EUROPEAN JOURNAL OF CANCER 48 (2012) 289–296

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.ejconline.com

A phase I and pharmacokinetic study of plitidepsin in children with advanced solid tumours: An Innovative Therapies for Children with Cancer (ITCC) study ☆

Birgit Geoerger [a,*,i], *Edward J. Estlin* [b,i], *Isabelle Aerts* [c,i], *Pamela Kearns* [d,i], *Brenda Gibson* [e,i], *Nadège Corradini* [f,i], *François Doz* [c,g,i], *Pilar Lardelli* [h,i], *Bernardo De Miguel* [h,i], *Arturo Soto* [h,i], *Raquel Prados* [h,i], *Gilles Vassal* [a,i]

[a] *Institut Gustave Roussy, Université Paris Sud XI, Villejuif, France*
[b] *Royal Manchester Children's Hospital, Manchester, United Kingdom*
[c] *Institut Curie, Paris, France*
[d] *School of Cancer Sciences, University of Birmingham, United Kingdom*
[e] *Royal Hospital for Sick Children, Glasgow, United Kingdom*
[f] *Centre Hospitalier Universitaire Nantes, Nantes, France*
[g] *Institut Curie, Université Paris Descartes, Sorbonne Paris Cité, France*
[h] *Pharma Mar, Colmenar Viejo, Madrid, Spain*

ARTICLE INFO

Article history:
Available online 24 November 2011

Keywords:
Plitidepsin
Children
Paediatric
Solid tumours
Pharmacokinetics
Phase I

ABSTRACT

Aims: To determine the maximum tolerated dose, the recommended dose (RD) for phase II studies, dose-limiting toxicities and pharmacokinetics (PK) for plitidepsin administered as a 3-h intravenous infusion every 2 weeks (one cycle) to children with refractory or relapsed solid tumours.

Methods: Consecutive cohorts of patients were treated according to a standard '3 + 3' design with escalating doses of plitidepsin at 4, 5 and 6 mg/m^2. Additional 15 patients were recruited at the RD to further evaluate safety and pharmacokinetic associations with respect to age, dose level and toxicity.

Results: Thirty-eight of 41 patients registered received plitidepsin. Dose-limiting toxicities during the first three treatment cycles related to myalgia, elevated creatine phosphokinase, transaminase increase and nausea/vomiting. The RD for plitidepsin is 5 mg/m^2. PK analyses revealed high inter-patient variability in plasma, but a similar clearance of plitidepsin in children and adolescents. One partial response confirmed at 4 weeks in a patient with neuroblastoma and one unconfirmed partial response in a pancreatoblastoma were observed; four other patients with neuroblastoma, medulloblastoma, glioblastoma and rhabdoid tumour had disease stabilisations lasting $\geqslant$3 months.

Conclusion: Plitidepsin administered to children as a 3-h infusion every 2 weeks is received with manageable toxicity for children with cancer, and the RD is 5 mg/m^2. Pharmacokinetic parameters in children and adolescents are comparable to adults. Future phase II studies of

☆ Presented in part at the 42nd and 44th Annual Meeting of the American Society of Clinical Cancer Research in Atlanta, GA in 2006 and in Chicago, IL in 2008, respectively, and at the Annual Meeting of the 38th Annual Congress of the SIOP, Geneva, Switzerland in 2006.

* *Corresponding author:* Address: Institut Gustave Roussy, Department of Paediatrics, 114 Rue Edouard Vaillant, 94805 Villejuif Cedex, France. Tel.: +33 1 42 11 46 61; fax: +33 1 42 11 52 75.
E-mail address: geoerger@igr.fr (B. Geoerger).
[i] On behalf of the European consortium Innovative Therapies for Children with Cancer (ITCC).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.10.036

plitidepsin are warranted, and our results suggest that plitidepsin could be appropriately developed in combination with other antitumour where myelosuppression is dose-limiting.

1. Introduction

Plitidepsin (Aplidin®) is a cyclic depsipeptide originally isolated from the Mediterranean tunicate *Aplidium albicans* and currently produced by chemical synthesis.[1] Plitidepsin has been proven active at nanomolar concentrations against various human malignant cell lines and tumour specimens, such as breast, melanoma and non-small-cell lung cancers.[2] The primary mechanism for cytotoxicity for plitidepsin has not been fully elucidated, but proposed mechanisms include oxidative stress resulting in cellular apoptosis,[3] induction of apoptosis via activation of the Rac1-JNK pathway[3–5] and increased levels of cell membrane phospholipid oxidation and DNA oxidation.[6] Plitidepsin has also antiangiogenic properties characterised by vascular endothelial growth factor (VEGF) and its receptor VEGFR-1 genes expression inhibition in preclinical models.[7–9]

