PM1183-A-019-20

An Open-Label, Multicenter Study to Assess the Potential Effects of Bosentan (a Moderate CYP3A4 Inducer) on the Pharmacokinetics of Lurbinectedin in Patients with Advanced Solid Tumors

CLINICAL TRIAL PROTOCOL

INVESTIGATIONAL MEDICINAL PRODUCTS: Lurbinectedin and Bosentan.
Protocol No.: PM1183-A-019-20
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-Confidential-

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This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

Confidentiality statement

PRINCIPAL INVESTIGATORS

A full list of Investigators will be available as a separate document.

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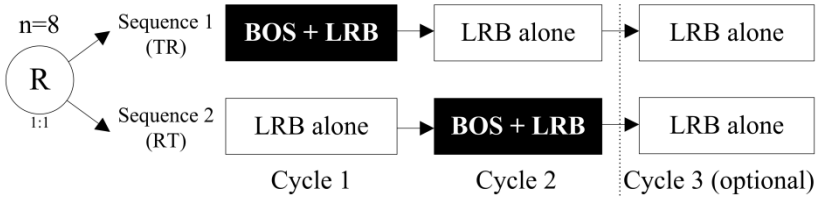
SYNOPSIS

TITLE	An Open-Label, Multicenter Study to Assess the Potential Effects of Bosentan (a moderate CYP3A4 inducer) on the Pharmacokinetics of Lurbinectedin in Patients with Advanced Solid Tumors.
PROTOCOL CODE	PM1183-A-019-20
INVESTIGATORS	A full list of investigators will be available as a separate document.
NUMBER OF SITES/ TRIAL LOCATION	Approximately two sites in Spain are expected to participate in this study.
STUDY OBJECTIVES	**Primary:** • To assess the effect of bosentan on lurbinectedin total plasma exposure in patients with advanced solid tumors. **Secondary:** • To assess the effect of bosentan on lurbinectedin unbound plasma exposure. • To assess the effect of bosentan on lurbinectedin major metabolites (i.e., M1 and M4). • To assess the effect of bosentan on the safety profile of lurbinectedin. • To collect and store a blood sample for germline DNA extraction for future pharmacogenetic (PGt) analysis of variations on genes that may influence exposure and response (i.e., disposition, metabolism and safety) to lurbinectedin.
STUDY DESIGN	Prospective, open-label, two-way crossover, phase Ib drug-drug interaction study in patients with advanced solid tumors. The study will include a pre-treatment (screening) phase followed by a treatment phase consisting of two lurbinectedin cycles, one cycle in combination with bosentan and one cycle as single agent (in different order depending on the study sequence), and one additional third cycle of lurbinectedin as a single agent for patients who meet the continuation criteria and obtain a clinical benefit after the first two cycles, and then follow-up of adverse events if any. Patients who meet the continuation criteria and obtain a clinical benefit after the first two cycles according to the Investigator's criteria will have the opportunity to continue treatment under a Compassionate Use Agreement after the completion of the optional third study cycle. Patients will be treated as outpatients. At the discretion of the Investigator, patients may be admitted to the study center on Day –1 or Day 1 and monitored, at least, until completion of the Day 1 pharmacokinetic (PK) blood sample collections. All patients will receive a maximum of three cycles: two consecutive cycles of lurbinectedin, one cycle with and one cycle without

	bosentan co-administration (in different order depending on the study Sequence 1 or Sequence 2 of treatment), followed by a third cycle with lurbinectedin alone (this last optional for patients with clinical benefit). Lurbinectedin will be administered as a 1-hour (-5/+20 min) intravenous (i.v.) infusion every three weeks (q3wk) via a central or peripheral vein. The dose of lurbinectedin will be 3.2 mg/m² for all patients when administered with and without bosentan. If toxicity occurs, the appropriate intra-patient dose level (DL) reductions will be implemented in the subsequent cycle. This DL reduction will be according to Table S3. Patients will be randomized in a 1:1 ratio (Figure S1) to Sequence 1 (TR: Test-Reference; lurbinectedin + bosentan in Cycle 1) or Sequence 2 (RT: Reference-Test; lurbinectedin + bosentan in Cycle 2). **Figure S1.** Study design.  BOS, bosentan; LRB, lurbinectedin; TR, test-reference; R; randomized; RT, reference-test. The enrollment of the patients will be simultaneous. However, if once the first three patients enrolled have completed Cycle 1 and Cycle 2, total lurbinectedin exposure does not allow an adequate PK assessment and if no unacceptable or life-threatening toxicities have occurred, the dose of lurbinectedin to be co-administered with bosentan in the remaining five patients can be adjusted accordingly. This decision will be made by the Sponsor and the study Investigators. Therefore, the planned dose of lurbinectedin, when given with bosentan for the remaining five patients, will be based on the acceptability of the PK and safety results from the first three patients. If the initial three patients do not experience adverse events (AEs) which might require a dose-reduction, the dose of lurbinectedin may still be adjusted (based on the assumption of dose-proportional pharmacokinetics) to produce plasma lurbinectedin area under the curve (AUC) values that are comparable to those when lurbinectedin is given in the absence of bosentan. However, if toxicity occurs in the initial three patients, the appropriate dose-reduction of lurbinectedin will be implemented in the remaining five patients accordingly. Patients will receive lurbinectedin until disease progression, unacceptable toxicity, consent withdrawal or while it is considered to be in their best interest, and for a maximum of three cycles. Treatment with lurbinectedin outside this study could be continued under a Compassionate Use Agreement after the completion of the optional third study cycle.
STUDY POPULATION **Inclusion criteria**	All patients must fulfill the following inclusion criteria to be enrolled in the study: 1) Voluntary signed and dated written informed consent prior to any specific study procedure.

	2) Male or female with age ≥ 18 years. 3) Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 1 (Appendix 1). 4) Life expectancy > 3 months. 5) Pathologically confirmed diagnosis of advanced solid tumors [except for primary central nervous system (CNS) tumors], for which no approved therapy exists. 6) Recovery to grade ≤ 1 from drug-related adverse events (AEs) of previous treatments, excluding alopecia and grade ≤2 asthenia or fatigue, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE v.5). 7) Laboratory values within fourteen days prior to registration: a) Absolute neutrophil count (ANC) ≥ 2.0 x 10^9/L, platelet count ≥ 120 x 10^9/L and hemoglobin ≥ 9.0 g/dL (patients may be transfused as clinically indicated prior to study entry). b) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x upper limit of normal (ULN). c) Serum total bilirubin ≤ 1.0 x ULN. If total bilirubin is > 1.0 x ULN, but ≤ 1.5 x ULN, direct bilirubin must be ≤ 1.0 x ULN. d) Albumin ≥ 3.5 g/dL. e) Creatinine clearance (CLcr) ≥ 30 mL/min (using Cockcroft and Gault's formula) (Appendix 4). f) Creatine phosphokinase (CPK) ≤ 2.5 x ULN. 8) Left ventricular ejection fraction (LVEF) by echocardiography (ECHO) or multiple-gated acquisition (MUGA) within normal range (according to institutional standards). 9) Evidence of non-childbearing status for women of childbearing potential (WOCBP). WOCBP must agree to use a highly effective contraceptive measure up to six months after treatment discontinuation. Valid methods to determine the childbearing potential, adequate contraception and requirements for WOCBP partners are described in Appendix 2. As bosentan may render hormonal contraceptives ineffective, and taking into account the teratogenic effects observed in animals, hormonal contraceptives cannot be the sole method of contraception during treatment with bosentan. Fertile male patients with WOCBP partners should use condoms during treatment and for four months following the last investigational medicinal product (IMP) dose.
Exclusion criteria	All patients who meet any of the following criteria will be excluded from participating in the study: 1) Concomitant diseases/conditions: a) History or presence of unstable angina, myocardial infarction, congestive heart failure, or clinically significant valvular disease within last year. b) Symptomatic arrhythmia or any uncontrolled arrhythmia requiring ongoing treatment.

	c) Known cirrhosis, alcohol induced steatosis, or chronic active hepatitis. For hepatitis B, this includes positive test for both Hepatitis B surface antigen (HBsAg) and quantitative Hepatitis B polymerase chain reaction (PCR or HVB-DNA+). For hepatitis C, this includes positive test for both Hepatitis C antibody and quantitative Hepatitis C by PCR (or HVC-RNA+). d) History of obstructive cholestatic liver disease (suitable for stenting procedure) or biliary sepsis in the past 2 months. e) Active COVID-19 disease (this includes positive test for SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs or nasal swabs by PCR). 2) Symptomatic, progressive or corticosteroids-requiring documented brain metastases or leptomeningeal disease involvement. Patients with asymptomatic documented stable brain metastases not requiring corticosteroids during the last four weeks are allowed. 3) Use of (strong or moderate) inhibitors or inducers of CYP3A4 activity within three weeks prior to Day 1 of Cycle 1 (Appendix 3). 4) Use of CYP3A4 substrates for which concomitant administration with moderate CYP3A4 inductor is contraindicated (Appendix 3). 5) Treatment with any investigational product within the 30 days before Day 1 of Cycle 1. 6) Women who are pregnant or breast-feeding and fertile patients (men and women) who are not using an effective method of contraception (Appendix 2). 7) Psychiatric illness/social situations that would limit compliance with study requirements.
PATIENTS FOR THE PHARMACOGE-NETIC EVALUATIONS	Only patients who voluntarily sign the written ICF for the pharmacogenetic sub-study will participate in it. Refusal to participate in this sub-study will not affect patient participation in the clinical study PM1183-A-019-20.
EXPECTED NUMBER OF PATIENTS	Eight patients will be enrolled. Additional patient(s) may be enrolled if any patient(s) does not complete the required assessments, including the PK blood sample collections of the first and second cycles.
REPLACEMENT OF PATIENTS	Patients must be replaced if they are not evaluable for the assessment of the primary endpoint (e.g., if they have not sufficient and interpretable PK parameters). All replaced patients who received the study treatment will be included in the general safety analysis.

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<td>STUDY DRUGS FORMULATION</td>
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Bosentan：
Commercially available bosentan film-coated tablets (with strengths of 125 mg) will be provided by the Sponsor.
Lurbinectedin:
Lurbinectedin 4 mg drug product (DP) is presented as a lyophilized powder for concentrate for solution for infusion in 30 mL vials and will be supplied by the Sponsor for the purposes of this study.
Before use, the 4 mg DP should be reconstituted with 8 mL of water for injection to give a solution containing 0.5 mg/mL of lurbinectedin. For administration to patients as an i.v. infusion, reconstituted vials are diluted with glucose 50 mg/mL (5%) solution for infusion or sodium chloride 9 mg/mL (0.9%) solution for infusion. The full composition of the lurbinectedin 4 mg DP and the reconstituted solution per mL is shown in Table S1.
Table S1. Composition of lurbinectedin (PM01183) vials.
<table>
<tr><th>Component</th><th>Concentration/vial</th><th>Concentration/vial after reconstitution</th></tr>
<tr><td>Lurbinectedin</td><td>4.0 mg</td><td>0.5 mg/mL</td></tr>
<tr><td>Sucrose</td><td>800 mg</td><td>100 mg/mL</td></tr>
<tr><td>Lactic acid</td><td>22.08 mg</td><td>2.76 mg/mL</td></tr>
<tr><td>Sodium hydroxide</td><td>5.12 mg</td><td>0.64 mg/mL</td></tr>
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<td>TREATMENT SCHEDULE AND ADMINISTRA-TION ROUTE</td>
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Patients will receive lurbinectedin as a 1-hour (-5/+20 min) i.v. infusion on Day 1 every three weeks (q3wk), over a minimum of 100 mL dilution on 5% glucose or 0.9% sodium chloride via a central line (or a minimum of 250 mL dilution if a peripheral line is used).
Patients will receive a maximum of three cycles: two consecutive cycles of lurbinectedin, one cycle with and one cycle without bosentan co-administration followed by a third cycle with lurbinectedin alone (this last optional for patients with clinical benefit).
In the co-administration cycles, bosentan will be administered orally twice daily in the morning and evening during five consecutive days, self-administered at home from Day -5 to Day -1 (i.e., five days before lurbinectedin infusion), and once daily on Day 1 (i.e., the day of lurbinectedin infusion), following recommendations at the Summary of Product Characteristics (SmPC). On Day 1 (i.e., day of lurbinectedin infusion), bosentan will be given immediately prior to the start of the lurbinectedin infusion. In fact, bosentan should be administered after obtaining the bosentan pharmacokinetic (PK) sample #1 and before the start of lurbinectedin infusion (-15 min to -1 min).
In case of lurbinectedin delay (≤2 days), bosentan could be administered twice daily during a maximum of seven consecutive days before lurbinectedin infusion, and supplied at the study center once daily on Day 1 (before lurbinectedin infusion).
All patients will be randomly assigned to the corresponding sequences:
<u>Sequence 1 (TR)</u>:

Cycle 1: Bosentan + lurbinectedin
Cycle 2: Lurbinectedin alone
Cycle 3: Lurbinectedin alone (optional)

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	Sequence 2 (RT): • Cycle 1: Lurbinectedin alone • Cycle 2: Bosentan + lurbinectedin • Cycle 3: Lurbinectedin alone (optional)
DOSE	**Bosentan co-administration cycle:** • Bosentan: 125 mg (one film-coated tablet of 125 mg) orally (p.o.) twice daily in the morning and in the evening during the prior five consecutive days before the day of lurbinectedin infusion (Day 1), and once daily on Day 1 (before lurbinectedin infusion). • Lurbinectedin: 3.2 mg/m² as a 1-hour (-5/+20 min) i.v. infusion on Day 1 in first three patients. Dose for remaining five patients will depend on PK and safety outcomes in first three patients. **Single agent lurbinectedin cycle:** 3.2 mg/m² as a 1-hour (-5/+20 min) i.v. infusion on Day 1.
PROPHYLACTIC MEDICATION	All patients, will receive standard antiemetic prophylaxis before each treatment infusion (i.e., 30 ± 5 minutes before lurbinectedin administration), i.v. as follows: • Corticosteroids i.v. (according to institutional standard antiemetic doses, at least dexamethasone i.v. 8 mg, or equivalent, maximum 20 mg/day of dexamethasone or equivalent). If feasible, the medication and dose administered in Cycle 1 should be maintained in Cycle 2. • Serotonin (5-HT_3) antagonists (ondansetron at least 8 mg i.v. and no more than 16 mg of ondansetron). If necessary, in addition to the above, the duration of treatment with 5-HT_3 antagonists and/or dexamethasone could be extended orally (if needed) (i.e., 4-8 mg/day for three consecutive days) and/or 10 mg of metoclopramide orally every eight hours could be added. **Aprepitant or any other NK-1 antagonist or related Substance P-antagonists (except for rolapitant) are forbidden while on lurbinectedin treatment.**
ALLOWED MEDICATIONS/ THERAPIES	• Therapies for pre-existing and treatment-emergent medical conditions, including pain management and local management of mucositis/stomatitis. • Blood products and transfusions, as clinically indicated. • Bisphosphonates. • In case of nausea or vomiting, extended symptomatic treatment for emesis will be allowed (with the exception of aprepitant and equivalent agents) • Erythropoietin treatment according to the American Society of Clinical Oncology (ASCO) guidelines. • Low-molecular weight heparin (LMWH) and/or any other anticoagulants, as clinically indicated. Oral anticoagulants must be carefully monitored. • Treatment or secondary prophylaxis with granulocyte colony-stimulating factors (G-CSF) according to ASCO guidelines.

	• CNS irradiation if required, and/or limited field bone radiotherapy for pain control outside the thoracic wall. • Megestrol acetate for appetite stimulation. • Contraceptives. As bosentan may render hormonal contraceptives ineffective, and taking into account the teratogenic effects observed in animals, hormonal contraceptives cannot be the sole method of contraception during treatment with bosentan.
PROHIBITED MEDICATIONS/ THERAPIES	• Concomitant administration of any other antineoplastic therapy. • Any other investigational agents. • Immunosuppressive therapies other than corticosteroids for antiemetic prophylaxis or pain control, or low-dose replacement in patients requiring this approach. • Aprepitant or any other NK-1 antagonist or related Substance P-antagonists (except for rolapitant). • Primary G-CSF prophylaxis. • CYP3A4 inhibitors such as ketoconazole, fluconazole, voriconazole, telithromycin, clarithromycin, erythromycin, nafcillin, aprepitant, fosaprepitant, verapamil, modafinil, nefazodone, or grapefruit juice. • CYP3A enzyme inducers and/or inhibitors (unless strictly necessary and when there is no therapeutic alternative treatments) (see Appendix 3). • Use of any prescription or non-prescription herbal and/or dietary supplements within 14 days prior to the first dose of study medication and until 31 days after the last administration of lurbinectedin, unless the Investigators, with the Sponsor agreement, consider it will not interfere with study procedures of patient safety.
DRUG-DRUG INTERACTIONS	*In vitro* studies with human microsomes have shown that CYP3A4 is the major CYP isoform involved in the metabolism of lurbinectedin. The estimated contribution of the other CYP isoenzymes to the lurbinectedin metabolism is considered to be negligible. Therefore, concomitant drugs which induce or inhibit any of these cytochromes, especially CYP3A4, should be carefully monitored or avoided, whenever is possible. A potentially significant interaction with aprepitant is suggested by available phase II data from ovarian cancer patients and phase I data from a combination trial (PM1183-A-008-13) with lurbinectedin and cisplatin. Lurbinectedin clearance was reduced by around 30%, approximately, in the presence of aprepitant. Although all patients eventually recovered, the use of aprepitant is currently forbidden in all phase II/III lurbinectedin studies.
EVALUABILITY OF PATIENTS	An evaluable patient for the main objective of the study (e.g., assessment of lurbinectedin PK) should have provided sufficient and interpretable PK parameters (e.g., AUC_{0-t} should cover at least 80% of $AUC_{0-\infty}$) of Cycle 1 and 2. Evaluable patients should have received

	the first two complete cycles regardless dose delays or reductions. The compliance of bosentan will be confirmed based on a patient's diary, the drug accountability and the expected individual plasma concentration at the steady state.
EVALUATION CRITERIA **Primary endpoint**	**<u>Plasma exposure to lurbinectedin</u>:** Plasma dose-normalized C_{max} and $AUC_{0-\infty}$ of lurbinectedin will be compared between Cycle 1 and Cycle 2. Pharmacokinetic analyses will be evaluated in plasma by standard non-compartmental methods, or population methods, if necessary.
Secondary endpoints	• **<u>Secondary PK parameters:</u>** o Differences in dose-normalized total AUC_{0-t} and C_{max} and in Cl, V_{ss} and $T_{1/2}$ of lurbinectedin between Cycle 1 and Cycle 2 will be explored. o Differences in dose-normalized unbound $AUC_{u,0-\infty}$, $AUC_{u,0-t}$ and $C_{u,max}$ and in CL_u, $V_{ss,u}$ and $T_{1/2,u}$ of lurbinectedin between Cycle 1 and Cycle 2 will be explored. o Differences in ratios between total $AUC_{0-\infty}$, AUC_{0-t} and C_{max}, of main lurbinectedin metabolites relative to parent drug between Cycle 1 and Cycle 2 will be explored. Additional PK parameters will be calculated if deemed appropriate. • **<u>Safety</u>:** patients will be evaluable for safety if they have received at least one partial or complete infusion of lurbinectedin. AEs, serious adverse events (SAEs) and laboratory abnormalities will be graded according to the NCI-CTCAE v.5. Additionally, treatment compliance, in particular dose reductions requirements and/or treatment delays due to AEs, and reasons for treatment discontinuation will also be described. • **<u>Pharmacogenetics:</u>** the presence or absence of PGt polymorphisms in genes relevant for lurbinectedin disposition (distribution, metabolism and excretion) from a single blood sample collected at any time during the trial (but preferably at the same time as the pre-treatments PK sample on Day 1 of Cycle 1), which will be stored to explain individual variability in main PK parameters in future analyses.
CRITERIA FOR TREATMENT CONTINUATION	Patients may continue treatment with lurbinectedin as long as no unacceptable toxicity and/or progression of the disease occurred, and for a maximum of three cycles. Thereafter, treatment with lurbinectedin can continue under a Compassionate Use Agreement. • **<u>Patients assigned to Sequence 1 (TR)</u>** (lurbinectedin + bosentan in Cycle 1), after Cycle 1 the administration of a new cycle should be delayed if the criteria in Table S2 are not met on the corresponding Day 1. Re-assessments will be performed periodically at intervals of at least 72 hours but not more than one week. Lurbinectedin administration should be delayed until recovery of these parameters. A maximum delay of 14 days from the theoretical due date will be allowed for recovery from treatment-related adverse events. If recovery has not occurred

after that period, the patient should discontinue the treatment, except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor.

- **Patients assigned to Sequence 2 (RT)** (lurbinectedin + bosentan in Cycle 2), criteria for treatment continuation should be investigated on Day 15 of Cycle 1 in order to confirm bosentan administration start on Day -5 of Cycle 2, assuring that the continuation criteria will be finally met on Day 1 of Cycle 2 assessment. In case of detected abnormalities on Day 15 in sequence RT, these assessments should be repeated every 48-72 hours until recovery to retreatment criteria. Initiation of bosentan will be delayed for a maximum of 14 days to ensure that pre-treatment with bosentan is fulfilled. If recovery has not occurred after that period, the patient should discontinue the treatment, except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor.

  Re-treatment criteria for lurbinectedin administration should be met on Day 1 of Cycle 2. If they were not met, treatment will be delayed for a maximum of 2 days to evaluate continuation of lurbinectedin administration, with re-treatment criteria being checked every 24 hours. During this time the patient will continue receiving bosentan. If after 2 days of delay the patient does not meet the criteria for treatment continuation, bosentan will be stopped and the patient will be considered not evaluable for the trial.

Table S2. Criteria for lurbinectedin treatment continuation.

Variable	Day 1
ECOG PS	≤ 1
ANC	≥ 1.5 x 10^9/l
Platelets	≥ 100 x 10^9/l
Hemoglobin [a]	≥ 8.0 g/dl
Total bilirubin	≤ 1.0 x ULN. If it is > 1.0 x ULN but ≤ 1.5 x ULN, direct bilirubin must be ≤ 1.0 x ULN
Albumin	≥ 3 g/dl
AST / ALT	< 3.0 x ULN
CPK	≤ 2.5 x ULN (≤ 5.0 x ULN is acceptable if elevation is disease-related)
Serum Creatinine levels/ Calculated CLcr (Cockcroft and Gault's formula)	Serum creatinine levels ≤ ULN or calculated CLcr ≥ 30 mL/min, if abnormal (>ULN) serum creatinine levels
Other non-hematological drug-related AEs (except isolated increased GGT and/or AP; or grade 2 alopecia, asthenia, peripheral neuropathy and not optimally treated nausea and/or vomiting)	Grade ≤ 1

[a] Patients may receive PRBC transfusion and/or EPO treatment if clinically indicated to increase/maintain adequate hemoglobin levels.

