions of the first cycle with a limited sampling schedule of eight samples. PK parameters will be calculated using population methods, after pooling data from this study with data obtained during phase I studies.

The sampling schedule during each infusion will be as follows:

Sample number	Optimal time point	Adequate time frame
1	Pre SOI	Pre SOI
2	5 min before EOI	5-1 min before EOI
3	30 min after EOI	30 min-1 h after EOI
4	2 h after EOI	1.5-3 h after EOI
5	6 h after EOI	6-8 h after EOI
6	24 h after EOI	20-28 h after EOI
7	48 h after EOI	40-72 h after EOI
8*	168 h after EOI	120-168 h after EOI

*Sample number 8 has to be taken BEFORE the start of the next infusion.
SOI, start of infusion; EOI, end of infusion.

PHARMACODYNAMIC AND PHARMACOGENOMIC SUBSTUDY EVALUATIONS

The aim of this investigation is to identify and validate molecular markers whose expression may be associated with the clinical outcome of patients treated with Zalypsis®. These molecular markers might allow the identification of those patients who will benefit from the treatment with Zalypsis®, thus improving health care by an individualized medicine.

The following analyses will be done on tumor and blood samples from consenting patients treated with Zalypsis®:

- Quantitation of mRNA expression in paraffin-embedded tumor tissue by quantitative real-time retrotranscriptase polymerase chain reaction (qRT-PCR) of genes identified during *in vitro* studies as potential biomarkers of response to Zalypsis®. These genes will be selected from PDGFRa and PARP and other genes related to the mechanism of action (MoA) of Zalypsis®.
- Quantitation of protein expression by immunohistochemistry in tissue microarrays constructed from the patient's paraffin-embedded tumor tissue blocks. The proteins to be determined include PDGFRa and PARP, and other proteins related to the MoA of Zalypsis®.
- Fluorescence *in situ* hybridization (FISH) and retrotranscriptase polymerase chain reaction (RT-PCR) analysis of EWS-FLI1 and EWS-ERG translocations and fusion protein variants in paraffin-embedded tissue.
- Detection of the presence of CTCs by retrotranscriptase polymerase chain reaction (RT-PCR) analysis of EWS-FLI1 and EWS-ERG translocations in the blood of patients.

STATISTICAL METHODS

Primary Endpoint:

- Overall response rate (ORR), defined as the percentage of patients with confirmed OR, either CR or PR, according to the RECIST v 1.1.

Secondary Endpoints:

- Duration of response (DOR), defined as the time between the date when the response criteria (PR or CR, whichever is first reached) are fulfilled and the first date when disease progression, recurrence or death is objectively documented

	(taking the smallest measurements documented since the treatment started as reference for PD). • Progression-free survival (PFS), defined as the time from the first day of study treatment to the day of negative assessment (progression or death) or last tumor evaluation. PFS at 3 and PFS at 6 months are defined as the Kaplan-Meier estimates of PFS at these time points. • Overall survival (OS), defined as the time from the first day of treatment to the date of death (or the last day when the patient is known to be alive). Survival will be followed every three months until death, or until the date of study termination, whichever occurs first. OS at 12 months is defined as the Kaplan-Meier estimates of OS at this timepoint. • Safety profile. • Pharmacokinetic profile. • Pharmacodynamic profile • Pharmacogenomic profile. **Sample Size Considerations:** The optimum two-stage design to test the null hypothesis that the ORR is $\leq$ 3% versus the alternative that ORR $\geq$ 20% was selected. After testing the drug on 12 patients in the first stage, the trial will be terminated if there is not any responder. If the trial goes on to the second stage, a total of 29 patients will be studied, 12 from the first stage and 17 from the second stage. If the total number of responding patients is $\leq$ 2, the drug will be considered not interesting in the setting of patients treated with this disease and with this schedule. This design has an expected sample size of 17.20 and a probability of early termination of 0.69. If the drug is actually not effective, there is a 0.05 probability of concluding that it is (type I error). If the drug is actually effective, there is a 0.1 probability of concluding that it is not (type II error). **Methods of Analysis:** Binomial estimates with exact 95% confidence intervals will be calculated for the analysis of the main endpoint (ORR). Time-to-event endpoints (DOR, PFS and OS, PFS rates at 3, 6 months and OS rates at 12 months) will be analyzed according to the Kaplan-Meier method. Baseline characteristics, AEs, serious adverse events (SAEs), laboratory evaluations, deaths and the reason for study discontinuations will be analyzed. Continuous variables will be tabulated and presented with summary statistics (i.e., mean, standard deviation, median and range). Categorical variables will be summarized in frequency tables by means of counts and percentages.
DURATION OF STUDY PERIOD (per patient)	Patients will be evaluated at scheduled visits in up to three study periods:

	• **Pre-treatment:** from signature of informed consent to the first infusion of Zalypsis®.
	• **Treatment:** from the first infusion of Zalypsis® to the last administration of the study drug plus 30 days, or onset of subsequent therapy, or death, whichever occurs first. An end-of-treatment visit will be performed within 30 days after last dose administration.
	• **Follow-up:** all patients will be followed after the end of treatment:
	  • Patients who discontinue treatment without progression will be followed every three months until disease progression, other antitumor therapy, or death, or until the date of study termination, whichever occurs first.
	  • After disease progression or after other antitumor therapy, patients will be followed every three months until death, or until the date of study termination, whichever occurs first.
	Study termination (clinical cutoff) will vary depending on the development of the trial. Planned study termination will occur:
	▪ Depending on the development/extension of the trial:
	  o Three months after the last treatment visit of the last evaluable patient if the study ends at the first stage.
	  o Six months after the last treatment visit of the last evaluable patient, if the study proceeds to the second stage.
	▪ Or 12 months after the last patient included in the study, if neither of the above have occurred.
	Patients will be considered to be **on-study** from the signature of the informed consent to the end of follow-up period. Patients will be considered to be **on-treatment** for the duration of their treatment and in the first 30 days following the date of the last study drug administration, or until the onset of subsequent therapy or death, whichever occurs first. **End of treatment** is defined as the day of the last study drug dose administration plus 30 days, or the onset date of subsequent therapy or the date of death, whichever occurs first.
	Patients will receive Zalypsis® while it is considered to be in their best interest. Specifically, treatment will continue until:
	• Disease progression.
	• Unacceptable toxicity (including any toxicity leading to the need for a third dose reduction or severe hypersensitivity reactions, except in the event of obvious clinical benefit, in which case the patient could be allowed to remain on treatment after having discussed and agreed on the case with the Sponsor(s)).

	• Patient or patient's representative refusal and/or non compliance with study requirements. • Intercurrent serious illness. • Protocol deviation with an effect on the risk/benefit ratio of the clinical trial. • Treatment delay > 2 weeks or impossibility to administer both Day 8 and Day 15 doses on a given cycle (except in case of clear clinical benefit, with the Sponsor(s)'s approval). • Requirement of > 2 dose reductions (except in case of clear clinical benefit, with the Sponsor(s)'s approval). • Administrative reasons or Sponsor(s)'s decision. Any subsequent therapies for the patients may be provided off-study according to the Investigator's criteria.
REPLACEMENT OF PATIENTS	Patients must be replaced if they are considered not evaluable for efficacy, i.e., if they are withdrawn from the study due to significant clinical deterioration of unknown reason, hypersensitivity reactions, patient refusal or unrelated AEs without any tumor assessments after the start of study treatment.
PLANNED TRIAL PERIODS	The total duration of the study will be approximately 36 months, including about 24 months of active enrollment. Consenting patients will be followed until death, or until the date of study termination, whichever occurs first. • Planned start date (first patient on study): 1Q11. • Planned enrollment period: 24 months. • Planned study termination will occur: ▪ Depending on the development/extension of the trial: o Three months after the last treatment visit of the last evaluable patient if the study ends at the first stage. o Six months after the last treatment visit of the last evaluable patient, if the study proceeds to the second stage. ▪ Or 12 months after the last patient included in the study, if neither of the above have occurred.

SCHEDULE OF ASSESSMENTS AND PROCEDURES

	PRE-TREATMENT	**TREATMENT (Cycle 1 and further cycles)**						**FOLLOW-UP** (1)
Study day		**D1**	**D8**	**D15**	**D22**	**D28 (=D1 of next cycle)**	**End of treatment (last dose + 30 days)**	**Every 3 months**
Study visit window	**-**	Within 2 days of pre-specified date						±1 week
Written informed consent	Before any study procedure	-	-	-	-	-	-	-
Medical history	D–28 to D1	-	-	-	-	-	-	-
Complete physical examination (2)	D–28 to D1	•	Repeat if clinically indicated				•	•
ECOG-PS(2)	D–28 to D1	•	Repeat if clinically indicated				•	•
Vital signs (2, 3)	D–28 to D1	•	•	•	-	•	•	•
Weight, height and BSA	D–28 to D1	•	Repeat if clinically indicated			•	Repeat if clinically indicated	
Hematology (4)	D–7 to D1	•	•	•	-	•	•	•
Biochemistry A (4, 5)	D–7 to D1	•	•	•	-	•	•	•
Biochemistry B (6)	D–7 to D1	•	Repeat if clinically indicated			•	•	•
Calculated creatinine clearance (6)	D–7 to D1	•	Repeat if clinically indicated			•	Repeat if clinically indicated	
Pregnancy test (7)	D–14 to D1	Every 4 weeks during treatment and 3 months after last dose administration						
ECG (5, 8)	D–28 to D1	Before each Zalypsis® infusion					•	•
LVEF (ECHO) (5, 9)	D–28 to D1	Every other cycle					•	•
Concomitant diseases and treatments	D–28 to D1	Throughout the "on-treatment" period						•
Adverse events	NA	Throughout the "on-treatment" period						•
Tumor assessment (10)	D–28 to D1	Every other cycle (± 1 week) until PD					•	•
Pharmacokinetics (11)	D1	•	•	-	-	-	-	-
Pharmacodynamic and pharmacogenomic samples, if patient consented (12, 13)	D–28 to D1	•	-	-	-	•	-	-
Other tests	D–28 to D1	When indicated, according to the clinical and laboratory context					•	•

See notes on next page →

SCHEDULE OF ASSESSMENTS AND PROCEDURES **(CONTINUED)**

On-treatment period = from first infusion of Zalypsis® to End of treatment.

End of treatment = 30 days after the day of last dose of study drug administration, unless the patient starts a new antitumor therapy or dies (whichever occurs first), in which case the date of administration of this new therapy or the date of death will be considered the date of end of treatment.

1. Patients withdrawn with Zalypsis®-related ongoing adverse events (AEs) must be followed up with the appropriate tests until event resolution.
2. Complete physical examination, ECOG PS, vital signs, and weight must be repeated on Day 1 of Cycle 1, prior to administration of the first Zalypsis® infusion.
3. Before any Zalypsis® infusion.
4. Repeat on Day 1 of Cycle 1 prior to treatment with Zalypsis®, if the treatment is administered more than one week after the pre-treatment tests. In subsequent administrations, repeat (with a −2 day window) before Zalypsis® infusion. If laboratory abnormalities grade ≥ 3 occur (according to the National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] v. 4.0), the appropriate test(s) must be done at least every 2-3 days until recovery recovery to grade ≤ 1 or baseline. In case of febrile neutropenia (any grade) absolute neutrophil count (ANC) determination must be repeated every day until resolution.
5. Follow-up of cardiac assessments (including ECG and LVEF) should be performed at three and six months after end of treatment. All troponin I determinations, ECGs and LVEFs will be reviewed by external cardiologists.
6. Repeat on Day 1 of Cycle 1 whenever clinically indicated. If laboratory abnormalities grade ≥ 3 occur (according to the NCI-CTCAE v. 4.0), the appropriate test(s) must be done at least every 2-3 days until recovery to grade ≤ 1 or baseline.
7. Pregnancy test, if applicable. Repeat on Day 1 of Cycle 1.
8. Electrocardiogram (ECG) must be repeated prior to the administration of each Zalypsis® infusion and if clinically indicated.
9. LVEF by ECHO to be performed every other cycle (with a 1-week window) and if clinically indicated.
10. Computed tomography (CT)-scan or magnetic resonance imaging (MRI) of all evaluable sites of disease, as per Response Evaluation Criteria In Solid Tumors (RECIST) v. 1.1, within four weeks prior to the first dose of Zalypsis®. Documentation of progressive disease (PD) is mandatory at study entry.
11. First two infusions in Cycle 1.
12. Circulating tumor cells (CTCs) will be collected on Day 1 before each Zalypsis® infusion for the first six cycles, and every three cycles thereafter.
13. Paraffin-embedded tumor tissue (obtained at diagnosis of the tumor) can be collected any time after registration.

Hematology: differential WBC (neutrophils, lymphocytes), hemoglobin and platelets.

Biochemistry A: liver function tests (ALT, AP, AST, LDH, total bilirubin), creatinine, CPK, troponin I, glucose, serum electrolytes (Na^+, Cl^-, K^+, Ca^{++} and Mg^{++}).

Biochemistry B: total protein and albumin.

AE, adverse event; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AP, alkaline phosphatase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase-fraction MB; CT, computed tomography; CTCs, circulating tumor cells; ECG, electrocardiogram; ECHO, echocardiogram; ECOG, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NA, not applicable; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; PD, progressive disease; PK, pharmacokinetics; RECIST, Response Evaluation Criteria In Solid Tumors; WBC, white blood cells.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE(s)	Adverse Event(s)
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
ASCO	American Society of Clinical Oncology
ASCT	Autologous Stem Cell Transplantation
AST	Aspartate Aminotransferase
βhCGs	Beta Human Chorionic Gonadotrophins
BSA	Body Surface Area
C_{max}	Maximum Plasma Concentration
CNS	Central Nervous System
CPK	Creatine Phosphokinase
CR	Complete Response
CRF	Case Report Form
CT	Computerized Tomography
CTC(s)	Circulating Tumor Cell(s)
D	Day
DSB(s)	Double Strand Break(s)
DNA	Deoxyribonucleic Acid
DOR	Duration of Response
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EFT	Ewing's Family of Tumors
EGFR	Epidermal Growth Factor Receptor
EI-CESS	European Intergroup Cooperative Ewing Sarcoma Studies
EOE	Extraosseous Ewing's Sarcoma
EOI	End of Infusion
FISH	Fluorescence *In Situ* Hybridization
G-CSF	Granulocyte Colony Stimulating Factor
GCP	Good Clinical Practice
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
GMT	Greenwich Meridian Time
h	Hour(s)
Hb	Hemoglobin
hERG	Human ERG Potassium Channel
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HRR	Homologous Recombination Repair
IB	Investigator's Brochure
IC_{50}	Half-maximal Inhibitory Concentration
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IUD	Intrauterine Contraceptive Device
i.v.	Intravenous(ly)
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
MedDRA	Medical Dictionary for Regulatory Activities
MoA	Mechanism of Action
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTD	Maximum Tolerated Dose

NA	Non Available/Non Applicable
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NER	Nucleotide Excision Repair
NYHA	New York Heart Association
OR	Objective Response
ORR	Overall Response Rate
OS	Overall Survival
RT-PCR	Retrotranscriptase Polymerase Chain Reaction
PD	Progressive Disease
PEN	Polyethylene Naphthalate
PFS	Progression-Free Survival
PGx	Pharmacogenomic(s)
PK	Pharmacokinetics
PM00104	Zalypsis®
PNET	Primitive Neuroectodermal Tumor
PR	Partial Response
PS	Performance Status
PTEN	Phosphatase and Tensin Homolog
q3wk	Every Three Weeks
q4wk	Every Four Weeks
q.d.	Every Day
qRT-PCR	quantitative Real-Time Retrotranscriptase Polymerase Chain Reaction
RD	Recommended Dose
RECIST	Response Evaluation Criteria In Solid Tumors
RNA	Ribonucleic Acid
RT	Radiotherapy
RT-PCR	Retrotranscriptase-Polymerase Chain Reaction
SAE(s)	Serious Adverse Event(s)
SD	Stable Disease
SOI	Start of Infusion
TTP	Time to Progression
ULN	Upper Limit of Normality
U.S./USA	United States/United States of America
WBC	White Blood Cells
WMA	World Medical Association

1. INTRODUCTION

1.1. EWING'S FAMILY OF TUMORS (EFT)

The Ewing's family of tumors, which include Ewing's sarcoma, primitive neuroectodermal tumor (PNET), Askin's tumor of the chest wall and extraosseous Ewing's sarcoma (EOE), are a group of tumors derived from the same primordial bone marrow-derived mesenchymal stem cell and have in almost all instances a clonal translocation in the long arms of chromosomes 11 and 22 (1).