Plitidepsin has selective concentration-dependent cytotoxicity towards childhood leukaemia cells while sparing normal blood cells.[10] Paediatric pre-clinical testing confirmed plitidepsin to be most active against acute lymphoblastic leukaemia cell lines *in vitro* and *in vivo*, while tumour growth inhibition was observed to a lesser extent in solid tumour xenografts.[11]

Phase I trials had established muscular and hepatic toxicities as the main dose-limiting toxicities (DLTs) associated with plitidepsin in adult cancer patients.[12–16] Remarkably, plitidepsin lacks severe bone marrow toxicity. A phase I programme in adults proposed plitidepsin administration as a 3-h intravenous infusion every 2 weeks at 5 mg/m^2 as the recommended dose (RD) for phase II studies.[17–21]

This dose-escalation, exploratory, phase I clinical trial aimed to define the maximum tolerated dose (MTD), the RD for phase II studies, dose-limiting toxicities and the preliminary investigation between pharmacokinetic disposition and pharmacodynamic observations such as toxicity, for plitidepsin administered as a 3-h intravenous infusion every 2 weeks in a paediatric population with refractory or relapsed malignant tumours. Exploration of anti-tumour activity was a secondary aim. However, because the toxicity profile for adults treated with plitidepsin is characterised by delayed muscular toxicity, the period of time that served to define DLT was extended to 6 weeks for all patients enrolled in this trial, and further experience was to be gained with additional patients treated at the defined RD.

2. Patients and methods

This multicenter trial followed the Good Clinical Practice requirements and the Declaration of Helsinki. The protocol was approved by the institutional review boards and independent ethic committees. A signed written informed consent of the legal representatives and assent of each patient, appropriate to the age, was obtained before any study procedure.

2.1. Eligibility criteria

Patients aged 1–18 years and diagnosed with refractory or relapsed malignant solid tumour; Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant 2$ or Lansky Play Scale $\geqslant 50\%$; life expectancy $\geqslant 8$ weeks; adequate haematological function (absolute neutrophil count $>1.0 \times 10^9$/L, platelets $\geqslant 100 \times 10^9$/L), adequate renal and hepatic function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] $\leqslant 2.5 \times$ the upper limit of normal [ULN]; total bilirubin $\leqslant 1.5 \times$ ULN; albumin $\geqslant 2.5$ g/dL); normal left ventricular ejection fraction (LVEF) were eligible.

Patients were excluded if they had received any investigational product, irradiation or chemotherapy within 4 weeks (6 weeks if nitrosourea), total body irradiation within 6 months, bone marrow transplantation within 3 months, or non-myelosuppressive biological therapy within 1 week prior to inclusion; previous treatment with doxorubicin at cumulative doses >400 mg/m^2 or mediastinal radiotherapy; suffered from any relevant medical condition; had organ toxicity $\geqslant$ Grade 2 according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC); or known hypersensitivity to plitidepsin or its reconstitution components (Cremophor®, mannitol and ethanol).

2.2. Study treatment

Escalating doses of plitidepsin (PharmaMar, Colmenar Viejo, Madrid, Spain) were administered as a 3-h intravenous infusion every 2 weeks (i.e. one cycle was 2 weeks). The starting dose was 4 mg/m^2, being 80% of the RD in adults.[22] Patients were re-treated if they had recovered from any toxicity to baseline or to CTC Grade 1 levels. Treatment cycles were repeated until disease progression, unacceptable toxicity, patient refusal or treatment delay for >2 weeks. Prophylactic treatment included serotonin (5-HT$_3$) receptor antagonists, glucocorticoids (dexamethasone), H$_1$ and H$_2$-receptor antagonists.[23]

2.3. Study design and assessments

The study followed a 3 + 3 design with cohorts of three patients treated per dose level and expanding to a maximum of six patients if one patient developed a DLT during the first three cycles of treatment. If none of the initial three patients had DLTs, further dose escalation proceeded with increments of 20–25%. The MTD was defined by the occurrence of a DLT in at least two of 3–6 patients treated at a given dose level, the RD as the dose level immediately below the MTD. An expansion phase of 15 patients followed at the RD.

The following toxicities, when occurring up to the end of Week 6 of treatment (i.e. three cycles) allowed definition of DLT: Grade 4 neutropenia and/or thrombocytopenia lasting ⩾7 days, Grade 4 neutropenia with documented infection, Grade 3/4 thrombocytopenia requiring transfusion for more than 7 days. Non-haematological DLTs comprising muscular toxicity (Grade 3/4 elevation of creatine phosphokinase [CPK], Grade 3/4 myalgia and muscular weakness, Grade 2 myalgia or muscular weakness lasting >2 weeks) and any other Grade 3/4 non-haematological toxicity (except Grade 3 fever, reversible transaminase elevation, hypersensitivity reaction and nausea/vomiting despite adequate prophylaxis).