Sequence 1 (TR):
If a patient does not meet the requirements for treatment continuation on Day 1 of further cycles, treatment will be withheld until recovery for a maximum of 14 days after the theoretical treatment date. If recovery has not occurred after a delay of > 14 days, discontinue treatment (except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor).

Sequence 2 (RT):
If a patient does not meet the requirements for treatment continuation on Day 15 of Cycle 1, treatment with bosentan will be withheld until recovery for a maximum of 14 days after the theoretical treatment date. If recovery has not occurred after a delay of > 14 days, discontinue treatment (except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor).
If a patient does not meet the requirements for treatment continuation on Day 1 of Cycle 2, treatment with lurbinectedin will be withheld until recovery for a maximum of 2 days after the theoretical treatment date. During this time, the patient will continue receiving bosentan. If recovery has not occurred after a delay of > 2 days, bosentan will be stopped and the patient will be considered not evaluable for the trial.

AEs, adverse events; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AP, alkaline phosphatase; AST, aspartate aminotransferase; CLcr, creatinine clearance; CPK, creatine phosphokinase; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EPO, erythropoietin; GGT, gamma glutamyltransferase; PRBC, packed red blood cells; ULN, upper limit of normal.

DOSE REDUCTION

Patients may continue in the study treatment at a lurbinectedin reduced dose if they present any of the following:

- Grade 4 thrombocytopenia or grade 3 thrombocytopenia concomitantly with grade ≥ 3 bleeding.
- Two dose delays or prolonged (> one week) dose delay due to treatment-related adverse events.
- Grade ≥ 3 treatment related non-hematological toxicity. Exceptions are: grade ≥ 3 nausea and/or vomiting not optimally treated, grade 3 fatigue lasting < two days, grade 3 diarrhea lasting < one day or non-optimally treated, isolated grade 3 transient ALT/AST elevations which are rapidly reversible and not leading to subsequent delays, and non-clinically relevant isolated biochemical abnormalities.
- Grade 4 neutropenia or any grade febrile neutropenia.

Up to two lurbinectedin dose reductions are allowed per patient. Patients who continue to experience treatment-related toxicity and/or dose delays after two dose reductions must be withdrawn from the study. Once the dose has been reduced for an individual patient, it will not be re-escalated under any circumstances. Lurbinectedin dose reduction levels are shown in Table S3.

Table S3. Levels of (intra-patient) lurbinectedin dose reduction in subsequent cycles.

Dose reduction	Lurbinectedin (q3wk) (mg/m^2)	
	Lurbinectedin alone	Bosentan co-administration cycle
1 (starting dose)	3.2	3.2
-1	2.6	2.6
-2	2.0	-

Lurbinectedin total doses in mg will be rounded to the first decimal, if necessary.
q3wk, every three weeks.

Patients who experience grade 3/4 hypersensitivity reactions and/or extravasation reactions will be withdrawn from the study treatment.

PHARMACOKINETIC EVALUATIONS

The plasma PK of lurbinectedin will be evaluated during the first two cycles with a schedule of 11 samples (see Table S4) in all patients. The plasma PK of bosentan will be evaluated during the bosentan co-administration cycle with a schedule of four samples (see Table S4) in

	all patients. **Table S4.** Lurbinectedin and bosentan blood sampling schedule and aliquot collection.

Sample No.	Day	Time (h) [c]	Sampling Time [d]	Lurbinectedin			Bosentan [e]	Time window
				Total	Unbound (f_u)	Metabo-lites		
#1 [a, b]	1	0	Preinfusion [f]	●	●	●	●	-15 to -1 min before SOI [g]
#2	1	0.917	5 min before EOI	●	●	●	-	±4 min
#3	1	1.5	30 min after EOI	●	-	●	-	± 5 min
#4	1	2	1 h after EOI	●	-	●	●	± 10 min
#5	1	3	2 h after EOI	●	-	●	-	± 10 min
#6	1	5	4 h after EOI	●	-	●	●	± 30 min
#7	1	7	6 h after EOI	●	-	●	-	± 30 min
#8	2	25	24 h after EOI	●	-	●	●	±2 h
#9	3	49	48 h after EOI	●	-	-	-	±2 h
#10	5	97	96 h after EOI	●	-	-	-	±24 h
#11	8	169	168 h after EOI	●	-	-	-	±48 h [h]

[a] Additional sample for AAG and IL-6.
[b] Additional sample for PGt, once only, if written IC given.
[c] Time relative to infusion start.
[d] Time relative to lurbinectedin end of infusion (except PK #1).
[e] Sampling for bosentan only applies in cycle with bosentan co-administration.
[f] 5 min before start of infusion.
[g] Bosentan should be administered after bosentan PK sample #1 and before the start of lurbinectedin infusion (-15 min to -1 min).
[h] If the scheduled sampling time for this sample should be modified within the allowed time window, there must be a difference of at least 20 hours between the collection times of samples PK#10 and PK#11.
AAG, alpha 1-acid glycoprotein; EOI, end of infusion; f_u, unbound fraction; h, hour; IC, informed consent; IL-6, Interleukin 6; EOI, end of infusion; min, minute; PGt, pharmacogenetic; PK, pharmacokinetic; SOI, start of infusion.

Pharmacokinetic parameters will be calculated using non-compartmental analysis (NCA).

UNBOUND FRACTION EVALUATION	On Day 1, plasma samples will be taken along with sample #1 (preinfusion) and sample #2 (5 min. before EOI) for the evaluation of lurbinectedin unbound fraction (f_u).
PHARMACOGE-NETICS	In order to explore factors that may help explain individual variability in the main PK parameters, the presence or absence of germline mutations or polymorphisms will be analyzed in leukocyte DNA extracted from a blood sample obtained at any time during the study, but preferably before infusion start along with PK sample #1 on Day 1 of Cycle 1.
STATISTICAL METHODS	This clinical pharmacology study is designed to assess the impact of bosentan co-administration on lurbinectedin PK parameters administered alone. **Analysis populations:** The PK population will include all patients enrolled who have sufficient and interpretable PK parameters to calculate the non-compartmental PK parameters. Only the patients who have completed the two cycles and have sufficient and interpretable PK assessments will be included in the statistical comparison to assess the effect of

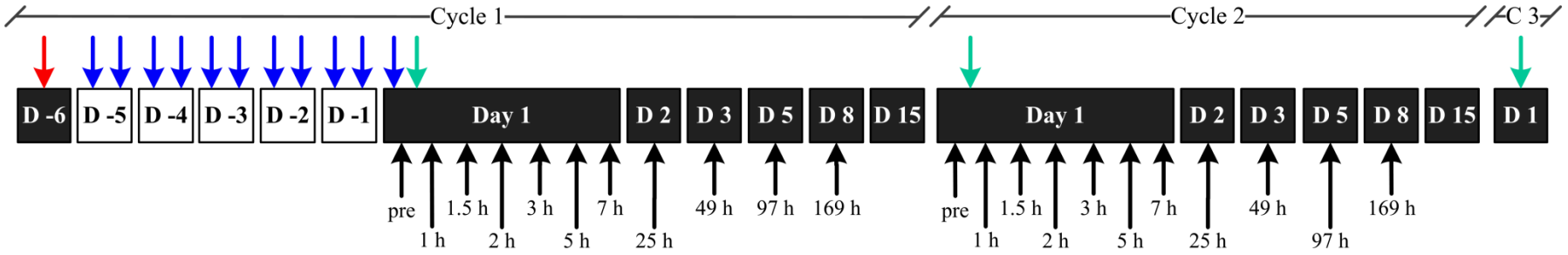
	bosentan on the PK of lurbinectedin. The safety population will include all patients who received at least one dose of lurbinectedin. Patients who have received at least one dose of bosentan but who did not receive any dose of lurbinectedin will be excluded from the safety population. The analysis of data from these patients will be performed separately (e.g., by means of narratives). The safety population will be used for all safety evaluations. **Sample size:** A block randomization (1:1 ratio) will be performed. At least 8 patients are expected to complete all study procedures, including the collection of sufficient and interpretable PK assessments. Although at least 8 patients will be enrolled in this study; it is estimated that complete data from eight patients will be sufficient to estimate the drug-drug interaction of bosentan on the PK of lurbinectedin. In case of patient replacement, a new patient number will be provided and he/she will be assigned to the same sequence as the patient being replaced. This study was designed to assess the potential effects of bosentan on the PK of lurbinectedin in patients with advanced malignancies. A sample size of eight patients was based on feasibility and clinical considerations. Based on previous studies, the intra-subject coefficient of variation (CV) of lurbinectedin PK parameters is estimated to be more than 30%. The precision (half-width) of the 90% CI for [(lurbinectedin + bosentan) / lurbinectedin alone] comparison on the log-scale will extend 0.389 from the observed differences in means, assuming that the intra-subject CV around 40%. This half-width corresponds to a 90% CI in the range of 70% and 147% assuming the ratio of the means equal to unity for each PK parameter. This 90% CI will be used to help with the interpretation of the results. **Plasma exposure to lurbinectedin:** Only patients who completed the study with sufficient and interpretable PK parameters will be included in the statistical comparison of plasma exposure to lurbinectedin. Descriptive statistics will be used to summarize pharmacokinetic data. The primary parameter of interest for the statistical analysis will be plasma dose-adjusted $AUC_{(0-\infty)}$ (if data do not permit so, $AUC_{(0-t)}$ will be used) of lurbinectedin. The analysis will compare the log-transformed AUC for lurbinectedin administered in combination with bosentan (Test) to the lurbinectedin alone (Reference). A mixed-effects model will be fit to the data with log-transformed AUC as the dependent variable, treatment (Test or Reference), period and sequence as fixed effects, and patient (sequence) as a random effect. The estimated least square means and intrasubject variability from the mixed-effects model will be used to construct 90% CIs for the difference in means on the log scale between treatments (Test or Reference). The adjusted mean differences and the 90% CIs will be exponentiated to obtain estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CIs for the ratios. A large difference (e.g. two-fold difference shown from CIs and least square means) will be considered to show evidence on whether bosentan co-administration has a clinically relevant effect on lurbinectedin exposure.

	<u>Secondary PK parameters</u>: Similar models used for the primary endpoint will be fit to the data with dose-normalized $AUC_{(0-t)}$ and C_{max} and in Cl, V_{ss} and $T_{1/2}$ of lurbinectedin or metabolite to parent exposure PK parameters ratio, as the dependent variable. Results will also be assessed graphically. **<u>Plasma protein binding</u>:** A similar model used for the primary endpoint will be fit to the data with dose-normalized AUC_u as the dependent variable. **<u>Safety</u>:** Descriptive statistics will be used to characterize the profiles of drug-related AEs, drug-related deaths, SAEs, clinical laboratory data, drug-related delays and/or treatment discontinuations. All AEs will be graded according to NCI-CTCAE v.5. Tables will be displayed by sequence and treatment (Test or Reference). **<u>Pharmacogenetics</u>:** Descriptive statistics will be used to summarize pharmacogenetic data at the time of planned analysis.
PLANNED TRIAL PERIODS (individually per patient)	Patients will be evaluated at scheduled visits in three study periods: • **Pre-treatment (screening):** from signature of the ICF to the day of first administration of the study treatment (bosentan or lurbinectedin). • **Treatment:** from first administration of the study treatment (bosentan or lurbinectedin) to the last dose of lurbinectedin plus 31 days (end-of-treatment, EOT) (maximum of three cycles). • **Follow-up:** after EOT, patients with treatment-related toxicities > grade 2 will be followed every four weeks until recovery to at least grade 1 or stabilization of all treatment-emergent AEs, or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first. Patients will be considered to be **on-study** from the signature of the ICF to the end of the follow-up period. Patients will be considered to be **on-treatment** for the duration of their treatment and until the day of EOT, immediately before the start of the follow-up period. An end-of-treatment (EOT) visit will be performed within 31 days (±10 days) after administration of the last dose of lurbinectedin, unless the patient dies or starts any new antitumor therapy outside this clinical study, in which case the EOT visit should be performed immediately before the start of the new therapy (ideally the day before or the same day).

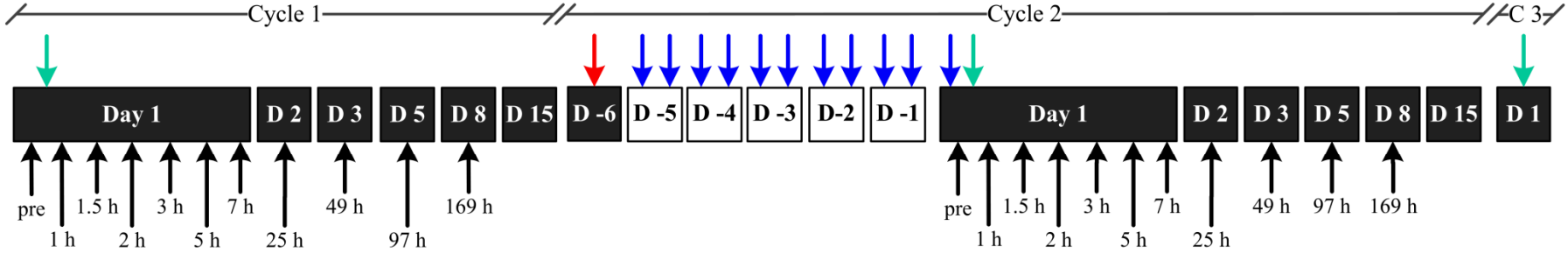
PLANNED TRIAL PERIODS (for the whole study)	The total duration of the study will be approximately 22 months, including approximately an 18-month enrollment period. **Planned start date** (first patient on study): 4Q2020. **Planned enrollment period**: approximately 18 months. **Planned end-of-study date** (clinical cutoff): 31 (±10) days after the date of the last lurbinectedin administration for last patient /last cycle, or when confirmation of evaluability of at least eight patients, whichever occurs last. Patients who meet the continuation criteria and obtain a clear clinical benefit with lurbinectedin after the completion of the first two study cycles, according to the Investigator's criteria may continue treatment outside this study under a Compassionate Use Agreement after the completion of the optional third study cycle. Should the patient continue under a Compassionate Use Agreement, the treating center must request authorization to the relevant Health Authorities and notify the Sponsor in due time.

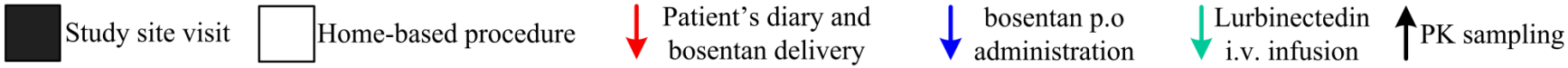
SCHEMATIC OF TRIAL DESIGN

Sequence 1 (TR)



Sequence 2 (RT)





C, cycle; D, day; h, hour; PK, pharmacokinetics; RT, reference-test sequence; TR, test-reference sequence.

Note: Please, be aware that in Sequence 2 (RT) the Day 15 (D15) of Cycle 1 and Day -6 (D-6) of Cycle 2 represents the same day/visit in absence of delays.

SCHEDULE OF ASSESSMENTS AND PROCEDURES

SEQUENCE 1 (TR):

Study Period►	Screening (days prior to registration)	Cycle 1												Cycle 2						Cycle 3	End of Treatment	Follow-up AEs
Cycle Day►		-6	-5	-4	-3	-2	-1	1*	2	3	5	8	15	1	2	3	5	8	15	1	31 (±10)	
Written IC	(Before any study procedure)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Demographic data	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Medical history/Baseline condition	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Primary diagnosis/Prior treatment(s)	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Complete physical examination, including weight, height and BSA [a]	-14	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	X	-
ECOG PS [a]	-14	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	X	-
Vital signs (HR, BP, Tª) [a]	-14	-	-	-	-	-	-	X	-	-	-	X	-	X	-	-	-	X	-	X	-	-
Hematology [b]	-14	-	-	-	-	-	-	X	-	-	-	X	X	X	-	-	-	X	X	X	X	-
Coagulation (INR) [c]	-14	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Biochemistry-A [b]	-14	-	-	-	-	-	-	X	-	-	-	X	X	X	-	-	-	X	X	X	X	-
Biochemistry-B [b]	-14	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	X	-
Calculated CLcr (Cockcroft and Gault´s formula) [b, d]	-14	-	-	-	-	-	-	X	-	-	-	X	X	X	-	-	-	X	X	X	X	-
Detection of SARS-CoV-2 (COVID-19) by PCR	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pregnancy test (serum β-hCG) (only if the patient is a WOCBP)	-14	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	X	-
ECG [e]	-14	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	-	X	-
LVEF (by ECHO or MUGA) [f]	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Randomization [g]	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bosentan delivery and patient's diary [h]	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prophylactic medication [i]	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	-	-
Lurbinectedin 1h (-5/+20 min) infusion	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	-	-
Bosentan oral administration	-	-	X	X	X	X	X	X [j]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Contact to patients (via telephone or other) [k]	-	-	X	X	X	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AAG blood sample collection	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	-	-	-
IL6 blood sample collection	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	-	-	-	-

Study Period ►	Screening (days prior to registration)	Cycle 1												Cycle 2						Cycle 3	End of Treatment	Follow-up AEs
Cycle Day ►		**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**1***	**2**	**3**	**5**	**8**	**15**	**1**	**2**	**3**	**5**	**8**	**15**	**1**	**31 (±10)**	
PGt blood sample collection (only if written IC given) [l]	-	-	-	-	-	-	-	One blood sample collected at any time during the study, but preferably just before lurbinectedin treatment start in Cycle 1												-	-	-
Lurbinectedin PK blood sample collection [m]	-	-	-	-	-	-	-	X	X	X	X	X	-	X	X	X	X	X	-	-	-	-
Bosentan PK blood sample collection [m]	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-
AEs [n]	Continuous [o]																					X [p]
Intercurrent events, concomitant disease and therapies	Continuous																					-

Registration = confirmation of eligibility and inclusion; Day 1 = the day of lurbinectedin administration (with infusion start) of cycle 1 or 2; Day 8 = seven days after the lurbinectedin administration of cycle 1 or 2 (±2 days); Day 15 = fourteen days after the lurbinectedin administration of cycle 1 or 2 (±2 days).

*** <u>Permitted windows for assessments *before* Day 1 of Cycle 1</u>**:
If physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Coagulation were assessed >10 days before Day 1 of Cycle 1, the tests must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).
If pregnancy test was done >7 days before Day 1 of Cycle 1, the test must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).
A patient should only be treated if eligibility criteria are still met according to assessments performed closest to treatment start.

<u>Permitted windows for assessments *after* Day 1 of Cycle 1</u>:
A ± 2-day time window will be allowed for Hematology, Biochemistry-A and CLcr (provided only if serum creatinine levels are above ULN) on Day 8 and Day 15, and for vital signs on Day 8 of Cycle 1.
A -1 day window will be allowed for physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, and Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Pregnancy test on Day 1 of Cycle 2 and further cycles (always prior to lurbinectedin infusion).
A + 2 days window for Day 1 lurbinectedin administration of Cycle 3.
If a drug administration is delayed beyond the permitted protocol window, assessments planned for the original administration day must be repeated on the day the dose is finally administered.

[a] Should treatment be administered more than ten days after the screening tests, repeat these tests prior to the first infusion and if clinically indicated. Height needs to be measured at baseline only. BSA will not be reported at the EOT. BSA calculated according to DuBois formula (see Appendix 4).
[b] Any patient having any grade ≥ 3 laboratory abnormalities and/or treatment-related AEs is suggested to have the relevant tests re-assessed at intervals of at least 72 hours but not more than one week until recovery to at least grade 2 has been documented.
[c] Repeat during treatment period and at EOT if clinically indicated.
[d] Calculated CLcr [Cockcroft and Gault's formula] should be provided only if serum creatinine levels are above ULN.
[e] ECG should be done in triplicate (at screening, Day 1 and EOT visit) and allow rhythm definition (at least 30 seconds of duration), PR interval and QT interval (raw and corrected by HR using Bazett's formula). On Day 1, 5 minutes before SOI (-25 to -1 min before preinfusion PK samples collection) and 1 hour after EOI (-15 to -1 min before the PK sample collection), both will be done in triplicate.
[f] A previous LVEF assessment could be used if was made within 28 days prior to screening and will be repeated if clinically indicated, and EOT.
[g] Randomization apply for all patients.
[h] Bosentan for self-administration and the patient's diary should be given to the patient on Day -6 with a -2/+1 day time window (i.e., from Day -8 to Day -5).
[i] Corticosteroids i.v. (according to institutional standard antiemetic doses, minimum dose of 8 mg/day and maximum dose of 20 mg/day of dexamethasone sodium phosphate or equivalent); If feasible, anti-emetic medication and dose administered in Cycle 1 should be maintained in Cycle 2. Serotonin ($5\text{-}HT_3$) antagonists (ondansetron at least 8 mg i.v. and no more than 16 mg of ondansetron) (i.e., 30 ± 5 minutes before lurbinectedin administration).
[j] If patient does not fulfill inclusion criteria (Cycle 1 in TR sequence) in Day 1 assessments and lurbinectedin administration is postponed no more than two days, bosentan should not be discontinued.

Study Period►	Screening (days prior to registration)	Cycle 1												Cycle 2						Cycle 3	End of Treatment	Follow-up AEs
Cycle Day►		-6	-5	-4	-3	-2	-1	1*	2	3	5	8	15	1	2	3	5	8	15	1	31 (±10)	

[k] The study center will contact the patients (via telephone or other methods) to remind them or confirm that they have taken the morning and evening dose of bosentan when they are not at the study center.

[l] Blood sample for genotyping will be collected at any time during the study but preferably just before infusion start in Cycle 1, only if written IC for the pharmacogenetic sub-study is given.

[m] Serial peripheral venous blood samples for determination of lurbinectedin (total and unbound), lurbinectedin metabolites (i.e., M1 and M4) and bosentan plasma concentrations will be collected at the time points indicated in Section 8.1.1, Table 9.

[n] Clinical assessment of the patient's signs and symptoms (including nurses' assessment and patient-reported issues) should continue on an ongoing basis throughout the study and be reported at least every cycle as AEs. Only information on SAEs that occurred after signature of the informed consent and up to Day -5 is required before treatment start. Grading should be as per the NCI-CTCAE v.5. Signs and symptoms will be assessed by the Sponsor as all the events ongoing during the screening period, except for prior history conditions, which should be reported in the medical history form. Ongoing events during the screening period are those present at any time between the informed consent date and the first drug administration, regardless of if they may be resolved or not at the date of Cycle 1 Day 1.

[o] The "AE form" will be used only for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines); and to report 'ongoing' baseline conditions in case of any significant change (improvement or worsening) during the study.

[p] Patients will be followed every four weeks until recovery to at least grade 1 or stabilization of all treatment-emergent AEs, or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first.