The median age of patients with EFT is 15 years, and more than 50% are adolescents. EFTs are rare in adults older than 30 years. Based on data from 1426 patients entered into the European Intergroup Cooperative Ewing Sarcoma Studies (EI-CESS), 59% of patients were male and 41% female. Primary sites included lower limbs (41%), pelvis (26%), chest wall (16%), upper limbs (9%), spine (6%), and skull (2%). For EOE, the most common sites are trunk (32%), limbs (26%), head and neck (18%), retroperitoneum (16%) and other sites (9%) (2).

The prognosis of EFT varies depending on primary tumor site, presence of metastases and tumor size. The overall 5-year disease-free survival rate for localized Ewing's sarcoma treated with surgery, radiation and multi-agent chemotherapy is 65-76% (3). However, the 5-year disease-free survival rate drops to 30% if metastases are present at diagnosis. Limited improvement of survival rates has been achieved for metastatic Ewing's sarcoma. Similarly, relapsed patients have a poor outcome, with very common failure to second-line therapy and low 5-year survival rates (13%) (4).

1.1.1. Therapeutic approach for EFT

A multidisciplinary approach, including surgery, chemotherapy, and radiotherapy (RT) is mandatory. Multi-agent chemotherapy is essential for Ewing's sarcoma due to the high risk of micrometastatic disease. Multi-drug chemotherapy for EFT always includes vincristine, doxorubicin, ifosfamide, and etoposide. Most protocols use cyclophosphamide as well and certain protocols incorporate dactinomycin. Duration of primary chemotherapy ranges from six months to one year (5).

In high-risk cases of Ewing's sarcoma, myeloablative chemotherapy followed by autologous stem cell transplant (ASCT) has been used as consolidation therapy. Although better survival than expected was reported with this approach, large prospective trials are needed to properly evaluate the potential utility of this therapy in Ewing's sarcoma patients (6).

The management of relapsed or recurrent disease is not standardized and, in most cases, consists of different combinations of the same agents used as adjuvant therapy. Patients are often treated with more than one regimen to reduce disease burden to the minimum. Ifosfamide and etoposide are active in treating Ewing's tumor of bone and should be considered for patients who have not previously received these agents. Combinations such as cyclophosphamide plus topotecan and irinotecan plus temozolomide have also been active in patients with recurrent or refractory disease. Radiotherapy of lung and bone, and surgical removal of metastases are also included in the management of these patients. However, patients with relapsed disease have a poor outcome; failure to second-line therapy is very common, with 5-year survival rates around 13% (4). In addition, the toxicity, morbi-mortality, and long-term complications of these agents

(cardiac, renal, pulmonary, gonadal and secondary neoplasias) are considerable. Therefore, there is an urgent need for new therapeutic agents with different mechanisms of action to manage this patient population.

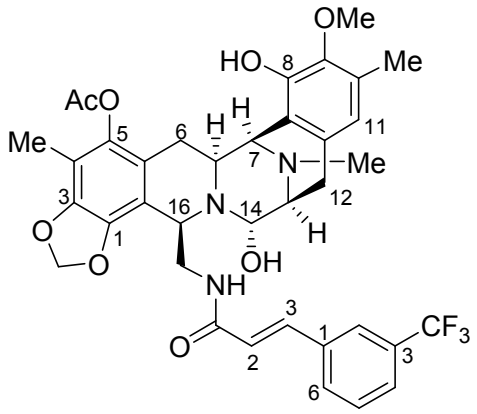
1.2. ZALYPSIS® (PM00104)

PM00104 (Zalypsis®) is a new synthetic alkaloid that was selected for clinical development as an antineoplastic agent because of its broad *in vitro* cytotoxic activity against human solid and non-solid tumor cell lines, its *in vivo* activity in human tumor xenografts, as well as an acceptable non-clinical toxicology profile. The main bases for the preclinical and clinical development of PM00104 are shown here, although complete details and references can be found in the Investigator's Brochure (IB), provided in a separate folder.

1.2.1. Chemical Structure and Formulation

PM00104 (Figure 1) is currently produced by chemical synthesis.

Figure 1. Chemical structure of PM00104.



Molecular formula: $C_{37}H_{38}F_3N_3O_8$.; Molecular weight: 709.708.

1.2.2. Mechanism of Action

Preliminary data on mechanism of action (MoA) suggest that PM00104 exerts effects on the cell cycle, and displays deoxyribonucleic acid (DNA)-binding properties as well as transcriptional inhibition (7).

Structure-activity relationship studies have led to the notion that the binding of PM00104 to DNA is a critical event in its cytotoxic action, but that an additional non-DNA target may be required to elicit an optimal antitumor response.

PM00104 displays strong inhibition of the transcriptional response, with a similar pattern at all the concentrations studied. In addition, PM00104 strongly inhibits the activation of the transcription of other genes such as MDR1, a gene critically involved in resistance to many chemotherapeutic agents, without affecting constitutive transcription. More details on MoA can be found in Section 1.2.6.1.

1.2.3. Preclinical Data

Zalypsis® has demonstrated antiproliferative *in vitro* activity against a broad spectrum of human solid and non-solid tumor types (Table 1). Regarding solid tumors, Zalypsis® exhibited *in vitro* antitumor activity (i.e., $IC_{50} \leq 10^{-8}$ M) against representative cell lines of bladder, colon, gastric, kidney, melanoma, pancreas, prostate, sarcoma, and thyroid; similar activities were noted against leukemia and lymphoma as representatives of non-solid tumors.

Zalypsis® also demonstrated *in vitro* antitumor activity, although to a lesser extent (i.e., IC_{50} ranging from 10^{-7} to 10^{-6} M), in representative cell lines of breast, lung, sarcoma (SK-LMS-1 and SW-684), and lymphoma (U937). *In vitro* activity (i.e., 10^{-6} to 10^{-5} M) was not seen, however, in a representative ovarian cell line (SK-OV-3).

Table 1. *In vitro* activity of Zalypsis®

Type	Tumor	Cell line	IC_{50} (M)
Solid	**Bladder**	5637	$8.6 \cdot 10^{-09} – 1.1 \cdot 10^{-09}$
	Breast	BT-474	$6.2 \cdot 10^{-07} – 8.2 \cdot 10^{-07}$
		MX-1	$4.7 \cdot 10^{-06} – 8.5 \cdot 10^{-06}$
	Colon	HT-29	$1.1 \cdot 10^{-08} – 7.7 \cdot 10^{-07}$
	Gastric	Hs746T	$2.1 \cdot 10^{-08} – 5.5 \cdot 10^{-11}$
	Kidney	768-O	$1.0 \cdot 10^{-08} – 2.1 \cdot 10^{-09}$
	Liver	SK-HEP-1	$2.2 \cdot 10^{-07} – 2.3 \cdot 10^{-07}$
	Lung	A459	$3.0 \cdot 10^{-06} – 4.8 \cdot 10^{-06}$
	Melanoma	SK-MEL-28	$1.7 \cdot 10^{-08} – 2.0 \cdot 10^{-09}$
	Ovarian	SK-OV-3	$1.7 \cdot 10^{-06} – 1.5 \cdot 10^{-05}$
	Pancreas	PANC-1	$7.9 \cdot 10^{-08} – 2.3 \cdot 10^{-09}$
	Prostate	DU-145	$2.7 \cdot 10^{-06} – 4.2 \cdot 10^{-06}$
		LNCaP	$7.5 \cdot 10^{-08} – 1.1 \cdot 10^{-08}$
		PC-3	$4.2 \cdot 10^{-08} – 6.8 \cdot 10^{-07}$
	Sarcoma	A-673	$1.6 \cdot 10^{-12} – 2.0 \cdot 10^{-12}$
		CHSA	$1.8 \cdot 10^{-08} – 1.0 \cdot 10^{-09}$
		OSA-FH	$1.5 \cdot 10^{-08} – 4.1 \cdot 10^{-07}$
		SK-LMS-1	$8.7 \cdot 10^{-07} – 2.2 \cdot 10^{-06}$
		SW-684	$2.7 \cdot 10^{-06} – 3.7 \cdot 10^{-06}$
	Thyroid	SW-579	$1.1 \cdot 10^{-08} – 2.3 \cdot 10^{-09}$
Non-solid	**Leukemia**	HL-60	$5.4 \cdot 10^{-08} – 8.6 \cdot 10^{-08}$
		K-562	$9.4 \cdot 10^{-09} – 1.3 \cdot 10^{-08}$
	Lymphoma	H9	$3.7 \cdot 10^{-11} – 2.0 \cdot 10^{-9}$
		MC116	$3.9 \cdot 10^{-08} – 2.5 \cdot 10^{-08}$
		U937	$1.4 \cdot 10^{-07} – 2.1 \cdot 10^{-07}$

Source: Investigator's Brochure.

In vivo tumor profiling of Zalypsis® was obtained by using a panel of six human tumor types, i.e., breast, colon, gastric, ovarian, prostate, and renal. The resulting tumor susceptibility was analyzed in xenografts grown in athymic mice when Zalypsis® was administered as single bolus intravenous (i.v.) injections (data from the IB).

Table 2**.** Antitumor activity of Zalypsis® in human athymic mouse xenografts

Tumor type, cell line	Route and Schedule	**Dose levels mg/kg (mg/m^2)**	**Antitumor Effect (Optimal Day)**
Breast, MX-1	i.v., qdx1	0.75 (2.25)	-64% ΔT/ΔC (Day 9)*
Colon, HT-29	i.v., qdx1	0.75 (2.25)	47% ΔT/ΔC (Day 7)§
Kidney, MRI-H-121	i.v., qdx1	0.75 (2.25)	7% ΔT/ΔC (Day 6)*
Ovary, SK-OV-3	i.v., qdx1	0.75 (2.25)	60% ΔT/ΔC (Day 6)
Prostate, PC-3	i.v., qdx1	0.75 (2.25)	2% ΔT/ΔC (Day 7)*
Stomach, MRI-H-254	i.v., qdx1	0.75 (2.25)	14% ΔT/ΔC (Day 15)*

* $p < 0.05$, statistically significant compared to control cohort
§ $p < 0.06$, trend to significance
ΔT/ΔC = % ratio of treated versus control tumor volumes; i.v., intravenous, qd, every day.

Zalypsis® demonstrated statistically significant antitumor activity against breast, gastric, and renal malignancies (Table 2). In colon tumors, only low significance was observed after Zalypsis® administration as a single i.v. bolus for the sample size tested (i.e., n = 5). With regard to gender-specific malignancies, Zalypsis® significantly inhibited prostate tumors, but was inactive against ovarian tumors. In summary, Zalypsis® demonstrated strong antitumor activity in breast, gastric, prostate, and renal, but had a more moderate antitumorigenic profile against colon.

The antitumor effectiveness of Zalypsis® was further analyzed in xenografts using the susceptible human breast tumor MX-1 after determination of single-day (i.e., qdx1) maximum tolerated dose (MTD) levels in mice, i.e., 0.75 mg/kg. Specifically, decreasing total doses were administered on a qdx1 i.v. bolus schedule (i.e., 1.0, 0.75, and 0.5 MTD_{qdx1}, respectively, each on Day 0), while increasing total levels were tested using a five-consecutive-day i.v. administration regimen (i.e., 1.0, 1.25, and 1.67 MTD_{qdx1}, respectively, each on Days 0-4). Interestingly, the qdx5 i.v. schedule appeared to have an optimal antitumor effect for Zalypsis® (data from the IB).

Zalypsis®, given by i.v. injection, produced toxicological effects typical of cytotoxic antitumor agents. Tissues containing cells with a high turnover rate were especially targeted in rats and dogs, i.e., bone marrow, reticuloendothelial system, and gastrointestinal tract, as well as the liver, the reproductive system and lesions in the injection site. Most toxicities were reversible or in repair at the end of an acute toxicity evaluation. Toxicological effects were more severe when the compound was given as a multicycle 24-hour infusion in rats and dogs. In the fractionated dose studies performed so far, Zalypsis® was well tolerated when administered to rats and dogs at levels based on dividing the MTD, calculated after single administration by a factor of five or three, for five consecutive days or three consecutive weeks, respectively. Zalypsis® displayed

less liver toxicity when administered using fractionated daily doses compared to single bolus administration. In rats receiving Zalypsis® at MTD levels (single administration), increases in liver function markers as well as histology findings related to liver injury were seen. However, these findings were not detected in those animals which received an equivalent total dose but in a fractionated five consecutive daily schedule of Zalypsis®. Regardless of the dosing schedule, toxicity related to hematopoietic and thymus systems were observed.

With respect to the safety pharmacology of Zalypsis®, none of the observations regarding the alterations triggered by Zalypsis® on either neurotoxicity, cardiovascular or respiratory function, raised any concern. For multicycle studies, Zalypsis® was well tolerated in rats and dogs at the MTD values when administered as a bolus. However, the administration of two (on Days 1 and 15) 24-hour infusions of Zalypsis® in rats resulted in deaths occurring at the higher dose levels studied. Histology evaluation revealed hemorrhage, necrosis and mineralization of the heart in many of the early dead animals, accompanied by degeneration in the animals treated at the highest doses. Zalypsis® appears to be more toxic after multiple 24-hour administration compared to either single 24-hour or single/multiple bolus administration in rats and dogs.

In the human ERG potassium channel (hERG) assay, after a 5-min exposure of HEK293 cells stably transfected with hERG complementary DNA (cDNA) to a concentration of 15 μM of Zalypsis®, inhibition of the hERG tail current was complete. Increasing concentrations of Zalypsis® (0.01, 0.03, 0.1, 0.3 and 3 μM) produced changes which were fitted into a sigmoidal function, with a calculated IC_{50} of 0.4 μM (0.28 μg/ml), well above the C_{max} found in animals.

As a measure of precaution and to ensure the safety of the patients, closer cardiac monitoring was performed during the phase I clinical development.

1.2.4. Metabolism

In the early phases of drug development, several *in vitro* studies were carried out to assess the biotransformation pathways of Zalypsis® in humans and other animals. These studies indicated that Zalypsis® underwent extensive hepatic microsomal-mediated metabolism in all animal species, including man. Measurement of Zalypsis® in urine samples was not possible due to the instability of this drug in this medium (8).

1.2.5. Clinical Data

The clinical development program includes four phase I clinical trials aimed to assess five different schedules of administration in patients with solid malignancies or lymphoma for which no standard therapy would reasonably be expected to result in cure or palliation.

One hundred and forty four patients have been included in phase I trials with Zalypsis® as of December 2009. Prophylactic antiemetic therapy with dexamethasone plus ondansetron or similar agents before the infusions was mandatory after the emetic events and dose-limiting toxicities (DLTs) were observed in the clinical trial PM104-A-001-04 (Table 3). A central catheter is mandatory since cases of infusion site reactions were also reported.

With respect to hematological events, decrease in blood cell counts have been observed (expected according to preclinical toxicology results), especially at the highest dose levels. In most cases, these hematological events were mild to moderate, transient and

not associated with clinical manifestations, such as fever, infection or hemorrhages.

With respect to biochemical events, severe increases in liver transaminases were rare and no grade 4 cases were reported. The laboratory abnormalities usually recovered within few days and no concomitant symptoms were reported.

As previously mentioned, closer cardiac monitoring was included in the clinical phase I program as a measure of precaution due to some findings in preclinical studies. Cardiac-related disorders found in these phase I studies have consisted of elevations of cardiac troponin I without other symptoms or relevant electrocardiogram (ECG)/echocardiogram (ECHO) alterations. Four patients treated in the study PM104-A-004-05, which evaluated the same schedule than that to be used in the current phase II trial (i.e., 1-hour Day 1, 8 and 15 every four weeks [q4wk]) had grade 4 troponin I increase at the highest dose levels: 2.0 mg/m^2 (n=2), 2.5 mg/m^2 (n=1) and 3.0 mg/m^2 (n=1). None of them had cardiac AEs, abnormal ECG or decrease in left ventricular ejection fraction (LVEF) at the same time than troponin I increase, except one patient treated with 3.0 mg/m2 who had grade 1 sinus tachycardia and a slight shortening of the QT interval. These events were considered "not relevant" by the Investigators and by an external cardiologist who evaluated these cases. ECGs performed as per study protocols before and after each infusion of Zalypsis® have been reviewed by an external cardiologist and no evidence of cardiotoxicity has been reported. However, close clinical and investigational monitoring of cardiac safety will be performed in the current study with Zalypsis®.