Patients evaluable for DLT must have received at least three plitidepsin infusions and have completed the full 6 weeks on a study that comprises this time, or have experienced DLT at any time from the point of the first administration of plitidepsin. All toxicities were graded following the NCI-CTC version 3.0 (http://ctep.cancer.gov/forms/CTCAEv3.pdf). Tumour response was assessed every 6 weeks and evaluated using the World Health Organization (WHO) criteria.[24]

2.4. *Pharmacokinetic analyses*

Serial blood samples for pharmacokinetics (PK) analysis of plitidepsin in plasma were taken during the first cycle prior to, 90 min after the start of the infusion, 10 min before the end of the infusion and then at times 0.25, 0.5, 3, 6, 24, 48 and 96 h after the infusion ended.

Heparinised samples were centrifuged at 4 °C at 1200*g*, and plasma stored at –20 °C until analysis using a validated high performance liquid chromatography system coupled with electrospray ionisation tandem mass spectrometry (HPLC–MS/MS) method.[25] The lower limit of quantification was 0.05 ng/mL. PK results were obtained using non-compartmental analysis and WinNonlin®.

3. Results

3.1. *Patient characteristics*

Between March 2004 and November 2007, 38 out of 41 patients were treated and evaluable for safety. Three registered patients discontinued before treatment initiation due to pain, disease progression or parental refusal. Patients and disease characteristics at baseline are shown in Table 1. The median age at inclusion was 10.0 years (range, 2–17 years), 90% had an ECOG PS of 0–1 and 73% were metastatic. All patients had received prior chemotherapy (median three lines, range 1–10) and 34 had received prior radiotherapy.

Table 1 – Patient and disease characteristics at baseline.

Parameters[a]		Number of patients (%)
No. of patients included/treated	–	41/38
Male/female	–	21 (51%)/20 (49%)
Age (years)	Median (range)	10.0 (2–17)
Performance status (ECOG) or equivalent Lansky play scale[b]	0	32 (80%)
	1	5 (13%)
	2	3 (7%)
Primary tumour histology	Neuroblastoma	8
	Nephroblastoma	4
	Osteosarcoma	4
	Ewing's sarcoma	4
	Medulloblastoma	3
	Rhabdoid tumour	3
	Rhabdomyosarcoma	3
	Anaplastic astrocytoma	2
	Ependymoma	2
	Hepatoblastoma	2
	Other sarcoma	1
	Pancreatoblastoma	1
	Choriod plexus carcinoma	1
	Glioblastoma	1
	Liponeurocytoma	1
	Brain stem glioma	1
Tumour extension[c]	Locally advanced	14 (34%)
	Metastatic	30 (73%)
Prior treatment	Chemotherapy	41 (100%)
	Surgery	40 (98%)
	Radiotherapy	34 (83%)
No. of lines of prior chemotherapy	Median (range)	3 (1–10)

[a] Data shown are n of patients except for median and range values.
[b] One included but not treated patient had no available information for performance status.
[c] Three patients had disease that was both locally advanced and metastatic at baseline. ECOG, Eastern Cooperative Oncology Group.

Table 2 – Distribution of patients and dose-limiting toxicities up to Week 6.

Plitidepsin dose level	Patients treated	Patients evaluable for DLT[a]	Patients with DLT	DLT
Level 1 (4 mg/m^2)	8	6	1	Grade 2 myalgia for more than 2 weeks at cycle 2
Level 2 (5 mg/m^2)	4	3	0	
Level 3 (6 mg/m^2)	7	5	2	Grade 4 CPK increase at cycle 2 Grade 3 ALT increase at cycle 2
Level 2 (5 mg/m^2)	4	3	1	Grade 3 nausea and G 3 vomiting at cycle 1
RD cohort expansion at 5 mg/m^2	15	9	7	Grade 3 myalgia and G 3 dysaesthesia at cycle 1 Grade 3 ALT increase at cycle 2 Grade 3 nausea and G 3 vomiting at cycle 1 Grade 3 CPK increase at cycle 3 Grade 3 CPK increase at cycle 2 Grade 3 CPK increase at cycle 3 Grade 3 CPK increase at cycle 3

ALT, alanine aminotransferase; CPK, creatine phosphokinase; DLT, dose-limiting toxicity; RD, recommended dose.

[a] Evaluable patients had to be treated with three complete plitidepsin administrations or experienced DLT; Non evaluable patients had early progression ($n = 8$) or stopped due to hypersensitivity reaction ($n = 4$); Grade 3 hypersensitivity reaction was considered as severe but not related to dose and thus not considered for dose-escalation procedure

3.2. *Plitidepsin treatment*

A total of 150 infusions were administered in 38 patients at three dose levels (4, 5 and 6 mg/m^2) with a median of three cycles per patient (range, 1–24 cycles) and a median relative dose intensity of 96% (range, 1.1–106%).