Hematology: Differential WBC (including neutrophils, monocytes and lymphocytes), erythrocytes, hemoglobin, hematocrit and platelets.
Coagulation: INR.
Biochemistry A: AST, ALT, AP, lactate dehydrogenase (LDH), creatinine, glucose, creatine phosphokinase (CPK), CPK-MB fraction (if CPK is elevated above the normal range) and gamma glutamyltransferase (GGT) and total bilirubin (direct bilirubin if total bilirubin is above the ULN).
Biochemistry B: Serum electrolytes (Na+, K+, Mg++), total calcium, total proteins, albumin, C-reactive protein (CRP).

AAG, alpha-1 acid glycoprotein; AE, adverse events; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; β-hCG, beta subunit of human chorionic gonadotropin; BP, blood pressure; BSA, body surface area; CLcr, Creatinine clearance; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase-isoenzyme MB; CRP, C-reactive protein; D, day; ECG, electrocardiogram; ECHO, echocardiogram; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EOT, end of treatment; GGT, gamma glutamyltransferase; HR, heart rate; IC, informed consent; ICF, informed consent form; ICH, International Conference on Harmonization; IL6, interleukin 6; INR, International Normalized Ratio; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; min, minutes; MUGA, multiple-gated acquisition scan; NCI-CTCAE, National Cancer Institute Common Toxicity Criteria; PCR, polymerase chain reaction; PGt; Pharmacogenetic; PK, pharmacokinetics; TR, test-reference sequence; SAE, serious adverse event; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SOI, start of infusion; T^a, temperature; ULN, upper limit of normal; WBC, white blood cells; WOCBP, women of childbearing potential.

SEQUENCE 2 (RT):

Study Period►	Screening (days prior to registration)	Cycle 1						Cycle 2											Cycle 3	End of Treatment	Follow-up AEs
Cycle Day►		1*	2	3	5	8	15	-5	-4	-3	-2	-1	1	2	3	5	8	15	1	31 (±10)	
Written IC	(Before any study procedure)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Demographic data	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Medical history/Baseline condition	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Primary diagnosis/Prior treatment(s)	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Complete physical examination, including weight and BSA [a]	-14	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
ECOG PS [a]	-14	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
Vital signs (HR, BP, Tª) [a]	-14	X	-	-	-	X	-	-	-	-	-	-	X	-	-	-	X	-	X	-	-
Hematology [b]	-14	X	-	-	-	X	X	-	-	-	-	-	X	-	-	-	X	X	X	X	-
Coagulation (INR) [c]	-14	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Biochemistry-A [b]	-14	X	-	-	-	X	X	-	-	-	-	-	X	-	-	-	X	X	X	X	-
Biochemistry-B [b]	-14	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
Calculated CLcr (Cockcroft and Gault´s formula) [b, d]	-14	X	-	-	-	X	X	-	-	-	-	-	X	-	-	-	X	X	X	X	-
Detection of SARS-CoV-2 (COVID-19) by PCR	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pregnancy test (serum β-hCG) (only if the patient is a WOCBP)	-14	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
ECG [e]	-14	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	X	-
LVEF (by ECHO or MUGA) [f]	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
Randomization [g]	-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bosentan delivery and patient's diary [h]	-	-	-	-	-	-	X		-	-	-	-	-	-	-	-	-	-	-	-	-
Prophylactic medication [i]	-	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
Lurbinectedin 1h (-5/+20 min) infusion	-	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
Bosentan oral administration	-	-	-	-	-	-	-	X	X	X	X	X	X [j]	-	-	-	-	-	-	-	-
Contact to patients (via telephone or other) [k]	-	-	-	-	-	-	-	X	X	X	X	X	-	-	-	-	-	-	-	-	-
AAG blood sample collection	-	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-
IL6 blood sample collection	-	X	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-
PGt blood sample collection (only if written IC given) [l]	-	One blood sample collected at any time during the study, but preferably just before lurbinectedin treatment start in Cycle 1																		-	-

Study Period ▶	Screening (days prior to registration)	Cycle 1						Cycle 2											Cycle 3	End of Treatment	Follow-up AEs
Cycle Day ▶		**1***	**2**	**3**	**5**	**8**	**15**	**-5**	**-4**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**5**	**8**	**15**	**1**	**31 (±10)**	
Lurbinectedin PK blood sample collection [m]	-	X	X	X	X	X	-	-	-	-	-	-	X	X	X	X	X	-	-	-	-
Bosentan PK blood sample collection [m]	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-
AEs [n]	Continuous [o]																				X [p]
Intercurrent events, concomitant disease and therapies	Continuous																				-

Registration = confirmation of eligibility and inclusion; Day 1 = the day of lurbinectedin administration (with infusion start) of cycle 1 or 2; Day 8 = seven days after the lurbinectedin administration of cycle 1 or 2 (±2 days); Day 15 = fourteen days after the lurbinectedin administration of cycle 1 o 2 (±2 days).

*** <u>Permitted windows for assessments *before* Day 1 of Cycle 1</u>**:
If physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Coagulation were assessed >10 days before Day 1 of Cycle 1, the tests must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).
If pregnancy test was done >7 days before Day 1 of Cycle 1, the test must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).
A patient should only be treated if eligibility criteria are still met according to assessments performed closest to treatment start.

<u>Permitted windows for assessments *after* Day 1 of Cycle 1</u>:
A ± 2-day time window will be allowed for Hematology, Biochemistry-A and CLcr (provided only if serum creatinine levels are above ULN) on Day 8 and Day 15, and for vital signs on Day 8 of Cycle 1.
A -1 day window will be allowed for physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, and Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Pregnancy test on Day 1 of Cycle 2 and further cycles (always prior to lurbinectedin infusion).
A + 2 days window for Day 1 lurbinectedin administration of Cycle 3.
If a drug administration is delayed beyond the permitted protocol window, assessments planned for the original administration day must be repeated on the day the dose is finally administered.

[a] Should treatment be administered more than ten days after the screening tests, repeat these tests prior to the first infusion and if clinically indicated. Height needs to be measured at baseline only. BSA will not be reported at the EOT. BSA calculated according to DuBois formula (see Appendix 4).
[b] In case of detected abnormalities on Day 15 in sequence RT, these assessments should be repeated every 48-72 hours until recovery to retreatment criteria. Initiation of cycle 2 will be delayed accordingly in order to ensure that pre-treatment with bosentan is fulfilled.
[c] Repeat during treatment period and at EOT if clinically indicated.
[d] Calculated CLcr [Cockcroft and Gault's formula] should be provided only if serum creatinine levels are above ULN.
[e] ECG should be done in triplicate (at screening, Day 1 and EOT visit) and allow rhythm definition (at least 30 seconds of duration), PR interval and QT interval (raw and corrected by HR using Bazett's formula). On Day 1, 5 minutes before SOI (-25 to -1 min before preinfusion PK samples collection) and 1 hour after EOI (-15 to -1 min before the PK sample collection), both will be done in triplicate.
[f] A previous LVEF assessment could be used if was made within 28 days prior to screening and will be repeated if clinically indicated, and EOT.
[g] Randomization apply for all patients.
[h] Bosentan for self-administration and the patient's diary should be given to the patient on Day 15 of Cycle 1 standing for Day -6 of Cycle 2 with a -2/+1 day time window (i.e., from Day 14 of Cycle 1 to Day -5 of Cycle 2) in absence of delays.
[i] Corticosteroids i.v. (according to institutional standard antiemetic doses, minimum dose of 8 mg/day and maximum dose of 20 mg/day of dexamethasone sodium phosphate or equivalent); If feasible, anti-emetic medication and dose administered in Cycle 1 should be maintained in Cycle 2. Serotonin ($5\text{-}HT_3$) antagonists (ondansetron at least 8 mg i.v. and no more than 16 mg of ondansetron) (i.e., 30 ± 5 minutes before lurbinectedin administration).
[j] If patient does not fulfill re-treatment criteria (Cycle 2 in RT sequence) in Day 1 assessments and lurbinectedin administration is postponed no more than two days, bosentan should not be

Study Period ▶	Screening (days prior to registration)	Cycle 1						Cycle 2											Cycle 3	End of Treatment	Follow-up AEs
Cycle Day ▶		1*	2	3	5	8	15	-5	-4	-3	-2	-1	1	2	3	5	8	15	1	31 (±10)	

discontinued.

[k] The study center will contact the patients (via telephone or other methods) to remind them or confirm that they have taken the morning and evening dose of bosentan when they are not at the study center.

[l] Blood sample for genotyping will be collected at any time during the study but preferably just before infusion start in Cycle 1, only if written IC for the pharmacogenetic sub-study is given.

[m] Serial peripheral venous blood samples for determination of lurbinectedin (total and unbound), lurbinectedin metabolites (i.e., M1 and M4) and bosentan plasma concentrations will be collected at the time points indicated in Section 8.1.1, Table 9.

[n] Clinical assessment of the patient's signs and symptoms (including nurses' assessment and patient-reported issues) should continue on an ongoing basis throughout the study and be reported at least every cycle as AEs. Only information on SAEs that occurred after signature of the informed consent and up to Day -1 is required before treatment start. Grading should be as per the NCI-CTCAE v.5. Signs and symptoms will be assessed by the Sponsor as all the events ongoing during the screening period, except for prior history conditions, which should be reported in the medical history form. Ongoing events during the screening period are those present at any time between the informed consent date and the first drug administration, regardless of if they may be resolved or not at the date of Cycle 1 Day 1.

[o] The "AE form" will be used only for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines); and to report 'ongoing' baseline conditions in case of any significant change (improvement or worsening) during the study.

[p] Patients will be followed every four weeks until recovery to at least grade 1 or stabilization of all treatment-emergent AEs, or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first.

Hematology: Differential WBC (including neutrophils, monocytes and lymphocytes), erythrocytes, hemoglobin, hematocrit and platelets.
Coagulation: INR
Biochemistry A: AST, ALT, AP, lactate dehydrogenase (LDH), creatinine, glucose, creatine phosphokinase (CPK), CPK-MB fraction (if CPK is elevated above the normal range) and gamma glutamyltransferase (GGT) and total bilirubin (direct bilirubin if total bilirubin is above the ULN).
Biochemistry B: Serum electrolytes (Na+, K+, Mg++), total calcium, total proteins, albumin, C-reactive protein (CRP).

AAG, alpha-1 acid glycoprotein; AE, adverse events; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; β-hCG, beta subunit of human chorionic gonadotropin; BP, blood pressure; BSA, body surface area; CLcr, Creatinine clearance; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase-isoenzyme MB; CRP, C-reactive protein; D, day; ECG, electrocardiogram; ECHO, echocardiogram; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EOT, end of treatment; GGT, gamma glutamyltransferase; HR, heart rate; IC, informed consent; ICF, informed consent form; ICH, International Conference on Harmonization; IL6, interleukin 6; INR, International Normalized Ratio; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; min, minutes; MUGA, multiple-gated acquisition scan; NCI-CTCAE, National Cancer Institute Common Toxicity Criteria; PCR, polymerase chain reaction; PGt; Pharmacogenetic; PK, pharmacokinetics; RT, reference-test sequence; SAE, serious adverse event; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOI, start of infusion; T^a, temperature; ULN, upper limit of normal; WBC, white blood cells; WOCBP, women of childbearing potential.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AAG	Alpha 1-acid Glycoprotein
AE(s)	Adverse Event(s)
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
AUC	Area Under the Concentration-time Curve
AUC_{last}	Area Under the Concentration-time Curve up to the last Measurable Concentration
AUCR	Area Under the Concentration-time Curve Ratio
β-hCG	Beta Subunit of Human Chorionic Gonadotropin
BEV	Bevacizumab
BM	Bone Marrow
BOS	Bosentan
BP	Blood Pressure
BSA	Body Surface Area
^{14}C	Radioactive Carbon
CI	Confidence Interval
CL	Clearance
CLcr	Creatinine Clearance
C_{max}	Maximum Plasma Concentration
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CPK	Creatine Phosphokinase
CPK-MB	Creatine Phosphokinase Isoenzyme MB
CRO	Contract Research Organization
CUP	Carcinoma of Unknown Primary Site
CV	Coefficient of Variation
CYP	Cytochrome P450
D	Day
DDI	Drug-drug Interaction
DOX	Doxorubicin
DP	Drug Product
DSBs	Double-strand Breaks
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG PS	Eastern Cooperative Oncology Group Performance Status
eCRF	Electronic Case Report Form
EFTs	Ewing's Family of Tumors
EMA	European Medicines Agency
EOI	End of Infusion
EOT	End-of-treatment
EPO	Erythropoietin
FD	Flat Dose
FDA	Food and Drug Administration
FiH	First-in-Human
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GCT	Germ Cell Tumor
GDPR	General Data Protection Regulation
GEM	Gemcitabine
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
GMT	Greenwich Mean Time
h	Hour(s)

Hb	Hemoglobin
HL	Half-life
HR	Heart Rate; Homologous Recombination
HRD	Homologous Recombination-deficient
5-HT$_3$	Serotonin
IB	Investigator's Brochure
IC	Informed Consent
IC$_{50}$	Half Maximal Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL-6	Interleukin 6
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
IUD	Intra-uterine Contraceptive Device
i.v.	Intravenous
L	Liter
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LDH	Lactate Dehydrogenase
LMWH	Low Molecular Weight Heparin
LRB	Lurbinectedin
LVEF	Left Ventricular Ejection Fraction
MBC	Metastatic Breast Cancer
Min	Minutes
Mg	Milligram
mL	Milliliter
MRT	Mean Residence Time
MUGA	Multiple-gated Acquisition Scan
NA	Not Applicable
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria
NER	Nucleotide Excision Repair
NET	Neuroendocrine Tumor
NHEJ	Non-homologous End Joining
NHP	Non-human Primates
NIHS	National Institute of Health Sciences
NSCLC	Non-small cell Lung Cancer
ORR	Overall Response Rate
OS	Overall Survival
OS6	Overall Survival Rate at Six Months
PBPK	Physiologically Based Pharmacokinetic Models
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PFS	Progression-free Survival
PGt	Pharmacogenetic
PK	Pharmacokinetics
PKPD	Pharmacokinetics and Pharmacodynamics
PLD	Pegylated Liposomal Doxorubicin
PN	Peripheral Neuropathy
PRBC	Packed Red Blood Cells
PS	Performance Status
PVC	Polyvinyl Chloride
q3wk	Every Three Weeks
qd	Daily
R	Randomized
RD	Recommended Dose
RECIST	Response Evaluation Criteria in Solid Tumors
RSI	Reference Safety Information
RT	Reference-Test Sequence
SAE(s)	Serious Adverse Event(s)

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCLC	Small Cell Lung Cancer
SD	Stable Disease
SDev	Standard Deviation
SmPC	Summary of Product Characteristics
SOI	Start of Infusion
SUSAR	Suspected Unexpected Serious Adverse Reaction
T^a	Temperature
$T_{1/2}$	Terminal Half-life
TAM	Tumor-associated Macrophage
T_{max}	Time at Which the Maximum Plasma Concentration is Observed
TR	Test-Reference Sequence
UK	Unknown
ULN	Upper Limit of Normal
Vs.	*Versus*
Vss	Volume of Distribution at Steady State
Vz	Volume of Distribution on the Terminal Phase
WBC	White Blood Cells
wk	Week(s)
WOCBP	Woman of Childbearing Potential.

1. INTRODUCTION

1.1 INFORMATION ON THE STUDY DRUG: LURBINECTEDIN

Lurbinectedin is a novel synthetic chemical entity composed of a pentacyclic skeleton formed by two fused tetrahydroisoquinoline rings with an additional tetrahydro-β-carboline moiety (Figure 1).

Lurbinectedin is an inhibitor of oncogenic transcription. It recognizes specific sequences in the minor groove of the DNA where it forms adducts that ultimately lead to the generation of double-strand breaks (DSBs). Additionally, it induces the specific degradation of transcribing RNA Pol II and the eviction of transcription factors from the promoters of actively transactivated genes. It is specifically active in tumors addicted to transcription. The generation of DSBs triggers an extended delay in the transition through phase S of the cell cycle with an arrest in the phases G2/M, leading to tumor cell death by apoptosis.

In vitro, lurbinectedin demonstrated cytotoxic effects against a broad selection of tumor types with half maximal inhibitory concentration (IC_{50}) values in the range of 1-10 nM. Although selectivity was also seen, a clustering of sensitive tumors has not been identified. Lurbinectedin also exhibited antitumor activity against different murine models of xenografted human-derived tumor types. Lurbinectedin has been tested as a single agent or in combination with different drugs in solid tumors; while antitumor activity in hematological tumors was deemed negligible, lurbinectedin has shown activity in different solid tumors; some of the most responsive tumor types were breast, small cell lung cancer (SCLC), ovarian and endometrial cancer.

Based on current clinical data, the toxicity of lurbinectedin is predictable, reversible and manageable. The most relevant toxicity is reversible myelosuppression with a nadir occurring in the middle of the second week after Day 1 infusion in an every-three-week cycle; overall, the incidence of febrile neutropenia is below 20% in all ongoing Phase II trials.

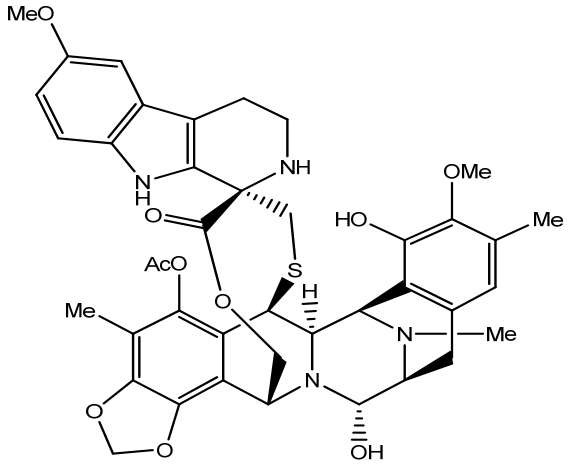
1.1.1 Name and Chemical Information

Lurbinectedin is produced by chemical synthesis and has the following properties:

Chemical Name	(1'R,6R,6aR,7R,13S,14S,16R)-8,14-dihydroxy-6',9-dimethoxy-4,10,23-trimethyl-19-oxo-2',3',4',6,7,9',12,13,14,16-decahydro-6aH-spiro[7,13-azano-6,16-(epithiopropanooxymethano)[1,3]dioxolo[7,8]isoquinolino[3,2-b][3]benzazocine-20,1'-pyrido[3,4-b]indol]-5-yl acetate
Molecular Formula	$C_{41}H_{44}N_4O_{10}S$
Molecular Weight	784.87g/mol

The structural formula of lurbinectedin is shown in Figure 1.

Figure 1. Structural formula of lurbinectedin.



Chirality/Stereochemistry: lurbinectedin is a single stereoisomer with the (1'R, 6R, 6aR, 7R, 13S, 14S, 16R) configuration

1.1.2 Non-clinical Data

The pentacyclic skeleton of lurbinectedin is mostly responsible for DNA minor groove recognition and binding. Lurbinectedin reacts with exocyclic amino group of guanines in the minor groove of DNA forming a covalent bond. Lurbinectedin preferentially binds the following triplet of bases: AGC>CGG>TGG>AGG>GGC (the middle guanine being the site for covalent adducts formation), in the promoter of actively transcribed genes [1].

From the mechanistic point of view, lurbinectedin is an inhibitor of oncogenic transcription, inducing the eviction of oncogenic transcription factors from their binding sites within the chromatin and their redistribution within the nucleus [2]. Secondarily, Lurbinectedin inhibits active transcription through specific and rapid degradation of transcribing RNA Pol II, preventing RNA Pol II elongation and consequently RNA synthesis, as observed in several human tumor cell lines [1].

Phosphorylated histone H2AX is detected in lurbinectedin-treated cells extracts after the disappearance of RNA Pol II, indicating the presence of DSBs. In the process of generation of DSBs, the Nucleotide Excision Repair (NER) machinery has a relevant role [1, 3]. The presence of the lurbinectedin-DNA adduct in a DNA strand causes that the XPF/ERCC1 nuclease of the NER system, instead of repairing the affected strand, generates breaks on the opposite DNA strand generating DSBs [1]. Indeed, lurbinectedin is less potent in NER-deficient cells than in NER-proficient cells. Resistance to DNA alkylators and platinum treatments is often accompanied by increased NER activity, suggesting that these platinum-resistant tumor cells could be sensitive to lurbinectedin. *In vitro*, lurbinectedin showed enhanced and similar activity towards two cisplatin-resistant ovarian carcinoma cell lines and oxaliplatin-resistant colon carcinoma cell lines [3, 4].

DSBs need to be repaired by the Homologous Recombination (HR) System. The Non-homologous End Joining (NHEJ) and HR repair pathways have been investigated in different cell systems in the activity of lurbinectedin [4, 5]. Lurbinectedin is more active against Homologous Recombination-deficient (HRD) cell lines such as BRCA mutated cells [4, 6, 7]. HR deficient cells have a sensitive profile, 50 to 100 times higher than

parental lines, possibly due to the persistence of unrepaired DSB during the S phase and the subsequent increase in apoptosis [5].

As a final consequence of its mechanism of action, lurbinectedin induces delayed transition through the phase S of the cell cycle and a final arrest in the G2/M phases, triggering tumor cell death by apoptosis [8].

Lurbinectedin has demonstrated strong *in vitro* antineoplastic activity in several human cancer cell lines, representative of prostate, pancreas, ovary, non-small cell lung cancer (NSCLC) and SCLC, liver, leukemia, lymphoma, kidney, gastric, colon and breast tumor cell lines, with 50% inhibitory concentration (IC_{50}) values ranging from 0.3 to 64.5 nM. Moreover, in a dedicated panel composed of 15 human SCLC cell lines, the compound showed compelling cytotoxic activity, with an average IC_{50} of 0.65 nM.

The *in vivo* antitumor activity of lurbinectedin was also demonstrated in a panel of different types of human-derived experimental tumors. Statistically significant tumor volume reduction has been observed in several xenograft models such as SCLC, NSCLC, glioma, breast, gastric, kidney, ovary and pancreas tumors. After treatment, molecular analysis of these tumors showed a significant increase of γ-H2AX as well as a reduction of tumor-associated macrophages (TAMs) [4]. The antitumor effect of the drug has been demonstrated in mice bearing different patient-derived xenografts of pancreatic ductal carcinoma and in mice bearing orthotopic tumors (pancreas, ovary and Ewing sarcoma): in all these models, the drug caused a significant tumor size reduction compared to placebo-treated animals.

Lurbinectedin presents a marked effect on tumor microenvironment by inhibiting transcription and secretion of tumor-growth promoting cytokines by TAMs [9]. *In vivo*, synergistic anti-tumor effect was demonstrated for the combination of lurbinectedin and immune checkpoint blockade (anti-PD1 antibody and anti-CTLA4 antibody) in a murine fibrosarcoma MCA205 model [10].

