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PHASE I STUDIES

First-in-human phase I study of the microtubule inhibitor plocabulin in patients with advanced solid tumors

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Summary

Background Plocabulin (PM060184) is a novel marine-derived microtubule inhibitor that acts as an antitumor agent. This first-in-human study evaluated dose-limiting toxicities (DLT) to define the maximum tolerated dose (MTD) and phase II recommended dose (RD) of plocabulin given as a 10-min infusion on Day (D) 1, D8 and D15 every four weeks. *Patients and methods* Forty-four patients with advanced solid tumors received plocabulin following an accelerated titration design. *Results* Plocabulin was escalated from 1.3 mg/m^2 to 14.5 mg/m^2, which was defined as the MTD. No RD was confirmed, because frequent dose delays and omissions resulted in low relative dose intensity (66%) at the 12.0 mg/m^2 expansion cohort. The main DLT was grade 3 peripheral sensory neuropathy (PSN); other DLTs were grade 4 tumor lysis syndrome, grade 4 cardiac failure and grade 3 myalgia. Toxicities were mainly mild to moderate, and included abdominal pain, myalgia, fatigue, nausea, and vomiting. Myelosuppression was transient and manageable. Plocabulin had a half-life of ~4 h and a wide diffusion to peripheral tissues. Antitumor response was observed in cervix carcinoma and heavily pretreated metastatic non-small cell lung cancer patients, and disease stabilization (≥3 months) in patients with colorectal, thymic, gastrointestinal stromal and breast tumors, among others. The clinical benefit rate was 33%. *Conclusion* The main DLT of plocabulin was PSN, as anticipated for a tubulin-binding agent. Since encouraging antitumor activity was observed, efforts to improve toxicity and to find the RD were planned in other trials evaluating D1&D8 and D1-D3 plus D15-D17 schedules.

Keywords Plocabulin · PM060184 · Microtubule inhibitor · First-in-human · Phase I · Solid tumors

Introduction

Microtubules are dynamic protein filaments assembled from tubulin subunits that play a key role in cell division and intracellular transport. Concerted growing and shrinking of microtubules is essential for correct chromosome segregation, and thus cell proliferation. Targeting microtubules and affecting their dynamics has proven to be a successful strategy for inhibiting proliferation of cancer cells. Microtubule inhibitors such as eribulin and vinca alkaloids bind to and sequester unassembled tubulin, thereby inhibiting microtubule polymerization; in contrast, microtubule-stabilizing drugs such as taxanes and epothilones bind to and hyperstabilize microtubules, thus preventing their depolymerization. Both strategies interfere with microtubule dynamics during mitotic spindle assembly, leading to cell cycle arrest and apoptosis, which ultimately translates into antitumor activity. Moreover, microtubule-targeting agents also have antiangiogenic and/or vascular-disrupting properties [1–3], which also interfere with tumor growth.

Plocabulin (PM060184) belongs to a new family of tubulin-binding agents originally isolated from the marine sponge *Lithoplocamia lithistoides*, currently produced by total chemical synthesis [4] and under evaluation in clinical trials with advanced cancer patients. Plocabulin binds to a site on β-tubulin different from that of vinca alkaloids, eribulin, or taxanes, and induces microtubule depolymerization through a distinct mechanism [5,

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6]. Apoptosis is induced by both caspase-dependent and non-classical apoptosis pathways [7]. Plocabulin has shown antitumor activity both in vitro against human cell lines [6] and in vivo in different patient-derived tumor xenograft models [7]. Remarkably, plocabulin has also shown antitumor activity in xenograft models expressing the P-gp multidrug efflux pump, which are resistant to vinorelbine and paclitaxel [7]. Plocabulin also affects the migration and invasion abilities of endothelial cells, which results in anti-angiogenic and vascular-disrupting effects in tumor xenograft models [8]. The goal of this first-in-human study was to establish a safe and tolerable dose and administration schedule for plocabulin monotherapy in patients with advanced solid tumors, and to evaluate its pharmacokinetic profile, safety, and preliminary antitumor activity.

Patients and methods

This study (EudraCT No: 2010–021855-15) was conducted at the Vall d'Hebron University Hospital (Barcelona, Spain) and the Institute Claudius Regaud (Toulouse, France) in accordance with the ICH Good Clinical Practice guidelines, and was approved by the respective Research Ethics Committees. Written informed consent was obtained before any procedure.

Eligibility criteria

Eligible patients were aged ≥18 years with histologically/cytologically confirmed advanced solid tumors refractory to standard therapy or with no standard therapy; recovered from previous toxicities to grade ≤ 1; with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score ≤ 1, and with adequate hematological, renal and hepatic function. In the expansion cohort, all patients had measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 and confirmed disease progression after last antitumor therapy.