A total of 35 cycles were delayed in 14 patients; 16 delays (median, 7 days; range, 1–21) were due to plitidepsin-related toxicity: CPK increase ($n = 7$), transaminase increase ($n = 6$), transaminase and CPK increase ($n = 1$), QTc segment prolongation ($n = 1$) and Grade 2 thrombocytopenia ($n = 1$). Nine patients had the plitidepsin dose reduced in 10 cycles due to CPK increase ($n = 6$) and transaminase increase ($n = 4$). The most common cause for discontinuation was disease progression (35 patients, 78%).

3.3. *Dose-limiting toxicities, maximum tolerated dose and recommended dose*

Of the first three patients treated at dose level 1 (4 mg/m^2), one patient experienced DLT with a Grade 2 myalgia lasting more than 2 weeks (Table 2). None of the three additional evaluable patients at this dose level experienced DLT. No DLTs occurred in the three evaluable patients at level 2 (5 mg/m^2). At level 3 (6 mg/m^2), dose-limiting toxicity (Grade 4 CPK and recurrent Grade 3 hepatic transaminase elevations) was observed in two of five evaluable patients. In consequence, three additional evaluable patients were treated at 5 mg/m^2. One of these experienced dose-limiting Grade 3 nausea/vomiting, but this was in the setting of known anticipatory vomiting. According to the 3 + 3 design, 5 mg/m^2 was considered the RD.

In the expanded cohort at 5 mg/m^2, 15 patients were recruited. Importantly, seven patients experienced Grade 3 CPK increase ($n = 4$), Grade 3 ALT increase ($n = 1$), Grade 3 nausea and vomiting ($n = 1$) and Grade 3 myalgia with dysesthesia ($n = 1$). Biochemical abnormalities that occurred at Cycle 2 or 3 were transient and not associated with clinical symptoms.

3.4. *Toxicity profile*

The adverse events (AEs) most commonly related to plitidepsin at all dose levels were myalgia, gastrointestinal

Table 3 – Treatment-related adverse events (⩾10% of patients)

Adverse events	Per patient ($n = 38$)			Per cycle ($n = 150$)		
	NCI-CTC grade					
	G 1 N (%)	G 2 N (%)	G 3 N (%)	G 1 N (%)	G 2 N (%)	G 3 N (%)
Abdominal pain	3 (8)	2 (5)	1 (3)	5 (3)	2 (1)	1 (1)
Fatigue	10 (26)	4 (11)	–	17 (11)	7 (5)	–
Hypersensitivity	1 (3)	3 (8)	4 (11)	2 (1)	3 (2)	4 (1)
Myalgia	16 (42)	6 (16)	1 (3)	36 (24)	8 (5)	1 (1)
Nausea	10 (26)	1 (3)	2 (5)	20 (13)	1 (1)	2 (1)
Pyrexia	8 (21)	2 (5)	1 (3)	11 (7)	3 (2)	1 (1)
Vomiting	9 (24)	4 (11)	3 (8)	18 (12)	5 (3)	3 (2)
Weight decreased	4 (11)	–	–	5 (3)	–	–

NCI-CTC, National Cancer Institute Common Toxicology Criteria.

Table 4 – Worst on-treatment laboratory abnormalities.

Laboratory abnormalities	Per patient (n = 38)				Per cycle (n = 150)			
	NCI-CTC grade							
	G 1 N (%)	G 2 N (%)	G 3 N (%)	G 4 N (%)	G 1 N (%)	G 2 N (%)	G 3 N (%)	G 4 N (%)
Haematological abnormalities[a]								
Haemoglobin	14 (38)	14 (38)	2 (5)	1 (3)	68 (46)	25 (17)	4 (3)	1 (1)
WBC	12 (32)	2 (5)	–	1 (3)	46 (31)	6 (4)	–	1 (1)
Lymphocytes	11 (30)	10 (27)	5 (14)	–	52 (35)	25 (17)	7 (5)	–
Neutrophils	2 (5)	3 (8)	2 (5)	1 (3)	9 (6)	10 (7)	3 (2)	1 (1)
Platelets	12 (32)	1 (3)	–	1 (3)	20 (13)	2 (1)	–	1 (1)
Biochemical abnormalities[b]								
ALT	11 (30)	6 (16)	12 (32)	–	49 (33)	28 (19)	22 (15)	–
AP	8 (22)	1 (3)	3 (8)	–	28 (19)	2 (1)	4 (3)	–
AST	18 (51)	5 (14)	2 (6)	1 (3)	51 (38)	8 (6)	5 (4)	1 (1)
CPK	6 (17)	2 (6)	5 (14)	1 (3)	12 (9)	2 (1)	6 (4)	1 (1)
Creatinine	7 (19)	–	–	–	10 (7)	–	–	–
Total bilirubin	6 (17)	1 (3)	–	–	7 (5)	1 (1)	–	–