Liver microsomal fractions from human, as well as from other animal species (i.e. mouse, rat, guinea pig, dog and non-human primates [NHP]), were incubated with unlabeled or $^{14}C_1$-lurbinectedin. Results showed that lurbinectedin was stable in the liver microsomes from these species in the absence of NADPH-regenerating system, indicating that the metabolism of lurbinectedin is NADPH-dependent. After the incubation period (10 min), $^{14}C_1$-lurbinectedin was nearly or completely metabolized in liver microsomes from NHP, mouse and human, indicating that lurbinectedin undergoes an intense and rapid NADPH-dependent microsome-mediated metabolism. The NHP was the preclinical specie that exhibit the most qualitatively and quantitatively human-similar microsome-mediated metabolism.

Experiments performed with human microsomes (both genders) and selective chemical inhibitors and inhibitory monoclonal antibodies directed against CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 pointed to CYP3A4 as the main CYP isoform involved in the phase I metabolism of lurbinectedin. The involvement of other CYPs in the microsome-mediated metabolism of lurbinectedin was demonstrated to be negligible [11, 12].

The interaction of lurbinectedin on several human ATP-binding cassette (ABC) efflux transporters, namely BCRP (ABCG2), BSEP (ABCB11) and MDR1 (ABCB1/P-gp), as well as several human uptake transporters, namely MATE1 (SLC47A1), OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), OAT1 (SLC22A6), OAT3 (SLC22A8), OCT1

(SLC22A1) and OCT2 (SLC22A2) were evaluated in *in vitro* studies. Results have shown lurbinectedin is substrate of MDR1 (ABCB1) transporter.

In vitro, the percent of lurbinectedin bound to human plasma proteins was very high (>99%) and independent of drug concentration. Lurbinectedin binds to both human serum albumin and α-1-acid glycoprotein (AAG), with higher affinity to AAG. In vitro binding to AAG is linear and non-saturable at clinically relevant concentrations.

In non-clinical species (rat, dog, and monkey), lurbinectedin exhibits low to moderate plasma clearance (from 1 to 10 L/h/kg) and large volume of distribution (from 2 to 56 L/Kg). The elimination half-life was long (from 9 to 22 h).

In non-clinical toxicity studies, lurbinectedin reversibly affected the hematopoietic system, mainly reducing reticulocytes and white blood cells and inducing a slight anemia, as well as bone marrow (BM) depletion and atrophy of the lymphoid system. Hepatotoxicity was observed mainly in rats and was characterized by markedly increased liver function tests, hepatocellular necrosis and biliary damage. Hepatotoxicity was less pronounced in dog studies.

Other target organs, mainly identified in rats, were atrophy of the gastrointestinal (GI) mucosa, adrenal gland cortical hypertrophy and kidney cortical tubular vacuolization. In summary, the main toxic effects of lurbinectedin – transient BM suppression and hepatotoxicity – are rather commonly seen with cytotoxic drugs.

Injection site lesions were also seen in studies regardless of the animal studies used. These lesions were characterized by perivascular hemorrhage, inflammation, edema and chronic phlebitis, necrosis and ulceration.

More information is available in the Investigator's Brochure (IB) of lurbinectedin.

1.1.3 Clinical Data

Up to the cut-off date of 15 January 2020 (date of last IB), a total of 2474 patients had been included in PharmaMar clinical studies evaluating lurbinectedin; 975 of them (39.4%) were treated with lurbinectedin as single-agent; three phase 1 studies in solid and hematological tumors at different regimes [day 1, days 1 and 8, and days 1-3 in cycles of 3 weeks (q3wk)], five phase 2 studies in different tumor types [pancreatic, ovarian, breast, NSCLC and selected aggressive solid tumors including SCLC] and one phase 3 study in ovarian cancer.

1.1.3.1 *Phase I Trials*

Currently, this program comprises four single-agent studies, three in solid tumors (one ongoing in Japanese patients) (Table 1) and one in acute leukemia patients, six phase Ib combination studies with gemcitabine (GEM), capecitabine, doxorubicin (DOX), cisplatin, paclitaxel with or without bevacizumab (BEV), and irinotecan in patients with selected advanced solid tumors.

The First-in-Human (FiH) study (PM1183-A-001-08) was conducted in patients with advanced and refractory solid tumors and evaluated lurbinectedin intravenously (i.v.) infused over one hour every three weeks (q3wk). Thirty-three patients were included and thirty-one patients were treated. The recommended dose (RD) was stablished at an equivalent dose of 4.0 mg/m^2/q3wk [13]. Since lurbinectedin clearance was found to be unrelated to body surface area (BSA), all patients in the RD expansion cohort were treated at a flat dose (FD) of 7.0 mg q3wk (equivalent to 4 mg/m^2). However, further

linear regression showed a strong correlation between neutropenia and its severity with the systemic exposure (area under the concentration-time curve – AUC), and for this reason, lurbinectedin prescriptions are currently calculated against BSA; moreover, phase Ib trials started with a FD and were amended to use a BSA-based dose. In this study (and subsequent studies), the median terminal plasma half-life ($T_{1/2}$) was around 60 hours, with a plasma clearance (CL) close to 12 L/h though large inter-individual variability exists. Urinary excretion of unaltered lurbinectedin was almost negligible regardless of dose; the mean percentage of unaltered drug recovered in urine at the recommended dose was 0.23% [13].

The most relevant toxicity found in the FiH trial was reversible myelosuppression, notably grade 4 non-febrile neutropenia in 40% of patients treated at the RD in Cycle 1. Neutropenia was generally predictable and short-lasting and rarely caused treatment delays with nadir usually occurring during the second week. No cases of febrile neutropenia occurred in this study, although patient selection might have played a role. Also, at the RD, treatment was generally well tolerated, with no grade 4 transaminase increases observed (in contrast to what occurred above the RD). No unexpected toxicities occurred in this study, and there were no signs of cumulative toxicity. All patients at this dose level were treated with standard antiemetic prophylaxis to control grade 2 nausea and/or vomiting [13].

There was one partial response as per the Response Evaluation Criteria in Solid Tumors (RECIST v. 1.0) in pancreatic cancer and three disease stabilizations (SD) at the RD lasting ≥ 4 months (two in soft tissue sarcomas, one in melanoma) [13].

To explore the feasibility of an alternative schedule (Day 1 and 8, q3wk), a second phase I trial (PM1183-A-005-11) was started in advanced non-colorectal cancer patients following a prospective FD escalation. The RD was 5 mg FD on Day 1 and 8 q3wk. Myelosuppression limited further dose escalation; in contrast to the FiH trial, grade 4 neutropenia lasted longer and there were more drug-related dose delays/omissions [14]. Based on the results from single-agent phase I clinical trials in solid tumors, the Day 1 q3wk schedule was assessed as more convenient with better compliance, and was selected for further clinical trials.

Table 1. Completed single-agent lurbinectedin phase I trials in solid tumor patients.

	PM1183-A-001-08	PM1183-A-005-11	Total
Participant sites and countries	(2): Spain, U.S.	(2): U.S.	
Schedule	1-h D1 q3wk	1-h D1, D8 q3wk	
Patients included	33	21	**54**
Treated patients	31	21	52
Evaluated patients	31	21	52
RD	4.0 mg/m^2 or 7.0 mg FD	5.0 mg FD	
Evaluable patients with DLTs at the RD (if applicable)	1 of 15: Myelosuppression	3 of 13: Myelosuppression and myelosuppression-related lack of compliance with treatment schedule	
Study status	Completed [13]	Completed [14]	

C, cycle; D, day 1; DLT, dose-limiting toxicity; FD, flat dose; h, hour; mg, milligrams; q3wk: every three weeks; RD, recommended dose; U.S., United States of America.

Lurbinectedin was also explored in combination with other drugs (DOX, GEM, capecitabine, paclitaxel with or without BEV, cisplatin with or without aprepitant, or irinotecan) in five clinical trials: PM1183-A-003-10, PM1183-A-004-10, PM1183-A-006-12, PM1183-A-007-13, PM1183-A-008-13 and PM1183-A-014-15 (Table 2).

Overall, antitumor activity has been observed in all combinations explored; breast, SCLC, ovarian and endometrial cancer were the most responsive tumor types in combination studies.

Table 2. Phase I combination lurbinectedin trials in solid tumor patients.

Study	Participant sites and countries	Schedule	Treated patients	RD	Evaluable patients with DLTs at the RD (if applicable)	Study status at cutoff
PM1183-A-003-10 [a]	7 Spain U.K.	PM01183 1-h D1 q3wk + i.v. DOX D1 q3wk	120	PM01183 4.0 mg FD + DOX 50.0 mg/m^2	8 of 60: Myelosuppression	Completed
				PM01183 2.0 mg/m^2 + DOX 40.0 mg/m^2	1 of 30: Myelosuppression	
PM1183-A-004-10	3 Spain U.K.	PM01183 1-h D1 and D8 q3wk + i.v. GEM 30-min D1, D8 q3wk	45	PM01183 3.0 mg FD (1.6 mg/m^2) + GEM 800 mg/m^2	None of 11	Completed
PM1183-A-006-12	4 Belgium Spain	PM01183 1-h D1, D8 + capecitabine p.o. b.i.d. D1-D14 q3wk	15	PM01183 2.0 mg FD + capecitabine 1650 mg/m^2/D b.i.d.	None of 9	Completed
		PM01183 1-h D1 q3wk + capecitabine p.o. b.i.d. D1-D14 q3wk	66	PM01183 4.0 mg FD (or 2.2 mg/m^2) + capecitabine 1650 mg/m^2/D b.i.d.	3 of 36: Myelosuppression-related lack of compliance with schedule	
PM1183-A-007-13	3 Spain Switzerland U.S.	PM01183 1-h D1 + paclitaxel 1-h D1, D8 q3wk	55	PM01183 4.0 mg FD/D (or 2.2 mg/m^2) + paclitaxel 80 mg/m^2/week	6 of 34: Myelosuppression	Completed
		PM01183 1-h D1 + paclitaxel 1-h D1, D8 + BEV D1 q3wk	12	PM01183 2.2 mg/m^2 + paclitaxel 80 mg/m^2/week + BEV 15 mg/kg	3 of 12 Myelosuppression and non-hematological (G4 colon perforation; n=1)	
PM1183-A-008-13	4 Switzerland U.K.	PM01183 1-h D1 + cisplatin 90-min D1 q3wk	24	PM01183 1.4 mg/m^2 + cisplatin 60 mg/m^2	1 of 3 Fatigue	Completed
		PM01183 1-h D1 + cisplatin 90-min D1 q3wk + Aprepitant	17	PM01183 1.1 mg/m^2 + cisplatin 60 mg/m^2	1 of 3 Rhabdomyolysis	
PM1183-A-014-15	3 USA Spain	PM01183 1-h D1 + Irinotecan 90-min D1, D8 q3wk	22	PM01183 1.5 mg/m^2 + Irinotecan 75 mg/m^2	1 of 4 Febrile neutropenia	Ongoing
		PM01183 1-h D1 + Irinotecan 90-	55	PM01183 2.0mg/m^2 + Irinotecan 75	3 of 12 Myelosuppression	

Study	Participant sites and countries	Schedule	Treated patients	RD	Evaluable patients with DLTs at the RD (if applicable)	Study status at cutoff
		min D1, D8 q3wk + G-CSF		mg/m^2		
		PM01183 1-h D1 + Irinotecan 90-min D1, D8 q3wk (irinotecan escalation group)	16	RD not defined at cutoff	.	
Total			327			

[a] Two patients > 75 years were included in study PM1183-A-003-10 before a protocol amendment limited age to a maximum of 75 years. Data from these two patients have been excluded from safety analyses, but included in efficacy analyses.
BEV, bevacizumab; b.i.d., twice daily; D, day; DLT, dose-limiting toxicities; DOX, doxorubicin; FD, flat dose; G, grade as per NCI-CTCAE v.3.0 (in PM1183-A-001-08 study) and v. 4.03 (in all the rest of the studies); GEM, gemcitabine; h, hour; i.v., intravenous; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; MDS, myelodysplastic syndrome; min, minute; mg, milligram; PM01183, lurbinectedin; p.o., orally; q3wk, every three weeks; RD, recommended dose; U.K., United Kingdom; U.S., United States of America.

With regards to lurbinectedin antitumor activity in hematological tumors, the only available information comes from one phase I trial (PM1183-A-002-10), where the clinical activity of lurbinectedin was considered negligible from the clinical point of view in patients with advanced acute leukemia. For this reason, drug development in this hematological disease is not warranted.

1.1.3.2 *Human Mass Balance Study*

In the human mass balance study (PM1183-A-015-16), near complete recovery of administered radioactivity (94.3±8.7%) was achieved within 500-hour post-dose following a single mass dose of 4.29±0.23 mg of [^{14}C$_1$]-lurbinectedin (radioactive dose of 74.28±8.62 μCi) administered as 1-hour i.v. infusion. The majority of radioactivity was excreted in feces (88.7±10.1%) with minimal excretion in urine (5.6±2.0%). Whole blood to plasma ratio of parent-related [^{14}C]-radioactivity based upon mean $AUC_{0\text{-last}}$ were found to be 0.68 indicating larger distribution in plasma.

The mean plasma lurbinectedin time to peak (t_{max}) and plasma [^{14}C]-radioactivity was found to be similar (~1.2 h). Mean plasma [^{14}C]-radioactivity C_{max}, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ were 1.4, 1.6 and 1.9-fold higher as compared to parent compound. Mean plasma [^{14}C]-radioactivity $t_{1/2}$ (55-hour) was 1.2-fold longer than plasma lurbinectedin $t_{1/2}$ (47-hour). Mean plasma clearance and volume of distribution of parent compound were found to be 1.7- and 2.1-fold higher than plasma [^{14}C]-radioactivity. According to $AUC_{0\text{-last}}$ comparison, lurbinectedin constituted approximately 62% of the total [^{14}C]-radioactivity in plasma, thus suggesting that parent compound is the main circulating moiety in plasma.

Among the five known lurbinectedin metabolites (M1 to M5), all were detected but only four (M1, M4 (PM030047), M3 (PM01158), and M2 (PM030036)) were quantified in plasma. M5 (PM030779) could not be quantified, but its presence in plasma, at low levels (around its LLOQ of 0.1 mg/mL), was demonstrated.

Up to 10-hour after the end of infusion, 88.7% of the total [^{14}C]-radioactivity in plasma can be attributed to lurbinectedin (70.2%) plus these four metabolites (18.5%). Based on these results, M1 and M4 (PM030047) were the major metabolites accounting for 10% and 7.3% of circulating radioactivity, respectively, thus representing 14.3% and 10.4% of the parent compound in plasma.

Whole blood to plasma ratio of parent-related [^{14}C]-radioactivity based upon mean $AUC_{0\text{-last}}$ were found to be 0.68 indicating larger distribution in plasma.

1.1.3.3 *Phase II Trials*

As of 15 January of 2020, five phase II clinical studies had explored the efficacy of lurbinectedin in specific solid tumor types and settings (Table 3): PM1183-B-001-10 in pancreatic cancer as second-line therapy post GEM-based failure; PM1183-B-002-11 in platinum-resistant/refractory ovarian cancer; PM1183-B-003-11 in previously treated BRCA1/2-associated metastatic breast cancer (MBC); PM1183-B-004-13 in NSCLC, and PM1183-B-005-14 in selected advanced solid tumors.

Four of these studies initially evaluated single-agent lurbinectedin at the RD and schedule determined in the FiH study (PM1183-A-001-08), i.e., 7.0 mg FD as a 1-h i.v. infusion q3wk. In addition, study PM1183-B-004-13 evaluated lurbinectedin in combination with GEM at the RD and schedule defined in a previous phase I study (PM1183-A-004-10), i.e., lurbinectedin 3.0 mg FD + GEM 800 mg/m^2 both on Day 1 and Day 8 q3wk. However, protocol amendments were implemented to change the fixed dose in these studies to a BSA-based dose, as a conservative approach after knowledge of data suggesting BSA-related toxicity. The fifth study (PM1183-B-005-14) evaluated lurbinectedin at a BSA-based dose of 3.2 mg/m^2 as a 1-h i.v. infusion q3wk.

Table 3. Phase II lurbinectedin trials in solid tumor patients.

Study Indication	Participant sites and countries	Drug dose and schedule	Treated patients	Primary endpoint	Results	Study status at cutoff
Non-randomized trials						
PM1183-B-001-10 Second-line metastatic pancreatic cancer	8 Spain U.K.	PM01183 7.0 mg FD 1-h D1 q3wk	44	OS6 rate	OS6: 33%	Completed
PM1183-B-003-11 BRCA 1/2-associated or unselected MBC	11 Spain U.S.	PM01183 7.0 mg FD or 3.5 mg/m^2 as 1-h infusion D1 q3wk	BRCA-mutated 54	Confirmed response as per RECIST v 1.1	BRCA-mutated ORR: 41%	Ongoing[a]
			BRCA-mutated + prior PARPi 20		BRCA-mutated + prior PARPi ORR: 5%	
			BRCA-unselected 35		BRCA-unselected ORR: 9%	
PM1183-B-005-14 Selected advanced solid tumors [b]	40 Belgium France Italy Spain Sweden Switzerland U.K. U.S.	PM01183 3.2 mg/m^2 as 1-h D1 q3wk	335 [c]	Confirmed response as per RECIST v 1.1	SCLC ORR: 36% BRCA-mutated MBC ORR: 29% EFTs ORR: 16% Endometrial ORR: 11% NETs ORR: 7% Biliary tract ORR: 6% GCTs ORR: 5%	Ongoing [a]
Total			**488**			

Study Indication	Participant sites and countries	Drug dose and schedule	Treated patients	Primary endpoint	Results	Study status at cutoff
Randomized trials						
PM1183-B-002-11 PRROC	9 France Spain	PM01183 7.0 mg FD as 1-h D1 q3wk	81 PM01183: 52 Topotecan: 29 [d]	Confirmed response as per RECIST v 1.1 and/or Rustin criteria	PM01183: ORR: 23% Topotecan: ORR: 0%	Completed
PM1183-B-004-13 Unresectable second-line NSCLC	14 Belgium France Italy Spain U.S.	PM01183 7.0 mg FD or 3.2 mg/m^2 as 1-h D1 q3wk	21	PFS4 rate	PFS4: 16%	Completed
		PM01183 3.0 mg FD or 1.6 mg/m^2 as 1-h + GEM 800 mg/m^2 both on D1, D8 q3wk	25		PFS4: 26%	
		Docetaxel 75 mg/m^2 as 1-h D1 q3wk	22		PFS4: 27%	
Total			**149**			
Total Phase II trials			**637**			
Total lurbinectedin single-agent (including 15 patients crossed over from topotecan)			**576**			

[a] Closed recruitment.
[b] SCLC, H&N, NETs, biliary tract carcinoma, endometrial carcinoma, BRCA1/2-associated metastatic breast carcinoma, CUP, GCTs and EFTs.
[c] Thirty nine of these patients were also included in the PM1183-B-005-14-QT sub-study, to evaluate the potential effects of lurbinectedin on the duration of the QTc interval.
[d] Fifteen of these patients crossed over to the lurbinectedin arm. CUP, carcinoma of unknown primary site; D, day; EFTs, Ewing's family of tumors; FD, flat dose; GCT, germ cell tumor; GEM, gemcitabine; h, hour; H&N, head and neck carcinoma; mg, milligrams; NET, neuroendocrine tumor; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS6, overall survival rate at six months; PD, progressive disease; PFS, progression-free survival; PFS4, progression-free survival rate at four months; PM01183, lurbinectedin; PRROC, platinum-resistant/refractory advanced ovarian cancer; q3wk, every three weeks; RECIST, Response Evaluation Criteria In Solid Tumors; SCLC, small cell lung cancer; SD, stable disease; U.K., United Kingdom; U.S., United States of America.

1.1.3.4 *Phase III Program*

As of 15 January 2020, one completed phase III study (PM1183-C-004-14, CORAIL) had compared the efficacy of lurbinectedin in platinum-resistant ovarian cancer against standard topotecan or pegylated liposomal doxorubicin (PLD). No significant differences between treatment arms were found for secondary efficacy endpoints of this trial. Lurbinectedin showed an improvement in terms of safety profile compared to the control arm.

The other phase III clinical study is currently ongoing (PM1183-C-003-14, ATLANTIS). This study had completed recruitment. The aim of this study is to compare the combination of lurbinectedin with DOX *vs.* treating physician choice (topotecan or CAV) as a second line in SCLC.

1.1.3.5 *Population Pharmacokinetic and Pharmacodynamic (PKPD) Modeling*

A population pharmacokinetic model of lurbinectedin was developed by pooling data from all studies (including drug combination studies) and it is regularly updated.

A population pharmacokinetic three-compartment model with linear elimination and linear distribution adequately fits the PK data. The model estimated a mean (standard deviation [SDev]) CL of 12.3 (7.0) L/h, a half-life (HL) of 47.2 (31.5) hours and a wide volume of distribution at steady state (V_{ss}) of 436 (250) L. All these PK parameters showed high variability (coefficient of variation [CV] about 50%) among patients. The lurbinectedin PK showed linearity among the dose range explored (0.5-4.5 mg/m^2).

Baseline covariates affecting lurbinectedin CL are albumin, alpha 1-acid glycoprotein, BSA, the presence of strong (voriconazole) or moderate (aprepitant or fluconazole) CYP3A4 inhibitors, and cardiac function measured by left ventricular ejection fraction (LVEF). The use of strong (voriconazole) or moderate (fluconazole or aprepitant) CYP3A4 inhibitors reduced lurbinectedin CL by approximately 40% and 20%, respectively. Therefore, the use of CYP3A4 inhibitors should be carefully monitored or avoided, whenever possible.

Renal elimination of lurbinectedin was minor in the FiH trial. In addition, the population PK model did not establish any relationship between lurbinectedin CL and CLcr or creatinine levels. Age and race were found to have no effect over lurbinectedin PK, although the number of elderly patients (>75 years) and non-Caucasian patients was small.

Lurbinectedin-induced neutropenia and thrombocytopenia population PKPD models showed that baseline ANC and platelet count are by far the covariates with the greatest effect on the incidence of neutropenia and thrombocytopenia. With a baseline absolute neutrophil count (ANC) value of 2.0 x 10^9/L (currently the lower limit of ANC for inclusion is 1.5 x 10^9/L), and a platelet count of 135 x 10^9 (currently the lower limit of platelet count for inclusion is 100 x 10^9/L), the incidence of grade 3/4 neutropenia and thrombocytopenia were estimated to be around 69% and 13%, respectively, in the first cycle. Simulations performed at 3.2 mg/m^2 showed a lower neutropenia and thrombocytopenia incidence compared to the previous RD of 7.0 mg FD. The results of the simulation also showed that BSA-dosing reduced the variability caused by BSA compared to 5.7 mg FD. The presence of ascites or the use of CYP3A4 inhibitors would increase the incidence of neutropenia, while the use of granulocyte colony-stimulating factor (G-CSF) would decrease it markedly. Additionally, breast cancer patients showed less risk of developing thrombocytopenia, while the use of strong CYP3A4 inhibitors, ascites or pancreatic cancer increased this risk. The population PK model of lurbinectedin showed that CL increases with BSA, and the population PKPD models for neutropenia and thrombocytopenia showed myelosuppression would be decreased by BSA dosing. Therefore, the use of a BSA-dosing strategy for lurbinectedin has been resumed in ongoing and planned trials.