No toxic deaths have occurred in the phase I studies with Zalypsis®.

The recommended dose (RD) for phase II studies, 2 mg/m^2, to be used in the current clinical trial (PM104-B-003-10) was determined by the study PM104-A-004-05 which used the same schedule (d1, d8 and d15; q4wk). A summary of the results of this finalized study can be found in Table 3.

Table 3. Summary of the phase I study PM104-A-004-05

Dose escalation cohorts		Patients included /patients with DLTs (n=49)	DLTs observed per patient
Dose level	**Dose (mg/m^2)**		
I	**0.07**	1 / 0	.
II	**0.15**	1 / 0	.
III	**0.3**	1 / 0	.
IV	**0.6**	4 / 0*	.
V	**0.9**	3 / 0	.
VI	**1.35**	3 / 0	.
VII	**2.0 (RD)**	24 / 1	• Grade 4 lipase increase and grade 3 diarrhea NOS
VIII	**3.0 (HDR)**	4 / 2	• Grade 4 fatigue • Grade 3 fatigue, grade 4 neutropenia lasting > 5 days, grade 4 thrombocytopenia and grade 3 cardiac troponin I increase**
IX	**2.5 (MTD)**	8 / 4	• Grade 1 thrombocytopenia and grade 2 neutropenia with two dose omissions • Grade 4 febrile neutropenia • Grade 4 cardiac troponin I increase** • Grade 3 fatigue

The shadowed row corresponds to data from the RD.

*At dose level IV, due to grade 3 transaminases (ALT and AST) increase in patient #104 (early disease progression) and drug-related grade 2 neutropenia in patient #107, in the subsequent dose levels cohorts were expanded to at least three patients and dose escalation was reduced to 50%.

**Two episodes of grade 3/4 cardiac troponin I increase occurred (patients #220 and #128), both were considered by the investigators as DLT despite not meeting all criteria established for DLT definition in the protocol (i.e., they were not associated with any evidence of cardiac damage by ECG or ECHO).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; DLT, dose-limiting toxicity; ECG, electrocardiogram; ECHO, echocardiography; HDR, highest dose reached; MTD, maximum tolerated dose; NOS, not otherwise specified; RD, recommended dose.

1.2.5.1. Clinical Trial PM104-A-004-05

Forty nine patients (29 males and 20 females) with malignant solid tumors have been enrolled and treated at the participating institutions (Institut Gustave Roussy, Paris, France, and Newcastle General Hospital-Northern Centre for Cancer, Newcastle, United Kingdom). The median age of the patients was 58 years (range, 22-76 years).

Table 3 summarizes the dose escalation, the patients treated at each cohort and the DLTs observed. The patients were distributed in nine dose levels. The starting dose was 0.07 mg/m^2. According to an accelerated design with escalating doses of Zalypsis®, one patient per dose was treated and dose was escalated by 100% from dose level I (0.07 mg/m^2) to dose level III (0.3 mg/m^2). The first patient treated at dose level IV (0.6 mg/m^2) had non-drug related grade 3 ALT and AST increase due to early disease progression. For safety reasons, this dose level was expanded to four patients. Another patient at this dose level had grade 2 drug-related neutropenia in the first treatment cycle. Due to the aforementioned events (nevertheless, not evaluated as DLTs), the

study continued with expanded cohorts of at least three patients and with dose escalation by 50% in dose level V (0.9 mg/m^2) and afterwards.

No DLTs were observed from dose level I (0.07 mg/m^2) to dose level VI (1.35 mg/m^2). After four consecutive dose increases by 50%, the first two DLTs were reported in dose level VIII (3.0 mg/m^2, the highest dose reached). Upon agreement with the investigators, an intermediate dose between this and the next lowest level (dose level VII; 2.0 mg/m^2) was proposed, and subsequent patients were enrolled and treated at a dose of 2.5 mg/m^2 (dose level IX). Four of eight patients treated with this dose had DLTs; therefore, 2.5 mg/m^2 was declared to be the MTD and 2.0 mg/m^2 (one DLT in 24 treated patients) was defined as the RD.

The tumor types included in this trial were gastrointestinal: colorectal adenocarcinoma (n=14), gastric carcinoma (n=5), esophagus carcinoma (n=4), pancreas adenocarcinoma (n=3), cholangiocarcinoma (n=2) and anal carcinoma (n=1); genitourinary: ovarian adenocarcinoma (n=2), prostate adenocarcinoma (n=2) and bladder carcinoma, cervix carcinoma and endometrial adenocarcinoma (n=1 each); respiratory: pleural mesothelioma (n=3) and non-small cell lung cancer (NSCL) (n=2); and others: malignant melanoma (n=4), soft tissue sarcoma (n=3) and lachrymal adenoid carcinoma (n=1).

At the recommended dose (RD) (2.0 mg/m^2), the total number of cycles administered was 24, with a median number of cycles administered per patient of 2 (range, 1-5 cycles). The median cumulative dose was 2.9 mg/m^2 (range, 0.7-8.0 mg/m^2), the median dose intensity was 1.2 mg/m^2 (range, 0.15-2.6 mg/m^2) and the median relative dose intensity was 77.9% (range, 33.2-103.2%).

1.2.5.1.1. Safety Data

The most common treatment-related AEs that occurred at the RD were nausea (87.5% of patients/77.4% of cycles), vomiting (83.3% of patients/62.3% of cycles) and fatigue (75.0% of patients/62.3% of cycles).

Five patients treated at the RD had six drug-related serious adverse events: grade 3 nausea and grade 3 neutropenic infection (both occurring in one patient); grade 3 diarrhea, grade 3 tumor flare, grade 3 tumor pain, and grade 1 pyrexia (one patient each).

No cardiac events or troponin increases were observed at the recommended dose.

The most frequent hematological abnormality at the RD was anemia, which was observed in all patients, although in most cases of mild degree. Nine patients (37.5%) worsened their hemoglobin values to grade 2 and only one patient (4.2%) reached grade 3 during one cycle. Other severe hematological toxicities at the RD consisted of grade 3 thrombocytopenia in one patient (4.2%) during one cycle, grade 3 leukopenia (8.3%) and grade 3/4 neutropenia (8.3%). These hematological abnormalities were reversible (lasted 1-7 days) and non-cumulative.

The most common severe non-hematological laboratory disorder at the RD was grade 3 transaminase increase (ALT, 4.2%; AST, 8.3%), followed by grade 3 AP increase (8.3%) and grade 3 total bilirubin increase (4.2%). No cases of grade 4 biochemical abnormalities were found at the RD. All cases of severe non-hematological toxicities found at this dose level were reversible and asymptomatic.

1.2.5.1.2. Efficacy Data

Four disease stabilizations lasting longer than 3 months were observed at different dose levels in patients with cervix carcinoma, colorectal adenocarcinoma, lachrymal adenoid carcinoma and bladder carcinoma (Table 4).

Table 4. PM104-A-004-05. Stable disease lasting ≥ 3 months.

Tumor type	Gender	Age (years)	Zalypsis® dose level (mg/m^2)	No. of prior regimens	Agent/s of last prior therapy	Best response to prior treatment / TTP (months)	No. of Zalypsis® cycles received	TTP with Zalypsis® (months)
Cervix carcinoma	Female	51	**II** (0.15)	1	Cisplatin	NA/NA	4	3.4
Colorectal adenocarcinoma	Male	57	**V** (0.9)	6	Erbitux/irinotecan	SD/5.9	5	5.1
Lachrymal adenoid carcinoma	Male	70	**VI** (1.35)	2	Imanitib	SD/1.8	8	9.5
Bladder carcinoma	Male	45	**IX (MTD)** (2.5)	2	5-fluorouracil/ cisplatin/epirubicin	SD/14.0	5	6.5

NA, not available; MTD, maximum tolerated dose; TTP, time to progression.

In addition, a phase II clinical trial (PM104-B-001-09) has been started to evaluate the antitumor activity of Zalypsis® administered with the same dose and schedule (1-h d 1, 8 and 15 q4wk) to that which will be used in the current study, in patients with advanced and/or metastatic endometrial or cervical cancer previously treated with one line of systemic chemotherapy. As of 31 May 2010, 16 patients have been recruited in this study.

1.2.5.2. Pharmacokinetic Data

The main pharmacokinetic (PK) characteristics of Zalypsis® are: prolonged half life (up to 50 hours), wide distribution (around 800 l), high interpatient variability and linear PK, except for doses higher than 3000 µg/m² infused in 1 hour.

1.2.6. Pharmacogenomic Data

For the past 30 years medical oncologists have focused on optimizing the outcome of cancer patients, developing new antitumor agents and defining new prognostic factors as well as integrating more effective supportive care measures. However, clinical anticancer strategies indicate that conceptually active therapies benefit just a small proportion of patients. As a consequence, a large cohort of patients needs to be exposed to antitumor treatments to obtain benefit in just a fraction of them.

Molecular targeted therapies and personalized cancer therapies are concepts that are raising expectations in anticancer drug development as a consequence of the genomics

technology developed and our improved understanding of cancer at the molecular level.

Pharmacogenomic (PGx) studies are aimed at identifying prognostic biomarkers that can help to define subpopulations of patients who will benefit from a particular therapy. These molecular markers of response to the drugs are not exclusive of the so-called "targeted therapies", but have also been identified in widely used cytotoxic agents. Representative examples include the relation between thymilydate synthase mRNA expression and response and survival with antifolates (9), beta tubulin III mRNA levels and response to tubulin interacting agents (10), Phosphatase and Tensin Homolog (PTEN) methylation and resistance to irinotecan (11), and STAT3 overexpression and resistance to epidermal growth factor receptor (EGFR) interacting agents (12).

For the last five years, the Sponsor(s) have implemented a translational research program oriented to the identification of molecular markers correlated with the response to the anticancer agents in development. Based on the experimental studies that indicate that there is an increased sensitivity to trabectedin in tumors with efficient nucleotide excision repair (NER) and deficient homologous recombination repair (HRR) DNA repair machinery, the expression of a set of DNA repair genes was analyzed in a retrospective cohort of sarcoma patient tumor samples (13). The main result obtained was the identification of the levels of expression of the gene BRCA1 as a marker that correlates with longer progression-free survival (PFS) and overall survival (OS) after the treatment with trabectedin (14). This fact, which nowadays has been validated in samples from a larger cohort of patients, opens the possibility of identifying those patients that would benefit from the treatment of trabectedin in a prospective fashion.

These very promising results are the bases for a more exhaustive research for molecular markers of tumor response to treatment that will in the future allow a real personalized antitumoral medicine.

1.2.6.1. Rationale for the Pharmacogenomic Substudy Associated to PM104-B-003-10

Zalypsis® is a novel synthetic antineoplastic agent currently in Phase II clinical development. It has strong antitumor activity in a wide variety of tumor lines *in vitro* and *in vivo*. Preliminary *in vitro* data suggest that Zalypsis® has DNA binding properties, induces cell cycle arrest and inhibits transcription. Although the precise mechanism of action of Zalypsis® has not been fully elucidated, there are increasing experimental data of Zalypsis® antitumor activity. Three main molecular characteristics describe the antitumor activity of Zalypsis®:

1. Zalypsis® is a DNA binder: Zalypsis® binds to the minor groove of DNA. This binding occurs in preferred trinucleotide sequences GC rich, preferably to GCG trinucleotide (from C. Bailey, unpublished PharmaMar internal report). The binding of Zalypsis® to the DNA produces a stabilization of the DNA duplex, with notable increase (13–18ºC) in the melting temperatures of DNA oligonucleotides containing either single or tandemly arranged binding sites (15). This stabilization could account for the need of the same DNA repair machinery that usually deals with inter-strand cross-links and involves proteins from both HRR and NER machineries (16).
2. Zalypsis® produces DNA double strand breaks (15): In cell lines, the Zalypsis®-DNA adducts provoked DNA double-strand breaks (DSBs), evidenced by an increase in phospho-γ-H2AX and phospho-CHK2. In addition, treatment of cells

with Zalypsis® led to cell cycle delay in S phase, activation of the DNA damage checkpoint and apoptotic cell death. More interestingly, Zalypsis® has shown a potent anti-myeloma action on cell lines and patient plasma cells, as well as on xenografted myeloma cells in mice (7). The action of Zalypsis® in plasma cells involved cell cycle blockade and apoptosis. The latter occurred by both caspase-dependent and independent routes. Zalypsis® provoked DNA DSBs, which were evidenced by an increase in phospho histone H2AX, causing up-regulation of p53 in myeloma cell lines bearing wild type forms of this protein. This increase in p53 was followed by augmented levels in p53-regulated proteins such p21, Noxa or Bak. Also, *Schizosaccharomyces pombe* containing a RAD51 mutation were found to be extremely sensitive to Zalypsis®, suggesting that the compound induces DSBs.

3. Zalypsis® interferes with DNA repair: Experimental data reveal that the DNA damage repair machinery is essential to overcome Zalypsis® induced DNA damage and suggest that this damage is mainly due to DSBs. It has been described that the antiproliferative activity of Zalypsis® does not depend on transcription-coupled NER. Zalypsis® induces phosphorilated histone γ-H2AX foci with the same efficiency in NER-deficient or NER-proficient cells. Moreover, the formation of γ-H2AX foci is replication dependent for Zalypsis®, effects suggestive of stalled transcription and replication forks (17). In addition, the dependency of the cytotoxic effects of the drug on an intact NER system have been studied on cells deficient in RAD13, which is the *Schizosaccharomyces pombe* counterpart of human XPG, a NER protein that has been shown to mediate, at least in part, the MoA of other agents in this class in human cells (18). RAD13 haploid deletion mutants were found to be as sensitive to Zalypsis® as wild-type cells indicating the independence of the cytotoxic effect of this compound to the lack of a functional nucleotide excision repair system. This evidence is in contrast with the pattern noted with other antitumors with DNA binding activity as trabectedin and cisplatin that are highly dependent on the activity of this repair pathway. The same study determined the sensitivity to Zalypsis® in a collection of 5000 haploid deletion mutants of the yeast *Saccharomyces cerevisiae* as a model. We have identified approximately 40 hypersensitive mutants and about 90 mutants resistant to Zalypsis® activity. Among the genes which deletion produced sensitivity to Zalypsis® we found a set of genes involved in sensing/repairing DSB, such as components of the MRX complex, several members of the homologous recombination proteins of the Rad52 epistasis group (recruited to the sites of DNA damage specifically during S-phase and G2), the Swi/Snf complex and a chromatin remodeling complex conserved in humans. Swi/Snf inactivation results in inefficient DSB repair and increased DNA damage sensitivity as well as a large defect in γ-H2AX phosphorylation strain with a deletion in SUMO ligase, Siz1 was one of the most resistant mutants to Zalypsis®. A role for this protein in the inhibition of homologous recombination at replication forks has been described recently, indicating again that the main lesions induced by Zalypsis® are DSBs.

4. Zalypsis® interferes with transcription: PM0104 inhibits the *in vitro* transcription of RNA both at the initiation and at the elongation phases (J.M. Egly personal communication). The inhibition of transcription induced by Zalypsis® is 10-fold more potent that induced by trabectedin. Studies conducted in a panel of sarcoma cell lines have identified a very significant correlation associating the expression of PDGFR-α and phosphorylation of c-kit with the *in vitro* sensitivity to Zalypsis®

(19). This association has been confirmed in a larger panel of cell lines of epithelial origin outside from the sarcoma indication, both *in vitro* and *in vivo* mice xenografts. Although it is not well understood how these data fit into the MoA of Zalypsis®, this strong correlation deserves a clinical confirmatory study.

Based on the available experimental data, it seems reasonable to develop studies to analyze the correlations between the tumor/patient and genes/proteins determinants in the efficiency/deficiency of the biological pathways shown above and the outcome of patients exposed to Zalypsis®. The ultimate goal has been the identification of molecular biomarkers present on patients that shall be prone to respond to Zalypsis® in order to implement a customized therapy in the future.