Total number of patients and cycles with data available for each parameter.
ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; NCI-CTC, National Cancer Institute Common Toxicity Criteria; WBC, white blood cells.
[a] Patients: n = 37 and cycles: n = 149 (haemoglobin, WBC, lymphocytes, neutrophils and platelets).
[b] Patients: n = 37 and cycles: n = 148 (ALT); Patients: n = 37 and cycles: n = 146 (AP); Patients: n = 35 and cycles: n = 134 (AST); Patients: n = 37 and cycles: n = 139 (CPK); Patients: n = 37 and cycles: n = 148 (creatinine); Patients: n = 35 and cycles: n = 146 (total bilirubin).

disturbance and non-specific concerns such as fatigue and pyrexia. CTC Grade 3 treatment-related AEs comprised hypersensitivity, vomiting, nausea, abdominal pain, back pain, myalgia, pyrexia and dysaesthesia (Table 3).

It is important to note that eight patients (21.1%) experienced nine (6% of cycles) plitidepsin-related hypersensitivity adverse events, despite the implementation of premedication. It reached Grade 3 in four patients who received one cycle each at a dose of 5 mg/m^2. Two patients experienced G3 allergic reaction early during the administration of Cycle 1, four events occurred during Cycle 2 and three during Cycle 3. In one patient, G1 allergic reaction was noted at Cycle 2 and Grade 3 during Cycle 3. As a result of hypersensitivity adverse events, two patients were withdrawn from the study; two further patients did not receive further plitidepsin treatment due to progressive disease. Four patients had received additional administration of plitidepsin (median 2.5 cycles; range 1–21) after these events and did not experience further allergic reactions.

Reversible transaminase increases were the most common severe biochemical abnormality at all dose levels (Table 4). Most episodes of severe transaminase increase returned to Grade $\leqslant$2 levels within one week. When these were observed, elevations of CPK appeared at 7–14 days after administration of Cycle 2 or 3 and returned to Grade 0/1 within 6–10 days. The most common Grade 3–4 haematological abnormalities,

Table 5A – Non-compartmental pharmacokinetic parameters of plitidepsin in 26 children and adolescents.

Dose level (n)		C_{max} (ng/mL)	Area under the Curve ($AUC_{t-\infty}$) (h*ng/mL)	Plitidepsin clearance (CL L/h/m^2)	HL (h)	Volume of distribution at steady-state (Vss L/m^2)
4 mg/m^2	Mean	22.5	161.9	29.3	20.1	516
(7)	S.Dev.	7.1	61.6	12.3	11.1	263
	Min.	11.4	80.6	15.1	2.1	104
	Max.	34.3	275.8	49.4	35.0	948
	CV %	31	38	42	55	51
5 mg/m^2	Mean	22.3	282.7	23.0	25.6	628
(13)	S.Dev.	8.9	145.8	11.4	7.5	226
	Min.	8.5	105.9	8.8	5.9	234
	Max.	40.3	563.7	47.8	36.8	977
	CV %	40	52	69	29	36
6 mg/m^2	Mean	32.8	278.9	24.3	42.3	902
(5)	S.Dev.	13.1	87.5	9.6	10.2	449
	Min.	13.3	143.2	15.2	27.0	477
	Max.	49.7	396.5	42.1	53.2	1712
	CV %	40	31	40	24	50

HL, half-life; cv%, coefficient of variation (%); C_{max}, maximum concentration.

irrespective of dose level, were Grade 3 lymphopenia, anaemia and neutropenia (Table 4).

3.5. *Efficacy*

Thirty-seven out of 38 treated patients were evaluable for efficacy; one patient was withdrawn from the study due to a plitidepsin-related allergic reaction at Cycle 1.

One partial response (PR), confirmed radiologically at 4 weeks, was found in a 2-year-old boy with metastatic neuroblastoma treated with plitidepsin at an initial dose of 6 mg/m^2. He received a total of nine plitidepsin cycles with a progression-free survival (PFS) of 4.6 months. In addition, a 14-year-old male patient with locally advanced pancreatoblastoma received nine plitidepsin cycles at 4 mg/m^2 and had PR after Cycle 3 which was not confirmed by subsequent imaging. Two patients with neuroblastoma, and one patient with each of hepatoblastoma, medulloblastoma, glioblastoma and rhabdoid tumour had stable disease (SD) as their best response. Disease stabilisation lasted for $\geqslant$3 months for three patients (one each of neuroblastoma, medulloblastoma and glioblastoma), and exceeding 6 months in a malignant teratoid rhabdoid tumour.

3.6. *Pharmacokinetics*

Twenty-six patients had complete plasma samples for PK. Non-compartmental PK evaluation showed high inter-patient variability of plitidepsin exposure, which appeared independent of dose at the three dose levels tested (Table 5A). Furthermore, the PK variability for plitidepsin did not relate to the occurrence of dose-limited toxicity for this patient population (Fig. 1).