Patients were excluded if they had received any other antitumor therapy within 3 weeks or any investigational drugs within 30 days before the first plocabulin infusion; had progressive or symptomatic brain or leptomeningeal metastases; were pregnant or lactating women; or had an increased cardiac risk. A protocol amendment added two new exclusion criteria: prior treatment with oxaliplatin or a history of grade 2/3 peripheral neuropathy (PN) due to prior therapy.

Study design and treatment

This was a first-in-human dose-escalation phase I study to identify dose-limiting toxicities (DLTs) and determine the maximum tolerated dose (MTD) and the recommended dose for phase II trials (RD) of plocabulin in patients with advanced solid tumors. Plocabulin was supplied by PharmaMar as a powder concentrate in 15 mg vials and reconstituted in 6 mL of sterile water for injection (2.5 mg/mL), further diluted with dextrose 5% solution before infusion and kept under light protection. Plocabulin was administered intravenously (i.v.) over 10 min on Day 1 (D1), D8 and D15 every four weeks (q4wk). Starting at dose level 3 (DL3, 5.2 mg/m^2), patients received primary antiemetic prophylaxis with steroids and 5-HT$_3$ antagonists 20–30 min before plocabulin administration, following the American Society for Clinical Oncology guidelines [9]. Plocabulin was given until disease progression, unacceptable toxicity, treatment delay >2 weeks due to toxicity, >2 dose reductions (except if clinical benefit was observed), intercurrent illness precluding safe participation, protocol deviation with a negative effect on the risk/benefit ratio, or inability to comply with study procedures.

Dose escalation and dose-limiting toxicities

The starting dose was 1.3 mg/m^2, which was 1/6 of the MTD found in the most sensitive animal species tested in preclinical studies. The study started with an accelerated dose-escalation phase, during which cohorts of at least one evaluable patient were treated at each dose level and dose escalation proceeded at 100% dose increments. In the event of clinically relevant drug-related grade ≥ 2 toxicities (except asthenia, anemia, diarrhea of <24 h duration or nausea/vomiting without optimal treatment), cohorts of at least 3 patients were treated at each dose level and up to 50% dose increments were allowed if no DLTs had occurred. Once a DLT was observed, dose escalation switched to a classical 3 + 3 design with up to 25% dose increments. The MTD was the lowest dose level at which more than one third of patients had DLTs, whereas the RD was the highest dose level at which one third or less of patients had DLTs. Once a dose had been defined as the RD, it was to be confirmed in an expansion cohort of at least 12 evaluable patients.

DLTs were determined in Cycle 1 and were defined as: grade 4 neutropenia lasting ≥7 days or with fever (≥38.5 °C), sepsis or severe infection; grade 4 thrombocytopenia; any grade ≥ 3 non-hematological toxicity (excluding nausea/vomiting, untreated grade 3 diarrhea lasting <24 h, grade 3 asthenia lasting <5 days, hypersensitivity reactions and non-clinically relevant isolated biochemical abnormalities), or treatment delay >2 weeks due to toxicity. Toxicities occurring after Cycle 1 or non-compliance with the intended dose intensity could also qualify as DLTs and/or affect the proposed RD.

Study assessments

Medical history, physical examination, ECOG PS score, vital signs, laboratory tests evaluating renal, liver and hematological function, electrocardiogram (ECG) and left ventricular ejection fraction (LVEF) were assessed at baseline. Patients were evaluated weekly while on plocabulin treatment. Serial ECGs were done in Cycle 1, and LVEF was assessed every two cycles. All

adverse events (AEs) were assessed throughout treatment, and laboratory values that reached grade ≥ 3 were re-assessed at least every 2–3 days until recovery. AEs and laboratory abnormalities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCTI-CTCAE) v.4, and were coded using the Medical Dictionary for Regulatory Activities (MedDRA) v.14.1. Antitumor activity was radiologically evaluated according to the RECIST v.1.1 every 8 weeks. Optionally, descriptive analysis of tumor markers was performed every 8 weeks.

Pharmacokinetic analyses

Blood samples were obtained on D1 of Cycle 1 from all patients and on D15 of Cycle 1 from patients treated in the expansion cohort. Urine produced during D1 and D15 of Cycle 1 was also collected in the latter subset of patients. Plasma and urine concentrations of plocabulin were determined using validated ultra-performance liquid chromatography-tandem mass spectrometry method. The lower limit of quantification was 0.1 ng/mL.