1.2 OVERALL STUDY RATIONALE

Lurbinectedin is extensively metabolized by the cytochrome P450 enzymes, primarily CYP3A4. Thus, potent inducers or inhibitors of this enzyme may alter the plasma concentrations of lurbinectedin. This study is designed to examine the PK and safety of lurbinectedin when co-administered with bosentan, a moderate CYP3A4 inducer, in comparison with lurbinectedin alone. The results of this study may be used to support future clinical studies in patients and prescribing information in future labeling.

1.3 PHARMACOGENETIC SUB-STUDY RATIONALE

Germline genetic variants may influence exposure and toxicity (i.e. disposition, metabolism and safety) to lurbinectedin. In order to explore the potential impact of genetic factors that may account for inter individual variability in the main PK parameters, blood sample for germline DNA extraction will be collected and stored for future pharmacogenetic (PGt) analysis. One blood sample obtained at any time during the study, but preferably just before infusion start in Cycle PK sample #1 on Day 1 of Cycle 1 will be obtained.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To assess the effect of bosentan on lurbinectedin total plasma exposure in patients with advanced solid tumors.

2.2 SECONDARY OBJECTIVES

- To assess the effect of bosentan on lurbinectedin unbound plasma exposure.
- To assess the effect of bosentan on lurbinectedin major metabolites (i.e., M1 and M4).
- To assess the effect of bosentan on the safety profile of lurbinectedin.
- To collect and store a blood sample for germline DNA extraction for future pharmacogenetic (PGt) analysis of variations on genes that may influence exposure and response (i.e., disposition, metabolism and safety) to lurbinectedin.

3. OVERALL STUDY DESIGN

3.1 STUDY DESIGN

This is a prospective, open-label, two-way crossover, phase Ib drug-drug interaction study in patients with advanced solid tumors.

The study will include a pre-treatment (screening) phase followed by a treatment phase consisting of two lurbinectedin cycles, one cycle in combination with bosentan and one cycle of lurbinectedin as single agent (in different order depending on the study sequence), and one additional third cycle of lurbinectedin as a single agent for patients who meet the continuation criteria and obtain a clinical benefit after the first two cycles, and then follow-up of adverse events if any.

Patients who meet the continuation criteria and obtain a clinical benefit after the first two cycles according to the Investigator's criteria will have the opportunity to continue treatment under a Compassionate Use Agreement after the completion of the optional third study cycle.

Patients will be treated as outpatients. At the discretion of the Investigator, patients may be admitted to the study center on Day –1 or Day 1 and monitored, at least, until completion of the Day 1 PK blood sample collections.

Patients will receive a maximum of three cycles: two consecutive cycles of lurbinectedin, one cycle with and one cycle without bosentan co-administration (in different order depending on the study Sequence 1 or Sequence 2 of treatment), followed by a third cycle with lurbinectedin alone (this last optional for patients with clinical benefit). Lurbinectedin will be administered as a 1-hour (-5/+20 min) i.v. infusion q3wk via a central or peripheral vein.

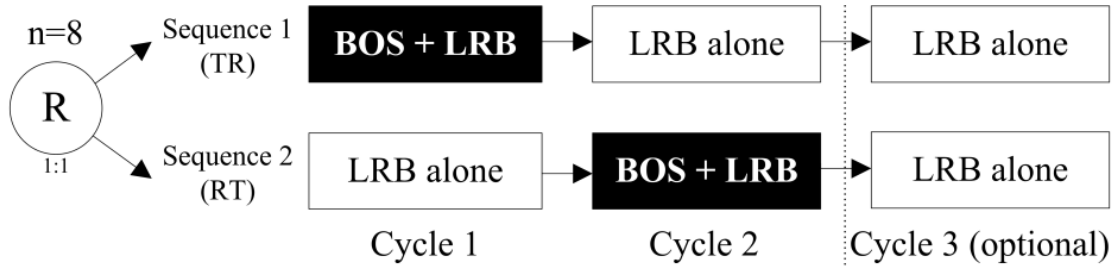
In the co-administration cycles, bosentan will be administered orally twice daily in the morning and evening during the prior five consecutive days, self-administered at home from Day -5 to Day -1 (i.e., five days before lurbinectedin infusion), and once daily on

Day 1 (i.e., the day of lurbinectedin infusion), following recommendations at the Summary of Product Characteristics (SmPC). On Day 1 (i.e., the day of lurbinectedin infusion), bosentan will be given immediately prior to starting the lurbinectedin infusion. In fact, bosentan should be administered after obtaining the bosentan pharmacokinetic (PK) sample #1 and before the start of lurbinectedin infusion (-15 min to -1 min).

In case of lurbinectedin delay (≤ 2 days), bosentan could be administered twice daily during a maximum of seven consecutive days before lurbinectedin infusion, and supplied at the study center once daily on Day 1 (before lurbinectedin infusion).

All patients will receive a maximum of three cycles: two consecutive cycles of lurbinectedin, one cycle with and one cycle without bosentan co-administration (in different order depending on the study Sequence 1 or Sequence 2 of treatment), followed by a third cycle with lurbinectedin alone (this last optional for patients with clinical benefit). Patients will be randomized in a 1:1 ratio (Figure 2) to Sequence 1 (TR: Test- Reference; lurbinectedin + bosentan in Cycle 1) or Sequence 2 (RT: Reference-Test; lurbinectedin + bosentan in Cycle 2). Lurbinectedin will be administered as a 1-hour (-5/+20 min) intravenous (i.v.) infusion every three weeks (q3wk) via a central or peripheral vein. The dose of lurbinectedin will be 3.2 mg/m² for all patients when administered with and without bosentan. If toxicity occurs, the appropriate intra-patient dose level (DL) reductions will be implemented in the subsequent cycle. This DL reduction will be according to Table 8.

Figure 2. Study design.



BOS, bosentan; LRB, lurbinectedin; TR, test-reference; R; randomized; RT, reference-test.

The enrollment of the patients will be simultaneous. However, if once the first three patients enrolled have completed Cycle 1 and Cycle 2, total lurbinectedin exposure does not allow an adequate PK assessment and if no unacceptable or life-threatening toxicities have occurred, the dose of lurbinectedin to be co-administered with bosentan in the remaining five patients can be adjusted accordingly.

This decision will be made by the Sponsor and the study Investigators. Therefore, the planned dose of lurbinectedin, when given with bosentan for the remaining five patients, will be based on the acceptability of the PK and safety results from the first three patients. If the initial three patients do not experience adverse events (AEs) which might require a dose-reduction, the dose of lurbinectedin may still be adjusted (based on the assumption of dose-proportional pharmacokinetics) to produce plasma lurbinectedin area under the curve (AUC) values that are comparable to those when lurbinectedin is given in the absence of bosentan. However, if toxicity occurs in the initial three patients, the appropriate dose-reduction of lurbinectedin will be implemented in the remaining five patients accordingly.

Patients will receive lurbinectedin until disease progression, unacceptable toxicity, consent withdrawal or while it is considered to be in their best interest, and for a maximum of three cycles. Treatment with lurbinectedin outside this study could be continued under a Compassionate Use Agreement after the completion of the optional third study cycle.

All patients will be randomly assigned to the corresponding sequences:

- Sequence 1 (TR):
  - Cycle 1: Bosentan + lurbinectedin
  - Cycle 2: Lurbinectedin alone
  - Cycle 3: Lurbinectedin alone (optional)

- Sequence 2 (RT):
  - Cycle 1: Lurbinectedin alone
  - Cycle 2: Bosentan + lurbinectedin
  - Cycle 3: Lurbinectedin alone (optional)

Lurbinectedin will be administered to eight evaluable patients and for a maximum of three cycles, while considered to be on the patient's best interest or until PD, unacceptable toxicity, intercurrent illness of sufficient magnitude to preclude safe continuation of the study, patient's refusal and/or non-compliance with study requirements, a protocol deviation with an effect on the risk/benefit ratio of the clinical study, more than two lurbinectedin dose reduction due to AEs related to lurbinectedin (unless clear clinical benefit has been documented and always with the Sponsor's agreement), or any other reason at the physician's judgment that precludes lurbinectedin continuation.

If the patient meets the continuation criteria and shows a clear clinical benefit after the first two cycles according to Investigator criteria, treatment with lurbinectedin may continue outside this study under a Compassionate Use Agreement at the same dose based on Investigator's decision and upon agreement with the Sponsor. Then, the treating center must request authorization to the relevant Health Authorities and notify the Sponsor in due time. In order to avoid a treatment discontinuation, during the Compassionate Use Agreement authorization an additional third cycle with lurbinectedin is allowed.

All adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.5. Treatment delays, dose reduction requirements and reasons for treatment discontinuation will be monitored throughout the study. The safety profile of patients will be monitored throughout the treatment and up to 31 days (±10 days) after the last lurbinectedin infusion (end of treatment, EOT), until the patient starts a new antitumor therapy or until the date of death, whichever occurs first. Any treatment-emergent AEs will be followed until recovery to at least grade 1 or stabilization of symptoms or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first. After treatment discontinuation, patients will be followed until resolution or stabilization of all toxicities, if any.

Patients will be evaluated at scheduled visits on three study periods: pre-treatment (screening), treatment (one cycle of lurbinectedin in combination with bosentan and two

as a single agent and the third cycle optional) and follow-up of adverse events if any (see Section Schedule of Assessments and Procedures).

3.2 STUDY DESIGN RATIONALE

Lurbinectedin is a genotoxic agent and should not be administered to healthy subjects. Therefore, this study will enroll patients with locally advanced or metastatic solid tumors. The crossover design reduces treatment bias and permits intra-subject comparisons and control.

The dose of 3.2 mg/m^2 of lurbinectedin administered as a 1-hour (-5/+20 min) infusion every 3 weeks will be given to patients when they are not given the bosentan co-administration. Studies of single-agent lurbinectedin given as same dose regimen have shown activity in several tumor types including endometrial cancer, breast cancer, and specially SCLC.

In the bosentan co-administration cycle, the twice daily oral administration in the morning and evening following recommendations at the SmPC will start with self-administration at home on Day -5 (five days before lurbinectedin infusion) and until Day -1 (the day before lurbinectedin infusion), and supplied at the study center on Day 1. On Day 1 (day of lurbinectedin infusion), bosentan will be given immediately prior to the start of the lurbinectedin infusion. In case of lurbinectedin delay (≤2 days), bosentan could be administered during a maximum of 7 and a half days.

The proposed bosentan regimen is expected to produce sustained induction of CYP3A4 activity over the entire lurbinectedin PK profile. The total daily dose given in a single dose (i.e. 125 mg twice daily) on the morning and on the evening during 5 and a half days (only morning dose on the day of lurbinectedin infusion start) will produce a consistent degree of induction of CYP3A4 activity during the day of lurbinectedin-bosentan co-administration and beyond, since it takes time for the enzyme's activity to return to normal following the inducer's removal.

The exposure of lurbinectedin given as a 1-hour i.v. infusion when co-administered with bosentan, a moderate CYP3A4 inhibitor, will be assessed. Previously, a dose of 3.2 mg/m^2 administered over 1 hour without concomitant administration of inhibitors of CYP3A4 activity, produced lurbinectedin concentrations in plasma that were measurable for at least 160 hours after start of the infusion (Basket study, PM1183-B-005-14). A 3.2 mg/m^2 dose of lurbinectedin co-administered with bosentan is expected to produce measurable concentrations in samples collected for at least the initial 80 hours.

The PK (and safety) of bosentan will be assessed in the first three patients in order to confirm the above statements.

Patients will receive i.v. corticosteroids (according to institutional standard antiemetic doses, minimum dose of 8 mg/day and maximum dose of 20 mg/day of dexamethasone sodium phosphate or equivalent) 30 ± 5 minutes before the infusion of lurbinectedin during Cycles 1 and 2. The optional treatment with additional antiemetic drugs on Day 1 of Cycles 1 and 2 is allowed, but should be identical across Cycle 1 and 2 for a given patient when possible to minimize intra-subject variability in the PK of lurbinectedin. Ondansetron is the recommended serotonin (5-HT$_3$) antagonist, since it is metabolized by multiple hepatic cytochrome P450 enzymes. Its metabolic clearance is less likely to

be influenced by the co-administration of bosentan, relative to a serotonin receptor antagonist that is predominately metabolized by a single cytochrome P450 enzyme.

A 168-hour blood sample collection period is sufficient to assess the potential effects of bosentan on lurbinectedin systemic exposure. In order to confirm the presence of measurable bosentan exposure and provide an abbreviated profile of plasma concentrations of bosentan, four samples will be collected in the cycle during which patients receive bosentan, as detailed in the Schedule of Assessments and Procedures section.

3.3 PRIMARY ENDPOINT

3.3.1 Plasma exposure to lurbinectedin

Plasma dose-normalized C_{max} and $AUC_{0-\infty}$ of lurbinectedin will be compared between Cycle 1 and Cycle 2. Pharmacokinetic analyses will be evaluated in plasma by standard non-compartmental methods, or population methods, if necessary.

3.4 SECONDARY ENDPOINTS

3.4.1 Secondary PK parameters

- Differences in dose-normalized total AUC_{0-t} and C_{max} and in Cl, V_{ss} and $T_{1/2}$ of lurbinectedin between Cycle 1 and Cycle 2 will be explored.
- Differences in dose-normalized unbound $AUC_{u,0-\infty}$, $AUC_{u,0-t}$ and $C_{u,max}$ and in CL_u, $V_{ss,u}$ and $T_{1/2,u}$ of lurbinectedin between Cycle 1 and Cycle 2 will be explored.
- Differences in ratios between total $AUC_{0-\infty}$, AUC_{0-t} and C_{max}, of main lurbinectedin metabolites relative to parent drug between Cycle 1 and Cycle 2 will be explored. Additional PK parameters will be calculated if deemed appropriate.

3.4.2 Safety Profile

Treatment safety, including AEs, serious adverse events (SAEs) and laboratory abnormalities will be graded according to the NCI-CTCAE v.5. Additionally, treatment compliance, in particular dose reductions requirements and/or treatment delays due to AEs, and reasons for treatment discontinuation will also be described. Patients will be evaluable for safety if they have received at least one partial or complete infusion of lurbinectedin.

3.4.3 Pharmacogenetics

The presence or absence of PGt polymorphisms in genes relevant for lurbinectedin disposition (distribution, metabolism and excretion) from a single blood sample collected (only if written IC given) at any time during the trial (but preferably at the same time as the pre-treatments PK sample on Day 1 of Cycle 1), which will be stored to explain individual variability in main PK parameters in future analyses.

4. SELECTION OF PATIENTS

4.1 INCLUSION CRITERIA

All patients must fulfill the following inclusion criteria to be enrolled in the study:

1) Voluntary signed and dated written informed consent prior to any specific study procedure.
2) Male or female with age ≥ 18 years.
3) Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 1 (Appendix 1).
4) Life expectancy > 3 months.
5) Pathologically confirmed diagnosis of advanced solid tumors [except for primary central nervous system (CNS) tumors], for which no approved therapy exists.
6) Recovery to grade ≤ 1 from drug-related adverse events (AEs) of previous treatments, excluding alopecia and grade ≤2 asthenia or fatigue, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE v.5).
7) Laboratory values within fourteen days prior to registration:
   a) Absolute neutrophil count (ANC) ≥ 2.0 x 10^9/L, platelet count ≥ 120 x 10^9/L and hemoglobin ≥ 9.0 g/dL (patients may be transfused as clinically indicated prior to study entry).
   b) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x upper limit of normal (ULN).
   c) Serum total bilirubin ≤ 1.0 x ULN.
   If total bilirubin is > 1.0 x ULN, but ≤ 1.5 x ULN, direct bilirubin must be ≤ 1.0 x ULN.
   d) Albumin ≥ 3.5 g/dL.
   e) Creatinine clearance (CLcr) ≥ 30 mL/min (using Cockcroft and Gault's formula) (Appendix 4).
   f) Creatine phosphokinase (CPK) ≤ 2.5 x ULN.
8) Left ventricular ejection fraction (LVEF) by echocardiography (ECHO) or multiple-gated acquisition (MUGA) within normal range (according to institutional standards).
9) Evidence of non-childbearing status for women of childbearing potential (WOCBP). WOCBP must agree to use a highly effective contraceptive measure up to six months after treatment discontinuation. Valid methods to determine the childbearing potential, adequate contraception and requirements for WOCBP partners are described in Appendix 2. As bosentan may render hormonal contraceptives ineffective, and taking into account the teratogenic effects observed in animals, hormonal contraceptives cannot be the sole method of contraception during treatment with bosentan. Fertile male patients with WOCBP partners should use condoms during treatment and for four months following the last investigational medicinal product (IMP) dose.

4.2 EXCLUSION CRITERIA

All patients who meet any of the following criteria will be excluded from participating in the study:

1) Concomitant diseases/conditions:
   a) History or presence of unstable angina, myocardial infarction, congestive heart failure, or clinically significant valvular disease within last year.
   b) Symptomatic arrhythmia or any uncontrolled arrhythmia requiring ongoing treatment.
   c) Known cirrhosis, alcohol induced steatosis, or chronic active hepatitis. For hepatitis B, this includes positive test for both Hepatitis B surface antigen (HBsAg) and quantitative Hepatitis B polymerase chain reaction (PCR or HVB-DNA+). For hepatitis C, this includes positive test for both Hepatitis C antibody and quantitative Hepatitis C by PCR (or HVC-RNA+).
   d) History of obstructive cholestatic liver disease (suitable for stenting procedure) or biliary sepsis in the past 2 months.
   e) Active COVID-19 disease (this includes positive test for SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs or nasal swabs by PCR).
2) Symptomatic, progressive or corticosteroids-requiring documented brain metastases or leptomeningeal disease involvement. Patients with asymptomatic documented stable brain metastases not requiring corticosteroids during the last four weeks are allowed.
3) Use of (strong or moderate) inhibitors or inducers of CYP3A4 activity within three weeks prior to Day 1 of Cycle 1 (Appendix 3).
4) Use of CYP3A4 substrates for which concomitant administration with moderate CYP3A4 inductor is contraindicated (Appendix 3).
5) Treatment with any investigational product within the 30 days before Day 1 of Cycle 1.
6) Women who are pregnant or breast feeding and fertile patients (men and women) who are not using an effective method of contraception (Appendix 2).
7) Psychiatric illness/social situations that would limit compliance with study requirements.

4.3 PATIENTS FOR THE PHARMACOGENETICS SUB-STUDY

Only patients who voluntarily sign the written ICF for the pharmacogenetic sub-study will participate in it. Refusal to participate in this sub-study will not affect patient participation in the clinical study PM1183-A-019-20.

5. PLAN OF THE STUDY

5.1 PLANNED STUDY PERIODS (FOR THE WHOLE STUDY)

The total duration of the study will be approximately 22 months, including approximately an 18-month enrollment period.

- **Planned start date** (first patient on study): 4Q2020.
- **Planned enrollment period**: approximately 18 months.
- **Planned end-of-study date** (clinical cutoff): 31 (±10) days after the date of the last lurbinectedin administration for last patient /last cycle, or when confirmation of evaluability of at least eight patients, whichever occurs last.

  Patients who meet the continuation criteria and obtain a clear clinical benefit with lurbinectedin after the completion of the first two study cycles, according to the Investigator's criteria may continue treatment outside this study under a Compassionate Use Agreement after the completion of the optional third study cycle. Should the patient continue under a Compassionate Use Agreement, the treating center must request authorization to the relevant Health Authorities and notify the Sponsor in due time.

5.2 PLANNED STUDY PERIODS (INDIVIDUALLY PER PATIENT)

Patients will be evaluated at scheduled visits in three study periods:

- **Pre-treatment (screening):** from signature of the ICF to the day of first administration of the study treatment (bosentan or lurbinectedin).
- **Treatment:** from first administration of the study treatment (bosentan or lurbinectedin) to the last dose of lurbinectedin plus 31 days (end-of-treatment, EOT) (maximum of three cycles).
- **Follow-up of adverse events:** after EOT, patients with treatment-related toxicities > grade 2 will be followed every four weeks until recovery to at least grade 1 or stabilization of all treatment-emergent AEs, or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first.

Patients will be considered to be **on-study** from the signature of the ICF to the end of the follow-up period. Patients will be considered to be **on-treatment** for the duration of their treatment and until the day of EOT, immediately before the start of the follow-up period.

An end-of-treatment visit will be performed within 31 days (±10 days) after administration of the last dose of lurbinectedin, unless the patient dies or starts any new antitumor therapy outside this clinical study, in which case the EOT visit should be performed immediately before the start of the new therapy (ideally the day before or the same day).

5.2.1 Discontinuations

5.2.1.1 *Treatment Discontinuation*

Treatment discontinuation occurs when an enrolled patient ceases to receive the study treatment regardless of the circumstances. By convention, the date of EOT is defined as 31 days after the day of the last dose of lurbinectedin (treatment discontinuation), the start of a new antitumor therapy or death, 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 EOT.

The primary reason for any treatment discontinuation will be recorded on the patient's electronic Case Report Form (eCRF).

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

5.2.1.2 *Reasons for Treatment Discontinuation*

Patients will receive the study medication for a maximum of three cycles and while it is considered to be in their best interest. Specifically, treatment will continue for a maximum of three cycles until:

- Disease progression.
- Unacceptable toxicity.
- Intercurrent illness of sufficient magnitude to preclude safe continuation of the study.
- Patient's refusal and/or non-compliance with study requirements.
- A protocol deviation with an effect on the risk/benefit ratio of the clinical study.
- Delay > 14 days due to treatment-related adverse events (unless clear clinical benefit has been documented and always with the Sponsor's agreement).
- More than two lurbinectedin dose reductions due to adverse events related to lurbinectedin (unless clear clinical benefit has been documented and always with the Sponsor's agreement).
- Any other reason at the physician's judgment that precludes lurbinectedin continuation.

5.2.1.3 *Study Discontinuation*

Study discontinuation occurs when an enrolled patient ceases to participate in the study, regardless of the circumstances, prior to completion of the study. Patients have the right to withdraw consent at any time; if this is the case, no further study procedures should be performed. The Investigator must determine the primary reason for discontinuation. The date and reason for a patient's discontinuation from the study will be recorded on the eCRF.

5.2.2 Protocol Deviations

A protocol deviation is defined as any departure from what is described in the protocol of a clinical study approved by an Independent Ethics Committee (IEC) and a Competent Authority. Therefore, it 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 Good Clinical Practice (GCP) guidelines or ethical issues (e.g., issues related to obtaining patients' informed consent, data reporting or Investigator's responsibilities).

Deviations with no effects on the risk/benefit ratio of the clinical study (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 study objectives, such as those involving relevant inclusion/exclusion criteria (which could mean that the patient is not eligible for the study) 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 study treatment 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 informed consent or not following the terms established for reporting serious adverse events (SAEs), etc.