There was no significant difference in plitidepsin clearance (Cl) in children ($\leqslant$12 years of age when entering study) with respect to adolescents (>12 but less than 18 years) as described in Table 5B. The elimination of the parent compound, as reflected by half-life, and the volume of distribution at steady-state (Vss) appeared comparable in children and adolescents.

4. Discussion

This first-in-child phase I clinical study of plitidepsin has identified the MTD, RD for phase II, DLT and PK for plitidepsin given as a 3-h intravenous infusion every 2 weeks in patients with refractory or relapsed advanced solid tumours. The starting dose of 4 mg/m^2, equivalent to 80% of the RD found for this schedule in adults, was successively escalated to plitidepsin 5 and 6 mg/m^2.

The recognition that muscular toxicity can have a delayed onset for adults receiving plitidepsin led to our caution in the design of this first clinical study to involve children. The period time that toxicity could determine the DLT and MTD/RD for plitidepsin was lengthened from the traditional cut-off of 3–4 weeks following first exposure to drug, and defined *a priori* as an initial 6 weeks (three completed cycles of treatment) of observation. This did not influence the selection of the RD during the cohort escalation phase, but did serve to highlight the tendency for muscle-related toxicity to occur between weeks 4 and 6. A high number of patients were considered as non-evaluable for DLT, for reasons of not having completed their first three cycles of plitidepsin either because of early tumour progression (n = 8) or hypersensitivity reactions (n = 4). However, this problem was mainly encountered in the second phase of this present study, where an additional 15 patients were treated at the RD to further characterise the toxicity profiles and any PK associations.

All of the DLT's encountered by patients in this phase I trial with expansion at the RD were reversible and of a non-cumulative pattern, and comprised Grade 3–4 CPK elevations, Grade 3 transaminase elevations, nausea/vomiting (found only for two patients with known problems with anticipatory vomiting) and Grade 2–3 myalgia. Of note, transient and clinically asymptomatic Grade 3–4 CPK and transaminase increase were the reason for all dose reductions and the majority of dose delays observed during the conduct of this study.

The safety profile of plitidepsin and the DLTs found here were comparable to those reported for plitidepsin in adults.[14–16,26] Myalgia, vomiting, nausea, fatigue and pyrexia were the most observed AEs but they reached Grade 3 in very few cases; whereas lymphocytopaenia and an increase in

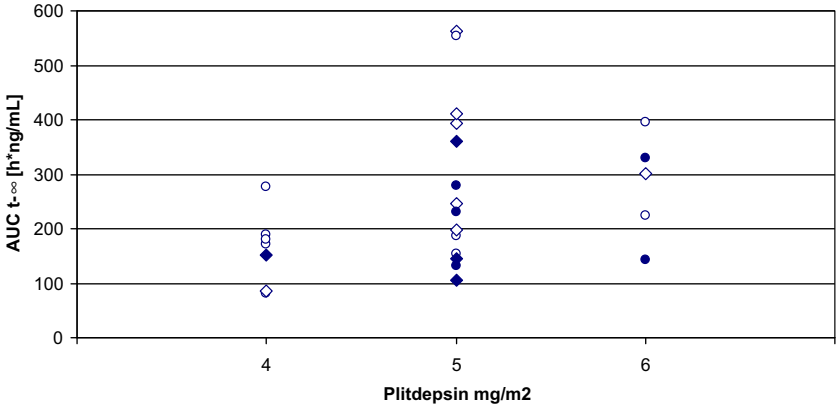

Fig. 1 – Non-compartmental analysis of Area under the Curve (AUC) until infinity of plitidepsin stratified by dose level in 26 patients after the first plitidepsin administration. Children ($\leqslant$12 years of age) are marked in diamonds, adolescents (>12 but less than 18 years) in circles. Patients experiencing dose-limiting toxicity are marked as filled symbols.

Table 5B – Non-compartmental pharmacokinetic parameters of plitidepsin according to age in 14 children (⩽12 years of age) and 12 adolescents (>12 but less than 18 years).

Age		Plitidepsin clearance (CL L/h/m^2)	HL (h)	Volume of distribution at steady-state (Vss L/m^2)
All	Mean	24.9	27.3	777
(26)	S.Dev.	11.7	11.9	408
	Min.	8.8	2.1	123
	Max.	49.4	53.2	1593
	CV %	47	44	53
Children	Mean	23.0	29.1	632
(14)	S.Dev.	11.4	14.5	390
	Min.	8.8	2.1	123
	Max.	47.8	53.2	1593
	CV %	51	50	62
Adolescents	Mean	24.8	25.2	947
(12)	S.Dev.	10.4	7.3	360
	Min.	8.8	7.7	459
	Max.	49.4	35.0	1518
	CV %	42	29	38

transaminases and CPK were frequent laboratory abnormalities attributed to plitidepsin in this study.