Statistical analysis

Categorical variables were presented in frequency tables and continuous variables by mean, median, and range. Descriptive statistics were used for the evaluation of safety. Time-related efficacy parameters were analyzed according to the Kaplan-Meier method. SAS® v.9.2 (SAS Institute Inc., Cary, NC, USA) was used to generate these outputs. Non-compartmental pharmacokinetic parameters were calculated using Phoenix WinNonlin v.6.3 (Certara USA Inc., Princeton, NJ, USA). The potential influence on selected pharmacokinetic parameters and demographic and clinical dichotomous variables were evaluated by a Student's T test or a Mann-Whitney's U test, as appropriate. For multinomial variables, an analysis of variance was used. For selected continuous demographic and clinical variables the relationship with selected pharmacokinetic parameters was graphically explored and assessed using correlation and regression methods. Ninety-five percent confidence intervals for the slope and p test with a level of significance of 0.05 were calculated to detect differences and correlations.

Results

Patient characteristics and treatment

Forty-seven patients were enrolled between January 2011 and November 2014, of whom 44 were treated and evaluable for DLTs. Patients' characteristics at baseline are shown in Table 1. Median age was 53 years (range, 22–72 years) and all had advanced/metastatic disease. The most common primary tumor type was colorectal adenocarcinoma ($n = 11$, 25%), followed by non-small cell lung cancer (NSCLC),

Table 1 Baseline characteristics of patients

	Total ($n = 44$)
Gender	
Male	23 (52%)
Female	21 (48%)
Median age (range; years)	53 (22–72)
ECOG performance status	
0	21 (47.7)
1	23 (52.3)
Median BSA (range; m^2)	1.8 (1.3–2.5)
Primary tumor	
Colorectal adenocarcinoma	11 (25%)
Breast carcinoma	5 (11%)
Cervix carcinoma	5 (11%)
NSCLC	5 (11%)
GIST	3 (7%)
Pancreas adenocarcinoma	3 (7%)
Soft tissue sarcoma	3 (7%)
Other [a]	9 (21%)
Median no. of metastatic sites (range)	4 (1–11)
Most common sites of metastatic disease	
Lung	23 (52%)
Liver	21 (48%)
Lymph node	17 (39%)
Bone	9 (21%)
Prior systemic treatment	
Chemotherapy	44 (100%)
Platinum compound	32 (73%)
Pyrimidine analogues	30 (68%)
Biological therapy	28 (64%)
Taxanes	18 (41%)
Anthracyclines and related	15 (34%)
Vinca alkaloids	12 (27%)
Investigational drug	9 (21%)
Other antineoplastic agents	35 (80%)
No. of prior lines of anticancer therapies	
Median (range)	4 (1–14)
≤ 2	8 (18%)
3	10 (23%)
≥ 4	26 (59%)

BSA body surface area, *ECOG* Eastern Cooperative Oncology Group, *GIST* gastrointestinal stromal tumor, *no.* number; *NSCLC* non-small cell lung cancer

[a] Cholangiocarcinoma, endometrium adenocarcinoma, germinal testicular carcinoma, head and neck carcinoma, malignant melanoma, ovarian adenocarcinoma, parotid adenocystic carcinoma, thymus carcinoma, and urothelial carcinoma ($n = 1$ each)

breast and cervix cancer ($n = 5$ each, 11%). Each patient had received a median of 4 lines (range, 1–14 lines) of prior anticancer therapy for advanced disease. Overall, 73% of patients were exposed to platinum compounds, 68% to pyrimidine analogues, 64% to biological therapy, 41% to taxanes, 34% to anthracyclines and related drugs, and 27% to vinca alkaloids.

A total of 120 plocabulin cycles were administered, with a median of 2 cycles (range, 1–12 cycles) per patient. Five patients (11%) received 6 or more cycles each. Most treatment discontinuations were due to disease progression ($n = 25$; 57%).

Dose-limiting toxicities and recommended dose

During dose escalation, plocabulin was administered as a 10-min i.v. infusion on D1, D8 and D15 q4wk, with a starting dose of 1.3 mg/m^2. Table 2 summarizes the dose escalation and the patients treated at each cohort. Two cases of grade 3 peripheral sensory neuropathy (PSN) defined the MTD at 14.5 mg/m^2. An intermediate dose level (DL8, 12.0 mg/m^2) was expanded to confirm it as the RD. Three DLTs were observed in one patient each in this expanded cohort: grade 3 myalgia, grade 3 PSN, and grade 4 cardiac failure (the latter was a delayed DLT that appeared during Cycle 2 in a patient with LVEF 46%, which was detected at the end of Cycle 1). Dose level DL8 was considered unfeasible because non-severe toxicity caused frequent dose omissions and delays on D8 or D15, which in turn resulted in low treatment compliance (median relative dose intensity = 66%). Therefore, the 12.0 mg/m^2 dose was tested with plocabulin administered as a 3-h infusion (DL9); however, although no formal DLTs were observed, neither compliance (median relative dose intensity = 57%) nor toxicity improved. Hence, the 10-min infusion duration was reestablished and a lower dose level was evaluated (DL10, 9.0 mg/m^2). At this dose, one of 3 patients experienced a DLT (grade 4 tumor lysis syndrome). Dose escalation was terminated without the RD being defined.