No deviations that may have an effect on the risk/benefit ratio of the clinical study will be authorized. All protocol deviations detected during the study will be appropriately documented, and those considered particularly relevant (i.e., those related to ethical issues, fulfillment of GCP guidelines and with an effect on the risk/benefit ratio) will be notified to the pertinent IEC and, if applicable, to the Competent Authorities as established by local regulations.

5.3 REPLACEMENT OF PATIENTS

Patients must be replaced if they are not evaluable for the assessment of the primary endpoint (e.g., if they have not sufficient and interpretable PK parameters).

Evaluable patients for the main objective of the study (e.g., assessment of lurbinectedin PK) should have provided sufficient and interpretable PK parameters (e.g., AUC_{0-t} should cover at least 80% of $AUC_{0-\infty}$) of Cycle 1 and 2. Evaluable patients should have received the first two complete cycles regardless dose delays or reductions.

The compliance of bosentan will be confirmed based on a patient's diary, the drug accountability and the expected individual plasma concentration at the steady state.

Any replacement subject during the treatment phase will be given a new subject number and assigned to the same sequence as the not-evaluable subject being replaced. All replaced patients who received the study treatment will be included in the general safety.

6. STUDY ASSESSMENTS AND PROCEDURES

6.1 ELIGIBILITY ASSESSMENTS

The patient will be allocated a patient number in the eCRF after signing the ICF and screening for eligibility begins. This patient number should be used on all future documentation and correspondence referring to this patient. During the screening period, the Investigator will assess the patient's eligibility for inclusion in the study by conducting the assessments summarized below (Table 4).

Table 4. Screening period assessments.

	ASSESSMENT	TIME
1. Written informed consents (general and PGt sub-study)	The informed consent process involves an explanation and discussion with the patient including time for questions and answers, and culminates in signing and dating the consent form if the patient agrees. Document registration in the patient's medical chart as well. Sub-study participation is optional and requires a separate signed consent.-	**Sequence 1 (TR) and Sequence 2 (RT):** Before any study-specific procedures.
2. Medical and cancer history/ clinical examination	◆ **Demographic data** (race/ethnicity [if permitted], age, gender). ◆ **Primary diagnosis and prior treatment(s)** (with best response, when available)**.** ◆ **Medical and cancer history/baseline condition.** ◆ **Complete physical examination**, including weight, height and calculation of BSA (Appendix 4). ◆ **ECOG** performance status (Appendix 1). ◆ **Vital signs:** heart rate, blood pressure, and body temperature.	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]
3. Laboratory tests	◆ **Hematology:** Differential WBC (including neutrophils, monocytes and lymphocytes), erythrocytes, hemoglobin, hematocrit and platelets. ◆ **Biochemistry-A:** AST, AP, ALT, lactate dehydrogenase (LDH), creatinine, glucose, creatine phosphokinase (CPK), CPK-MB fraction (if CPK is elevated above the normal range) and gamma glutamyltransferase (GGT) and total bilirubin (direct bilirubin if total bilirubin is above the ULN). ◆ **Biochemistry-B:** Serum electrolytes (Na+, K+, Mg++), total calcium, total proteins, albumin, CRP. ◆ **Coagulation:** INR. ◆ **Calculated CLcr** (Cockcroft-Gault formula) (Appendix 4). [b]	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]
4.Detection of SARS-CoV-2 (COVID-19)	◆ PCR for SARS-CoV-2 (COVID-19) in nasopharyngeal/oropharyngeal swabs and nasal swabs.	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]
5. Pregnancy test	Assessment of β-hCG (serum) only if the patient is a WOCBP (Appendix 2).	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]
6. Cardiac assessment	◆ **ECG**: Cardiac rhythm will be done in triplicate and identified in ECG intervals of at least 30 seconds of duration, PR interval, QT interval (raw and corrected by HR using Bazett's formula)	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]
	◆ **LVEF:** ECHO or MUGA scan.	**Sequence 1 (TR) and Sequence 2 (RT):** A previous LVEF assessment could be used if was made within 28 days prior to screening, if no previous assessment available; perform within 14 days prior to day of registration. [a]
7. Bosentan delivery and patient's diary	-	**Only in Sequence 1 (TR):** Day -6 with a -2/+1 day time window (i.e., from Day -8 to Day -5), but after the registration.

	ASSESSMENT	TIME
8. Intercurrent events, concomitant disease and therapies	-	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]
9. Adverse events	Clinical assessment of the patient's signs and symptoms. [c] Only information on SAEs that occurred after signature of the informed consent is required before treatment start. Grading should be as per the NCI-CTCAE v.5.	**Sequence 1 (TR) and Sequence 2 (RT):** Within 14 days prior to day of registration. [a]

Permitted windows for assessments before Day 1 of Cycle 1:

If physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Coagulation were assessed >10 days before Day 1 of Cycle 1, the tests must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).

If pregnancy test was done >7 days before Day 1 of Cycle 1, the test must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).

A patient should only be treated if eligibility criteria are still met according to assessments performed closest to treatment start.

[a] Sequence 1 (TR)/ Sequence 2 (RT): day of registration, confirmation of eligibility.

[b] Calculated CLcr [Cockcroft and Gault's formula] should be provided only if serum creatinine levels are above ULN.

[c] Signs and symptoms will be assessed by the Sponsor as all the events ongoing during the screening period, except for prior history conditions, which should be reported in the medical history form. Ongoing events during the screening period are those present at any time between the informed consent date and the first drug administration, regardless of if they may be resolved or not at the date of Cycle 1 Day 1.

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; β-hCG, beta subunit of human chorionic gonadotropin; BSA, body surface area; CLcr, Creatinine clearance; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase-isoenzyme MB; CRP, C-reactive protein; ECG, electrocardiogram; ECHO, echocardiogram; ECOG PS, Eastern Cooperative Oncology Group Performance Status; GGT, gamma glutamyltransferase; INR, International Normalized Ratio; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MUGA, multiple-gated acquisition scan; NCI-CTCAE, National Cancer Institute-Common Terminology Criteria for Adverse Events; PCR, polymerase chain reaction; PGt; pharmacogenetic; RT, reference-test; SAE, serious adverse event; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TR, test-reference sequence; ULN, upper limit of normal; WBC, white blood cells; WOCBP, woman of childbearing potential.

6.2 PATIENT REGISTRATION

Eligibility will be checked before registration confirmed by the Sponsor. The patient will be a screening failure if not all inclusion criteria are met, if any exclusion criterion is met, or if the Sponsor does not approve registration. Regardless of circumstances, Investigators will not be allowed to treat any patient before appropriate receipt of the Sponsor's agreement to proceed with registration. A patient should only be treated if eligibility criteria are still met according to assessments performed closest to first drug administration.

A patient who has been treated without the Sponsor's agreement may not be considered evaluable for the primary endpoint of the study and may need to be replaced (although can continue in treatment provided this does not pose a risk to the patient until discontinuation for any other reason).

For patients included in the study (all registered patients) but never treated, only the applicable forms at baseline and off-study visit modules of the eCRF should be completed but serious adverse events (SAEs) occurring during screening period need to be reported (using paper SAE forms).

For screen failures (patients who started screening but were not finally registered), only the screening form of the eCRF should be completed but SAEs occurring during screening need to be reported (using paper SAE forms).

6.3 PATIENT RANDOMIZATION

This is an open-label study; therefore, blinding of treatment will not be performed.

Block randomization will be used to avoid bias in the assignment of patients to treatment sequence group, to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment sequence groups, and to enhance the validity of statistical comparisons across treatment sequence groups. Randomization will apply for all patients.

6.4 EVALUATIONS DURING TREATMENT

The following assessments will be done while patient is on treatment (Table 5). For a more graphic display, please refer to the table Schedule of Assessments and Procedures.

Table 5. Evaluations during treatment.

	ASSESSMENT	TIME
1. Clinical examination	◆ Complete physical examination, including weight and calculation of BSA. [a]	**Sequence 1 (TR) and Sequence 2 (RT):** Day 1 of each cycle (always prior to lurbinectedin infusion). Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment.
	◆ Vital signs: heart rate, blood pressure and body temperature.	**Sequence 1 (TR) and Sequence 2 (RT):** Day 1 of each cycle (always prior to lurbinectedin infusion), and Day 8 in Cycles 1 and 2. Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment.
	◆ **ECOG** performance status (Appendix 1).	**Sequence 1 (TR) and Sequence 2 (RT):** Day 1 of each cycle (always prior to lurbinectedin infusion). Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment.
2. Laboratory tests [b]	◆ **Hematology:** Differential WBC (including neutrophils, monocytes and lymphocytes), erythrocytes, hemoglobin, hematocrit and platelets. ◆ **Biochemistry A:** AST, AP, ALT, lactate dehydrogenase (LDH), creatinine, glucose, creatine phosphokinase (CPK), CPK-MB fraction (if CPK is elevated above the normal range) and gamma glutamyltransferase (GGT) and total bilirubin (direct bilirubin if total bilirubin is above the ULN).	**Sequence 1 (TR) and Sequence 2 (RT):** Cycle 1: Day 8 and Day 15. Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment. Cycle 2: Day 1, Day 8 and Day 15. Cycle 3: Day 1. (always prior to lurbinectedin infusion).
	◆ **Biochemistry B:** Serum electrolytes (Na+, K+, Mg++), total calcium, total proteins, albumin, CRP.	**Sequence 1 (TR) and Sequence 2 (RT):** Cycle 2 and Cycle 3: Day 1 of each cycle (always prior to lurbinectedin infusion). Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment.

	ASSESSMENT	TIME
	◆ **Coagulation (INR):**	**Sequence 1 (TR) and Sequence 2 (RT):** Repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment. Also repeat if clinically indicated.
	◆ **Calculated CLcr:** Cockcroft and Gault´s formula (Appendix 4). [c]	**Sequence 1 (TR) and Sequence 2 (RT):** Cycle 1: Day 8 and Day 15. Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than ten days have passed since the screening assessment. Cycle 2: Day 1, Day 8 and Day 15. Cycle 3: Day 1. (always prior to lurbinectedin infusion).
3. Pregnancy test	Only applicable to WOCBP. Assessment of β-hCG (in serum).	During the on-treatment period, testing should be done be every cycle (always prior to lurbinectedin infusion). Also repeat on Day 1 of Cycle 1 (prior to lurbinectedin infusion) if more than seven days have passed since the screening assessment.
4. Pharmacokinetics	◆ **Cycle 1 and Cycle 2**: Eleven blood samples each (before lurbinectedin infusion start, 5 min before end of lurbinectedin infusion and 30 min, 1 hour, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours, 96 hours, 168 hours after the end of lurbinectedin infusion) will be collected for pharmacokinetic analyses (see details in Section 8.1.1).	**Sequence 1 (TR) and Sequence 2 (RT):** Cycle 1 and Cycle 2. PK sampling for bosentan will be done in Cycle 1 for patients assigned to Sequence 1 (TR), and in Cycle 2 for assigned to Sequence 2 (RT). See Table 9.
5. AAG and IL6 collection	One blood sample per cycle.	**Sequence 1 (TR) and Sequence 2 (RT):** On Day 1 of Cycle 1 and Cycle 2 (always prior to lurbinectedin infusion).
6. Pharmacogenetics (only if written informed consent given)	One blood sample.	**Sequence 1 (TR) and Sequence 2 (RT):** At any time during the study, but preferably just before lurbinectedin infusion start in Cycle 1.
7. ECG	ECG should be done in triplicate and cardiac rhythm will be identified in ECG intervals of at least 30 seconds of duration, PR interval, QT interval (raw and corrected by HR using Bazett's formula).	**Sequence 1 (TR) and Sequence 2 (RT):** On Day 1 of Cycle 1 and Cycle 2, 5 minutes before SOI (-25 to -1 min before preinfusion PK samples collection) and 1 hour after EOI (-15 to -1 min before the PK sample collection).
8. LVEF	LVEF measured by MUGA or ECHO.	**Sequence 1 (TR) and Sequence 2 (RT):** Repeat if clinically indicated.
9. Bosentan delivery and patient's diary	-	**Sequence 2 (RT):** Day -6 of Cycle 2 with a -2/+1 day time window (i.e., from Day 14 of Cycle 1 to Day -5 of Cycle 2).
10. Contact to patients	The study center will contact the patients daily (via telephone or other methods) to remind them or confirm that they have taken the morning and evening dose of bosentan when they are not at the study center.	**Sequence 1 (TR):** Day -5, -4, -3, -2 and -1 of Cycle 1. **Sequence 2 (RT):** Day -5, -4, -3, -2 and -1 of Cycle 2.
11. Intercurrent events, concomitant disease and therapies	-	Throughout the "on treatment" period. [d]
12. Adverse events	As per NCI-CTCAE v.5. [e]	Throughout the "on treatment" period. [d]

Registration = confirmation of eligibility and inclusion; Day 1 = the day of lurbinectedin administration (with infusion start) of cycle 1 or 2; Day 8 = seven days after the lurbinectedin administration of cycle 1 or 2 (±2 days); Day 15 = fourteen days after the lurbinectedin administration of cycle 1 or 2 (±2 days).

	ASSESSMENT	TIME

Permitted windows for assessments *before* Day 1 of Cycle 1:
If physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Coagulation were assessed >10 days before Day 1 of Cycle 1, the tests must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).
If pregnancy test was done >7 days before Day 1 of Cycle 1, the test must be repeated the day before or the Day 1 (always prior to lurbinectedin infusion).
A patient should only be treated if eligibility criteria are still met according to assessments performed closest to treatment start.
Permitted windows for assessments *after* Day 1 of Cycle 1:
A ± 2-day time window will be allowed for Hematology, Biochemistry-A and CLcr (provided only if serum creatinine levels are above ULN) on Day 8 and Day 15, and for vital signs on Day 8 of Cycle 1.
A -1 day window will be allowed for physical examination, ECOG PS, vital signs, Hematology, Biochemistry-A, Biochemistry-B, CLcr (provided only if serum creatinine levels are above ULN) and Pregnancy test on Day 1 of Cycle 2 and further cycles (always prior to lurbinectedin infusion).
A + 2 days window for Day 1 lurbinectedin administration of Cycle 3.
If a drug administration is delayed beyond the permitted protocol window, assessments planned for the original administration day must be repeated on the day the dose is finally administered.

[a] BSA calculated according to DuBois formula (see Appendix 4).
[b] Any patient having any grade ≥ 3 laboratory abnormalities and/or treatment-related AEs is suggested to have the relevant tests re-assessed at intervals of at least 72 hours but not more than one week (for patients allocated to Sequence 1) or at least every 48-72 hours (for patients allocated to Sequence 2) until recovery to at least grade 2 has been documented.
[c] Calculated CLcr [Cockcroft and Gault's formula] should be provided only if serum creatinine levels are above ULN.
[d] "On treatment period" = from first administration of the study treatment (bosentan or lurbinectedin) to the last lurbinectedin administration (and maximum of three cycles) plus 31 days (EOT). Follow-up period will be extended every four weeks until recovery to at least grade 1 or stabilization of all treatment-emergent AEs, or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first.
[e] The "AE form" will be used only for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines); and to report 'ongoing' baseline conditions in case of any significant change (improvement or worsening) during the study.

AAG, alpha 1-acid glycoprotein; AEs, adverse events; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BSA, body surface area; CLcr, creatinine clearance; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase-isoenzyme MB; CRP, C-reactive protein; ECG, electrocardiogram; ECHO, echocardiogram; EOI, end of infusion; EOT, end of treatment; GGT, gamma glutamyltransferase; ICH, International Conference on Harmonization; IL-6, interleukin 6; INR, International Normalized Ratio; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MUGA, multiple-gated acquisition scan; NCI-CTCAE, National Cancer Institute-Common Terminology Criteria for Adverse Events; PK, pharmacokinetics; RT, reference-test; SOI, start of infusion; TR, test-reference sequence; ULN, upper limit of normal; WBC, white blood cells; WOCBP, woman/women of childbearing potential.

6.5 EVALUATIONS AT THE END OF TREATMENT

The end-of-treatment (EOT) visit will be scheduled within 31 days (±10 days) after administration of the last dose of lurbinectedin, unless the patient starts a subsequent antitumor therapy, in which case the end-of-treatment visit should be performed immediately before the start of the new therapy (ideally the day before or the same day).

Patients who meet the continuation criteria and obtain a clear clinical benefit with lurbinectedin after the completion of the first two study cycles, may continue treatment outside this study under a Compassionate Use Agreement after the completion of the optional third study cycle. Should the patient continue under a Compassionate Use Agreement, the treating center must request authorization to the relevant Health Authorities and notify the Sponsor in due time. Patients, regardless of the reason for ending the treatment, will have to undergo at the end of treatment the following assessments:

- Complete physical examination (BSA and height not needed).
- ECOG PS.
- Laboratory tests (hematology and biochemistry A and B, and Calculated CLcr). Calculated CLcr [Cockcroft and Gault's formula] should be provided only if serum creatinine levels are above ULN.
- Coagulation (INR) will be repeated only if clinically indicated.
- ECG (in triplicate) and LVEF assessment.
- Adverse Events.
- Intercurrent events, concomitant disease and therapies.
- Pregnancy test in WOCBP.

Adverse events must be reported for 31 days after the last study treatment administration. All serious adverse events (SAEs) occurring within 31 days of the last study treatment administration or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first, will be reported. Beyond this period of time, only those suspected to be treatment-related SAEs will be reported (Section 8.5.2).
The Sponsor will evaluate all safety information that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

6.6 FOLLOW-UP AFTER END-OF-TREATMENT VISIT

After EOT, patients with treatment-related toxicities > grade 2 will be followed every four weeks until recovery to at least grade 1 or stabilization of all treatment-emergent AEs, or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first.

7. TREATMENT

7.1 DESCRIPTION OF TREATMENT

All patients will receive two consecutive cycles of lurbinectedin, one cycle with and one cycle without bosentan co-administration (in different order depending on the study sequence of treatment), followed by a third cycle with lurbinectedin alone (this last cycle optional for patients with clinical benefit).

7.1.1 Drug Formulation and Supply

Bosentan

Commercially available bosentan film-coated tablets (with strengths of 125 mg) will be provided by the Sponsor.

Lurbinectedin

Lurbinectedin 4 mg drug product (DP) is presented as a lyophilized powder for concentrate for solution for infusion in 30 mL vials and will be supplied by the Sponsor for the purposes of this study.

Before use, the 4 mg DP should be reconstituted with 8 mL of water for injection to give a solution containing 0.5 mg/mL of lurbinectedin. For administration to patients

as an i.v. infusion, reconstituted vials should be diluted with either glucose 50 mg/mL (5%) or sodium chloride 9 mg/mL (0.9%) solution for infusion.

The full composition of the lurbinectedin 4 mg DP and the reconstituted solution per mL is shown below:

Table 6. Composition of lurbinectedin Drug Product reconstituted solution.

Component	Concentration/vial	Concentration/vial after reconstitution
Lurbinectedin	4.0 mg	0.5 mg/mL
Sucrose	800 mg	100 mg/mL
Lactic acid	22.08 mg	2.76 mg/mL
Sodium hydroxide	5.12 mg	0.64 mg/mL

For detailed instructions on the reconstitution and dilution, refer to the latest lurbinectedin IB and the "Preparation Guide for Infusion" document.

7.1.2 Storage Conditions

Bosentan

Bosentan film-coated tablets should be stored following the instructions described in the SmPC.

Lurbinectedin

Until more information is available from ongoing stability studies, lurbinectedin DP 4 mg should be stored at +5º ± 3ºC.

7.2 ADMINISTRATION OF THE STUDY MEDICATION

Bosentan

Bosentan will be administered orally twice daily as 125 mg film-coated tablets.

In the co-administration cycles, bosentan will be administered orally twice daily in the morning and in the evening from Day -5 to Day -1 (i.e., five days before lurbinectedin infusion), and once daily on Day 1 (i.e., the day of lurbinectedin infusion), following recommendations at the Summary of Product Characteristics (SmPC). On Day 1 (i.e., day of lurbinectedin infusion), bosentan will be given immediately prior to the start of the lurbinectedin infusion. In fact, bosentan should be administered after obtaining the bosentan PK sample #1 and before the start of lurbinectedin infusion (-15 min to -1 min). In case of lurbinectedin delay (≤2 days), bosentan could be administered twice daily during a maximum of seven consecutive days before lurbinectedin infusion, and supplied at the study center once daily on Day 1 (before lurbinectedin infusion).

In Sequence 1 (TR), bosentan for self-administration and the patient's diary should be given to the patient on Day -6 with a -2/+1 day time window (i.e., from Day -8 to Day -5). In Sequence 2 (RT), bosentan and the patient's diary should be given to the patient on Day -6 of Cycle 2 with a -2/+1 day time window (i.e., from Day 14 of Cycle 1 to Day -5 of Cycle 2).

In addition, the study center will contact the patients (via telephone or other methods) daily during bosentan intake to remind them or confirm that they have taken the morning and evening dose of bosentan when they are not at the study center.

Lurbinectedin

Lurbinectedin will be administered as a 1-hour (-5/+20 min) i.v. infusion on Day 1 q3wk, over a minimum of 100 mL dilution on 5% glucose or 0.9% sodium chloride via a central line (or a minimum of 250 mL dilution if a peripheral line is used).

Patients will receive a maximum of three cycles: two consecutive cycles of lurbinectedin, one cycle with and one cycle without bosentan co-administration (in different order depending on the Sequence 1 or Sequence 2 of treatment), followed by a third cycle with lurbinectedin alone (this last cycle optional for patients with clinical benefit).

The dose of lurbinectedin when given in combination with bosentan will be 3.2 mg/m². Lurbinectedin dose will be based on the acceptability of the PK and safety results from the first three patients.

In cycles with lurbinectedin as single agent, lurbinectedin will we administered at 3.2 mg/m^2.

7.3 PROPHYLACTIC MEDICATION

All patients will receive standard antiemetic prophylaxis before each treatment infusion (i.e., 30 ± 5 minutes before lurbinectedin administration), i.v. as follows:

- Corticosteroids i.v. (according to institutional standard antiemetic doses, minimum dose of dexamethasone 8 mg/day and maximum dose of 20 mg/day of dexamethasone or equivalent). If feasible, the medication and dose administered in Cycle 1 should be maintained in Cycle 2.
- Serotonin (5-HT_3) antagonists (ondansetron at least 8 mg i.v. and no more than 16 mg of ondansetron).

If necessary, in addition to the above, the duration of treatment with 5-HT_3 antagonists and/or dexamethasone could be extended orally (if needed) (i.e., 4-8 mg/day for three consecutive days) and/or 10 mg of metoclopramide orally every eight hours could be added (according to institutional guidelines).

For the purpose of safety evaluations, optimum prophylaxis is defined as all the aforementioned medications at their respective maximum dose.