No unexpected toxicities or toxic deaths were reported in children. Nevertheless, a higher incidence of Grade 3 hypersensitivity reactions (10% of patients) was observed in children with respect to adults (3%), despite the mandatory prophylactic medication.

For the previous trials conducted in the adult cancer setting, plitidepsin-induced musculoskeletal disorders (muscle cramps and weakness associated with type II diffuse muscular fibre atrophy and late increase in CPK),[26] that had been the most commonly reported DLT, a phenomenon that may have related to concomitant therapy with dexamethasone[20] and could be modulated by the administration of L-carnitine.[15,27] For this present paediatric study, most episodes of muscular toxicity were mild-to-moderate, and only one patient at 5 mg/m^2 presented self-limiting Grade 3 myalgia, which resolved without sequelae. Episodes of CPK elevation were not associated with clinical symptoms of muscular or renal toxicity.

Plitidepsin was otherwise well tolerated by the children recruited to this study. Grade 3–4 haematological abnormalities were infrequent and, as with the other toxicities described, there was no evidence of a dose-relationship and with little effect on treatment.

Investigation of the PK disposition of plitidepsin in children revealed that there were no differences seen between children and adolescents for Cl and Vss corrected by BSA derived from non-compartmental analysis, and which were comparable to those reported in adults.[15] Plitidepsin exposure was independent from the three dose levels and not correlated with DLT occurrence.

In keeping with the general experience of phase I studies in the paediatric setting,[28] some evidence of clinical benefit in terms of disease response or stability was found for eight of the 38 children that were evaluable for response.

In conclusion, this phase I study has demonstrated that it is feasible to administer plitidepsin in the setting of children and adolescents with advanced solid tumours, and that, although the toxicities encountered were in keeping with the adult experience described previously, there were fewer reports of significant clinical muscular toxicities for children. The pharmacokinetic disposition of plitidepsin is not age-dependent, is similar to that found for adults and does not relate to the occurrence of DLT. The RD for plitidepsin, administered as a 3-h infusion every 2 weeks to paediatric cancer patients, is 5 mg/m^2. Future phase II studies of plitidepsin are warranted, and our results suggest that plitidepsin could be appropriately developed in combination with other antitumour where myelosuppression is dose-limiting.

Conflict of interest statement

None declared.

Acknowledgments

The authors would like to thank all patients and their parents who participated in the trial, and to acknowledge the work done by Adnan Tanovic (medical writer, Pharma Mar), Antonio Nieto (statistician, Pharma Mar), Arturo Soto (clinical pharmacology manager Pharma Mar) and Iratxe Pérez (clinical research associate, Pharma Mar).

Supported by Pharma Mar, Colmenar Viejo, Madrid, Spain.

REFERENCES

1. Rinehart KL, Lithgow-Berelloni AM. Novel Antiviral and Cytotoxic Agent. *PCT Int. Pat. Appl.* WO 91.04985, Apr. 18, 1991; GB Appl. 89/22,026, Sept. 29, 1989. Chem Abstr. 1991,115.
2. Depenbrock H, Peter R, Faircloth GT, et al. In vitro activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells. *Br J Cancer* 1998;**78**:739–44.
3. Garcia-Fernandez LF, Losada A, Alcaide V, et al. Aplidin induces the mitochondrial apoptotic pathway via oxidative stress-mediated JNK and p38 activation and protein kinase C delta. *Oncogene* 2002;**21**:7533–44.
4. Muñoz-Alonso MJ, González-Santiago L, Zarich N, et al. Plitidepsin has a dual effect inhibiting cell cycle and inducing apoptosis via Rac1/c-Jun NH2-terminal kinase activation in human melanoma cells. *J Pharmacol Exp Ther* 2008 Mar;**324**:1093–101.
5. Cuadrado A, Garcia-Fernandez LF, Gonzalez L, et al. Aplidin induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and p38 MAPK. *J Biol Chem* 2003;**278**:241–50.
6. Gonzalez-Santiago L, Suarez Y, Zarich N, et al. Aplidin induces JNK-dependent apoptosis in human breast cancer cells via alteration of glutathione homeostasis, Rac1 GTPase activation, and MKP-1 phosphatase downregulation. *Cell Death Differ* 2006;**13**:1968–81.
7. Broggini M, Marchini SV, Galliera E, et al. Aplidine, a new anticancer agent of marine origin, inhibits vascular endothelial growth factor (VEGF) secretion and blocks VEGF-