Toxicity profile

All 44 treated patients were evaluable for safety. Peripheral sensory neuropathy (PSN) was one of the main toxicities (Table 3), which reached grade 3 in 3 patients: 2 treated at the MTD (14.5 mg/m^2) and one at 12.0 mg/m^2. One of these patients had a previous history of grade 3 PSN due to having received prior oxaliplatin for 28 cycles, while the other had received prior oxaliplatin for 12 cycles. The severity of PSN was reversible in all 3 cases. Median latency of grade 3 PSN was 3 days after the first plocabulin dose (range, 3–10 days) and lasted a median of 40 days (range, 27–40 days). Only 2 of 14 patients with previous oxaliplatin therapy had grade 3 PSN (both treated at the MTD), and one patient had grade 2 PSN. Other common treatment-related AEs (or with unknown relationship) at all dose levels were fatigue (61% grade 1/2; 16% grade 3), alopecia (57% grade 1/2), diarrhea (50% grade 1/2),

Table 2 Dose escalation scheme and dose-limiting toxicity

Dose level plocabulin (mg/m^2)	Pts with DLTs/pts. treated [a]	DLT description
10-min i.v. infusion		
DL1 (1.3)	0/1	–
DL2 (2.6)	0/1	–
DL3 (5.2)	0/1	–
DL4 (7.5)	0/5	–
DL5 (9.3)	0/4	–
DL6 (11.6)	0/3	–
DL7 (14.5)	2/2	Grade 3 PSN
		Grade 3 PSN
DL8 (12.0)	3/20	Grade 3 PSN
		Grade 4 cardiac failure [b]
		Grade 3 myalgia
DL10 (9.0)	1/3	Grade 4 tumor lysis syndrome
3-h i.v. infusion		
DL9 (12.0)	0/4	–

DL dose level, *DLT* dose-limiting toxicity, *i.v.* intravenous, *LVEF* left ventricular ejection fraction, *min.* minute, *PSN* peripheral sensory neuropathy, *pts.* patients

[a] All patients treated were evaluable for DLTs

[b] Delayed DLT experienced in Cycle 2 but with reduced cardiac function (LVEF of 46%) detected at end of Cycle 1. Despite this LVEF value, the D1 Cycle 2 infusion was administered the next day

Table 3 Laboratory abnormalities (hematological and biochemical) and drug-related (or unknown relationship) adverse events in ≥10% of patients at all dose levels ($n = 44$)

NCI-CTCAE grade	1–2	3	4	Total
Drug-related adverse events (or with unknown relationship)				
Peripheral sensory neuropathy	31 (71%)	3 (7%)		34 (77%)
Fatigue	27 (61%)	7 (16%)		34 (77%)
Nausea	26 (59%)			26 (59%)
Alopecia	25 (57%)			25 (57%)
Diarrhea	22 (50%)			22 (50%)
Anorexia	15 (34%)	1 (2%)		16 (36%)
Vomiting	16 (36%)			16 (36%)
Abdominal pain	13 (30%)	2 (5%)		15 (34%)
Myalgia [a]	12 (27%)	1 (2%)		13 (30%)
Constipation	10 (23%)			10 (23%)
Dyspnea	5 (11%)	1 (2%)	1 (2%) [b]	7 (16%)
Weight decreased	7 (16%)			7 (16%)
Pyrexia	6 (14%)			6 (14%)
Tumor pain	2 (5%)	3 (7%)		5 (11%)
Headache	4 (9%)	1 (2%)		5 (11%)
Hematological abnormalities (all causalities)				
Anemia	33 (75%)	10 (23%)		43 (98%)
Neutropenia	17 (39%)	2 (5%)		19 (43%)
Thrombocytopenia	18 (41%)			18 (41%)
Biochemical abnormalities (all causalities)				
ALT increased	23 (52%)	1 (2%)		24 (55%)
AST increased	30 (68%)	2 (5%)	1 (2%)	33 (75%)
Creatinine increased	6 (14%)			6 (14%)
Hypoalbuminemia	21 (49%)	2 (5%)		23 (54%)
Total bilirubin increased	9 (21%)			9 (21%)

As no recommended dose was established, this table contains all AEs experienced by ≥10% of patients (pts) treated at all dose levels and infusion times ($n = 44$). The number of patients with each AE (worst case) is specified. Hematological and biochemical abnormalities are shown regardless of their relationship to treatment and also include patients with abnormalities present at baseline

AE adverse event *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *NCI-CTCAE* National Cancer Institute Common Terminology Criteria for Adverse Events

[a] Includes cases of myalgia and musculoskeletal pain

[b] Transient dyspnea in a patient with previous history of dyspnea and lung infection, which had been treated with antibiotics. Transient dyspnea was possibly related to the disease under study but causality with plocabulin could not be ruled out

anorexia (34% grade 1/2; 2% grade3), abdominal pain (30% grade 1/2; 5% grade 3), and myalgia (27% grade 1/2; 2% grade 3). Of note, treatment-related nausea and/or vomiting were mild-moderate, but occurred in 59 and 36% of patients, respectively (Table 3), thereby justifying the administration of primary antiemetic prophylaxis, as received by patients in this study from DL3 (5.2 mg/m^2) onwards.