1.3. RATIONALE FOR THIS PHASE II EXPLORATORY TRIAL OF ZALYPSIS® IN EFT

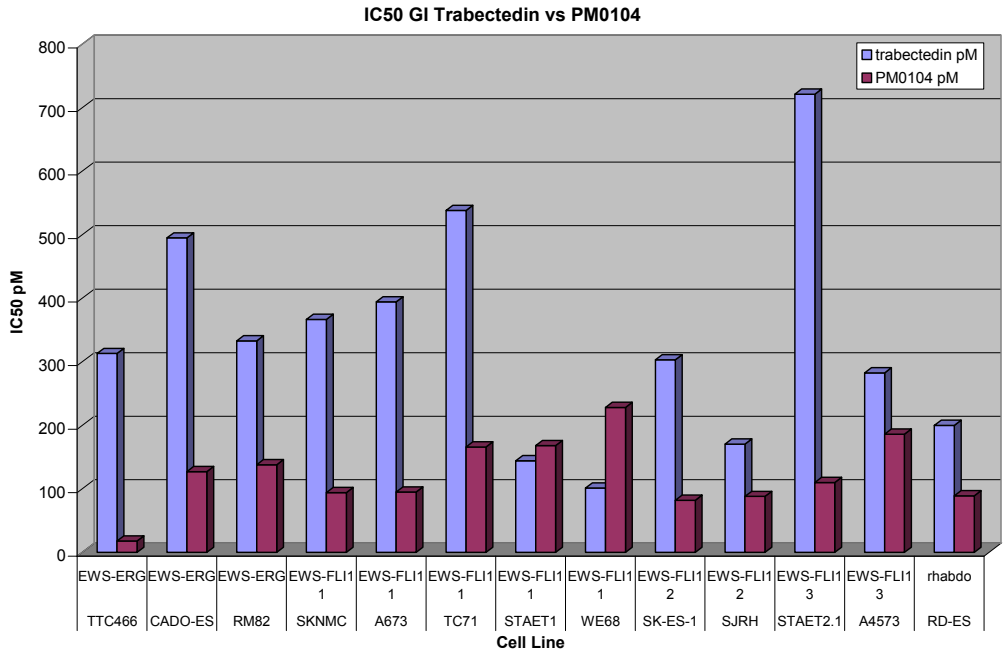
Ewing's sarcoma is an aggressive neoplasm of the bone and soft tissues that arises primarily in adolescence and young adulthood. It is characterized by the occurrence of non-random gene rearrangements between the EWS gene on chromosome 22q12 with various members of the ETS gene family (20, 21). Although the introduction of chemotherapy has significantly improved the chance of survival of non-metastatic patients, shifting the 5-year survival rate to around 50–60% (22-24), in the last two decades the survival rate of Ewing's sarcoma patients has reached a plateau phase (25, 26). Particularly, limited improvement of survival rates has been achieved for metastatic and relapsed Ewing's sarcoma, with 5-year survival rates dropping to 13% in the latter; therefore, relapsed Ewing´s sarcoma represents an unmet medical need.

There is evidence that sarcomas associated to translocations which deregulate transcription factors have high response rates to treatment with DNA binders such as trabectedin or Zalypsis® compounds.

- Trabectedin has shown higher efficacy in the treatment of sarcomas having specific translocations producing deregulated transcription factors including FUS-CHOP myxoid/round cell liposarcoma (27-29) and Ewing's sarcoma (27, 30-32) although the specific mechanisms of action are still under investigation.
- Interestingly, they share TET-family genes (EWS or FUS) as gene fusion components. The fusion proteins contain a DNA binding domain and a transcriptional activation domain under the control of a constitutively expressed promoter that makes the fusion protein a deregulated transcription factor.
- Trabectedin has been described to impede the binding of FUS-CHOP fusion protein to some promoters regulated by the chimerical protein, interfering with the expression of downstream proteins (33).
- Both Zalypsis® and trabectedin have been claimed to interfere with the transcriptional activity of EWS-FLI1, downregulating the expression of some EWS-FLI1 regulated genes such as NR0B1 (DAX1) (34).
- Zalypsis® is a DNA binder that binds to DNA at the minor groove with a stronger effect in transcription regulation than in DNA repair as compared to trabectedin (7).
- Zalypsis® shows an increased sensitivity compared to that of trabectedin in a panel of Ewing's sarcoma cell lines characterized by chromosomal translocations producing deregulated transcription factors (35). The effects of Zalypsis® and trabectedin on growth inhibition are shown in Figure 2.

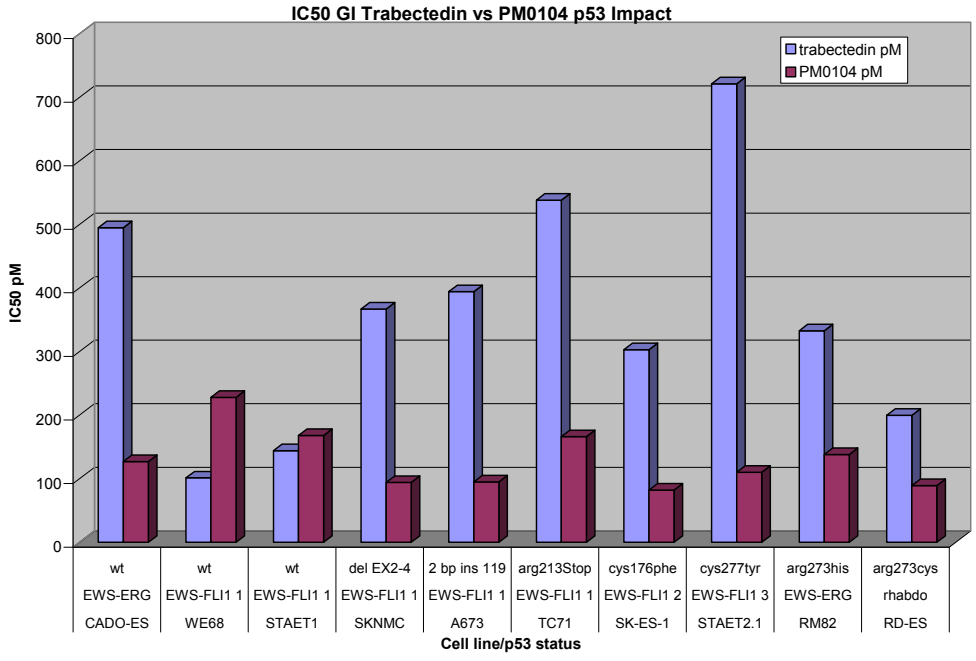
- Zalypsis® increased sensitivity in the Ewing's sarcoma cell line panel is independent of the translocation/chimerical transcription factor variants. Wild-type p53 cell lines are more sensitive to trabectedin than to Zalypsis® (35) (see Figure 3).

Figure 2. Effects of Zalypsis® and trabectedin on growth inhibition.



Increased sensitivity in terms of growth inhibition IC_{50} to Zalypsis® compared to trabectedin in a panel of Ewing's sarcoma cell lines containing specific chromosomal translocations.

Figure 3. Effects of Zalypsis® and trabectedin on p53 impact



Increased sensitivity in terms of p53 IC_{50} to Zalypsis® compared to trabectedin in a panel of Ewing's sarcoma cell lines containing specific chromosomal translocations.

2. STUDY OBJECTIVES

2.1. PRIMARY OBJECTIVE

• To determine the antitumor activity of Zalypsis® administered as a 1-h i.v. infusion on d1, d8 and d15, q4wk, to patients with advanced and/or metastatic EFT.

2.2. SECONDARY OBJECTIVES

- To determine time-to-event efficacy parameters.
- To characterize the safety profile and tolerability of Zalypsis® in patients with unresectable advanced and/or metastatic EFT.
- To characterize the PK of Zalypsis® when administered as a single-agent to patients with EFT.
- To determine the pharmacodynamic profile by measuring the effect of Zalypsis® on the number of Ewing's sarcoma CTCs at different times of treatment and its correlation with the clinical outcome.
- To determine the PGx profile. Hypothesis-generating exploratory PGx analyses will be conducted to correlate the molecular parameters found in the tumor and blood samples of the patients with the clinical results achieved with Zalypsis®.

3. OVERALL STUDY DESIGN

This is a multicenter, open-label, single-arm, non-comparative, phase II clinical trial with single-agent Zalypsis® given as a 1-h i.v. infusion on d1, d8 and d15, q4wk, to patients with advanced and/or metastatic EFT who failed to standard chemotherapy.

The primary endpoint of the study is the overall response rate (ORR), defined as the percentage of patients with objective response (OR), either complete response (CR) or partial response (PR), as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

The study will consist of two stages. If in the first stage there are no responders after testing the drug on 12 evaluable patients, the trial will be terminated. If there are ≥ 1 responders the trial will go on to the second stage and 17 additional evaluable patients will be accrued. A total of 29 evaluable patients will be studied. If the total number of responders is ≤ 2, the drug will be considered as not interesting in the setting of patients treated for this disease with this schedule.

Treatment will be administered in the absence of disease progression and/or unacceptable toxicity. In case of obtaining a CR, two additional cycles will be administered and then the treatment will be stopped.

A disease evaluation will be performed at baseline and every other cycle (±1 week) until evidence of disease progression.

Patients will be evaluated using clinical and laboratory assessments before and after each treatment cycle. Any treatment-related AEs will be followed-up until the events or their sequelae resolve or stabilize at a level acceptable to the Investigator and the Sponsor(s). Patients no longer receiving study treatment will be followed up for survival (see Section 5.2).

4. SELECTION OF PATIENTS

4.1. INCLUSION CRITERIA

In order to be included into the trial, patients have to fulfill all of the following criteria:

1. Voluntary written informed consent, obtained from the patient or his/her representative before the beginning of any specific study procedures.
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) score ≤ 2.
7. Life expectancy ≥ 3 months.
8. Complete recovery from the effects of drug-related AEs derived from previous treatments, excluding alopecia and grade 1 peripheral neuropathy, according to the NCI-CTCAE v. 4.0.
9. At least one measurable lesion ("target lesion" according to the 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 will be designated as "non-target" lesions unless progression is 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 or normality (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 ≥ 25 g/l.
13. Left ventricular ejection fraction (LVEF) within normal limits (LVEF of at least 50%).
14. Women of childbearing potential must have a negative serum pregnancy test before study entry. Both women and men must agree to use a medically acceptable method of contraception throughout the treatment period and for three months after discontinuation of treatment. Acceptable methods of contraception include complete abstinence, intrauterine device (IUD), oral contraceptive, subdermal implant and double barrier (condom with a contraceptive sponge or contraceptive suppository).

4.2. EXCLUSION CRITERIA

Patients fulfilling any of the following criteria will not be included into the trial:

1. Prior therapy with Zalypsis®.
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 spine, provided that acute effects of radiation treatment have resolved (Appendix 2). Hormonal therapy and palliative radiation therapy (i.e., for control of pain from bone metastases) must be discontinued before study entry.
5. Patients with a prior invasive malignancy (except non-melanoma skin cancer and *in situ* cervix carcinoma) who have 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 (CNS) 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 ECG, i.e., patients with the following are 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) Known hepatitis B virus (HBV) or hepatitis C virus (HCV) infection.
   f) Immunocompromised patients, including those known to be infected with the human immunodeficiency virus (HIV).
   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, will 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 must 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®.

4.3. PATIENTS FOR THE PHARMACOGENOMIC SUBSTUDY

4.3.1. Inclusion Criteria

1. All patients included in trial PM104-B-003-10 will be eligible.
2. Only those patients who have themselves or their representatives voluntarily signed the Informed Consent Form for the PGx study will participate.

4.3.2. Exclusion Criteria

1. Patients who do not consent to participate in this substudy (by themselves or through their representatives). Refusal to participate in the PGx substudy will not affect participation in the main trial PM104-B-003-10.

5. PLAN OF THE STUDY

5.1. DURATION OF STUDY (WHOLE POPULATION)

The total duration of the study will be approximately 36 months, including about 24 months of active enrollment.

Consenting patients will be followed until death, or until the date of study termination, whichever occurs first.

- Planned start date (first patient on study): 1Q11.
- Planned enrollment period: 24 months.
- Planned study termination will occur:
  - Depending on the development/extension of the trial:
    - Three months after the last treatment visit of the last evaluable patient if the study ends at the first stage.
    - Six months after the last treatment visit of the last evaluable patient, if the study proceeds to the second stage.
  - Or 12 months after the last patient included in the study, if neither of the above have occurred.

5.2. DURATION OF STUDY AND TREATMENT (PER PATIENT)

Patients will receive study treatment as long as it is considered to be in their own benefit (see Section 7.2.3). Patients will be evaluated at scheduled visits in up to three study periods:

- **Pre-treatment:** from the signature of informed consent to the first infusion of Zalypsis®.
- **Treatment:** from the first infusion of Zalypsis® to the last administration of study drug plus 30 days, or onset of subsequent therapy or death, whichever occurs first. An end-of-treatment visit will be performed within 30 days after last dose administration.
- **Follow-up:** all patients will be followed up after the end of treatment:
  - Patients who discontinue treatment without progression will be followed every three months until disease progression, other antitumor therapy or death or until the date of study termination, whichever occurs first.
  - After disease progression or after other antitumor therapy, patients will be followed every three months until death, or until the date of study termination, whichever occurs first.

Patients may withdraw their consent at any time; no further study activities will be conducted on them.

Patients will be considered to be **on-study** from the signature of the informed consent to the end of follow-up period. Patients will be considered to be **on-treatment** for the duration of their treatment and in the first 30 days following the date of the last study drug administration, or until the onset of subsequent therapy or death, whichever occurs first. **End of treatment** is defined as the day of the last study drug dose administration plus 30 days, or onset date of subsequent therapy or the date of death, whichever occurs first.

5.2.1. Discontinuations

5.2.1.1. Treatment Discontinuation

The end of treatment occurs when an enrolled patient ceases to receive the study medication, regardless of the circumstances. The primary reason for any discontinuation will be recorded on the patient's Case Report Form (CRF). By convention, the date of end of treatment will be the day of the last Zalypsis® administration plus 30 days, or the date of the onset of subsequent therapy or the date of death, whichever occurs first.

If a patient discontinues treatment, every effort should be made to complete the scheduled assessments.

5.2.1.2. Reasons for Treatment Discontinuation

Administration of the study treatment should be discontinued if this is considered to be in the best interest of the patient. More specifically, treatment will be discontinued due to any of the following reasons:

- Disease progression.
- Unacceptable toxicity (including any toxicity leading to the need for a third dose reduction or severe hypersensitivity reactions, except in the event of obvious clinical benefit, in which case the patient could be allowed to remain on treatment after having discussed and agreed on the case with the Sponsor(s)).
- Patient's representative refusal and/or non compliance with study requirements.

- Intercurrent serious illness.
- Protocol deviation with an effect on the risk/benefit ratio of the clinical trial.
- Treatment delay > 2 weeks or impossibility to administer both Day 8 and Day 15 doses on a given cycle (except in case of clear clinical benefit, with the Sponsor(s)'s approval).
- Requirement of > 2 dose reductions (except in case of clear clinical benefit, with the Sponsor(s)'s approval).
- Administrative reasons or Sponsor(s)'s decision.

Regardless of the reason, patients who discontinue the treatment must not be re-treated at any time.

Any subsequent therapies for the patients may be provided off-study according to the Investigator's criteria.

5.2.1.3. Study Discontinuation

Study discontinuation occurs when an enrolled patient ceases to participate in the study, regardless of the reason. Patients have the right to withdraw consent at any time; if this is the case, no further follow-up should be performed.

The date and reason for study discontinuation will be clearly documented on the patient's CRF.

5.2.2. Protocol Deviations

A protocol deviation is defined as any departure from what is described in the protocol of a clinical trial approved by an Independent Ethics Committee/Institutional Review Board (IEC/IRB) and Competent Authorities. Therefore, this applies to deviations related to patient inclusion and clinical procedures (e.g., assessments to be conducted or parameters to be determined), and also to other procedures described in the protocol that concern the Good Clinical Practice (GCP) guidelines or ethical issues (e.g., issues related to obtaining the patients' Informed Consent, data reporting, the responsibilities of the Investigator, etc.).