Aprepitant or any other NK-1 antagonist or related Substance P-antagonists (except rolapitant) are forbidden while on lurbinectedin treatment.

7.4 CRITERIA FOR TREATMENT CONTINUATION

Patients may continue treatment with lurbinectedin as long as no unacceptable toxicity and/or PD occurred, and for a maximum of three cycles. Thereafter, treatment with lurbinectedin can continue under a Compassionate Use Agreement.

- **Patients assigned to Sequence 1 (TR)** (lurbinectedin + bosentan in Cycle 1), after Cycle 1 the administration of a new cycle should be delayed if the criteria in Table 7 are not met on the corresponding Day 1. Re-assessments will be performed periodically at intervals of at least 72 hours but not more than one week. Lurbinectedin administration should be delayed until recovery of these parameters. A maximum delay of 14 days from the theoretical due date will be allowed for recovery from treatment-related AEs. If recovery has not occurred

after that period, the patient should discontinue the treatment, except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor.

- **Patients assigned to Sequence 2 (RT)** (lurbinectedin + bosentan in Cycle 2), criteria for treatment continuation should be investigated on Day 15 of Cycle 1 in order to confirm bosentan administration start on Day -5 of Cycle 2, assuring that the continuation criteria will be finally met on Day 1 of Cycle 2 assessment. In case of detected abnormalities on Day 15 in sequence RT, these assessments should be repeated every 48-72 hours until recovery to retreatment criteria. Initiation of bosentan will be delayed for a maximum of 14 days to ensure that pre-treatment with bosentan is fulfilled. If recovery has not occurred after that period, the patient should discontinue the treatment, except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor.

  Re-treatment criteria for lurbinectedin administration should be met on Day 1 of Cycle 2. If they were not met, treatment will be delayed for a maximum of 2 days to evaluate continuation of lurbinectedin administration, with re-treatment criteria being checked every 24 hours. During this time the patient will continue receiving bosentan. If after 2 days of delay the patient does not meet the criteria for treatment continuation, bosentan will be stopped and the patient will be considered not evaluable for the trial.

If the criteria for treatment continuation are not met as described above, treatment must be discontinued and the EOT visit performed.

Table 7. Criteria for lurbinectedin treatment continuation.

Variable	Lurbinectedin (Day 1)
ECOG PS	≤ 1
ANC	≥ 1.5 x 10^9/L
Platelets	≥ 100 x 10^9/L
Hemoglobin [a]	≥ 8.0 g/dL
Total bilirubin	≤ 1.0 x ULN. If it is > 1.0 x ULN but ≤ 1.5 x ULN, direct bilirubin must be ≤ 1.0 x ULN
Albumin	≥ 3.0 g/dL
AST/ALT	≤ 3.0 x ULN
CPK	≤ 2.5 x ULN (≤ 5.0 x ULN is acceptable if elevation is disease-related)
Serum Creatinine levels/ Calculated CLcr (Cockcroft and Gault's formula)	Serum creatinine levels ≤ ULN or calculated CLcr ≥ 30 mL/min, if abnormal (>ULN) serum creatinine levels
Other non-hematological drug-related AEs (except isolated increased GGT and/or AP; or grade 2 alopecia, asthenia, peripheral neuropathy and non-optimally treated nausea and/or vomiting)	Grade ≤ 1

[a] Patients may receive PRBC transfusions and/or EPO treatment if clinically indicated to increase/maintain adequate hemoglobin levels.

Sequence 1 (TR):
If a patient does not meet the requirements for treatment continuation on Day 1 of further cycles, treatment will be withheld until recovery for a maximum of 14 days after the theoretical treatment date. If recovery has not occurred after a delay of > 14 days, discontinue treatment (except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor).

Variable	Lurbinectedin (Day 1)

Sequence 2 (RT):
If a patient does not meet the requirements for treatment continuation on Day 15 of Cycle 1, treatment with bosentan will be withheld until recovery for a maximum of 14 days after the theoretical treatment date. If recovery has not occurred after a delay of > 14 days, discontinue treatment (except in case of obvious patient benefit at the criteria of the Investigator and upon agreement with the Sponsor).
If a patient does not meet the requirements for treatment continuation on Day 1 of Cycle 2, treatment with lurbinectedin will be withheld until recovery for a maximum of 2 days after the theoretical treatment date. During this time, the patient will continue receiving bosentan. If recovery has not occurred after a delay of > 2 days, bosentan will be stopped and the patient will be considered not evaluable for the trial.

AEs, adverse events; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AP, alkaline phosphatase; AST, aspartate aminotransferase; CLcr, creatinine clearance; CPK, creatine phosphokinase; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EPO, erythropoietin; GGT, gamma glutamyltransferase; PRBC, packed red blood cells; UULN, upper limit of normal.

7.5 DOSE REDUCTION

Patients may continue in the study treatment at a lurbinectedin reduced dose if they present any of the following:

- Grade 4 thrombocytopenia or grade 3 thrombocytopenia concomitantly with grade ≥ 3 bleeding.
- Two dose delays or prolonged (> one week) dose delay due to treatment-related adverse events.
- Grade ≥ 3 treatment related non-hematological toxicity. Exceptions are: grade ≥ 3 nausea and/or vomiting not optimally treated, grade 3 fatigue lasting < two days, grade 3 diarrhea lasting < one day or non-optimally treated, isolated grade 3 transient ALT/AST elevations which are rapidly reversible and not leading to subsequent delays and, non-clinically relevant isolated biochemical abnormalities.
- Grade 4 neutropenia or any grade febrile neutropenia.

Up to two dose reductions are allowed per patient. Patients who continue to experience treatment-related toxicity and/or dose delays after two dose reductions must be withdrawn from the study. Once the dose has been reduced for an individual patient, it will not be re-escalated under any circumstances.

Lurbinectedin dose reduction levels are shown in Table 8.

Table 8. Levels of (intra-patient) lurbinectedin dose reduction in subsequent cycles.

Dose reduction	Lurbinectedin (q3wk) (mg/m^2)	
	Lurbinectedin alone	Bosentan co-administration cycle
1 (starting dose)	3.2	3.2
-1	2.6	2.6
-2	2.0	-

Lurbinectedin total doses in mg will be rounded to the first decimal, if necessary.
q3wk, every three weeks.

Patients who experience any treatment-related grade 3/4 hypersensitivity and/or extravasation reactions will permanently discontinue treatment.

7.6 CONCOMITANT MEDICATION

All treatments received by the patient during the on-treatment period of the trial must be documented in the eCRF.

7.6.1 Allowed Medications/Therapies

- Therapies for pre-existing and treatment-emergent medical conditions, including pain management and local management of mucositis/stomatitis.
- Blood products and transfusions, as clinically indicated.
- Bisphosphonates.
- In case of nausea or vomiting, extended symptomatic treatment for emesis will be allowed (with the exception of aprepitant equivalent agents).
- Erythropoietin treatment according to the ASCO guidelines.
- Low-molecular weight heparin (LMWH) and/or any other anticoagulants, as clinically indicated. Oral anticoagulants must be carefully monitored.
- Treatment or secondary prophylaxis with G-CSF according to ASCO guidelines.
- CNS irradiation if required, and/or limited field bone radiotherapy for pain control outside the thoracic wall.
- Megestrol acetate for wasting syndrome.
- Contraceptives.

7.6.2 Prohibited Medications/Therapies

- Concomitant administration of any other antineoplastic therapy.
- Any other investigational agents.
- Immunosuppressive therapies other than corticosteroids for antiemetic prophylaxis, pain control, or low-dose replacement in patients requiring this approach.
- Aprepitant or any other NK-1 antagonist or related Substance P-antagonists (except rolapitant)**.**
- Primary G-CSF prophylaxis.
- CYP3A4 inhibitors such as ketoconazole, fluconazole, voriconazole, telithromycin, clarithromycin, erythromycin, nafcillin, aprepitant, fosaprepitant, verapamil, modafinil, nefazodone, or grapefruit juice.
- CYP3A enzyme inducers and/or inhibitors (unless strictly necessary and when there is no therapeutic alternative treatments) (see Appendix 3).
- Use of any prescription or non-prescription herbal and/or dietary supplements within 14 days prior to the first dose of study medication and until 31 days after the last administration of lurbinectedin, unless the Investigators, with the Sponsor agreement, consider it will not interfere with study procedures of patient safety.

7.6.3 Drug-Drug Interactions

In vitro studies with human microsomes have shown that CYP3A4 is the major CYP isoform involved in the metabolism of lurbinectedin. The estimated contribution of the other CYP isoenzymes to the lurbinectedin metabolism is considered to be negligible.

Therefore, concomitant drugs which induce or inhibit any of these cytochromes, especially CYP3A4, should be carefully monitored or avoided, whenever is possible.

A potentially significant interaction with aprepitant is suggested by available phase II data from ovarian cancer patients and phase I data from a combination trial with lurbinectedin and cisplatin (PM1183-A-008-13). Lurbinectedin clearance was reduced by around 30%, approximately, in the presence of aprepitant. Although all patients eventually recovered, the use of aprepitant is currently forbidden in all phase II/III lurbinectedin studies.

7.7 DRUG ACCOUNTABILITY

The Investigator at each study site (or center) will be the person ultimately responsible for drug accountability at the site. The Investigator site will appoint the designee as product storage manager and delegate the control of and accountability for the trial drugs to him/her. After receipt of the investigational product, he/she will keep records to allow a comparison of quantities of drug received and used at each site for monitoring purposes.

The investigator or designated study personnel will maintain a log of all bosentan drug dispensed and returned. Drug supplies for each subject will be inventoried and accounted for throughout the study.

All unused drug supplied by the Sponsor will be properly destroyed at the study site. Documentation of this procedure must be provided to the Sponsor (or its designee). If the Sponsor (or its designee) agrees, unused drug supplies may be returned to the drug repository.

7.8 TREATMENT COMPLIANCE

Lurbinectedin will be administered as an i.v. infusion by qualified staff. The morning and evening doses of bosentan should be administered following the SmPC specifications. Patients will self-administer the morning and evening daily doses of bosentan when not at the study center. The study center will contact the patients (via telephone or other methods) to remind them or confirm that they have taken the morning and evening dose of bosentan when they are not at the study center. When the subject is at the study center, the administration of bosentan is to be supervised and documented by the staff. Patients will be requested to keep records of bosentan when administered at home. The patients will be given a diary to record the dates and times when bosentan was administered at home. Diaries will be reviewed at each study visit and recorded in the source data system. The compliance of bosentan will be confirmed based on a patient's diary, the drug accountability and the expected individual plasma concentration at the steady state.

8. STUDY EVALUATIONS

8.1 PHARMACOKINETIC EVALUATIONS

8.1.1 Pharmacokinetic Sample Collection and Handling

Serial peripheral venous blood samples for determination of lurbinectedin (total and unbound), lurbinectedin metabolites (i.e., M1 and M4) and bosentan plasma

concentrations will be collected at the time points indicated in Table 9 and also in the Schedule of Assessments and Procedures. The exact dates and times of blood sample collection must be recorded in the eCRF.

PK sampling for lurbinectedin will be done always in Cycle 1 and Cycle 2 with a schedule of eleven samples in all patients. PK sampling for bosentan will be done in Cycle 1 of Sequence 1 (TR), and in Cycle 2 of Sequence 2 (RT) with a schedule of four samples in all patients (Table 9).

Plasma samples for PK analysis of lurbinectedin and bosentan will be obtained through a peripheral vein located in the contra-lateral side to that of the administration of lurbinectedin. In any case, the sampling vein has to be different to that in which drugs are being administered. Even the last sample must never be collected from the catheter used for the drug administration.

If the blood sample is obtained from a catheter, the first milliliter (mL) of blood will be discarded to avoid the dilution of the sample with the solution used to keep it clean. Heparin (10 U/mL in normal saline solution) or a slow drip of normal saline solution (10 mL/h) can be used to keep the catheter permeable between extractions.

The laboratory manual provides further information regarding handling and shipment of plasma samples. Samples will be stored at –80 (±10) ºC.

Table 9. Lurbinectedin and bosentan blood sampling schedule and aliquot collection.

Sample No.	Day	Time (h)[c]	Sampling Time [d]	Lurbinectedin			Bosentan[e]	Time window
				Total	Unbound (f_u)	Metabolites		
#1 [a, b]	1	0	Preinfusion [f]	●	●	●	●	-15 to -1 min before SOI [g]
#2	1	0.917	5 min before EOI	●	●	●	-	±4 min
#3	1	1.5	30 min after EOI	●	-	●	-	± 5 min
#4	1	2	1 h after EOI	●	-	●	●	± 10 min
#5	1	3	2 h after EOI	●	-	●	-	± 10 min
#6	1	5	4 h after EOI	●	-	●	●	± 30 min
#7	1	7	6 h after EOI	●	-	●	-	± 30 min
#8	2	25	24 h after EOI	●	-	●	●	±2 h
#9	3	49	48 h after EOI	●	-	-	-	±2 h
#10	5	97	96 h after EOI	●	-	-	-	±24 h
#11	8	169	168 h after EOI	●	-	-	-	±48 h [h]

[a] Additional sample for AAG and IL-6.
[b] Additional sample for PGt, once only, if written IC given.
[c] Time relative to infusion start.
[d] Time relative to lurbinectedin end of infusion (except PK #1).
[e] Sampling for bosentan only applies in cycle with bosentan co-administration.
[f] 5 min before start of infusion.
[g] Bosentan should be administered after bosentan PK sample #1 and before the start of lurbinectedin infusion (-15 min to -1 min).
[h] If the scheduled sampling time for this sample should be modified within the allowed time window, there must be a difference of at least 20 hours between the collection times of samples PK#10 and PK#11.
AAG, alpha 1-acid glycoprotein; EOI, end of infusion; f_u, unbound fraction; h, hour; IC, informed consent; IL-6, Interleukin 6; EOI, end of infusion; min, minute; PGt, pharmacogenetic; PK, pharmacokinetic; SOI, start of infusion.

8.1.2 Analytical Procedures

HPLC-MS/MS determinations of total, unbound plasma concentrations of lurbinectedin and total plasma concentration of its main metabolites (i.e., M1 and M4) will be performed using validated assays.

A validated bioanalytical method will be used to measure the bosentan plasma concentrations.

8.1.3 Pharmacokinetic Parameters

The time course of metabolite concentration –relative to parent drug, and if possible, absolute– will be elucidated up to the maximum possible extent, to obtain the best available estimate for metabolite exposure.

Total and unbound plasma concentration time course of the lurbinectedin and its main metabolites will be analyzed by standard non-compartmental methods. Bosentan total plasma concentration time course also will be analyzed by standard non-compartmental methods. The area under the concentration-time curve (AUC) will be calculated using the linear-log trapezoidal rule with extrapolation to infinity. The maximum plasma concentration (C_{max}) will be obtained directly from the experimental data. Terminal half-life ($T_{1/2}$) will be obtained from the terminal rate constant calculated by linear regression using at least 3 observations. Other parameters will be calculated including:

Mean residence time extrapolated to infinity (MRT) corrected by the infusion time. It is calculated by dividing the area under the moment curve extrapolated to infinity by the area under the curve extrapolated to infinity and subtracting half the duration of drug infusion.

Total body clearance (CL), calculated by dividing the administered dose by the AUC with extrapolation to infinity.

Volume of distribution (both at steady state and based on the terminal phase) (V_{ss} and $V_{z,}$ respectively): V_{ss} is an estimate that equals mean residence time times total body clearance. V_z, calculated dividing the administered dose by the product of the AUC with extrapolation to infinity by the terminal rate constant.

The same analysis will be applied to the metabolites for which the obtained experimental data allow it. The potential relevance of the identified metabolites will be explored by additional preclinical experiments that will be decided on the basis of the results and, if possible, by opportunistic observations based on the study data.

8.2 PHARMACOGENETICS

Patients will be evaluable for pharmacogenetics if they have signed the ICF and a valid 9-mL blood sample will be taken for genetic analysis at any time during the study, but preferably before infusion start along with PK sample #1 on Day 1 of Cycle 1.

A genotype evaluation in genes relevant for lurbinectedin metabolism and transport will be investigated and reported in an independent report in order to determine whether the observed differences between patients in PK parameters are related to these genetic features.

8.3 SAFETY

Patients will be evaluable for safety if they have received at least part of one lurbinectedin infusion. AEs will be graded according to the NCI-CTCAE v. 5.

Treatment compliance, i.e., dose delays, dose reductions and reasons for treatment discontinuation, will be monitored throughout the study.

The safety profile of patients will be monitored throughout the treatment and up to 31 days (±10 days) after the last treatment infusion (EOT), or until the patient starts a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or until the date of death, whichever occurs first.

Any treatment-emergent AEs will be followed until recovery to at least grade 1 or stabilization of symptoms or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurs first. After treatment discontinuation, patients will be followed until resolution or stabilization of all toxicities, if any. Patients having any treatment-related grade ≥ 3 AEs should have relevant tests re-assessed at intervals of at least 72 hours but not more than one week (for patients allocated to Sequence 1) or at least every 48-72 hours (for patients allocated to Sequence 2) until recovery to at least grade 2.

8.4 ADVERSE EVENT DEFINITIONS

8.4.1 Adverse Event

An AE is any untoward medical occurrence in a patient or 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.

Illnesses with onset during the study or exacerbations of pre-existing illnesses, including but not limited to clinically significant changes in physical examination findings and abnormal objective tests/procedures findings (e.g., X-ray, ECG) should be recorded. 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 (see definition below), and/or
- The test result is considered to be clinically relevant by the Investigator.

For the purpose of this protocol, PD or worsening of the underlying malignancy should not be reported as AEs.

8.4.2 Serious Adverse Event

A SAE is defined as 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, or
- is medically significant.
- Any suspected transmission via a medicinal product of an infectious agent.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as 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 (including associated signs and symptoms) or appearance of new tumor lesions cannot be reported as a SAE.

8.4.3 Death

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

8.4.4 Life Threatening Event

A life threatening event is defined as any event in which the subject is 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.

8.4.5 Hospitalization/Prolongation of Hospitalization

Any event requiring hospitalization (or prolongation of hospitalization) that occurs or worsens during the course of a patient's participation in a clinical study must be reported as a SAE unless exempted from SAE reporting. 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 treating physician.

Hospitalizations that do not meet criteria for SAE reporting are:

- Reasons described in the protocol (e.g., investigational medicinal product [IMP] administration, protocol-required investigations). However, events requiring hospitalization or prolongation of hospitalization as a result of a complication of therapy administration or clinical study procedure will be reported as a SAE.
- Hospitalization or prolonged hospitalization for technical, practical or social reasons, in the absence of an AE.
- 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:

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

8.4.6 Unexpected/Unlisted Adverse Event

An AE is considered unexpected/unlisted when the nature or severity of which is not consistent with the applicable reference safety information. The Sponsor will use as the reference safety information (RSI) for the evaluation of listedness/expectedness the RSI of the most updated IB for lurbinectedin, and the SmPC for commercially-available bosentan.

8.4.7 Adverse Reaction

All untoward and unintended response to an IMP related to any dose administered. This definition also covers medication errors and uses outside what is foreseen in the protocol, including overdose, misuse and abuse of the product.

8.4.8 Adverse Events Related to the Study Drug

An AE is considered related to a study drug/IMP if the Investigator and/or the Sponsor's assessment of causal relationship to the IMP(s) is "Y (yes)" (see Section 8.4.10). The Investigator will assess the causal relationship of the IMP(s) to the SAE. The Sponsor will consider related to the study drug(s)/IMP(s) those events for which the Investigator and/or the Sponsor assesses the causal relationship with the IMP(s) as "Uk (unknown)" when it cannot rule out a role of the IMP(s) in the event.

8.4.9 Expedited Reporting

The Sponsor is responsible for appropriate expedited reporting to the Competent Authorities, the Investigators and the IEC according to current legislation.

8.4.10 Assessment of Causal Relationship to the Study Drug

The Investigator must provide an assessment of causality for the IMP according to the following criteria:

- Y (yes): there is a reasonable possibility that the IMP(s) caused the SAE.
- N (not): there is NO a reasonable possibility that the IMP(s) caused the SAE and other causes are more probable.
- UK (Unknown): only to be used in special situations where the Investigator has insufficient information (i.e., the patient was not seen at his/her center). If none of the above can be used.

8.5 ADVERSE EVENT REPORTING PROCEDURES

8.5.1 Reporting of Adverse Events

The Sponsor will collect AEs until 31-day (±10 days) after administration of the last dose of lurbinectedin or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or until the date of death, whichever occurs first. All AEs suspected to be related to the study drug(s)/IMP(s) must be followed-up 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.

All AEs, including medication errors and uses outside what is foreseen in the protocol, must be recorded in English using medical terminology in the source document and the

eCRF. 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.5 and assign a relationship to each study drug(s)/IMP(s); and 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 or its designated representative. The Investigator must provide any relevant information as requested by the Sponsor in addition to that on the eCRF.

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

- Is associated with clinically significant symptoms, and/or
- Leads to a change in study dosing (omission, delay or reduction) 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 eCRF (e.g. biochemistry, hematology).

Signs and symptoms: The Sponsor will consider signs and symptoms all the events ongoing during the screening period, except for prior history conditions, which should be reported in the medical history form. Ongoing events during the screening period are those present at any time between the informed consent date and the first drug administration, regardless of if they may be resolved or not at the date of Cycle 1 Day 1.

The "Adverse event form" will be used only for events that occur after the first drug infusion or any event related to a study procedure within the study period (according to ICH guidelines); and to report 'ongoing' baseline conditions in case of any significant change (improvement or worsening) during the study.

8.5.2 Reporting of Serious Adverse Events

The Sponsor will collect SAEs from the time of signing of the ICF and until 31 (±10) days after administration of the last dose of lurbinectedin or until the start of a new antitumor therapy, until the continuation of treatment outside this study under a Compassionate Use Agreement or death, whichever occurred first. Beyond this period of time, only those SAEs suspected to be related to the IMPs will be collected. Nonetheless, the Sponsor must evaluate any safety information related to the clinical study that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

All SAEs (as defined above) that have occurred after patient registration, regardless of relationship to the study drug(s)/IMP(s), must be reported immediately, and always within 24 hours to the Pharma Mar S.A. Pharmacovigilance Department electronically by completing the applicable eCRF section. Only in situations of electronic system failure or pregnancy exposure during the clinical trial, can SAEs be reported using paper on a "SAE form by fax (+34 91 846 6004), e-mail (AEincoming@pharmamar.comphv@pharmamar.com) or telephone (+34 91 823 4562). Out of office hours [Greenwich Meridian Time (GMT)], assistance on SAE reporting can be obtained by calling the Pharmacovigilance Department at +34 681 263 592. In case of electronic system failure or pregnancy exposure during the clinical trial, SAEs

initially reported by alternative methods (not electronically), must be followed by a completed electronic SAE reporting on eCRF from the investigational staff within one working day.

SAEs occurring during the screening phase (from ICF signature to the first study drug administration) and after off-study will be reported using a paper "SAE form" that must be forwarded as mentioned above always within 24 hours to the Pharma Mar S.A. Pharmacovigilance Department by fax or email.