Grade 4 AEs occurred only in 3 patients (7%), and comprised one episode each of dyspnea (of unknown causality, in one patient with metastatic lung cancer and a previous history of dyspnea), cardiac failure, and tumor lysis syndrome; all three events resolved without sequelae. No treatment-related deaths occurred.

The main hematological toxicity was anemia (23% grade 3). Neutropenia and thrombocytopenia were mostly mild and moderate, except in 2 patients (5%) who had grade 3 neutropenia. No cases of febrile neutropenia occurred. Most biochemical abnormalities were grade 1 or 2. The 4 patients with transaminase increases had hepatic metastases. Altogether, laboratory abnormalities were transient and manageable.

At the expansion cohort, 21 (13%) infusions were omitted and 12% of cycles were delayed due to toxicity, while dose reductions (4%) were rare. Non-hematological toxicities (PSN, fatigue) were the most common reasons for dose omissions, delays and reductions.

Efficacy

Thirty-six patients were evaluable for efficacy as per RECIST v.1.1, of whom 21 (58%) had previously been treated with microtubule inhibitors. Administration of plocabulin 12 mg/m^2 as a 10-min i.v. infusion resulted in 2 confirmed partial responses (Table 4). One of these responses (44% tumor shrinkage) was found in one patient with cervix carcinoma that had progressed to first-line treatment with paclitaxel-carboplatin (Fig. 1), after which the Investigator decided to discontinue treatment due to a possible pneumonia. The other partial response (36% tumor shrinkage) was observed in one patient with metastatic NSCLC heavily pretreated with 6 prior lines, of which 3 included microtubule inhibitors (paclitaxel, docetaxel and vinorelbine). In addition, 10 patients (28%) had disease stabilization for ≥3 months, including patients with colorectal carcinoma ($n = 4$), gastrointestinal stromal tumor ($n = 2$; with progression-free survival [PFS] of 3.1 and 6.6 months), and breast, cervix, thymus or parotid adenocystic carcinoma ($n = 1$ each) (Table 4). Clinical benefit (i.e., response or disease stabilization for ≥3 months) was therefore observed in 12 of 36 patients (33%).

Four of 6 colorectal patients evaluable for efficacy had disease stabilization for ≥3 months, received a median of 4 lines (range, 2–5 lines) of prior therapy each, and had a PFS ranging from 3.7 to 12.8 months. In 3 of these patients, this was correlated with a reduction (between 18 and 80%) in the expression of the tumor marker carcinoembryonic antigen (CEA); the 80% reduction occurred in a patient with a PFS of 12.8 months.

Five of 10 patients with disease stabilization achieved a better response with plocabulin compared to their previous line of antitumor therapy (Table 4). Seven patients with disease stabilization stopped treatment due to disease progression, 2 stopped due to a decision by the Investigator, and one refused further treatment.

Pharmacokinetics

Pharmacokinetic data were obtained from 43 patients treated on D1 and from 17 patients treated on D15 of Cycle 1. As shown in Fig. 2, pharmacokinetic profiles for plocabulin administered as 10-min and 3-h infusions showed linearity, as they overlap when normalized by dose level. Table 5 lists pharmacokinetic parameters by dose level and day. Total body clearance (CL) was independent of dose. No significant differences were found between the area under the concentration-time curve (AUC) and maximum plasma concentration (C_{max}) on D1 and D15 of patients receiving 12.0 mg/m^2, suggesting that drug accumulation is unlikely.

The impact of baseline demographic, hematological and biochemical variables on pharmacokinetic parameters was evaluated. Body weight and body surface area (BSA) at baseline were found to affect volume of distribution at steady state

Table 4 Characteristics of patients with clinical benefit

Plocabulin treatment					Last prior treatment		
Dose level (mg/m^2)	Tumor type	Cycles received	Best response	PFS (mo)	Prior lines [a]	Best response	TTP (mo)
DL3 (5.2)	CRC	2	SD	4.3+	5	PD	1.7
DL4 (7.5)	CRC	6	SD	5.1	3	SD	11.0
DL5 (9.3)	Parotid ACC	6	SD	5.8+	1	PD	4.8
DL6 (11.6)	CRC	4	SD	3.7	2	PR	10.4
DL8 (12.0)	CRC	12	SD	12.8	5	SD	4.6
	GIST	6	SD	6.6	9	PR	16.8
	Breast cancer	6	SD	6.0+	10	PD	2.7
	Thymus cancer	4	SD	3.9	3	PD	1.7
	Cervix cancer	2	PR	3.6+	1	CR	11.9
	NSCLC	4	PR	3.3	5	Uk	9.0
	GIST	3	SD	3.1	8	SD	3.2
DL10 (9.0)	Cervix cancer	4	SD	4.1	3	PD	5.7