Deviations with no effects on the risk/benefit ratio of the clinical trial (such as minimal delays in assessments or visits) will be distinguished from those that might have an effect on this risk/benefit ratio, such as:

- Deviations that might affect the clinical trial objectives, such as those involving the inclusion/exclusion criteria (which could mean that the patient is not eligible for the trial) and those having an effect on patient evaluability.
- Deviations that might affect the patient's well-being and/or safety, such as an incorrect dosing of the investigational medicinal product (IMP, this is Zalypsis®) due to not following dose adjustment specifications or an incorrect preparation of the medication.
- Deviations related to the following of GCP guidelines as described in the protocol and regulations in force, such as deviations when obtaining the Informed Consent or not following the terms established for reporting serious adverse events, etc.

As a general rule, NO deviations that may have an effect on the risk/benefit ratio of the clinical trial will be authorized. All protocol deviations considered particularly relevant according to regulations, which are related to ethical issues, fulfillment of GCP

guidelines and trial procedures, will be notified to the pertinent IEC/IRB and, if pertinent, to the competent authorities as established by local regulations.

5.3. REPLACEMENT OF PATIENTS

Patients must be replaced if they are considered not evaluable for efficacy, i.e., if they are withdrawn from the study due to significant clinical deterioration of unknown reason, hypersensitivity reactions, patient refusal or unrelated AEs without any tumor assessments after the start of study treatment.

5.4. STUDY TERMINATION (CLINICAL CUTOFF)

Study termination will occur:

- Depending on the development/extension of the trial:
    - Three months after the last treatment visit of the last evaluable patient if the study ends during the first stage.
    - Six months after the last treatment visit of the last evaluable patient, if the study proceeds to the second stage.
- Or 12 months after the last patient included in the study, if neither of the above have occurred.

5.5. PRE-TREATMENT EVALUATION

During the pre-treatment period, and once the patient or his/her representative has signed the Informed Consent Form, the Investigator will confirm the patient's eligibility for the study by conducting the following assessments (Table 5).

Additional information on the collection and processing of pharmacodynamic and PGx samples will be provided as a separate document in the "Guide for Identification, Packaging and Shipment of Pharmacodynamic and Pharmacogenomic Samples".

Table 5. Pre-treatment assessments

	PRE-TREATMENT ASSESSMENT	TIME
1. History and clinical examination	◆ Written informed consent.	Prior to any specific study procedures.
	◆ Medical history: o Date of diagnosis of the primary disease. o Demographic information (race/ethnicity, age). o Prior treatments (surgery, radiotherapy, chemotherapy, immunotherapy), specifying the best response and the date of PD. ◆ Concomitant diseases and treatments. ◆ Weight, height and BSA. ◆ Complete physical examination. ◆ Vital signs: heart rate, blood pressure and body temperature. ◆ ECOG PS (Appendix 1).	All within four weeks prior to Day 1 of Cycle 1. Complete physical examination, ECOG PS, vital signs, and weight must be repeated on Day 1 of Cycle 1, prior to administration of the first Zalypsis® infusion.
	◆ ECG* ◆LVEF* by ECHO.	Within four weeks prior to Day 1 of Cycle 1; ECG must be repeated on Day 1 of Cycle 1, before the administration of the first Zalypsis® infusion.
2. Laboratory tests	◆ **Hematology:** differential WBC (neutrophils, lymphocytes), hemoglobin and platelets. ◆ **Biochemistry A:** liver function tests (ALT, AP, AST, LDH, total bilirubin), creatinine, CPK, troponin I*, glucose, and serum electrolytes (Na^+, Cl^-, K^+, Ca^{++}, Mg^{++}). ◆ **Biochemistry B:** total protein and albumin.	All within one week prior to Day 1 of Cycle 1. Repeat Hematology and Biochemistry A on Day 1 of Cycle 1 prior to treatment with Zalypsis® (a window of −2 days allowed), if the treatment is administered more than one week after the pre-treatment test. Repeat Biochemistry B on Day 1 of Cycle 1 whenever clinically indicated.
3. Calculated creatinine clearance	Calculated according to Cockcroft and Gault's formula (Appendix 4).	Within one week prior to Day 1 of Cycle 1. Repeat on Day 1 of Cycle 1 whenever clinically indicated.
4. Pregnancy test, if applicable		Within two weeks prior to registration and repeat on Day 1 of Cycle 1.
5. Tumor assessment	CT-scan or MRI of all measurable/evaluable sites of disease, as per RECIST v. 1.1. Documentation of PD is mandatory.	Within four weeks prior to the first dose of Zalypsis®.
6. PK samples	See Section 6.	Blood samples will be collected on Day 1 of Cycle 1: one before Zalypsis® infusion, and one 5 min before the end of infusion.
7. Pharmacodynamic and pharmacogenomic samples, from consenting patients	See Section 8.4 and 8.5.	CTCs will be collected on Day 1 of Cycle 1 before Zalypsis® infusion. Paraffin-embedded tumor tissue (obtained at diagnosis of the tumor) can be collected any time after registration.
8. Other tests	When indicated by the clinical and laboratory context.	Within four weeks prior to Day 1 of Cycle 1.

* All troponin I determinations, ECGs and LVEFs will be reviewed by external cardiologists.

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BSA, body surface area; CPK, creatine phosphokinase; CT, computed tomography; CTCs, circulating tumor cell(s); ECG, electrocardiogram; ECHO, echocardiography; ECOG, Eastern Cooperative Oncology Group;; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; PD, progressive disease; PK, pharmacokinetics; PS, performance status; RECIST, Response Evaluation Criteria In Solid Tumors; WBC, white blood cells.

5.6. PATIENT REGISTRATION

After ensuring that the patient meets all eligibility criteria and the patient or his/her representative has given written informed consent, he/she will be entered into the trial by contacting the clinical trial monitor designated by the Sponsor(s) and faxing the completed Patient Registration Form (Fast Fact Sheet). The Sponsor(s) will check this Registration Form and confirm eligibility. A patient number will be provided to the site of enrolment within one working day. This patient number should be used on all future documentation and correspondence referred to this patient.

5.7. EVALUATIONS DURING TREATMENT

The following assessments will be done while the patient is on treatment (Table 6). A 2-day window is allowed for the different tests and procedures (except where otherwise specified).

Table 6. Evaluations during treatment

	ASSESSMENT	TIME
1. Clinical examination	◆ Complete physical examination. ◆ ECOG PS (Appendix 1).	Day 1 of Cycle 1 prior to the Zalypsis® infusion, and then whenever clinically indicated.
	◆ Vital signs: heart rate, blood pressure and body temperature.	Before any Zalypsis® infusion.
	◆ Weight and BSA.	Repeat on Day 1 of each cycle prior to the Zalypsis® infusion, and then whenever clinically indicated.
	◆ Concomitant disease and treatments.	Throughout the "on-treatment" period*.
2. Laboratory tests	◆ **Hematology:** differential WBC (neutrophils, lymphocytes), hemoglobin and platelets. ◆ **Biochemistry A:** liver function tests (ALT, AP, AST, LDH, total bilirubin), creatinine, CPK, troponin I**, glucose, and serum electrolytes (Na^+, Cl^-, K^+, Ca^{++}, Mg^{++}) .	Repeat on Days 1, 8 and 15 of each cycle prior to Zalypsis® infusion. If NCI-CTCAE grade ≥ 3 occurs, the appropriate test(s) should be repeated at least every 2-3 days until recovery to grade ≤ 1 or baseline. In case of febrile neutropenia (any grade) ANC determination must be repeated every day until resolution.
	◆ **Biochemistry B:** total protein and albumin.	Repeat on Day 1 of each cycle prior to Zalypsis® infusion, and then whenever clinically indicated. If NCI-CTCAE grade ≥ 3 occurs, the appropriate test(s) should be repeated at least every 2-3 days until recovery to grade ≤ 1 or baseline.
3. Calculated creatinine clearance	Calculated according to Cockcroft and Gault's formula (Appendix 4).	Repeat on Day 1 of each cycle prior to the Zalypsis® infusion, and then whenever clinically indicated.
4. ECG**		ECG must be repeated prior to administration of each Zalypsis® infusion and if clinically indicated.
5. LVEF**	ECHO.	Repeat every other cycle, with a 1-week window, and if clinically indicated.
6. Pregnancy test, if applicable		Every four weeks during treatment and three months after last Zalypsis® administration.

	ASSESSMENT	TIME
7. AEs	As per NCI-CTCAE v. 4.0.	Throughout the "on-treatment" period*. Patients withdrawn with Zalypsis®-related ongoing AEs must be followed up with the appropriate tests until event resolution.
8. Tumor Assessment	CT-scan or MRI of all evaluable sites of disease, as per RECIST v. 1.1.	Every other cycle (± 1 week) until PD.
9. PK samples	See Section 6.	Blood samples will be collected on Day 1 and 8 of Cycle 1, two before Zalypsis® end of infusion and six therafter, at 30 min, 2 h, 6 h, 24 h, 48 h and 168 h after end of infusion.
10. Pharmacodynamic samples, from consenting patients	See Section 8.4.	On Day 1 before each Zalypsis® infusion for the first six cycles, and every three cycles thereafter.
11. Other tests		When indicated, according to the clinical and laboratory context.

*On-treatment period = from first infusion of Zalypsis® to End of treatment.

End of treatment = 30 days after the day of last dose of study drug administration, unless the patient starts a new antitumor therapy or dies (whichever occurs first), in which case the date of administration of this new therapy or the date of death will be considered the date of end of treatment.

** All troponin I determinations, ECGs and LVEFs will be reviewed by external cardiologists.

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BSA, body surface area; CPK, creatine phosphokinase; CT; computed tomography; ECG, electrocardiogram; ECHO, echocardiogram; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; PD, progressive disease; PK, pharmacokinetics; PS, performance status; RECIST, Response Evaluation Criteria In Solid Tumors; WBC, white blood cells.

5.8. EVALUATION AT END OF TREATMENT

The end-of-treatment visit will be scheduled at 30 days after the last Zalypsis® infusion (a window of ± 2 days is allowed).

Regardless of the reason for the discontinuation, the same complete workup conducted before study entry (except for medical history) will have to be done at this end-of-treatment visit. This will include the following assessments:

- Complete physical examination.
- Vital signs.
- ECOG PS.
- Weight and BSA, if clinically indicated.
- Hematology.
- Biochemistry A.
- Biochemistry B.
- Calculated creatinine clearance, if clinically indicated.
- Pregnancy test, if applicable.
- ECG and LVEF by ECHO.

- Concomitant diseases and treatments.
- Safety assessment (AEs).
- Tumor assessment.
- Other tests (when indicated, according to the clinical and laboratory context).

Adverse events must be reported until 30 days after the last study drug administration. All serious adverse events (SAEs) occurring within 30 days of the last study drug administration will be reported. Beyond this period of time, only those SAEs suspected to be treatment-related will be reported (see Section 9).

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

5.9. FOLLOW-UP AFTER END-OF-TREATMENT VISIT

The date and reason of the study discontinuation will be recorded on the patient's CRF (see Section 5.2.1.1).

Patients who discontinue treatment without progression will be followed every three months until disease progression, other antitumor therapy or death or until the date of study termination, whichever occurs first. After disease progression or after other antitumor therapy, patients will be followed every three months until death, or until the date of study termination, whichever occurs first.

Patients' follow-up determinations will include: ECOG PS, vital signs, complete physical examination, weight if clinically indicated, hematology, biochemistry A and B, concomitant diseases and treatment, calculated creatinine clearance if clinically indicated, and tumor assessment.

Follow-up of cardiac assessments (including ECG and LVEF) should be performed at three and six months after end of treatment.

Patients who withdraw consent will not be followed with any study procedures.

Patients withdrawn with ongoing Zalypsis®-related AEs (including SAEs) will be followed-up until the events or their sequelae resolve or stabilize at a level acceptable to the Investigator and the Sponsor(s).

Additional parameters will be assessed and/or the frequency of observations will be increased at the Investigator's discretion and according to the nature of the observed AEs. In case of death, when available, autopsy data should be provided.

6. PHARMACOKINETICS

All patients included in this study will be sampled for PK during the first two infusions of the first cycle. Blood samples for the measurement of the Zalypsis® plasma concentration will be obtained following the sampling schedule detailed in Table 7.

6.1. SAMPLE COLLECTION, STORAGE, AND SHIPPING OF THE PK SAMPLES

All PK sample collection dates and times will be recorded on the PK sheet.

In the treatment infusions with plasma sampling for PK, the infusion rate will be established so as to ensure that the total dose is infused in 1 hour. The drug will be infused at a constant rate throughout the 1-hour period. In order to obtain reliable PK information, the infusion rate should not be modified once it has begun. **If a variation in the infusion time occurred, it must be recorded on the CRF and PK sheet**, stating

clearly the time of the beginning and the end of the infusion. The infusion rate should not be changed to maintain the intended duration of infusion. It would be enough to record the actual duration on the CRF and on the PK sampling sheet for those cycles in which PK sampling is performed.

Blood samples for PK analysis will be obtained through a peripheral vein located in the contralateral side to the study drug infusion. **No sample (even the last one) must ever be collected from the catheter employed to administer Zalypsis®**. If a blood sample is obtained from another catheter, the first milliliter (ml) of blood will be discarded to avoid dilution of the sample with the solution used to keep it permeable.

Five-ml blood samples will be collected in sodium heparin tubes. Sample tubes will be gently inverted several times to ensure adequate mixing and immediately centrifuged at 2500 x g for 15 min at +4°C to separate the plasma. If immediate centrifugation is not possible, the tubes containing the blood samples must be placed in an ice bath at 0-4°C for a maximum of 30 minutes. After centrifugation, the plasma will be transferred to the provided polypropylene tubes (one per sample) and stored at -20ºC until shipped to the analysis laboratory. The cell pellet should be discarded. Blood and plasma tubes will be provided by the Sponsor(s).

Blood samples will be collected on Day 1 and 8 of Cycle 1, at the time points shown in Table 7.

Table 7. Plasma pharmacokinetic sampling schedule

Sample number	Optimal time point	Adequate time frame
1	Pre-SOI	Pre-SOI
2	5 min before EOI	5-1 min before EOI
3	30 min after EOI	30 min-1 h after EOI
4	2 h after EOI	1.5-3 h after EOI
5	6 h after EOI	6-8 h after EOI
6	24 h after EOI	20-28 h after EOI
7	48 h after EOI	40-72 h after EOI
8*	168 h after EOI	120-168 h after EOI

*Sample number 8 has to be taken BEFORE the start of the next infusion.
SOI, start of infusion; EOI, end of infusion.

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

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

Samples will be sent for analysis in the boxes provided for this purpose, filled with dry ice, by the study courier service to the following address:

Mr. Alan Gibbs
Icon Development Solutions
Manchester Bioanalytical Laboratory
Parkway One; Parkway Business Centre
300 Princess Road
Manchester
M14 7QU, United Kingdom

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

Samples will be destroyed following the appropriate laboratory procedures, after the approval of the final analytical study report by the Sponsor(s).

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

7. TREATMENT

7.1. DESCRIPTION OF TREATMENT

Zalypsis® will be administered as a 1-hour i.v. infusion on d1, 8, 15, q4wk, to patients with advanced and/or metastatic Ewing's sarcoma previously treated with at least one prior line of chemotherapy.

For instructions regarding drug inventory, handling, reconstitution, dilution, storage, accountability and disposal, please refer to the Preparation Guide for Zalypsis® and the Zalypsis® IB, both provided as separate documents.

7.2. ADMINISTRATION OF STUDY MEDICATION

7.2.1. Formulation

PM001004 (Zalypsis®, 2.5 mg/vial) is provided as a powder for concentrate for solution for infusion in only one strength of 2.5 mg.

7.2.2. Dose Schedule

PM001004 (Zalypsis®) will be administered at a dose of 2 mg/m^2. A treatment cycle consists of the drug administration on Days 1, 8 and 15, plus one week of follow-up (study evaluations should be completed during each cycle prior to subsequent Zalypsis® infusion). Treatment cycles will be repeated every four weeks.

7.2.3. Criteria for Treatment Continuation

Patients will remain on treatment in the absence of confirmed or unacceptable toxicity that is not resolved after applying the appropriate dose reductions.