All SAEs suspected to be related to the study drug must be followed until the event or its sequelae resolves or stabilizes to at least grade 1, or to an acceptable level according to the Investigator and the Sponsor or his/her designated representative.

8.5.3 Reporting of Pregnancy Cases Occurring During the Clinical Study

National regulations require that the Sponsor collect information on pregnancies occurring during clinical trials, in which exposure to the study drug 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 31 days of the patient's discontinuation visit, are considered an 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 study drug is suspected.
- Possible exposure of a pregnant woman.
- All reports of elevated/questionable or indeterminate beta human chorionic gonadotropins (β-hCGs).

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 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 the Pharmacovigilance Department at PharmaMar immediately by facsimile using the Pregnancy Report Form.

The Investigator will follow the pregnancy until completion/termination, and must notify the outcome of the pregnancy to the Pharmacovigilance Department at PharmaMar within 24 hours of first knowledge as a follow-up to the initial Pregnancy 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 Sponsor, Pharmacovigilance by facsimile within 24 h of the Investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, regardless of causality, as SAEs. In addition, any infant death at any time thereafter that the Investigator suspects as related to the in utero exposure to the study drug should also be reported to the Pharmacovigilance Department at PharmaMar by facsimile within 24 h of the Investigators' knowledge of the event.

8.6 ADVERSE EVENTS MONITORING

Safety review will be performed at Pharma Mar, S.A. once the SAE forms have been received and the eCRFs have been completed by the Investigator.

Periodic safety review of clinical data will be performed; however, no formal Data Safety Monitoring Board has been appointed for this trial. AEs will be monitored by the Investigators and by the study team at Pharma Mar, S.A. The personnel in charge of this process are defined in the section "Study Contacts" of this protocol. In general, a clinical oncologist, together with a member of the Pharma Mar, S.A. Pharmacovigilance Department 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.

Non-serious AEs will be checked for accuracy against the eCRF during monitoring visits by the monitor.

8.7 EFFICACY

Not applicable.

9. STATISTICAL METHODS

This clinical pharmacology study is designed to assess the impact of bosentan co-administration on lurbinectedin PK parameters administered alone.

9.1 ANALYSIS POPULATIONS

The PK population will include all patients enrolled who have sufficient and interpretable PK parameters to calculate the non-compartmental PK parameters. Only the patients who have completed the two cycles and have sufficient and interpretable PK assessments will be included in the statistical comparison to assess the effect of bosentan on the PK of lurbinectedin.

The safety population will include all patients who received at least one dose of lurbinectedin. Patients who have received at least one dose of bosentan but who did not receive any dose of lurbinectedin will be excluded from the safety population. The analysis of data from these patients will be performed separately (e.g., by means of narratives). The safety population will be used for all safety evaluations.

9.2 SAMPLE SIZE

A block randomization (1:1 ratio) will be performed. At least 8 patients are expected to complete all study procedures, including the collection of sufficient and interpretable PK assessments. Although at least 8 patients will be enrolled in this study; it is estimated that complete data from eight patients will be sufficient to estimate the drug-

drug interaction of bosentan on the PK of lurbinectedin. In case of patient replacement, a new patient number will be provided and he/she will be assigned to the same sequence as the patient being replaced.

This study was designed to assess the potential effects of bosentan on the PK of lurbinectedin in patients with advanced malignancies. The 90% CI will be used to help with the interpretation of the results. A sample size of eight patients was based on feasibility and clinical considerations. Based on previous studies, the intra-subject coefficient of variation (CV) of lurbinectedin PK parameters is estimated to be more than 30%. The precision (half-width) of the 90% CI for [(lurbinectedin + bosentan) / lurbinectedin alone] comparison on the log-scale will extend 0.389 from the observed differences in means, assuming that the intra-subject CV around 40%. This half-width corresponds to a 90% CI in the range of 70% and 147% assuming the ratio of the means equal to unity for each PK parameter. This 90% CI will be used to help with the interpretation of the results.

9.3 STATISTICAL ANALYSIS

Descriptive statistics (arithmetic and geometric means, median, SDev, CV% and 95% confidence interval [CI], range of value, frequencies and percentages) will be used to summarize the data.

9.3.1 Demographics

Descriptive statistics will be used to summarize the patients' baseline characteristics.

9.3.2 Safety

All AEs will be graded according to NCI-CTCAE v.5.

Treatment-emergent adverse events (TEAEs) are any adverse event aggravated in severity from baseline or having their onset between the first dose of the study drug and 31-day (±10 days) after the last treatment dose, death or date of further therapy, whichever came first. AEs related to the study treatment or with unknown relationship occurring more than 31 days after the last dose were also taken into account as TEAEs.

Descriptive statistics will be used to characterize the profiles of drug-related AEs, drug-related deaths, SAEs, clinical laboratory data, drug-related delays and/or treatment discontinuations. Tables will be displayed by sequence and treatment (Test or Reference).

9.3.3 Efficacy

Not applicable.

9.3.4 Pharmacokinetics

Plasma exposure to lurbinectedin

Only patients who completed the study with sufficient and interpretable PK parameters will be included in the statistical comparison of plasma exposure to lurbinectedin.

Descriptive statistics will be used to summarize pharmacokinetic data.

The primary parameter of interest for the statistical analysis will be plasma dose-adjusted $AUC_{(0-\infty)}$ (if data do not permit so, $AUC_{(0-t)}$ will be used) of lurbinectedin. The

analysis will compare the log-transformed AUC for lurbinectedin administered in combination with bosentan (Test) to the lurbinectedin alone (Reference).

A mixed-effects model will be fit to the data with log-transformed AUC as the dependent variable, treatment (Test or Reference), period and sequence as fixed effects, and patient (sequence) as a random effect. The estimated least square means and intrasubject variability from the mixed-effects model will be used to construct 90% CIs for the difference in means on the log scale between treatments (Test or Reference). The adjusted mean differences and the 90% CIs will be exponentiated to obtain estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CIs for the ratios.

A large difference (e.g. two-fold difference shown from CIs and least square means) will be considered to show evidence on whether bosentan co-administration has a clinically relevant effect on lurbinectedin exposure.

Secondary PK parameters

Similar models used for the primary endpoint will be fit to the data with dose-normalized $AUC_{(0\text{-}t)}$ and C_{max} and in Cl, V_{ss} and $T_{1/2}$ of lurbinectedin or metabolite to parent exposure PK parameters ratio, as the dependent variable. Results will also be assessed graphically.

Plasma protein binding

A similar model used for the primary endpoint will be fit to the data with dose-normalized AUC_u as the dependent variable.

9.3.5 Pharmacogenetics

Descriptive statistics will be used to summarize pharmacogenetic data in a separate report.

10. ADMINISTRATIVE SECTION

10.1 ETHICS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki (Appendix 5) and will be consistent with GCP and other applicable regulatory requirements.

The study personnel involved in conducting this study 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, the patient's diary and the patient ICF will receive IEC approval/favorable opinion prior to initiation. The decision of the IEC 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 before commencement of the study.

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

All protocol amendments will be agreed upon by the Sponsor 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.

10.2 MONITORING, AUDITING AND INSPECTING

The study will be monitored by regular site visits and telephone calls to the Investigator by the clinical study monitor designated by Pharma Mar, S.A. Source document verification may be performed remotely, if required.

During site visits, the study monitor should review original patient records, drug accountability records and document retention (study file). Additionally, the monitor should observe study procedures and will discuss any problem 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 Guideline for Good Clinical Practice, GCP) to source documents (i.e., 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-GCP.

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

At any time during the course or at the end of the study, the Clinical Quality Assurance Department of PharmaMar or external auditors contracted by the Sponsor may conduct an onsite or remote audit visit to the centers (ICH-GCP).

Participation in this study implies acceptance of potential onsite or remote inspections by national or foreign Competent Authorities.

10.3 PATIENT INFORMED CONSENT

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 ICF 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 with one copy of the Informed Consent Form (ICF). This copy must provide written full information about the clinical trial, in a language that is non-technical and easily understood. The Investigator should allow the necessary time for the patient or his/her legally acceptable representative to inquire about the details of the clinical study; then, the ICF must be freely signed and personally dated by the patient and by the person who conducted the Informed Consent discussion before the beginning of the study. The patient should receive a copy of the signed ICFs and any other written information provided to study patients prior to participation in the trial.

Participation in the pharmacogenetic sub-study is optional and will need an additional ICF signed by the patient. Should the patient decide not to participate in the pharmacogenetic sub-study, this will not affect his/her rights to participate in the current study.

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.

10.4 CONFIDENTIALITY/ PATIENTS IDENTIFICATION

The collection and processing of personal data from patients enrolled in this study will be limited to data that are necessary to investigate the efficacy, safety, quality, and usefulness of the IMP(s) used in this study. It is the Investigator's responsibility that sufficient information pertaining to the patient's identity be retained.

The study monitor, the Sponsor's auditor, the IEC and the Competent Authorities should have direct access to all requested study-related records and agree to keep the identity of the 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, if applicable, and this consent should also address the transfer of the data to other entities and countries.

The Sponsor will comply with General Data Protection Regulation (GDPR) (EU) 2016/679 effective from 25 May 2018 (repealing Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995), and applicable regulations on the protection of individuals with regards to the processing of personal data and on the free movement of such data.

10.5 ELECTRONIC CASE REPORT FORMS

Electronic case report forms (eCRFs) will be used to record all data for each patient. It is the responsibility of the Investigator to ensure that the eCRFs are properly and fully completed in English. ECRFs must be completed for all patients who have given informed consent and have been enrolled into the study.

A patient's source documentation comprises all medical records (including but not limited to physician/hospital notes, nurses notes, IMP preparation records including reconstitution and dilution, IMP administration records) and any original document, and as such they should be maintained at the study site.

The data collected in the eCRF will be entered into the Sponsor's databases which comply with GDPR (EU) 2016/679 effective from 25 May 2018 (repealing 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 and on the free movement of such data.

10.6 INSURANCE

The Sponsor will provide insurance or indemnity in accordance with the applicable regulatory requirements.

10.7 RETENTION OF RECORDS

The Investigator/Institution should maintain study documents according to ICH 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.

10.8 USE OF INFORMATION AND PUBLICATION

Before the investigators of this study submit a paper or abstract for publication or otherwise publicly disclose information concerning the study drug or products, Pharma Mar, S.A. 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 Pharma Mar, S.A. 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. Relevant Sponsor personnel who have fully participated in the study must be considered for co-authorship of the publication. In general, the order of the investigator will reflect who recruits the highest number of patients with information finally available for data analysis.

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12. APPENDICES

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: CONTRACEPTION AND PREGNANCY TESTING

This document is based on the Heads of Medicines Agencies' Recommendations Related to Contraception and Pregnancy Testing in Clinical Trials, published by the Clinical Trial Facilitation Group (CTFG) on 21 September 2020 and available at http://www.hma.eu/ctfg.html (accessed 5 January 2021).

A woman is considered of childbearing potential (WOCBP) following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. Immediately after detecting a case of suspected pregnancy in a patient, the decision on her continued participation in the clinical trial will be jointly taken by the patient, the Investigator and the Sponsor, with the patient's best interest in mind. A decision to continue the pregnancy will require immediate discontinuation any investigational medicinal product (IMP).

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy. Fertile male patients included in this study should refrain from fathering a child or donating sperm during the study and for four months following the last IMP dose. If they have WOCBP partners the male subject should use condom during treatment and for four months following the last IMP dose.

Highly effective birth control methods are:

1. Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation [1]:
    a. oral
    b. intravaginal
    c. transdermal
2. Progestogen-only hormonal contraception associated with inhibition of ovulation [1]:
    a. oral
    b. injectable
    c. implantable [2]
3. Intrauterine device (IUD) [2]
4. Intrauterine hormone-releasing system (IUS) [2]
5. Bilateral tubal occlusion [2]
6. Vasectomized partner [2,3]
7. Sexual abstinence [4]
8. A combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods.

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method (see below).

[2] Contraception methods that are considered to have low user dependency.

[3] Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success.

[4] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together.

Contraception methods with low user dependency should preferably be used, in particular when contraception is introduced as a result of participation in this trial. It cannot be excluded that the IMP bosentan may reduce exposure to substrates of CYP3A through enzyme induction; the efficacy of hormonal contraceptives may be reduced if co-administered with this drug.

APPENDIX 3: LIST OF CYP1/CYP2/CYP3 INHIBITORS, INDUCERS AND SUBSTRATES

Table 1. Classification of *in vivo* inhibitors of CYP Enzymes (1).

CYP enzymes	Strong Inhibitors (2) ≥ 5-fold increase in AUC or > 80% decrease in CL	Moderate inhibitors (3) ≥ 2 but < 5-fold increase in AUC or 50-80% decrease in CL	Weak inhibitors (4) ≥ 1.25 but < 2-fold increase in AUC or 20-50% decrease in CL
CYP1A2	ciprofloxacin, enoxacin, fluvoxamine[a]	methoxsalen, mexiletine ,oral contraceptives	acyclovir, allopurinol, cimetidine, peginterferon alpha-2a, piperine, zileuton
CYP2B6	-	-	clopidogrel[b], tenofovir, ticlopidine[c], voriconazole[d]
CYP2C8	gemfibrozil[e]	clopidogrel[b], deferasirox, teriflunomide	trimethoprim
CYP2C9	-	amiodarone, fluconazole[f], miconazole, piperine	diosmin, disulfiram, fluvastatin, fluvoxamine[a], voriconazole
CYP2C19	fluconazole[f], fluoxetine[g], fluvoxamine[a], ticlopidine	felbamate	omeprazole, voriconazole
CYP2D6	bupropion, fluoxetine[g], paroxetine, quinidine[h], terbinafine	abiraterone, cinacalcet, duloxetine, lorcaserin, mirabegron	amiodarone, celecoxib, cimetidine, clobazam, cobicistat, escitalopram, fluvoxamine[a], labetalol, ritonavir[h,i,j], sertraline, vemurafenib
CYP3A	boceprevir, clarithromycin[h], cobicistat[h], danoprevir and ritonavir[j], elvitegravir and ritonavir[j], grapefruit juice[k], idelalisib, indinavir and ritonavir[j], itraconazole[h], ketoconazole, lopinavir and ritonavir[h,j], nefazodone, nelfinavir[h], paritaprevir and ritonavir and (ombitasvir and/or dasabuvir)[j], posaconazole, ritonavir(h,j), saquinavir and ritonavir[h,j], telaprevir[h], tipranavir and ritonavir[h,j], telithromycin, troleandomycin, voriconazole	aprepitant, ciprofloxacin, conivaptan[l], crizotinib, cyclosporine, diltiazem[m], dronedarone[h], erythromycin, fluconazole[f], fluvoxamine[a], imatinib, tofisopam, verapamil[h]	chlorzoxazone, cilostazol, cimetidine, clotrimazole, fosaprepitant, istradefylline, ivacaftor[h], lomitapide, ranitidine, ranolazine[h], ticagrelor[h]

1. Please note: For an updated list, see the following link: https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table3-2
2. A strong inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a substrate for that CYP by equal or more than 5-fold.
3. A moderate inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 5-fold but equal to or more than 2-fold.
4. A weak inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 2-fold but equal to or more than 5-fold.

[a] Strong inhibitor of CYP1A2 and CYP2C19. Moderate inhibitor of CYP3A and Weak inhibitor of CYP2D6.

[b] Moderate inhibitor of CYP2C8 and weak inhibitor of CYP2B6.
[c] Strong inhibitor of CYP2C19 and weak inhibitor of CYP2B6.
[d] Strong inhibitor of CYP2C19 and CYP3A, and weak inhibitor of CYP2B6.
[e] Strong inhibitor of CYP2C8 and inhibitor of OATP1B1 and OAT3.
[f] Strong inhibitor of CYP2C19 and moderate inhibitor of CYP2C9 and CYP3A.
[g] Strong inhibitors of CYP2C19 and CYP2D6.
[h] Inhibitor of P-gp (defined as those increasing AUC of digoxin to ≥1.25-fold).
[i] Strong inhibitors of CYP3A and weak inhibitor of CYP2D6.
[j] Ritonavir is usually given in combination with other anti-HIV or anti-HCV drugs in clinical practice. Caution should be used when extrapolating the observed effect of ritonavir alone to the effect of combination regimens on CYP3A activities.
[k] The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Studies have shown that it can be classified as a "strong CYP3A inhibitor" when a certain preparation was used (e.g., high dose, double strength) or as a "moderate CYP3A inhibitor" when another preparation was used (e.g., low dose, single strength).
[l] The classification is based on studies conducted with intravenously administered conivaptan.
[m] Diltiazem increased AUC of certain sensitive CYP3A substrates (e.g., buspirone) more than 5-fold.

Table 2. Classification of *in vivo* inducers of CYP Enzymes (1).

CYP enzymes	Strong Inducers ≥ 80% decrease in AUC	Moderate Inducers 50-80% decrease in AUC	Weak Inducers 20-50% decrease in AUC
CYP1A2	-	phenytoin[a] rifampin[b], ritonavir[c,d], smoking, teriflunomide	-
CYP2B6	carbamazepine[e]	efavirenz[e], rifampin[a]	nevirapine, ritonavir[c, d]
CYP2C8	-	rifampin[a]	-
CYP2C9	-	enzalutamide[g], rifampin[a]	apalutamide, aprepitant, carbamazepine[e], ritonavir[c, d]
CYP2C19	rifampin[a]	apalutamide, efavirenz[e,f], enzalutamide[g], phenytoin[b]	ritonavir[c, d]
CYP3A	apalutamide, carbamazepine[e], enzalutamide[g], mitotane, phenytoin[b], rifampin[a], St. John's wort[h]	bosentan, efavirenz[f], etravirine, phenobarbital, primidone	armodafinil, modafinil[i], rufinamide

1. Please note: For an updated list, see the following link: https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table3-3.

[a] Strong inducer of CYP3A and moderate inducer of CYP1A2, CYP2C19.
[b] Strong inducer of CYP2C19, CYP3A, and moderate inducer of CYP1A2, CYP2B6, CYP2C8, CYP2C9.
[c] Moderate inducer of CYP1A2 with dose of 800 mg/day ritonavir (not with other anti-HIV drugs). Effect on CYP1A2 at lower doses of ritonavir is unknown.
[d] Weak inducer of CYP2B6, CYP2C9, and CYP2C19. Classification is based on studies conducted with ritonavir itself (not with other anti-HIV drugs) at doses of 100-200 mg/day, although larger effects have been reported in literature for high doses of ritonavir.
[e] Strong inducer of CYP2B6, CYP3A, and weak inducer of CYP2C9.
[f] Moderate inducer of CYP2B6, CYP2C19 and CYP3A.
[g] Strong inducer of CYP3A and moderate inducer of CYP2C9, and CYP2C19.
[h] The effect of St. John's wort varies widely and is preparation-dependent.
[i] Based on effect of 200 mg/day modafinil. A higher dose (400 mg/day) modafinil had larger induction effect on CYP3A.

Table 3. Examples (1) of sensitive and moderate sensitive *in vivo* CYP substrates.

CYP enzymes	Sensitive substrates (2)	Moderate sensitive substrates (3)
CYP1A2	alosetron, caffeine, duloxetine, melatonin, ramelteon, tasimelteon, tizanidine	clozapine, pirfenidone, ramosetron, theophylline
CYP2B6	bupropion[a]	efavirenz[a]
CYP2C8	repaglinide[b]	montelukast, pioglitazone, rosiglitazone
CYP2C9	celecoxib[c]	glimepiride, phenytoin, tolbutamide, warfarin
CYP2C19	S-mephenytoin, omeprazole	diazepam, lansoprazole[d], rabeprazole, voriconazole
CYP2D6	atomoxetine, desipramine, dextromethorphan , eliglustat[e], nebivolol, nortriptyline, perphenazine, tolterodine, R-venlafaxine	encainide, imipramine, metoprolol, propafenone, propranolol, tramadol, trimipramine, S-venlafaxine
CYP3A (4)	alfentanil, avanafil, buspirone, conivaptan, darifenacin, darunavir[f], ebastine, everolimus, ibrutinib, lomitapide, lovastatin[g], midazolam, naloxegol, nisoldipine, saquinavir[f], simvastatin[g], sirolimus, tacrolimus, tipranavir[f], triazolam, vardenafil	alprazolam, aprepitant, atorvastatin[c], colchicine, eliglustat[e], pimozide, rilpivirine, rivaroxaban, tadalafil
	budesonide, dasatinib, dronedarone, eletriptan, eplerenone, felodipine, indinavir[f], lurasidone, maraviroc, quetiapine, sildenafil, ticagrelor, tolvaptan	

1. Please note: For an updated list, see the following link: https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table3-1.
2. Sensitive CYP substrates refer to drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a known CYP inhibitor.
3. Moderate sensitive substrates are drugs that demonstrate an increase in AUC of ≥2 to <5-fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies.
4. Sensitive substrates of CYP3A with ≥10-fold increase in AUC by co-administration of strong index inhibitors are shown above the dashed line.

[a] Listed based on an in vivo induction study and the observed effect might be partly attributable to induction of other pathway(s).
[b] OATP1B1 substrate.
[c] Listed based on pharmacogenetic studies.
[d] S-lansoprazole is a sensitive substrate in CYP2C19 EM subjects.
[e] Sensitive substrate of CYP2D6 and moderate sensitive substrate of CYP3A.
[f] Usually administered to patients in combination with ritonavir, a strong CYP3A inhibitor.
[g] Acid form is an OATP1B1 substrate.

APPENDIX 4: FORMULAS

Cockcroft-Gault Formula for Calculated Creatinine Clearance

$$\text{CLcr (mL/min)} = \frac{(140 - \text{Age}) \times \text{Weight (kg)}}{72 \times \text{Creatinine}_{\text{serum}}\text{(mg/dL)}} \times 0.85 \text{ if female}$$

Cockcroft D.W., Gault M.H. Prediction of Creatinine Clearance from Serum Creatinine. Nephron 1976;16:31–41 (DOI:10.1159/000180580)

DuBois Formula for Body Surface Area

$$\text{Body Surface Area } (m^2) = \text{Weight (kg)}^{0.425} \times \text{Height (cm)}^{0.725} \times 0.007184.$$

DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Medicine. 1916; 17:863-71.

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 DC, USA, October 2002 (Note of Clarification added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)

59th WMA General Assembly, Seoul, Republic of Korea, October 2008

64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

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 be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. 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."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. 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 proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must 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.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this 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.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

    All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. 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.

22. The design and performance of each research study involving human subjects must be clearly described and justified 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, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. 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 amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, 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, post-study provisions 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.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must 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 must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised 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 group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent 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 authorised representative. The potential subject's dissent should be respected.

30. 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 group. In such circumstances the physician must seek informed consent from the legally authorised 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 must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their 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 adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians

must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:
Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or
Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention
and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.
Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers 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. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised

representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.