ACC adenoid cystic carcinoma, *CR* complete response, *CRC* colorectal carcinoma, *DL* dose level, *GIST* gastrointestinal stromal tumor, *mo* months, *NSCLC* non-small cell lung cancer, *PD* progressive disease, *PFS* progression-free survival, *PR* partial response, *SD* stable disease ≥3 months, *TTP* time to progression, *Uk* unknown

[a] Prior lines for advanced disease, including all types of anticancer therapy (chemotherapy, biological therapy, hormone therapy)

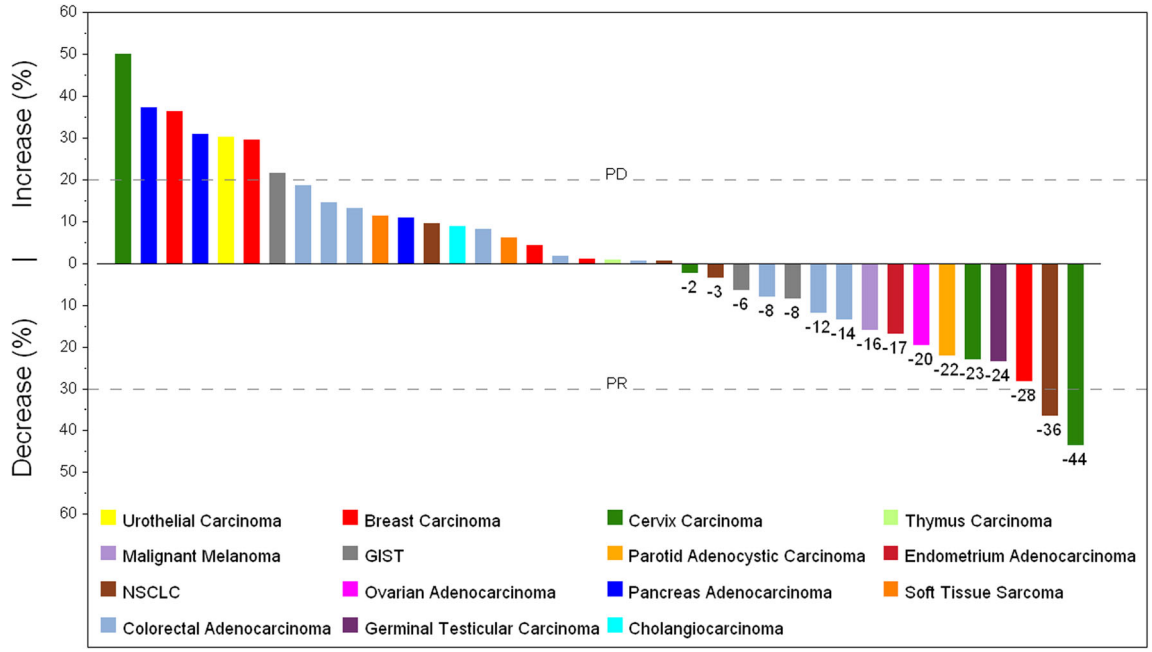

Fig. 1 Maximum variation in target lesions according to RECIST v.1.1. GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors

(V_{ss}), while the level of aspartate aminotransferase (AST) affected CL. No other relationship was found between baseline variables, such as renal function values, and pharmacokinetic parameters. The mean percentage of unaltered plocabulin recovered in urine was around 6%.

Discussion

This first-in-human study met its goal of defining the MTD of plocabulin as a 10-min infusion on D1, D8 and D15 q4wk, which was 14.5 mg/m^2. However, it failed to determine the RD. The RD was defined in the study protocol as a safe and tolerable dose tested in at least 12 patients treated at the expansion cohort. However, only 66% of the planned total dose was administered to the 20 patients treated at 12.0 mg/m^2. Increasing the infusion duration from 10 min to 3 h did not improve compliance or toxicity at this dose. Taking into account that 4 and 3 patients were respectively treated at doses of 9.3 mg/m^2 and 11.6 mg/m^2, that no DLTs were observed in these patients, and that higher dose levels resulted in significant toxicity and/or non-compliance, it may be assumed that the plocabulin RD might be within this dose range.