In order to be re-treated on Day 8 and Day 15 of the first cycle and on subsequent infusions, the patients will have to fulfill the following criteria:

a) Platelet count ≥ 75 x 10^9/l, hemoglobin ≥ 9 g/dl and ANC ≥ 1.0 x 10^9/l.
b) AP ≤ 2.5 x upper limit of normality (ULN) (≤ 5 x ULN in case of extensive bone involvement).
c) ALT and AST ≤ 3 x ULN (≤ 5 x ULN in case of hepatic metastases).
d) Renal function: patients with calculated creatinine clearance (using Cockcroft and Gault's formula) ≥ 30 ml/min.
e) Total bilirubin ≤ 1.5 x upper limit or normality (ULN), unless due to Gilbert's syndrome.
f) Albumin ≥ 2.5 g/dl.
g) Other non-hematological toxicities grade ≤ 1 (grade 2 in case of asthenia).

If these criteria are not met on Day 8, 15 or on subsequent infusions, study drug administration should be skipped and criteria reevaluated weekly. Treatment administration will be resumed upon recovery of these parameters, according to these same criteria.

If a patient has to skip two doses (at Day 8 and Day 15) or has a delay > 2 weeks from the theorical day of re-treatment, the patient should be discontinued from treatment, except in the event of obvious clinical benefit, in which case the patient may be allowed to remain on treatment only after having discussed and agreed on the case with the Sponsor(s), and upon subsequent recovery of all parameters according to the aforementioned criteria.

7.2.4. Dose Reduction

Zalypsis® dose reductions will take place based on the worst treatment-related toxicity found since the last dose administration. The criteria for dose reduction are:

- Grade 4 neutropenia that lasts > 5 days.
- Febrile neutropenia.
- Grade 4 thrombocytopenia.
- Grade ≥ 3 transaminase increase that lasts > 7 days.
- Any other grade 3-4 adverse event non-hematologic toxicity except nausea, vomiting and diarrhea (unless grade 3 or 4 nausea, vomiting and diarrhea persists despite use of adequate medication).
- In the event of a patient requiring dose reduction, the new Zalypsis® dose level is described in Table 8.

Table 8**.** Dose modification according to toxicity

Dose level	**Zalypsis® (mg/m^2)**
1	**2.0**
-1	1.6
-2	1.3

Following the resolution of any of the aforementioned toxicities, patients may continue treatment at one dose level below. If treatment-related toxicities reappeared in the following drug administrations, a second dose reduction to a level below is allowed.

No more than two dose reductions per patient will be allowed during the study. Patients requiring more than two dose reductions should discontinue the treatment, except in the event of obvious clinical benefit, in which case the patient could be allowed to remain on treatment after having discussed and agreed on the case with the Sponsor(s).

No dose escalations will be allowed in this study.

7.3. CONCOMITANT MEDICATION

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

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

7.3.1. Prophylactic Antiemetic Treatment

According to the American Society of Clinical Oncology (ASCO) guidelines for drugs with moderate emetic risk (36), before the infusion of Zalypsis® the patients will receive prophylactic treatment for emesis for adult patients consisting of dexamethasone 8 mg i.v. and 5-HT3 antagonists (ondansetron 8 mg i.v. or granisetron 1 mg i.v. or tropisetron 5 mg i.v.).

If necessary, one or both of the following may also apply:

- Adding 10 mg of metoclopramide orally every eight hours.
- Extending the duration of treatment with 5-HT3 antagonists and/or dexamethasone.

7.3.2. Concomitant Medication Permitted

- Therapies for preexisting and treatment-emergent medical conditions.
- Prophylactic antiemetic treatment (described in Section 7.3.1).
- Secondary prophylaxis with colony-stimulating factors such as granulocyte (G-CSF) or granulocyte-macrophage (GM-CSF) colony-stimulating factors are allowed according to the ASCO guidelines (37).
- Erythropoietin therapy or derivatives (38).
- Bisphosphonates.
- In case of diarrhea (39) and pruritus, appropriate treatment will be permitted, including topical and systemic corticosteroids and antibiotics.
- Palliative local radiation may be applied. The irradiated lesion will then not be considered an area of measurable/evaluable disease.

7.3.3. Concomitant Medication Prohibited

- Concomitant administration of any other antineoplastic therapy.
- Investigational agents.
- Immunosuppressive therapies, including systemic corticosteroids, unless used as therapy for preexisting and treatment-emergent medical conditions (e.g., emesis, rash, anorexia).
- Primary prophylaxis (i.e., before the intended preventable events occurs) with colony-stimulating factors such as granulocyte (G-CSF) or granulocyte-macrophage (GM-CSF) colony-stimulating factors. Their use as secondary prophylaxis is permitted according to the ASCO guidelines (37). However, dose reduction will be applied if indicated, regardless of the use of secondary prophylaxis with colony-stimulating factors.

7.4. PACKAGING AND LABELING

7.4.1. Zalypsis®

PM00104 (Zalypsis®) is provided as a sterile lyophilized powder for concentrate for solution for infusion. Each vial will contain 2.5 mg of PM00104.

The labels will contain information that will meet applicable regulatory requirements.

7.5. DRUG ACCOUNTABILITY

Proper drug accountability will be done by the clinical trial monitor. Each study site will keep records to allow a comparison of quantities of drug received and used at each site. The Investigator at each study site will be the person ultimately responsible for drug accountability at the site.

All unused drug supplied by the Sponsor(s) will be properly destroyed at the study site. Documentation of this procedure must be provided to the clinical trial monitor. If the Sponsor(s) agree, unused drug supplies may be returned to the drug repository.

7.6. TREATMENT COMPLIANCE

The Investigator is responsible for supervising compliance with the instructions described in this study protocol.

8. STUDY EVALUATIONS

8.1. EFFICACY

To be evaluable for efficacy, patients must have received at least four of the six infusions in the first two cycles (e.g., two infusions in each cycle, or three infusions in Cycle 1 and one infusion in Cycle 2), and have at least one disease measurement recorded not less than six weeks after treatment onset.

In addition, any eligible patient who receives at least two of the three infusions in one treatment cycle and presents disease progression or dies due to progressive disease (PD) prior to response evaluation will be considered evaluable for the main endpoint (ORR) and will be categorized as an “early progression”.

Patients withdrawn due to toxicity without any tumor assessment after the start of study treatment will be considered as “treatment failures” and will not be replaced.

Patients withdrawn due to significant clinical deterioration of unknown reason, or due to hypersensitivity reactions or unrelated AEs, and patients who refuse to continue on study for any reason without any tumor assessment after the start of study treatment will be considered not evaluable for efficacy and will be replaced.

Assessment of efficacy will be done using the RECIST v1.1 and will be essentially based on a set of measurable lesions identified at baseline as target lesions and followed until disease progression. A comprehensive workup will be performed at baseline and every other cycle (± 1 week) until evidence of PD. The same procedure will be used to evaluate each identified lesion both at baseline and throughout the treatment period.

In case of detection of an OR, either CR or PR, a confirmation assessment has to be performed a minimum of four weeks after the first documentation of the response.

Additionally, detection of the presence of circulating CTCs by retrotranscriptase polymerase chain reaction (RT-PCR) analysis of EWS-FLI1 and EWS-ERG translocations in the blood of patients will be performed.

The time-to-event parameters that will be analyzed are detailed in Section 10.2.2.

A reference to the RECIST v. 1.1 may be found in Appendix 3.

8.2. SAFETY

Patients will be evaluable for safety if they have received at least one total or partial infusion of Zalypsis®.

Safety will be evaluated using clinical examinations, which will comprise vital signs analysis, clinical assessment of AEs, changes in laboratory parameters (hematological and biochemical, including liver and cardiac function tests), and any other analyses that may be considered necessary.

All AEs will be classified according to the NCI-CTCAE, v. 4.0 and will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) v. 11.0.

8.3. CENTRAL CARDIOLOGICAL REVIEW

Because of safety reasons, an independent cardiologist will undertake a review of all the ECGs and ECHOs performed to the patients during the course of the study, in order to improve the consistency in the evaluation criteria applied.

Originals or copies of all ECGs, ECHOs, and laboratory records of troponin I, need to be provided by the Investigator to the Independent Reviewer on ongoing basis. Other patient clinical or laboratory records may need to be provided by the Investigator upon request from the Independent Reviewer.

In the event of disagreement between the Investigator's and the centralized assessment, discussion will take place between the two parties in order to reach a consensus. However, if it is not forthcoming, it is the assessment of the independent reviewer, which will be retained in the report of the Expert Review, and in intermediate and final reports.

8.4. PHARMACODYNAMIC SUBSTUDY

A secondary endpoint of the study is the enumeration of Ewing's sarcoma CTCs, not only as a prognostic factor but also as a pharmacodynamic marker of response to Zalypsis® treatment. A unique characteristic of the Ewing's sarcoma tumor cells is having a specific chromosomal translocation that can be determined with high specificity in blood samples by quantitative real-time retrotranscriptase polymerase chain reaction (qRT-PCR), even in the presence of an excess of normal blood cells of several orders of magnitude. Thus, the chromosomal translocation will be initially identified by FISH in tumor samples and latter assayed in the blood samples.

This pharmacodynamic substudy will analyze the presence of Ewing's sarcoma cells in the blood of patients before Zalypsis® infusion in each cycle of treatment. It is expected that most patients in the advanced metastatic setting will be positive for the presence of Ewing's sarcoma cells in their blood, as detected by qRT-PCR. It seems reasonable that an effective treatment with Zalypsis® would lead to the reduction of CTCs as it has been described for other first-line treatments in this tumor type (41). Similarly, the reappearance of the CTCs could be used as an early marker of PD.

To perform this pharmacodynamic substudy it is required the collection of 8 ml of

peripheral blood in Vacutainer® CPT™ tubes from the patient immediately before the infusion of Zalypsis® in each treatment cycle. The blood sample has to be immediately centrifuged to separate plasma and white blood cells and stored frozen until analysis.

The number of Ewing's sarcoma CTCs at each time point will be determined by qRT-PCR and correlated with the patient's outcome.

For detailed information on the collection and shipping of CTCs samples please refer to the Guide for Identification, Packaging and Shipment of Pharmacodynamic and Pharmacogenomic samples.

8.5. PHARMACOGENOMIC SUBSTUDY

The identification of PGx biomarkers of response to Zalypsis® is a secondary endpoint of this study to be evaluated in a PGx substudy. The aim of this PGx substudy is to identify and validate molecular markers whose expression may be associated with the clinical outcome of patients treated with Zalypsis®. These molecular markers will allow the identification of those patients who would benefit from the treatment with Zalypsis®, improving the health care by an individualized medicine.

The experimental data points out to Zalypsis® as a DNA binder that produces DSBs in the DNA that are repaired by homologous recombination repair. In addition, Zalypsis® has also been involved in transcription, interfering both with transcription initiation and elongation (PharmaMar internal report). Then, it seems rational to develop studies to conduct correlations between the outcome of tumor/patient exposed to Zalypsis® and genes/proteins determinant in the efficiency/deficiency of the DNA repair pathways and transcription. The ultimate goal is the characterization of such patients that may be prone to respond to Zalypsis® in order to implement a customized therapy in the future.

Several genes, which have been identified as related to the response to Zalypsis® in mammalian cell lines and in yeast models, will be analyzed as potential markers of response in patients treated with Zalypsis®. Initially PDGFRa and PARP genes will be analyzed. Additonal genes related to Zalypsis® MoA could be selected for analysis later on. The expression of those genes in patient paraffin-embedded tumor tissue will be determined, analyzing mRNA levels by qRT-PCR, and protein expression by immunohistochemistry (using specific antibodies).

The ideal sample for gene mRNA expression analysis is the paraffin-embedded tumor tissue obtained at the diagnosis of the disease, because it is frequently conserved in the pathology archives. Tumor tissue slices in polyethylene naphthalate (PEN) membrane slides are microdissected to isolate tumoral cells and total RNA is extracted and the mRNA expression of selected genes is performed by qRT-PCR using gene specific primers.

In addition, the same tissue block samples used in the mRNA expression analysis will be studied at the protein level by tissue microarrays. To do so, cylinders from the paraffin-embedded tissue blocks will be cut and arrayed in a new paraffin block to allow the simultaneous determination of the expression of proteins by immunohistochemistry using specific antibodies. Thus, the expression of several proteins and its histological localization can be analyzed and correlated with the clinical outcomes of the corresponding patients, in a technical setting more familiar for the Pathology Departments.

PharmaMar will provide a Guide for Identification, Packaging and Shipment of Pharmacodynamic and Pharmacogenomic Samples detailing the procedures to follow

for sample collection, labeling and shipment. PharmaMar is responsible for selecting reference laboratories with demonstrated skills to perform the tests planned.

A positive consequence in the upcoming development of Zalypsis® is to be expected if we are able to identify a correlation between the expression of RNA/protein in the tumor tissue and the patient clinical outcomes after Zalypsis® treatment, since this active drug could be then given in a customized way to advanced resistant cancer patients, thus maximizing the rate of clinical benefit.

9. ADVERSE EVENTS REPORTING

9.1. DEFINITIONS

9.1.1. Adverse Event

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

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

Any event involving adverse drug reactions, illnesses with onset during the study or exacerbations of pre-existing illnesses should be recorded, including but not limited to clinically significant changes in physical examination findings and abnormal objective test findings (e.g., ECG). The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

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

Tumor progression or appearance of new tumor lesions MUST NOT be reported as an AE.

9.1.2. Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse experience occurring at any dose that:

- Results in death (is fatal),
- Is life-threatening,
- Requires or prolongs inpatient hospitalization,
- Results in persistent or significant disability or incapacity,
- Is a congenital anomaly or birth defect,
- Is medically significant, or
- Is any suspected transmission of an infectious agent via a medicinal product.

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

Tumor progression or appearance of new tumor lesions MUST NOT be reported as a SAE.

9.1.2.1. Death

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

9.1.2.2. Life-threatening Event

Any event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

9.1.2.3. Hospitalization or Prolongation of Hospitalization

Any AE requiring hospitalization (or prolongation of hospitalization) that occurs or worsens during the course of a patient's participation in a clinical trial must be reported as a SAE unless exempted from SAE reporting (see Section 9.2.2). Prolongation of hospitalization is defined as any extension of an inpatient hospitalization beyond the stay anticipated/required for the initial admission, as determined by the Investigator or by the treating physician.

Hospitalizations that do not meet criteria for SAE reporting are:

a. Reasons described in protocol [e.g., investigational medicinal product (IMP) administration, protocol-required intervention/investigations, etc]. However, events requiring hospitalizations or prolongation of hospitalization as a result of a complication of therapy administration or clinical trial procedures will be reported as SAEs.
b. Hospitalization or prolonged hospitalization for technical, practical or social reasons, in absence of an AE.
c. Pre-planned hospitalizations: any pre-planned surgery or procedure must be documented in the source documentation. Only if the pre-planned surgery needs to be performed earlier due to a worsening of the condition, should this event (worsened condition) be reported as a SAE.

Other situations that MUST NOT be considered as hospitalizations are the following:

d. An emergency visit due to an accident where the patient is treated and discharged.
e. When the patient is held 24 hours for observation and finally is not admitted.
f. Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (e.g., laser eye surgery, arthroscopy, etc).

9.1.3. Unlisted/Unexpected Adverse Event

An AE, the nature or severity of which is not consistent with the applicable reference safety information. The Sponsor(s) will use as the reference safety information for the most updated IB for the studied IMP.

9.1.4. Assessment of Causal Relationship to the Study Drug/IMP

The Investigator must provide an assessment of causal relationship of the clinical trial IMPs to each SAE according to the following scale:

Y There is a reasonable possibility that the IMP caused the SAE.

N There is no reasonable possibility that the IMP caused the SAE and other causes are more probable.

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

9.1.5. Adverse Events Related to Study Drug (IMP)

An AE is considered related to a study drug/IMP if the Investigator's assessment of causal relationship to the IMP is "Y" (yes).

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

The Sponsor(s) will also consider related to the study drug/IMP those events for which the Investigator assesses the causal relationship with the IMP as "Uk" when it cannot rule out a role of the IMP in the event. See Section 9.1.4 for causality scale.

9.1.6. Expedited Reporting

The Sponsor(s) is responsible for the appropriate expedited reporting of SAEs to the Regulatory Authorities. The Sponsor(s) will also report all SAEs that are unlisted and related to the study drug (IMP), to the Investigators and to the IECs/IRBs according to the current legislation unless otherwise required and documented by the IECs/IRB.