VEGFR-1 (flt-1) autocrine loop in human leukemia cells MOLT-4. *Leukemia* 2003;**17**:52–9.
8. Straight AM, Oakley K, Moores R, et al. Aplidin reduces growth of anaplastic thyroid cancer xenografts and the expression of several angiogenic genes. *Cancer Chemother Pharmacol* 2006;**57**:7–14.
9. Biscardi M, Caporale R, Balestri F, et al. VEGF inhibition and cytotoxic effect of aplidin in leukemia cell lines and cells from acute myeloid leukemia. *Ann Oncol* 2005;**16**:1667–74.
10. Bresters D, Broekhuizen AJ, Kaaijk P, et al. In vitro cytotoxicity of aplidin and crossresistance with other cytotoxic drugs in childhood leukemic and normal bone marrow and blood samples: a rational basis for clinical development. *Leukemia* 2003;**17**:1338–43.
11. Morton CL, Houghton PJ, Gorlick R, et al. Initial testing of aplidin by the pediatric pre-clinical testing program. *Pediatr Blood Cancer* 2009;**53**:509–12.
12. Anthoney A, Paz-Ares L, Twelves C. Phase I and pharmacokinetic (PK) study of Aplidin (APL) using a 24-hour, weekly schedule. *Proc Am Soc Clin Oncol* 2000;**19**. abstr. 734.
13. Ciruelos EM, Twelves C, Dominguez MJ, Mckay H, Anthony A, Castellanos D. Phase I clinical and pharmacokinetic study of the marine compound Aplidin (APL) administered as a 3 hour infusion every 2 weeks. *Proc Am Soc Clin Oncol* 2002;**21**. abstr. 422.
14. Izquierdo MA, Bowman A, García M, et al. Phase I clinical and pharmacokinetic study of plitidepsin as a 1-hour weekly intravenous infusion in patients with advanced solid tumors. *Clin Cancer Res* 2008;**14**:3105–12.
15. Faivre S, Chieze S, Delbaldo C, et al. Phase I and pharmacokinetic study of aplidine, a new marine cyclodepsipeptide in patients with advanced malignancies. *J Clin Oncol* 2005;**23**:7871–80.
16. Maroun JA, Belanger K, Seymour L, et al. Phase I study of Aplidine in a dailyx5 one-hour infusion every 3 weeks in patients with solid tumors refractory to standard therapy. A National Cancer Institute of Canada Clinical Trials Group study: NCIC CTG IND 115. *Ann Oncol* 2006 Sep;**17**:1371–8.
17. Dumez H, Gallardo E, Culine S, et al. Phase II study of biweekly plitidepsin as second-line therapy for advanced or metastatic transitional cell carcinoma of the urothelium. *Mar Drugs* 2009;**7**:451–63.
18. Eisen T, Thomas J, Miller Jr WH, et al. Phase II study of biweekly plitidepsin as second-line therapy in patients with advanced malignant melanoma. *Melanoma Res* 2009;**19**:185–92.
19. Baudin E, Droz JP, Paz-Ares L, et al. Phase II study of plitidepsin 3-hour infusion every 2 weeks in patients with unresectable advanced medullary thyroid carcinoma. *Am J Clin Oncol* 2010 Feb;**33**:83–8.
20. Mateos MV, Cibeira MT, Richardson PG, et al. Phase II clinical and pharmacokinetic study of plitidepsin 3-hour infusion every two weeks alone or with dexamethasone in relapsed and refractory multiple myeloma. *Clin Cancer Res* 2010;**16**:3260–9.
21. Peschel C, Hartmann JT, Schmittel A, et al. Phase II study of plitidepsin in pretreated patients with locally advanced or metastatic non-small cell lung cancer. *Lung cancer* 2008;**60**:374–80.
22. Smith M, Bernstein M, Bleyer WA, et al. Conduct of phase I trials in children with cancer. *J Clin Oncol* 1998;**16**:966–78.
23. Gralla R, Osoba D, Kris M, et al. Recommendations for the use of antiemetics: evidence-based, clinical practice guidelines. American Society of Clinical Oncology. *J Clin Oncol* 1999;**17**:2971–94.
24. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;**47**:207–14.
25. Celli N, Mariani B, Di Carlo F, et al. Determination of aplidin, a marine-derived anticancer drug, in human plasma, whole blood and urine by liquid chromatography with electrospray ionisation tandem mass spectrometric detection. *J Pharm Biomed Anal* 2004;**34**:619–30.
26. Jimeno J, Lopez-Martin JA, Ruiz-Casado A, et al. Progress in the clinical development of new marine-derived anticancer compounds. *Anticancer Drugs* 2004;**15**:321–9.
27. Schoffski P, Guillem V, Garcia M, et al. Phase II randomized study of plitidepsin (aplidin), alone or in association with L-carnitine, in patients with unresectable advanced renal cell carcinoma. *Mar Drugs* 2009;**7**:57–70.
28. Estlin EJ, Cotterill S, Pratt CB, Pearson AD, Bernstein M. Phase I trials in pediatric oncology: perceptions of pediatricians from the United Kingdom Children's Cancer Study Group and the Pediatric Oncology Group. *J Clin Oncol* 2000;**18**:1900–5.