Regarding the safety profile of plocabulin, PN and fatigue were the most relevant toxicities, and also the most common reasons for study treatment modification. Chemotherapy-induced peripheral neuropathy (CIPN) is a common dose-limiting, dose-dependent, cumulative toxicity that has been described for many frequently used antineoplastic agents, such as platinum compounds and microtubule inhibitors, including taxanes, eribulin, epothilones and vinca alkaloids. Dorsal root ganglia neurons and peripheral axons lack an efficient

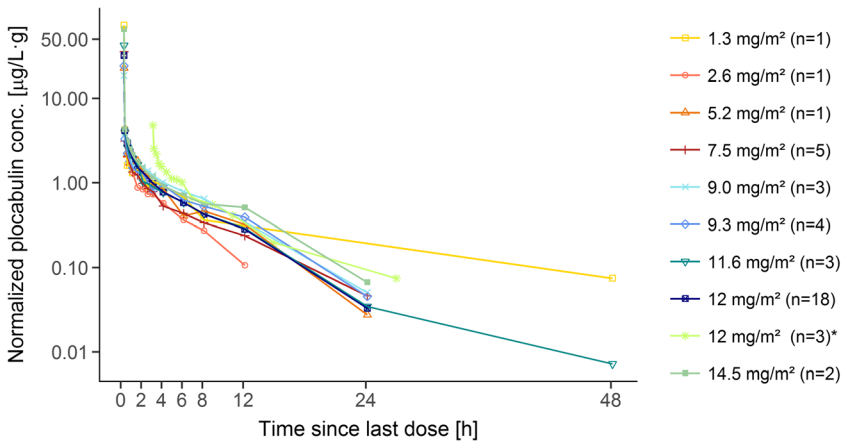

Fig. 2 Mean normalized plocabulin plasma concentration time course, by dose level and day of treatment. *3-h infusion scheme; h, hour

Table 5 Non-compartmental pharmacokinetic parameters of plocabulin administered as a 10-min and 3-h infusion

Infusion schedule	Dose level (mg/m^2)	Day	n	C_{max} (μg/L)	AUC (μ·h/L)	CL (L/h)	HL (h)	V_{ss} (L)	V_z (L)
10-min	1.3	1	1	211	107	27	15	244	584
	2.6	1	1	154	69	71	3	161	346
	5.2	1	1	256	191	59	4	240	338
	7.5	1	5	457 (286)	234 (88)	60 (19)	5 (2)	214 (134)	403 (175)
	7.5	15	1	118	214	54	3	245	248
	9.0	1	3	265 (230)	263 (133)	62 (24)	4 (1)	315 (129)	373 (178)
		15	1	508	224	57	3	110	227
	9.3	1	4	382 (135)	290 (47)	56 (9)	5 (1)	253 (66)	364 (119)
		15	1	199	170	98	3	306	378
	11.6	1	3	796 (255)	393 (86)	51 (15)	5 (1)	177 (81)	363 (70)
	12.0	1	20	614 (385)	381 (117)	61 (22)	4 (1)	223 (105)	347 (119)
		15	14	589 (235)	351 (77)	62 (20)	4 (1)	184 (63)	346 (108)
	14.5	1	2	1744 (1402)	772 (332)	40 (20)	5 (1)	170 (151)	307 (190)
3-h	12.0	1	3	97 (20)	376 (87)	57 (15)	6 (1)	291 (71)	477 (224)

Values are expressed as mean (standard deviation)

AUC area under the concentration-time curve, *CL* clearance, C_{max} maximum plasma concentration, *h* hours; *HL* half-life, *L* liter, *min* minute, *SD* standard deviation, V_{ss} volume of distribution at steady state, V_z volume of distribution associated with the terminal phase

neurovascular barrier and are thus exposed to anticancer drugs [10]. Chemotherapy might involve sensory and motor nerve damage or dysfunction; however, they predominantly appear as pure sensory symptoms, as motor weakness is always accompanied by sensory symptoms [10–12].