9.2. PROCEDURES

9.2.1. Reporting Adverse Events

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

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

Investigators must assess severity (grade) of the event following the NCI-CTCAE v. 4.0 and assign relationship to the study drug/IMP; pursue and obtain information adequate both to determine the outcome and to assess whether it meets the criteria for classification as a SAE requiring immediate notification to the Sponsor(s) or its designated representative. The Investigator must provide any relevant information as requested by the Sponsor(s) in addition to that on the CRF.

Disease progression should not be reported as an adverse event and will only be reported in the applicable CRF page.

Abnormal laboratory tests occurring during the study should be only recorded in the AE section of the CRF if the disorder:

- is associated with clinically significant symptoms, and/or
- leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment or other therapy, and/or
- leads to any of the outcomes included in the definition of a SAE.

Otherwise laboratory results should be reported in the corresponding section of the CRF (Biochemistry or Hematology).

9.2.2. Reporting Serious Adverse Events

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

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

All SAEs (as defined above) regardless of relationship to the study drug/IMP must be reported immediately and always within 24 hours to the PharmaMar Pharmacovigilance function by fax (+34 91 846 6004) or telephone (+34 91 846 6054). Out of office hours (GMT), assistance on SAE reporting can be obtained by calling the Pharmacovigilance Service at +34 91 823 4749.

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

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

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

9.2.3. Reporting Pregnancy Cases Occurring During the Clinical Trial

National regulations require that clinical trial Sponsor(s) collect information on pregnancies occurring during clinical trials, in which exposure to the IMPs at any time during pregnancy, via either maternal or paternal exposure, is suspected.

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

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

- Any occurrence of a pregnancy where any kind of exposure to the IMP/s is suspected.
- Possible exposure of a pregnant woman (this could involve a partner of a male patient or a pregnant female who came in contact with the clinical trial IMP/s).

- All reports of elevated/ questionable or indeterminate beta human chorionic gonadotrophins (βhCGs).

Immediately after detecting a case of suspected pregnancy in a female clinical trial patient, the decision on her continued participation in the clinical trial will be jointly taken by the trial patient, the Investigator and the Sponsor(s), with the patient's best interest in mind. A decision to continue the pregnancy will require immediate withdrawal from the trial.

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

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

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

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

9.2.4. Periodic Safety Review of Clinical Data

Although periodic safety review of clinical data will be performed, no formal Data Safety and Monitoring Board (DSMB) has been appointed for this exploratory, small sample-size trial. Adverse events will be monitored by the Investigators and by the study team at PharmaMar.

The personnel in charge of this process are defined on the section "Study Contacts" of the protocol. A medical oncologist will review the safety data of this trial on an ongoing basis.

SAEs will be collected, assessed and reported as per the applicable Regulations by the Pharmacovigilance Department / Regulatory Affairs Department.

In the case of an accumulation of unexpected SAEs, stopping guidelines have been established in the protocol (see Section 5.2).

As per the applicable regulations, the Sponsor(s) will report to IECs/IRBs, investigators and Regulatory Authorities:

- expeditedly: all serious, related, unexpected AEs or critical safety findings from this and any other clinical trial with the same study drug/ IMP, and
- periodically: within an Annual Report: all the relevant safety information generated in all clinical trials with the study drug/ IMP.

Non-serious AEs will be assessed during monitoring visits by the monitor who will discuss them with investigators. Any protocol deviation will also be discussed with the investigator during monitoring visits. Safety review will be performed at PharmaMar once CRFs have been monitored, collected and shipped to the Sponsor(s).

10. STATISTICAL METHODS

The statistical analysis will be done by the Sponsor(s) or under the Sponsor(s)'s authority.

10.1. SAMPLE SIZE

The optimum two-stage design to test the null hypothesis that the overall response rate (ORR) is ≤ 3% versus the alternative that ORR ≥ 20% was selected. After testing the drug on 12 patients in the first stage, the trial will be terminated if there is not any responder. If the trial goes on to the second stage, a total of 29 patients will be studied, 12 from the first stage and 17 from the second stage. If the total number responding is less than or equal to 2 (two patients), the drug will be considered not interesting in the setting of patients treated with this disease and with this schedule. This design has an expected sample size of 17.20 and a probability of early termination of 0.69. If the drug is actually not effective, there is a 0.05 probability of concluding that it is (type I error). If the drug is actually effective, there is a 0.1 probability of concluding that it is not (type II error).

10.2. ENDPOINTS

10.2.1. Primary Endpoints

- Overall response rate (ORR), defined as the percentage of patients with confirmed O), either CR or PR, according to the RECIST v1.1.

10.2.2. Secondary Endpoints

Efficacy:

- Duration of response (DOR), defined as the time between the date when the response criteria (PR or CR, whichever is first reached) are fulfilled and the first date when disease progression, recurrence or death is objectively documented (taking the smallest measurements documented since the treatment started as reference for PD).
- Progression-free survival (PFS), defined as the time from the first day of study treatment to the day of negative assessment (progression or death), start of subsequent antitumor therapy, or last tumor evaluation. PFS at 3 and PFS at 6 months are defined as the Kaplan-Meier estimates of PFS at these time points.
- Overall survival (OS), defined as the time from the first day of treatment to the date of death (or the last day when the patient is known to be alive). Survival will be followed for up to six months after the last treatment visit of the last patient or 12 months after the last patient is included, whichever occurs first. OS at 12 months is defined as the Kaplan-Meier estimates of OS at this time point.

Safety:

- Clinical examinations.
- Vital signs analysis.

- Clinical assessment of AEs and SAEs.
- Changes in laboratory parameters: hematological and biochemical (including liver and cardiac function tests).
- Reasons for study discontinuations, dose delays, omissions, and reductions.
- Any other analyses that may be considered necessary to characterize the safety profile of Zalypsis® in patients with advanced and/or metastatic EFT.

Others:

- Pharmacokinetic profile.
- Pharmacogenomic profile.
- Pharmacodynamic profile.

10.3. STATISTICAL ANALYSIS

Continuous variables will be tabulated and presented with summary statistics (i.e., mean, standard deviation, median and range). Categorical variables will be summarized in frequency tables by means of counts and percentages.

10.3.1. Efficacy Analysis

All patients who have received at least four of the six infusions in the first two cycles (i.e., two infusions in each cycle, or three infusions in Cycle 1 and one infusion in Cycle 2), and at least one disease measurement recorded not less than six weeks after treatment onset.

In addition, any eligible patient who receives at least two of the three infusions in one treatment cycle and experience disease progression or die due to progressive disease (PD) prior to response evaluation will be considered evaluable for the main endpoint (ORR) and will be categorized as an “early progression”.

Patients withdrawn due to toxicity without any tumor assessment after the start of study treatment will be considered as “treatment failures” and will not be replaced.

Patients withdrawn due to significant clinical deterioration of unknown reason, hypersensitivity reactions, who refuse to continue on study for any reason or unrelated AEs without any tumor assessment after the start of study treatment, will be considered not evaluable for efficacy and will have to be replaced.

Assessment of efficacy will be done using the RECIST v. 1.1 and will be essentially based on a set of measurable lesions identified at baseline as target lesions and followed until disease progression. A disease evaluation will be performed at baseline and every other cycle (± 1 week) until evidence of PD. The same procedure will be used to evaluate each identified lesion both at baseline and throughout the treatment period.

In case of detection of an OR, either CR or PR, a confirmation assessment has to be performed a minimum of four weeks after the first documentation of the response.

Binomial estimates with exact 95% confidence intervals will be calculated for the analysis of the main endpoint (ORR).

Time-to-event endpoints (DOR, PFS and OS, PFS at 3 and 6 months and OS rates at 12 months) will be analyzed according to the Kaplan-Meier method.

10.3.2. Safety Analysis

Patients will be evaluable for safety if they have received at least one total or partial infusion of Zalypsis®.

Safety will be evaluated using clinical examinations, which will comprise vital signs analysis, clinical assessment of AEs, changes in laboratory parameters (hematological and biochemical, including liver and cardiac function tests, deaths, reason for study discontinuations, dose delays, omissions and reductions, and any other analyses that may be considered necessary to characterize the safety profile of Zalypsis® in unresectable locally advanced and/or metastatic EFT. Cardiac tests as troponin I, ECG and LVEF measurements will be analyzed descriptively.

All AEs will be classified according to the NCI-CTCAE, v 4.0, and will be coded using the MedDRA, v. 11.0.

10.3.3. Pharmacokinetic Analysis

Pharmacokinetic parameters will be calculated using population methods, after pooling data from this study with data obtained during phase I studies.

10.3.4. Pharmacodynamic and Pharmacogenomic Analyses

The presence and the abundance of the translocation in blood will be correlated to the outcome of the patient, in search for a marker of response to the treatment or in anticipation of the progression. Also, the molecular parameters found in the tumor and blood samples will be correlated with the patients' clinical results.

11. ADMINISTRATIVE SECTION

11.1. ETHICS

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the World Medical Association (WMA) Declaration of Helsinki (see Appendix 5) and will be consistent with GCP guidelines and pertinent regulatory requirements.

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

The study will be conducted in compliance with the protocol. The protocol, any amendments and the patient informed consent will receive IEC/IRB approval/favorable opinion prior to initiation, according to pertinent regulations.

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

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

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

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

11.2. MONITORING, AUDITING AND INSPECTING

The study will be monitored by regular site visits and telephone calls to the Investigator by the PharmaMar clinical trial monitor.

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

Adequate time for these visits should be allocated by the Investigator. The Investigator should also ensure that the monitor is given direct access (as per International Conference on Harmonization [ICH] Topic E6 [R1] Guideline for Good Clinical Practice, Sections 4.9.7 and 6.10) to source documents (e.g., hospital or private charts, original laboratory records, appointment books, etc.) of the patient which support data entered in the case report forms, as defined in the ICH Topic E6 (R1) Guideline for Good Clinical Practice, Sections 1.51 and 1.52.

Systems and procedures will be implemented to ensure the quality of every aspect of the trial.

During the course of the trial, the Clinical Quality Assurance Department of the Sponsor(s) or external auditors contracted by the Sponsor(s) may conduct an onsite audit visit (ICH Topic E6 (R1) Guideline for Good Clinical Practice, Section 1.6).

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

11.3. PATIENT INFORMED CONSENT FORM

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

The Informed Consent Forms will include all elements required by ICH, GCP and applicable regulatory requirements.

Prior to inclusion into the trial, the Investigator or a person designated by the Investigator must provide the patient and/or his/her representative with one copy of the Informed Consent Form for the clinical trial and one copy of the Informed Consent Form for the substudy. Both copies must provide written fully information about the clinical trial and the substudy in a language that is non-technical and easily understood. The Investigator should allow time necessary for the patient and/or his/her legally acceptable representative to inquire about the details of the clinical trial and the substudy. Each Informed Consent Form must be freely signed and personally dated by the patient and/or his/her representative and by the person who conducted the informed consent discussion before the beginning of the study. The patient and/or representative should receive a copy of both the trial and the substudy signed Informed Consent Forms, and any other written information provided to study patients and their representatives prior to participation in the trial.

During a patient's participation in the trial, any updates to the consent forms and any updates to the written information will be provided to him/her.

If there is a need to obtain new consent from the patients, the Investigator or a person designated by the Investigator should inform the patients of any new information relevant to the patients' willingness to continue participation in the study, before obtaining the written consent.

11.4. CONFIDENTIALITY / IDENTIFICATION OF PATIENTS

The collection and processing of personal data from the patients enrolled in this clinical trial will be limited to those data that are necessary to investigate the efficacy, safety, quality and usefulness of the study drug used in this trial.

It is the Investigator's responsibility that sufficient information on the identity of the patients will be retained.

The trial monitor, the Sponsor(s)'s auditor, the IECs/IRBs and the regulatory authorities should have direct access to all requested trial-related records, and agree to keep the identity of study patients confidential.

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

Explicit consent for the processing of personal data will be obtained from the participating patient before data collection, and this consent should also address the transfer of the data to other entities and countries.

The Sponsor(s) shall comply with the Directive 95/46/EEC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

11.5. CASE REPORT FORMS

Case report forms (CRFs) will be used to record all data for each patient. It is the responsibility of the Investigator to ensure that the CRFs are properly and completely filled in. CRFs must be completed for all patients who have given their informed consent (by themselves or through their representatives) and have been enrolled into the study.

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

The data collected in the CRF will be entered into the Sponsor(s) databases, which comply with the Spanish Act implementing the Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data.

11.6. INSURANCE

The Sponsor(s) will provide insurance or indemnity in accordance with applicable regulatory requirements.

11.7. RETENTION OF RECORDS

The Investigator/Institution should maintain trial documents according to Section 8 of the ICH Topic E6 (R1) Guideline for Good Clinical Practice and as required by applicable regulatory requirements.

Essential documents should be retained as per the aforementioned ICH guideline or for a longer period of time, if required by the applicable regulations.

11.8. USE OF INFORMATION AND PUBLICATIONS

Before the investigators of this study submit a paper or abstract for publication or otherwise publicly disclose information concerning the study drug or products, PharmaMar must be provided with at least 60 days to revise and approve the proposed publication or disclosure to ensure that confidential and proprietary data are protected.

If PharmaMar determines that patentable patient matter is disclosed in the proposed publication or disclosure, the publication or disclosure will be withheld for a period of time considered convenient. If the study is part of a multicenter study, the first publication of the study shall be made in conjunction with the presentation of a joint, multicenter publication of the study results with the investigators and the institutions from all appropriate sites that are contributing data, analysis and comments. However, if such a multicenter publication is not submitted within 12 months after conclusion, abandonment or termination of the study at all sites, the present study may be published individually in accordance with the procedure established above.

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

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Appendix 1. ECOG Performance Status Assessment Scale

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

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

Appendix 2. Percent of Bone Marrow Irradiated

Percent of Normal Bone Marrow Irradiated Using Standard Radiation Ports

(From Ellis RE: Phys Med Biol 5:255, 1961)

Port	*Marrow Volume at Risk (%)*
Skull (not including mandible)	12
Upper limp girdle (unilateral, including the humeral head, scapulae, clavicle)	4
Sternum	2
Ribs (all)	8
Ribs (hemithorax)	4
Cervical vertebra (all)	3
Thoracic vertebra (all)	14
Lumbar vertebra (all)	11
Sacrum	14
Pelvis	26
Mantle	25
Upper para-aortic nodes	11
Inverted Y	45

Appendix 3. Response Evaluation Criteria in Solid Tumors (RECIST) v. 1.1

This document summarizes the main information contained in RECIST v. 1.1.

Further details can be found in the original article: Eisenhauer EA, Therasse P, Bogaerts J, et al.: New response evaluation criteria in solid tumours: revised RECIST guideline (v. 1.1). Eur J Cancer 2009; 45(2): 228-247.[1]

LIST OF ABBREVIATIONS

CR	Complete Response
CRF	Case Report Form
CT	Computerized Tomography
FDG-PET	Fluorodeoxyglucose-Positron Emission Tomography
MRI	Magnetic Resonance Imaging
NE	Not Evaluable
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression-free Survival
PR	Partial Response
PSA	Prostate-specific Antigen
RECIST	Response Evaluation Criteria in Solid Tumors
SD	Stable Disease
TTP	Time to Progression

LIST OF TABLES

[1] A summary of major changes from RECIST 1.0 to RECIST 1.1 can be found at the end of this document (**Table 4**).

[2] This table is named Appendix I in the original RECIST 1.1 article.

1. MEASURABILITY OF TUMOR LESIONS AT BASELINE

1.1 Definitions

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

1.1.1 Measurable

Tumor Lesions:

Must be accurately measured in at least one dimension (*longest* diameter in the plane of measurement is to be recorded) with a *minimum* size of:

- 10 mm by computerized tomography (CT) scan (irrespective of scanner type) and magnetic resonance imaging (MRI) (no less than double the slice thickness and a minimum of 10 mm).
- 10 mm caliper measurement by clinical exam (when superficial).
- 20 mm by chest X-ray (if clearly defined and surrounded by aerated lung).

Malignant Lymph Nodes:

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed (see Schwartz *et al.* Eur J Cancer. 2009; 45(2):261-267). See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

1.1.2 Non-measurable

All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as lesions considered truly non-measurable. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, and abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.1.3 Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone Lesions:

- Bone scan, positron emission tomography (PET) scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with *identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the *soft tissue component* meets the definition of measurability described above.

- Blastic bone lesions are non-measurable.

Cystic Lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with Prior Local Treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

1.2. Specifications by Methods of Measurement

1.2.1 Measurement of Lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than four weeks before the beginning of the treatment.

1.2.2 Method of Assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical Lesions:

Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-Ray:

Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung. See original article, Appendix II, for more details.

Computed Tomography (CT), Magnetic Resonance Imaging (MRI):

CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. As is described in original article (Appendix II), when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans). More details concerning the use of both CT and MRI for assessment of objective tumor response evaluation are provided in the original article, Appendix II.

Ultrasound:

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in the original article, Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy:

The use of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor Markers:

Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response (CR). Because tumor markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and prostate-specific antigen (PSA) response (in recurrent prostate cancer) have been published (Rustin *et al.* J Natl Cancer Inst 2004; 96:487–488; Bubley *et al.* J Clin Oncol 1999; 17:3461–3467; Scher *et al.* J Clin Oncol 2008; 26:1148–1159). In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria, which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer (Vergote *et al.* J Natl Cancer Inst 2000; 92:1534–1535).

Cytology, Histology:

These techniques can be used to differentiate between partial response (PR) and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g., with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met

criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

2. TUMOR RESPONSE EVALUATION

2.1 Assessment of Overall Tumor Burden and Measurable Disease

To assess objective response or future progression, it is necessary to estimate the overall *tumor burden at baseline* and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 1. Measurability of tumor at baseline). In studies where the primary endpoint is tumor progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

2.2 Baseline Documentation of "Target" and "Non-target" Lesions

When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as ***target lesions*** and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved that a *maximum* of two and four lesions will be recorded, respectively). For evidence to support the selection of only five target lesions, see analyses on a large prospective database in the article by Bogaerts *et al.* Eur J Cancer 2009;45:248–260.

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to *reproducible repeated measurements*. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance the next largest lesion which can be measured reproducibly should be selected. To illustrate this point see the example in the original article, Figure 3 of Appendix II.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. As noted in the previous section, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm x 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement (see also the example in the original article, Figure 4 of Appendix II). All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be

considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed

A *sum of the diameters* (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the *baseline sum diameters*. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g., 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

2.3 Response Criteria

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

2.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest sum diameters while on study.

2.3.2 Special Notes on the Assessment of Target Lesions

Lymph Nodes:

Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm. Case report forms (CRFs) or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis < 10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target Lesions that Become 'Too Small to Measure':

While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the CRF. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that Split or Coalesce on Treatment:

As noted in the original article, Appendix II, when non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

2.3.3 Evaluation of Non-target Lesions

This section provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. While some non-target lesions may actually be measurable,

they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression (see comments below) of existing non-target lesions (Note: the appearance of one or more new lesions is also considered progression).

2.3.4 Special Notes on Assessment of Progression of Non-target Disease

The concept of progression of non-target disease requires additional explanation as follows:

When the Patient Also Has Measurable Disease:

In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy (see examples in the original article, Appendix II and further details below). A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the Patient Has only Non-measurable Disease:

This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. Some illustrative examples are shown in the original article, Figures 5 and 6 of Appendix II. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

2.3.5 New Lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e., not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show PR or CR. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was *not* scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While fluorodeoxyglucose-positron emission tomography (FDG-PET) response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive[3] FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up:
    - If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
    - If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).
    - If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

[3] A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

2.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement (see Section 2.6. Confirmatory Measurement/Duration of Response). Specifically, in non-randomized trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

2.4.1 Time Point Response

It is assumed that at each protocol specified time point, a response assessment occurs. **Table 1** provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

Table 1. Time point response: patients with target (+/–non-target) disease.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non- PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR, complete response; NE, inevaluable; PD, progressive disease; PR, partial response; SD, stable disease.

When patients have non-measurable (therefore non-target) disease only, **Table 2** is to be used.

Table 2. Time point response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR, complete response, NE, inevaluable; PD, progressive disease.

[a] 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials; so, to assign this category when no lesions can be measured is not advised.

2.4.2 Missing Assessments and Inevaluable Designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

2.4.3 Best Overall Response: All Time Points

The best overall response is determined once all the data for the patient is known.

Best Response Determination in Trials Where Confirmation of Complete or Partial Response IS NOT Required:

Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered inevaluable.

Best Response Determination in Trials Where Confirmation of Complete or Partial Response IS Required:

Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally four weeks later). In this circumstance, the best overall response can be interpreted as in **Table 3**.

Table 3. Best overall response when confirmation of complete response (CR) and partial response (PR) is required.

Overall response. First time point	Overall response. Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met,

Overall response. First time point	Overall response. Subsequent time point	BEST overall response
		otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR, complete response; NE, inevaluable; PD, progressive disease; PR, partial response; SD, stable disease.

[a] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

2.4.4 Special Notes on Response Assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (< 10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the CRF.

In trials where confirmation of response is required, repeated 'NE' time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in **Tables 1–3**.

Conditions that define 'early progression, early death and inevaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

For equivocal findings of progression (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled

assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

2.5 Frequency of Tumor Re-evaluation

Frequency of tumor re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, in the context of phase II studies where the beneficial effect of therapy is not known, follow-up every 6–8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances. The protocol should specify which organ sites are to be evaluated at baseline (usually those most likely to be involved with metastatic disease for the tumor type under study) and how often evaluations are repeated. Normally, all target and non-target sites are evaluated at each assessment. In selected circumstances certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when CR is identified in target disease or when progression in bone is suspected.

After the end of the treatment, the need for repetitive tumor evaluations depends on whether the trial has as a goal the response rate or the time to an event (progression/death). If 'time to an event' (e.g., time to progression, disease-free survival, progression-free survival) is the main endpoint of the study, then routine scheduled re-evaluation of protocol specified sites of disease is warranted. In randomized comparative trials in particular, the scheduled assessments should be performed as identified on a calendar schedule (for example: every 6–8 weeks on treatment or every 3–4 months after treatment) and should not be affected by delays in therapy, drug holidays or any other events that might lead to imbalance in a treatment arm in the timing of disease assessment.

2.6 Confirmatory Measurement/Duration of Response

2.6.1 Confirmation

In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see Bogaerts *et al*. Eur J Cancer 2009; 45(2): 248-260). However, in all other circumstances, i.e., in randomized trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

2.6.2 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

2.6.3 Duration of Stable Disease

Stable disease is measured from the start of the treatment (in randomized trials, from date of randomization) until the criteria for progression are met, taking as reference the *smallest sum on study* (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of SD varies in different studies and diseases. If the proportion of patients achieving SD for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and SD as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between trials are to be made.

2.7 Progression-free Survival/Proportion Progression-free

2.7.1 Phase II Trials

This guideline is focused primarily on the use of objective response endpoints for phase II trials. In some circumstances, 'response rate' may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases 'progression-free survival' (PFS) or the 'proportion progression-free' at landmark time points, might be considered appropriate alternatives to provide an initial signal of biologic effect of new agents. It is clear, however, that in an uncontrolled trial, these measures are subject to criticism since an apparently promising observation may be related to biological factors such as patient selection and not the impact of the intervention. Thus, phase II screening trials utilizing these endpoints are best designed with a randomized control. Exceptions may exist where the behavior patterns of certain cancers are so consistent (and usually consistently poor), that a non-randomized trial is justifiable (see Van Glabbeke *et al.* Eur J Cancer 2002; 38(4): 543-549). However, in these cases it will be essential to document with care the basis for estimating the expected PFS or proportion progression-free in the absence of a treatment effect.

2.7.2 Phase III trials

Phase III trials in advanced cancers are increasingly designed to evaluate PFS or time to progression (TTP) as the primary outcome of interest. Assessment of progression is relatively straightforward if the protocol requires all patients to have measurable disease. However, restricting entry to this subset of patients is subject to criticism: it may result in a trial where the results are less likely to be generalizable if, in the disease under study, a substantial proportion of patients would be excluded. Moreover, the restriction to entry will slow recruitment to the study. Increasingly, therefore, trials allow entry of both patients with measurable disease as well as those with non-measurable disease only. In this circumstance, care must be taken to explicitly describe the findings which would qualify for progressive disease for those patients *without* measurable lesions. Furthermore, in this setting, protocols must indicate if the maximum number of recorded target lesions for those patients with measurable disease may be relaxed from five to three (based on the data found in Bogaerts *et al.* Eur J Cancer 2009; 45:248–260, and Moskowitz *et al.* Eur J Cancer 2009; 45:300–310). As found in the 'special notes on assessment of progression' (Section 2.3.4), these guidelines offer recommendations for assessment of progression in this setting. Furthermore, if available, validated tumor marker measures of progression (as has been proposed for ovarian cancer) may be useful to integrate into the definition of progression. Centralized blinded review of imaging studies or of source imaging reports to verify 'unequivocal progression' may be needed if important drug development or drug approval decisions are to be based on the study outcome. Finally, as noted earlier, because the date of progression is subject to ascertainment bias, timing of investigations in study arms should be the same. The article by Dancey *et al.* Eur J Cancer 2009; 45:281–289, provides a more detailed discussion of the assessment of progression in randomized trials.

2.8 Independent Review of Response and Progression

For trials where objective response (CR + PR) is the primary endpoint, and in particular where key drug development decisions are based on the observation of a minimum number of responders, it is recommended that all claimed responses be reviewed by an expert(s) independent of the study. If the study is a randomized trial, ideally reviewers should be blinded to treatment assignment. Simultaneous review of the patients' files and radiological images is the best approach.

Independent review of progression presents some more complex issues: for example, there are statistical problems with the use of central-review-based progression time in place of investigator-based progression time due to the potential introduction of informative censoring when the former precedes the latter. An overview of these factors and other lessons learned from independent review is provided in an article by Ford *et al.* Eur J Cancer 2009; 45:268–274.

2.9 Reporting Best Response Results

2.9.1 Phase II Trials

When response is the primary endpoint, and thus all patients must have measurable disease to enter the trial, all patients included in the study must be accounted for in the report of the results, even if there are major protocol treatment deviations or if they are not evaluable. Each patient will be assigned one of the following categories:

1. Complete response.
2. Partial response.
3. Stable disease.
4. Progression.
5. Inevaluable for response: specify reasons [for example: early death, malignant disease; early death, toxicity; tumor assessments not repeated/incomplete; other (specify)].

Normally, all eligible patients should be included in the denominator for the calculation of the response rate for phase II trials (in some protocols it will be appropriate to include all treated patients). It is generally preferred that 95% two-sided confidence limits are given for the calculated response rate. Trial conclusions should be based on the response rate for all eligible (or all treated) patients and should not be based on a selected 'evaluable' subset.

2.9.2 Phase III Trials

Response evaluation in phase III trials may be an indicator of the relative antitumor activity of the treatments evaluated and is almost always a secondary endpoint. Observed differences in response rate may not predict the clinically relevant therapeutic benefit for the population studied. If objective response is selected as a primary endpoint for a phase III study (only in circumstances where a direct relationship between objective tumor response and a clinically relevant therapeutic benefit can be unambiguously demonstrated for the population studied), the same criteria as those applying to phase II trials should be used and all patients entered should have at least one measurable lesion.

In those many cases where response is a secondary endpoint and not all trial patients have measurable disease, the method for reporting overall best response rates must be pre-specified in the protocol. In practice, response rate may be reported using either an 'intent to treat' analysis (all randomized patients in the denominator) or an analysis where only the subset of patients with measurable disease at baseline are included. The protocol should clearly specify how response results will be reported, including any subset analyses that are planned.

The original version of RECIST suggested that in phase III trials one could write protocols using a 'relaxed' interpretation of the RECIST guidelines (for example, reducing the number of lesions measured) but this should no longer be done since these revised guidelines have been amended in such a way that it is clear how these criteria should be applied for all trials in which anatomical assessment of tumor response or progression are endpoints.

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Table 4. Summary of major changes from RECIST 1.0 to RECIST 1.1.

RECIST v. 1.0		RECIST v. 1.1	Rationale
Minimum size measurable lesions	CT: 10 mm spiral 20 mm non-spiral	CT 10 mm; delete reference to spiral scan	Most scans used have 5 mm or less slice thickness. Clearer to give instruction based on slice interval if it is greater than 5 mm
	Clinical: 20 mm	Clinical: 10 mm (must be measurable with calipers)	Caliper measurement will make this reliable
	Lymph node: not mentioned	CT: ≥ 15 mm short axis for target ≥ 10–<15 mm for non-target < 10 mm is non-pathological	Since nodes are normal structure need to define pathological enlargement. Short axis is most sensitive
Special considerations on lesion measurability	–	Notes included on bone lesions, cystic lesions	Clarify frequently asked questions
Overall tumor burden	10 lesions (5 per organ)	5 lesions (2 per organ)	Data warehouse analysis shows no loss of information if lesion number reduced from 10 to 5. A maximum of 2 lesions per organ yields sufficient representation per disease site
Response criteria target disease	CR lymph node not mentioned	CR lymph nodes must be <10 mm short axis	In keeping with normal size of nodes
	PD 20% increase over smallest sum on study or new lesions	PD 20% increase over smallest sum on study (including baseline if that is smallest) and at least 5 mm increase or new lesions	Clarification that if baseline measurement is smaller than any on study measurement, it is reference against which PD is assessed 5 mm absolute increase to guard against over calling PD when total sum is very small and 20% increase is within measurement error
Response criteria non-target disease	'Unequivocal progression' considered as PD	More detailed description of 'unequivocal progression' to indicate that it should not normally trump target disease status. It must be representative of overall disease status change, not a single lesion increase	Confusion with RECIST 1.0 where some were considering PD if 'increase' in any non-target lesion, even when target disease is stable or responding
New lesions	–	New section on New lesions	To provide guidance on when a lesion is considered new (and thus PD)
Overall response	Table integrated target and non-target lesions	Two tables: one integrating target and non-target and the other of non-target only Special notes: How to assess and measure lymph nodes CR in face of residual tissue Discussion of 'equivocal' progression	To account for the fact that RECIST criteria are now being used in trials where PFS is the endpoint and not all patients have measurable (target) disease at baseline Frequently asked questions on these topics

RECIST v. 1.0		RECIST v. 1.1	Rationale
Confirmatory measure	For CR and PR: criteria must be met again 4 weeks after initial documentation	Retain this requirement ONLY for non-randomized trials with primary endpoint of response	Data warehouse shows that response rates rise when confirmation is eliminated, but the only circumstance where this is important is in trials where there is no concurrent comparative control and where this measure is the primary endpoint
Progression-free survival	General comments only	More specific comments on use of PFS (or proportion progression-free) as phase II endpoint Greater detail on PFS assessment in phase III trials	Increasing use of PFS in phase III trials requires guidance on assessment of PD in patients with non-measurable disease
Reporting of response results	9 categories suggested for reporting phase II results	Divided into phase II and phase III 9 categories collapsed into 5 In phase III, guidance given about reporting response	Simplifies reporting and clarifies how to report phase II and III data consistently
Response in phase III trials	More relaxed guidelines possible if protocol specified	This section removed and referenced in section above: no need to have different criteria for phase II and III	Simplification of response assessment by reducing number of lesions and eliminating need for confirmation in randomized studies where response is not the primary endpoint makes separate 'rules' unnecessary
Imaging appendix	Appendix I	Appendix II: updated with detailed guidance on use of MRI, PET/CT Other practical guidance included	Evolving use of newer modalities addressed. Enhanced guidance in response to frequent questions and from radiology review experience
New appendices		Appendix I: comparison of RECIST 1.0 and 1.1 Appendix III: frequently asked questions	

CR, complete response; CT, computed tomography; MRI, magnetic resonance imaging; RECIST, response evaluation criteria in solid tumors; PD, progressive disease; PET, positron emission tomography; PFS, progression-free survival; PR, partial response.

Appendix 4. Cockcroft and Gault's formula

For calculating creatinine clearance*

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

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

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

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

Appendix 5. Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)

55th WMA General Assembly, Tokyo 2004 (Note of Clarification on paragraph 30 added)

59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand

the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor(s) and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international

norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the

right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted

guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    a. The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    b. Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.