In this context, the peripheral neurotoxicity of plocabulin is an anticipated side effect that is dose-limiting and leads to treatment delays and discontinuations, just as is the case with eribulin, vinca alkaloids, taxanes and platinum compounds [10–14]. PN with plocabulin had an early onset of 3–10 days, and was mainly sensory; this might be due to the limited number of patients treated, and also to the fact that autonomic and neuromotor symptoms are frequently underrated, as they are less evident to patients and physicians. In this sense, the abdominal pain and diarrhea observed in this trial might be symptoms of autonomic PN, as has been reported with vinca alkaloids, whereas myalgia might be a symptom of motor PN. Data here presented suggest that severe (grade 3) PN with plocabulin is a dose-dependent toxicity, as has been observed with other PN-inducing chemotherapies. Peripheral neurotoxicity results in dose delays, omissions and reductions, a general problem of CIPN that might impact treatment efficacy. In the present study, severe PSN was found in 3 of 44 patients (7%) at all doses and in one of 24 patients (4%) at 12.0 mg/m^2; of note, all episodes of severe PSN were found at dose levels that were considered too high for future patient treatment. In comparison, grade 3 PN was observed in 12% of patients treated with three-weekly paclitaxel [15], and grade 3/4 PN in 5–14% of those treated with docetaxel [16] and in 6–17% of those treated with different oxaliplatin-combinations [17–22]. The symptoms of plocabulin-induced PSN were reversible, and were no longer severe within 6 weeks after treatment discontinuation. Remarkably, 2 of 3 patients experiencing severe PSN had received previous treatment with oxaliplatin for several cycles and one had a previous history of oxaliplatin-induced grade 3 PSN, thereby suggesting that patients with neuronal damage at study entry are more susceptible to have high-dose plocabulin-induced neuropathy. Given this suspicion, the study protocol was amended to exclude patients with this profile. However, a retrospective analysis of trial data could not confirm whether prior exposure to or a cumulative dose of oxaliplatin treatment per se is a risk factor for plocabulin-induced PSN, as the cumulative dose of oxaliplatin could not be obtained from the medical records of many patients at the time of the analysis, and only 2 of 14 patients with previous oxaliplatin therapy had plocabulin-induced grade 3 PSN (both at the MTD) and another one had grade 2 PSN. Moreover, 9 of 30 patients without previous oxaliplatin therapy had plocabulin-induced grade 2 PSN and one patient had grade 3 PSN. Thus, plocabulin-induced grade 2/3 PSN was found in 21% of patients with previous oxaliplatin therapy, and in 33% of patients without previous oxaliplatin therapy.

Other treatment-related toxicities, such as musculoskeletal and abdominal pain, nausea, and vomiting, were reversible and mainly mild or moderate. Given the high frequency of nausea and vomiting, the protocol was amended to make primary antiemetic prophylaxis compulsory to all patients treated with plocabulin. Hematological toxicity and laboratory abnormalities did not represent a safety concern either. Serious AEs related to plocabulin were scarce, and rarely resulted in changes in study treatment.

Plocabulin had a short half-life of ~4 h, a CL of ~60 L/h and a V_{ss} of ~250 L, thus implying a moderate extraction ratio and a wide diffusion to peripheral tissues. Interpatient variability of these parameters was low when compared to that of other antitumor agents. V_{ss} showed to be affected by body weight and BSA, which is a common finding, as body size metrics are related to the amount of extracellular water [23]. Renal excretion is a minor route of elimination of plocabulin. Thus, changes in renal function are very unlikely to have an impact on exposure to plocabulin.

The patient population evaluated in this trial was heavily pretreated, with a median of 4 prior lines for advanced disease per patient. Nevertheless, response to plocabulin was observed in two patients with cervix carcinoma and metastatic NSCLC patients; the patient with cervix carcinoma was still showing response at treatment discontinuation. Almost 60% of patients evaluable for efficacy (21 of 36 patients) had previously been treated with microtubule inhibitors. Moreover, disease stabilization for ≥3 months was observed in patients with colorectal cancer (CRC) ($n = 4$), gastrointestinal stromal tumor ($n = 2$), and cervix, breast, thymus and parotid adenocystic carcinoma ($n = 1$ each); of note, the latter 4 had shown disease progression after the last prior therapy. Most patients with disease stabilization stopped treatment with plocabulin due to disease progression.

The clinical benefit rate ($n = 4$ of 6 evaluable patients) observed among CRC patients treated with plocabulin was remarkable, as all 6 had received prior standard treatment with oxaliplatin, fluorouracil, irinotecan and bevacizumab, and 3 had received investigational drugs. Despite this heavy pretreatment, plocabulin improved the clinical benefit achieved with the last prior therapy line in 2 of 4 patients. In agreement with the radiological disease stabilization, the tumor marker CEA decreased in all 3 CRC patients analyzed.

In conclusion, as anticipated for a tubulin-binding agent, the main DLT identified for plocabulin in this first-in-human trial was PSN. Since encouraging antitumor activity was observed, efforts to improve toxicity and to find the RD were planned in another dose-escalation phase I trial evaluating a D1 plus D8 schedule and a D1-D3 plus D15-D17 schedule (NCT01299636). Furthermore, plocabulin is being evaluated in combination with gemcitabine in solid tumors (NCT02533674), and as single-agent in patients with advanced CRC (NCT03427268) or advanced hormone receptor positive/HER2-negative breast cancer (EudraCT 2015–002395-24). Once available, these results will provide valuable additional information on the safety, feasibility and pharmacokinetic behavior of plocabulin in a much wider patient population.

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Compliance with ethical standards

Conflicts of interest A Soto Matos-Pita, C Coronado, J Iglesias, S Fudio, and K Zaragoza are employees of Pharma Mar, S.A.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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